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CONTENT

Agronomy and Forestry Sciences

- 1 Effects of 6-benziladenine, thidiazuron, and 1-naphthaleneacetic acid on the *in vitro* callus formation and shoot multiplication from leaves of Mai vang (*Ochna integerrima*) HD01 line
Kiet C. Nguyen, Duy M. Pham, Van H. Phan, Tri M. Bui, & Hau T. Le

Animal Sciences, Veterinary Medicine, Aquaculture and Fisheries

- 13 Prevalence and antibiotic resistance of *Escherichia coli* isolated from the respiratory tract of goats in Can Tho city, Vietnam
Thuan K. Nguyen, Ninh T. K. Truong, Vy L. P. Nguyen, Trung T. Truong, & Thuong T. Nguyen
- 24 Classification of pet owners based on knowledge attitude and practice about rabies and its vaccination in Duc Hue, Long An province during the period 2021 - 2023
Bao D. Truong, Dung T. T. Nguyen, Mai T. Duong, Phuong H. T. To, Son H. Ly, Minh D. Vo, Loan B. P. Tran, Trang P. T. Nguyen, Thong Q. Le, Linh N. Nguyen, Tuyen V. Cao, Thanh Vo, Oanh K. T. Vo, & Khanh T. M. Nguyen

Biotechnology

- 38 Optimization of alkali-catalyzed organosolv treatment of spent coffee grounds for obtaining polysaccharides
Duong T. T. Nguyen, Sang V. Nguyen, Dong N. T. Le, Anh T. V. Nguyen, & Ly T. P. Trinh

Environment and Natural Resources

- 50 Aerogel materials from sugarcane bagasse: the effect of additives
Van T. T. Le, Ty H. Nguyen, Chien M. D. Vu, & Phuong T. Nguyen
- 57 Community-based ecotourism: A case study in Thanh Phu district, Ben Tre province, Vietnam
Thinh V. D. Nguyen, Vy T. H. Nguyen, Nhi T. C. Bui, & Diem T. N. Phan

Effects of 6-benziladenine, thidiazuron, and 1-naphthaleneacetic acid on the *in vitro* callus formation and shoot multiplication from leaves of Mai vang (*Ochna integerrima*) HD01 line

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ABSTRACT

Mai vang (*Ochna integerrima* (Lour.) Merr.) not only is a popular ornamental plant but also possesses numerous valuable herbal properties. An exceptional line, the HD01, was bred by adept florists at the Binh Loi Mai village, Ho Chi Minh City. The objective of this research was to determine the optimal 6-benziladenine (BA), thidiazuron (TDZ) and 1-naphthaleneacetic acid (NAA) concentrations appropriate for callus formation from leaf samples and regeneration of shoots from callus of the HD01 line in *in vitro* condition. The study including two experiments was arranged in a completely randomized design with two factors. For callus induction, the culture medium supplemented with concentrations of TDZ combined with concentrations of NAA was used, while to induce shoots, concentrations of BA combined with concentrations of NAA were added to the medium. The results indicated that 0.2 mg/L TDZ combined with 0.25 mg/L NAA on the base of MS (Murashige and Skoog) medium supplemented with 7 g/L agar and 30 g/L sugar gave 100% callus formation rate after 17.6 days, with the largest callus diameter (2.7 cm) and highest callus mass (2.4 g) after 60 days of culture. The leaf-derived callus produced the best results when cultured on MS medium supplemented with 7 g/L agar, 30 g/L sugar, and a combination of 1 mg/L BA and 0.25 mg/L NAA, forming shoot clusters with the height of 2.2 cm, an average of 8.5 shoots per cluster and 9.7 leaves per cluster after 60 days of culture. Based on these results, a preliminary protocol for Mai vang HD01 micropropagation was established.

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1. Introduction

The Mai vang tree (*Ochna integerrima*) is an important ornamental plant in Vietnam. Native to the South East Asia region, the plant is seen as a symbol for the Lunar New Year due to the flowering time usually falling in the holiday. Therefore, *O. integerrima* has become one of the most widely cultivated and traded ornamental plants during the Lunar New Year. Many elite lines and varieties are produced by breeders and growers every year, each with particular superior traits. Among them, the HD01 line selected at Huu Duc mai garden in Binh Loi Mai village has many outstanding characteristics, such as large flower diameter (up to 4 - 6 cm), extended flowering period, and darker petal and stamen color than other lines.

In conventional cultivation, *O. integerrima* is propagated mainly by seeds. However, genetic variation makes it difficult to create uniform seedlings, and sometimes the daughter plants no longer retain the value of the parents. Clonal propagation methods such as cutting, air-layering or grafting are also frequently employed for *O. integerrima*, but with low multiplication rates and efficiencies. *In vitro* propagation techniques have the advantage of high propagation rates as well as high uniformity of seedlings, so it has been applied to propagate many plant species with different economic values (Tran, 2005). There have been a few studies on *in vitro* propagation of *O. integerrima* both domestically and internationally, but few have managed to employ cell dedifferentiation and callus formation from leaves to create high propagation rates and utilize the more abundant starting materials. Furthermore, although there has been some success in *in vitro* propagation of *O. integerrima*, the protocols need adjustment, optimizing the mineral contents and plant growth regulators depending on each particular variety or line.

Among the plant growth regulators, auxin and cytokinin play an important role in plant *in vitro* morphogenesis, including callus, shoot and root formation. 6-benziladenine (BA), thidiazuron (TDZ) and 1-naphthaleneacetic acid (NAA) are common plant growth regulators frequently employed in micropropagation. The combinations of types and concentrations of these plant growth regulators are usually the deciding factor in the morphogenesis and regeneration of plant cell *in vitro*, particularly for *O. integerrima*. Each stage of micropropagation may require very different and specific combinations (Ho et al., 2019). Therefore, the objective of this study was to determine the most appropriate combination for establishing the calluses from leaves of the HD01 line and regeneration of these calluses to shoot clusters.

2. Materials and Methods

2.1. Time and place of the study

The study was carried out from March to July 2023, at the tissue culture lab of the Department of Physiology and Biochemistry, Faculty of Agronomy, Nong Lam University, Ho Chi Minh City, Vietnam.

2.2. Experimental design

2.2.1. Experiment 1: Effects of TDZ and NAA concentrations on the induction of callus formation from *O. integerrima* leaf samples

Experiment 1 had two factors: factor T (TDZ concentration): 0.1 mg/L; 0.2 mg/L; 0.3 mg/L; 0.4 mg/L, and factor N (NAA concentration): 0 mg/L; 0.25 mg/L; 0.5 mg/L. The experiment consisted of 12 treatments with 3 replications and arranged in a completely randomized design. Each replicate had 5 flasks. Each flask had 3 samples.

2.2.2. Experiment 2: Effects of BA and NAA concentrations on shoot regeneration from callus of *O. integerrima*

Experiment 2 had two factors: factor B (BA concentration): 0 mg/L; 1 mg/L; 2 mg/L; 3 mg/L, and factor N (NAA concentration): 0 mg/L; 0.25 mg/L; 0.5 mg/L. The experiment consisted of 12 treatments with 3 replications and arranged in a completely randomized design. Each replicate had 5 flasks. Each flask had 3 samples.

2.3. Experimental method

The base medium in both experiments was Murashige and Skoog (MS) supplemented with 7 g/L agar, 30 g/L sucrose, and plant growth regulators according to treatments.

In experiment 1, leaves of 8 - 10 cm in length and 4 - 5 cm in width were disinfected with sodium hypochlorite (made in Vietnam) for 20 min and rinsed 3 times with sterilized water. The undamaged sections of the leaves with veins were then cut into 1×1 cm and put in the flasks face-down on the medium and kept in the dark for 60 days.

In experiment 2, calluses were produced in large quantity following the best treatment in experiment 1. After 60 days in the dark, the calluses were then put under light (2500 ± 500 lux) for another 10 days. Green, compact and dry calluses were selected, and small samples of these calluses with the diameter of 0.5 cm were dissected and used as starting materials to be transplanted into the flasks for experiment 2. The calluses were grown for 60 days.

2.4. Parameters

2.4.1. Experiment 1

The samples were monitored every day and the day of first callus formation was recorded

for each treatment. Colors of the calluses were recorded every 10 days, and the calluses were considered dead when they turned dark brown. On day 60 of culture, the largest diameters (cm) and fresh weights (mg) of 10 random samples were measured. The callus formation rate (%) in each treatment on day 60 was calculated as the number of samples that successfully formed calluses divided by the total number of samples (45). Samples were considered successfully forming callus when undifferentiated, white or yellowish/yellow cell clusters could be observed on the leaf samples.

2.4.2. Experiment 2

The number of shoots and number of leaves per cluster and the height of each shoot cluster (cm) were recorded through the glass layer of the flask every 10 days. Time of shoot formation was defined as the day when more than 50% of the samples formed shoot buds in each treatment. Fresh weights of shoot clusters (mg) were recorded on day 60.

2.5. Data processing and analyses

Data were processed with Excel 2010 (Microsoft, USA). R (version 4.2.3) was used to perform ANOVA and rank the treatments with Duncan's multiple range test at $\alpha = 0.01$ or $\alpha = 0.05$. Data on the rate of callus formation in experiment 1 were transformed using the formula before statistical analysis, the value 0 replaced with $1/4 n$ and 100 replaced with $100 - 1/4 n$ (with $n = 15$ samples) before the formula was applied. Data on the diameter of callus in experiment 1, number of shoots, shoot cluster height and number of leaves in experiment 2 were transformed using the formula before statistical analysis (Ngo et al., 2013).

3. Results and Discussion

3.1. Effect of TDZ and NAA concentrations on the induction of callus from leaf samples of *O. integerrima*

3.1.1. Effect of TDZ and NAA concentrations on the rate and time of callus formation

Table 1. Effect of thidiazuron (TDZ) and 1-naphthaleneacetic acid (NAA) concentrations on the rate (%) and time (days) of callus formation from leaf samples of *O. integerrima* HD01

	TDZ concentration (mg/L)	NAA concentration (mg/L)			Average (T)
		0	0.25	0.5	
Rate of callus formation (%)	0.1	0	100	93.3	64.4
	0.2	0	100	84.4	61.5
	0.3	0	100	84.4	61.5
	0.4	0	100	82.2	60.7
Average (N)		0 ^c	100 ^a	86.1 ^b	
CV (%) = 17.4%; $F_T = 0.3^{ns}$; $F_N = 299.2^{**}$; $F_{T*N} = 0.3^{ns}$					
Time of callus formation (days)	0.1	-	18.2	17.8	-
	0.2	-	17.6	17.9	-
	0.3	-	17.7	17.8	-
	0.4	-	18.1	18.3	-
Average (N)		-	17.9	18.0	-

Within the same group, the same letter following the means represents statistically insignificant difference. ns: statistically insignificant difference; **: significant difference at $P \leq 0.01$.

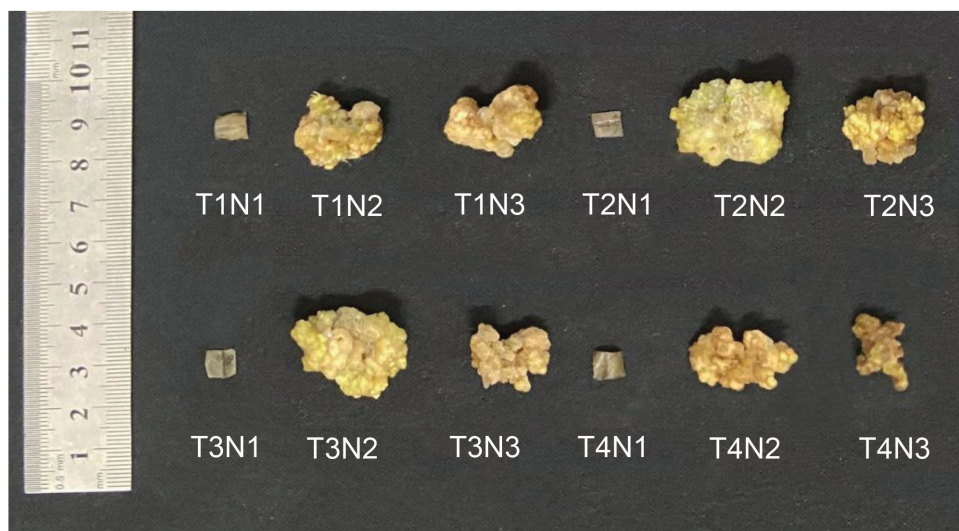


Figure 1. Callus samples of the treatments after 60 days of culture. T1-4: thidiazuron concentration of 0.1, 0.2, 0.3 or 0.4 mg/L, respectively; N1-3: 1-naphthaleneacetic acid concentration of 0, 0.25 or 0.5 mg/L, respectively.

On day 60, the results of Table 1 shows that the TDZ concentration had no effect on the rate of callus formation. However, the NAA concentration significantly affected the rate of callus formation (Figure 1). In particular, the concentration of 0.25 mg/L NAA gave the best result, achieving 100% callus formation rate, significantly higher than the rates of the remaining concentrations. Considering the interaction between TDZ and NAA concentrations, the rate of callus formation was not statistically different, ranging from 82.2% to 100%. The combination of TDZ and NAA has been reported to result in

high rates of callus formation in some *in vitro* plants (Verma, 2016; Karakas, 2020). The data of this experiment show similar results. Depending on the type of plants, the most appropriate ratio of TDZ and NAA for callus formation may differ (Lu et al., 2023).

Callus formation time in the treatments ranged from 17.6 days to 18.3 days. In particular, the treatment supplemented with 0.2 mg/L TDZ combined with 0.25 mg/L NAA gave the fastest callus formation time (17.6 days). However, no statistically significant differences were found.

3.1.2. Effect of TDZ and NAA concentrations on color of callus during culture period

Table 2. Effect of thidiazuron (TDZ) and 1-naphthaleneacetic acid (NAA) concentrations on color of callus from leaf samples of *O. integerrima* HD01

TDZ concentration (mg/L)		NAA concentration (mg/L)		
		0	0.25	0.5
Day 20	0.1	-	Light yellow	Light yellow
	0.2	-	Light yellow	Light yellow
	0.3	-	Light yellow	Light yellow
	0.4	-	Light yellow	Light yellow
Day 30	0.1	-	Light yellow	Light yellow
	0.2	-	Light yellow	Light yellow
	0.3	-	Light yellow	Light yellow
	0.4	-	Light yellow	Light yellow
Day 40	0.1	-	Yellow	Dark yellow
	0.2	-	Yellow	Dark yellow
	0.3	-	Yellow	Dark yellow
	0.4	-	Yellow	Dark yellow
Day 50	0.1	-	Yellow	Dark yellow
	0.2	-	Yellow	Dark yellow
	0.3	-	Yellow	Dark yellow
	0.4	-	Dark yellow	Dark yellow
Day 60	0.1	-	Dark yellow	Yellow-brown
	0.2	-	Yellow	Golden brown
	0.3	-	Yellow	Pale brown
	0.4	-	Yellow-brown	Brown

As indicated in Table 2, no callus formation was observed in treatments using only TDZ over 60 days of culture. After 20 - 30 days of culture, treatments with combination of TDZ and NAA at any concentrations produced light yellow calluses.

Most distinctions in callus color were observed on day 60, when calluses in all treatments using 0.5 mg/L NAA at any TDZ concentrations showed signs of browning, as well as calluses in treatment with 0.4 mg/L TDZ and 0.25 mg/L NAA which also turned yellow brown. This indicates high concentration of NAA or TDZ induced early aging of the calluses in *O. integririma*.

3.1.3. Effects of TDZ and NAA concentrations on diameter and fresh weight of callus

Both single factor and interaction between two factors had statistically significant effects on diameter and fresh weight of callus. Regarding the interaction between these two factors (Table 3), at the NAA concentration of 0.25 mg/L, both diameter and fresh weight of callus were improved as TDZ concentration increased from 0.1 mg/L to 0.2 or 0.3 mg/L. Interestingly, at the NAA concentration of 0.5 mg/L, no significant change in diameter and fresh weight of callus when TDZ concentration changed was observed. This could indicate that the effect of NAA at this concentration was too strong and masking the effect of TDZ.

Table 3. Effects of thidiazuron (TDZ) and 1-naphthaleneacetic acid (NAA) concentrations on diameter (cm) and fresh weight (g) of *O. integririma* HD01 samples after 60 days of culture

		NAA concentration (mg/L)			Average (T)
		0	0.25	0.5	
Callus diameter (cm)	0.1	0 ^c	1.8 ^b	1.9 ^b	1.2 ^{ab}
	0.2	0 ^c	2.7 ^a	1.8 ^b	1.5 ^a
	0.3	0 ^c	2.6 ^a	1.8 ^b	1.5 ^a
	0.4	0 ^c	1.9 ^b	1.6 ^b	1.2 ^b
Average (N)		0 ^c	2.3 ^a	1.8 ^b	
CV = 5.5%; F _T = 4.1 [*] ; F _N = 618.3 ^{**} ; F _{T*N} = 3.9 ^{**}					
Fresh weight of samples (g)	0.1	0 ^c	1.4 ^b	1.4 ^b	1.0 ^b
	0.2	0 ^c	2.4 ^a	1.4 ^b	1.3 ^a
	0.3	0 ^c	2.4 ^a	1.3 ^b	1.2 ^a
	0.4	0 ^c	1.5 ^b	1.2 ^b	0.9 ^b
Average (N)		0 ^c	1.9 ^a	1.3 ^b	
CV (%) = 11.6%; F _T = 20.9 ^{**} ; F _N = 731.6 ^{**} ; F _{T*N} = 19.7 ^{**}					

Within the same group, the same letter following the means represents statistically insignificant difference. *: significant difference at $0.01 < P \leq 0.05$; **: significant difference at $P \leq 0.01$.

On the other hand, the leaf samples all failed to form any callus when no NAA was supplemented, regardless of the TDZ concentration. For the formation of callus in many plant species, auxin and cytokinin concentrations need to be at a certain balance (Ikeuchi et al., 2013). The interaction between auxin and cytokinin is

especially important to control several processes of formation, development and maintenance of meristems necessary to create the plant body (Su et al., 2011). Nitsch (1986) asserted that only the simultaneous action of auxin and cytokinin can strongly stimulate DNA synthesis and induce cell division. While TDZ has been reported to exhibit

both cytokinin and auxin effects, the cytokinin activity is still much more potent, which is why TDZ is still widely considered a cytokinin (Ahmad & Faisal, 2018). This could explain why using TDZ alone was shown to be insufficient to generate callus in *O. integerrima* in this study.

3.2. Effects of the combination of BA and NAA on shoot regeneration from callus of *O. integerrima* HD01

3.2.1. Effects of BA and NAA concentrations on the number of shoots generated from callus

Table 4 shows the number of shoots in the shoot clusters generated from calluses of *O. integerrima* during the culture period of 60 days. On day 10 - 20, the shoot buds were too small to be distinguished accurately with naked eyes, therefore these data were omitted.

Table 4. Effects of 6-benziladenine (BA) and 1-naphthaleneacetic acid (NAA) concentrations on the number of shoots (shoots/cluster) generated from callus of *O. integerrima* HD01

Days of culture	BA concentrations (mg/L)	NAA concentrations (mg/L)			Average (B)
		0	0.25	0.5	
30	0	0 ^d	0.4 ^{cd}	0.5 ^c	0.3 ^c
	1	3.7 ^b	7.9 ^a	3.3 ^b	4.9 ^a
	2	2.9 ^b	3.3 ^b	0.2 ^{cd}	2.1 ^b
	3	3.0 ^b	3.1 ^b	0.8 ^{cd}	2.3 ^b
Average (N)		2.4 ^b	3.7 ^a	1.21 ^b	
CV (%) = 6.4%; $F_B = 311.4^{**}$; $F_N = 183.0^{**}$; $F_{B*N} = 42.8^{**}$					
40	0	0 ^e	0.4 ^d	0.5 ^d	0.3 ^c
	1	4.1 ^b	8.3 ^a	3.5 ^{bc}	5.3 ^a
	2	3.0 ^c	3.6 ^{bc}	0.3 ^{de}	2.3 ^b
	3	3.0 ^c	3.3 ^{bc}	0.3 ^{de}	2.2 ^b
Average (N)		2.5 ^b	3.88 ^a	1.13 ^c	
CV (%) = 5.8%; $F_B = 385.2^{**}$; $F_N = 221.5^{**}$; $F_{B*N} = 47.9^{**}$					
50	0		0.5 ^c	0.5 ^c	0.3 ^c
	1		8.3 ^a	3.6 ^b	5.4 ^a
	2		3.8 ^b	0.4 ^c	2.5 ^b
	3		3.7 ^b	0.3 ^c	2.4 ^b
Average (N)			4.1 ^a	1.2 ^c	
CV (%) = 8.2%; $F_B = 186.5^{**}$; $F_N = 108.7^{**}$; $F_{B*N} = 23.8^{**}$					
60	0	0 ^d	0.5 ^{cd}	0.7 ^c	0.4 ^c
	1	4.3 ^b	8.5 ^a	3.7 ^b	5.5 ^a
	2	3.3 ^b	3.9 ^b	0.5 ^{cd}	2.6 ^b
	3	3.2 ^b	3.8 ^b	0.4 ^{cd}	2.5 ^b
Average (N)		2.7 ^b	4.2 ^a	1.3 ^c	
CV (%) = 8.1%; $F_B = 177.8^{**}$; $F_N = 98.0^{**}$; $F_{B*N} = 23.6^{**}$					

Within the same group, the same letter following the means represents statistically insignificant difference; **: significant difference at $P \leq 0.01$.

The results show that as early as day 30, when the shoot buds were distinguishable, significant differences among treatments due to the effects of each factor and the interaction between two factors could be observed, and these differences maintained until the end of the culture period (day 60). The numbers of shoots only slightly increased in all treatments from day 30 to day 40, with the largest increase in treatment with 1 mg/L BA and no NAA (from 3.7 to 4.1 shoots, equivalent to a 10.8% increase), and beyond day 40, they remained largely unchanged. This indicates that the reprogramming of different *O. integerrima* callus cells to shoot meristems happened simultaneously soon after the exposure to the change in the growth regulator environment. Maintaining the growth regulators might still be necessary for the development of these shoots, but very few new shoot meristems were formed in the later half of the culture period. This information may be useful for future studies on the cellular and molecular mechanisms of the cell morphogenesis in *O. integerrima* under the effects of plant growth regulators.

The trend in the difference of number of shoots among treatments was also largely the same throughout the culture period. When

no BA was used, very few shoots were formed regardless of the NAA concentration, which demonstrates the important role of BA in reprogramming the unorganized callus cells into shoot meristems that could develop into shoot buds. At high concentrations of both BA (2 or 3 mg/L) and NAA (0.5 mg/L), the *O. integerrima* calluses also generate very low number of shoots, which suggests that these concentrations had become excessive for *O. integerrima* cells and inhibited the regeneration of shoots. The remaining combinations of BA and NAA concentrations resulted in statistically similar number of shoots regenerated from the calluses, except for the combination of 1 mg/L BA and 0.25 mg/L NAA, which gave the highest number of shoots regenerated (8.5 shoots/cluster on day 60), statistically higher than all other treatments (Figure 2). These results are consistent with the research of Ho et al. (2019) on stem cuttings, which showed the combination of 1.5 mg/L BA and 0.5 mg/L NAA achieved the highest number of shoots (3.4 shoots) regenerated from the original culture. Compared with the results of Mai & Lam (2013), the shoot multiplication rate in this study was much higher (8.5 compared to 2.3), which proves that the combination of BA and NAA gives better results than using BA alone.



Figure 2. *In vitro* *O. integerrima* shoots after 60 days of culture in different combinations of 6-benziladenine (BA) and 1-naphthaleneacetic acid (NAA) concentrations. B1-4: BA concentration of 0, 1, 2 or 3 mg/L, respectively; N1-3: NAA concentration of 0, 0.25 or 0.5 mg/L, respectively.

3.2.2. Effect of BA and NAA concentrations on the height and number of leaves of the shoot cluster generated from callus

Unlike the number of shoots, the height of shoot cluster, which represents the growth of shoots, shows gradual increase in some treatments, specifically treatments with 1

mg/L BA at all NAA concentrations, as well as treatment with 2 mg/L BA combined with 0.25 mg/L NAA, over the culture period of 60 days (Table 4). In the rest of the treatments, very low increase in shoot cluster height was observed, indicating that very little growth of the shoots happened in these treatments.

Table 5. Effects of 6-benziladenine (BA) and 1-naphthaleneacetic acid (NAA) on shoot cluster height (cm) of *O. integerrima* HD01

Days of culture	BA concentration (mg/L)	NAA concentration (mg/L)			Average (B)
		0	0.25	0.50	
30	0	0 ^c	0 ^c	0 ^c	0 ^b
	1	0.8 ^{ab}	1.2 ^a	0.5 ^{abc}	0.8 ^a
	2	0 ^c	0.4 ^{bc}	0 ^c	0.1 ^b
	3	0.1 ^c	0.1 ^c	0 ^c	0.1 ^b
	Average (N)	0.8 ^b	0.4 ^a	0.13 ^b	
CV (%) = 13.9%; $F_B = 22.8^{**}$; $F_N = 5.0^*$; $F_{B*N} = 1.1^{ns}$					
40	0	0 ^d	0 ^d	0 ^d	0 ^b
	1	1.0 ^{ab}	1.4 ^a	0.7 ^{abc}	1.0 ^a
	2	0 ^d	0.6 ^{bc}	0 ^d	0.2 ^b
	3	0.2 ^{cd}	0.3 ^{cd}	0 ^d	0.2 ^b
	Average (N)	0.3 ^b	0.6 ^a	0.2 ^b	
CV (%) = 13.1%; $F_B = 30.5^{**}$; $F_N = 7.4^{**}$; $F_{B*N} = 1.6^{ns}$					
50	0	0 ^c	0 ^c	0.1 ^c	0 ^c
	1	1.3 ^b	1.9 ^a	1.08 ^b	1.5 ^a
	2	0 ^c	0.9 ^b	0.05 ^c	0.3 ^b
	3	0.2 ^c	0.3 ^c	0.04 ^c	0.2 ^{bc}
	Average (N)	0.4 ^b	0.8 ^a	0.3 ^b	
CV (%) = 11.0%; $F_B = 69.6^{**}$; $F_N = 14.9^{**}$; $F_{B*N} = 3.6^*$					
60	0	0 ^c	0.1 ^c	0.1 ^c	0 ^c
	1	1.4 ^b	2.2 ^a	1.2 ^b	1.6 ^a
	2	0.0 ^c	0.9 ^b	0.1 ^c	0.3 ^b
	3	0.3 ^c	0.3 ^c	0.1 ^c	0.2 ^{bc}
	Average (N)	0.4 ^b	0.9 ^a	0.4 ^b	
CV (%) = 9.8%; $F_B = 95.9^{**}$; $F_N = 19.9^{**}$; $F_{B*N} = 4.5^{**}$					

Within the same group, the same letter following the means represents statistically insignificant difference; ns: nonsignificant difference; *: significant difference at $0.01 < P \leq 0.05$; **: significant difference at $P \leq 0.01$.

Despite the gradual increase in shoot cluster height over the culture period, the trend in the differences among treatments remained similar from day 30 to 60. The BA concentration of 1 mg/L was clearly the most appropriate for shoot growth, as at any NAA concentration, the treatment with 1 mg/L BA gave the highest results statistically compared to other BA concentrations. Among treatments with 1 mg/L BA, the combination with 0.25 mg/L NAA also give the highest results of cluster height (2.2 cm on day 60, equivalent to a

57.1% higher than the second-best treatment of 1 mg/L BA and no NAA), which coincides with the best number of shoots in this treatment, showing that this combination is the most appropriate for generation of shoots from *O. integerrima* leaf callus. Cytokinin stimulates shoot formation directly or indirectly on intact plants as well as on *in vitro* cultured plant tissue. This is more effective when combining cytokinin with auxin in an appropriate ratio (Nguyen & Le, 2006).

Table 6. Effects of 6-benziladenine (BA) and 1-naphthaleneacetic acid (NAA) concentrations on the number of leaves formed during shoot regeneration of *O. integerrima* HD01

Days after implantation	BA concentrations (mg/L)	NAA concentrations (mg/L)			Average (B)
		0	0.25	0.5	
30	0	0.0 ^f	0.4 ^{de}	0.2 ^{ef}	0.2 ^c
	1	1.5 ^{abc}	2.3 ^a	1.4 ^{bc}	1.8 ^a
	2	0.4 ^{ef}	2.2 ^{ab}	0.3 ^{ef}	1.0 ^{bc}
	3	1.3 ^{bc}	1.3 ^{cd}	0.4 ^{ef}	1.0 ^b
Average (N)		0.8 ^b	1.5 ^a	0.6 ^b	
CV (%) = 10.3%; $F_B = 39.8^{**}$; $F_N = 39.2^{**}$; $F_{B*N} = 6.9^{**}$					
40	0	0.0 ^d	0.2 ^d	0.1 ^d	0.1 ^c
	1	4.2 ^b	9.0 ^a	4.6 ^b	5.9 ^a
	2	0.1 ^d	7.5 ^a	0.2 ^d	2.6 ^b
	3	2.7 ^c	2.2 ^c	0.3 ^d	1.8 ^b
Average (N)		1.8 ^b	4.7 ^a	1.3 ^b	
CV (%) = 9.5%; $F_B = 211.2^{**}$; $F_N = 123.5^{**}$; $F_{B*N} = 42.5^{**}$					
50	0	0.0 ^d	0.2 ^d	0.1 ^d	0.1 ^c
	1	4.4 ^{bc}	9.6 ^a	4.7 ^{bc}	6.2 ^a
	2	0.1 ^d	7.6 ^{ab}	0.2 ^d	2.6 ^b
	3	2.9 ^c	2.3 ^c	0.3 ^d	1.8 ^b
Average (N)		1.8 ^b	4.9 ^a	1.3 ^c	
CV (%) = 10.5%; $F_B = 177.4^{**}$; $F_N = 111.1^{**}$; $F_{B*N} = 35.5^{**}$					
60	0	0.0 ^d	0.3 ^d	0.2 ^d	0.2 ^c
	1	4.4 ^b	9.7 ^a	4.8 ^b	6.3 ^a
	2	0.2 ^d	7.7 ^a	0.3 ^d	2.7 ^b
	3	3.0 ^c	2.3 ^c	0.3 ^d	1.9 ^b
Average (N)		1.9 ^b	5.0 ^a	1.4 ^b	
CV (%) = 10.0%; $F_B = 185.9^{**}$; $F_N = 115.2^{**}$; $F_{B*N} = 36.9^{**}$					

Within the same group, the same letter following the means represents statistically insignificant difference; **: significant difference at $P \leq 0.01$.

Data on the number of leaves (Table 5) also show that there were few changes in the differences among treatment from day 30 to 60, indicating that the effects of the growth regulator combinations were largely determined early in the culture period. This is despite the fact that the number of leaves still increased substantially from day 30 to day 40.

The BA concentration of 1 mg/L was also shown to be most appropriate for the development of *O. integerrima* shoots from callus, indicated by the highest number of leaves at this concentration compared to other BA concentrations within the same NAA concentration, although at the NAA concentration of 0.25 mg/L, there was no significant difference between 1 mg/L and 2 mg/L BA (Table 6). Leaves affect by these two treatments also gave the highest number (9.7 and 7.7 leaves on day 60, respectively).

Collectively, the results obtained show that the combination of 1 mg/L BA and 0.25 mg/L NAA is most appropriate for the rapid *in vitro* regeneration of *O. integerrima* shoots from leaf callus. The shoot clusters in this treatment had high number of shoots and developed well, contributing to a high propagation rate.

4. Conclusions

For the callus formation from leaf sample, the combination of 0.2 mg/L TDZ and 0.25 mg/L NAA gave the best results with a callus formation rate of 100%, the highest callus diameter and fresh weight, reaching 2.7 cm and 2.4 g, respectively, after 60 days of culture. The calluses remained yellow on day 60 of culture.

For the shoot regeneration from the calluses, using MS medium supplemented with 1 mg/L BA combined with 0.25 mg/L NAA gave shoot

clusters with the highest number of shoots (8.5 shoots), cluster height (2.2 cm) and number of leaves (9.7 leaves) after 60 days of culture.

Conflict of interest

The authors have no conflicts of interest to declare.

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References

- Ahmad, N., & Faisal, M. (2018). *Thidiazuron: From urea derivative to plant growth regulator*. Singapore: Springer Nature.
- Ho, N. T. C., Pham, H. N., & Nguyen, N. T. (2019). Process of the micropropagation of *Ochna integerrima* (Lour.) Merr. *Nguyen Tat Thanh University Journal of Science and Technology* 6, 28-32. <https://vjol.info.vn/index.php/dh-NTT/article/view/44697>.
- Ikeuchi, M., Sugimoto, K., & Iwase, A. (2013). Plant callus: Mechanisms of induction and repression. *The Plant Cell* 25(9), 3159-3173. <https://doi.org/10.1105/tpc.113.116053>.
- Karakas, P. F. (2020). Efficient plant regeneration and callus induction from nodal and hypocotyl explants of goji berry (*Lycium barbarum* L.) and comparison of phenolic profiles in calli formed under different combinations of plant growth regulators. *Plant Physiology and Biochemistry* 146, 384-391. <https://doi.org/10.1016/j.plaphy.2019.11.009>.
- Lu, X., Fei, D. L., Li, Y., Du, L. J., Ma, W. W., Huang, J. H., & Wang, L. J. (2023). Effect of different plant growth regulators on callus and adventitious shoots induction, polysaccharides accumulation

- and antioxidant activity of *Rhodiola dumulosa*. *Chinese Herbal Medicines* 15(2), 271-277. <https://doi.org/10.1016/j.chmed.2022.07.005>.
- Mai, D. V., & Lam, P. N. (2013). *In vitro* multiple shoot and root induction, acclimatization of Vietnamese mickey mouse plant (*Ochna integerrima* (lour.) merr.). In *Proceedings of The National Biotechnology Science Conference: Gene Technology, Enzyme Technology and Biochemistry, Medical and Pharmaceutical Biotechnology, Animal Biotechnology* (761-765). Ha Noi, Vietnam: Institute of Biotechnology. Retrieved April 20, 2023, from https://sti.vista.gov.vn/file_DuLieu/dataTLKHCN//Vd1278-2015/2013/Vd1278-2015Q520130761.pdf.
- Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with Tobacco tissue cultures. *Physiology Plantarum* 15(3), 473-497. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>.
- Ngo, P. D., Huynh, T. T. H., Nguyen, N. D., Tran, M. V., & Tran, T. H. (2013). *Irrigation and drainage, guide for using mstatc, sas and excel 2007 in experimental processing for agriculture and water management*. Nong Lam University, Ho Chi Minh City, Vietnam.
- Nguyen, L. D., & Le, T. T. T. (2006). *Cell technology*. Ho Chi Minh City, Vietnam: Vietnam National University Ho Chi Minh City Press.
- Nitsch, C. (1968). *In vitro* induction of flowering in a short-day plant: *Plumbago indica* L. *Annals of Natural Sciences: Botany* 9(12), 1-91.
- Su, K. C., Takaki, T., & Petronczki, M. (2011). Targeting of the RhoGEF Ect2 to the equatorial membrane controls cleavage furrow formation during cytokinesis. *Developmental Cell* 21(6), 1104-1115. <https://doi.org/10.1016/j.devcel.2011.11.003>.
- Tran, M. V. (2005). *Plant cell biotechnology textbook*. Institute of Tropical Biology, Ho Chi Minh City, Vietnam.
- Verma, K. S., Das, K. A., Cingoz, S. G., Uslu, E., & Gurel, E., (2016). Influence of nutrient media on callus induction, somatic embryogenesis and plant regeneration in selected Turkish crocus species. *Biotechnology Reports* 10, 66-74. <https://doi.org/10.1016/j.btre.2016.03.006>.

Prevalence and antibiotic resistance of *Escherichia coli* isolated from the respiratory tract of goats in Can Tho city, Vietnam

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ARTICLE INFO	ABSTRACT
Research Paper Received: January 27, 2024 Revised: March 01, 2024 Accepted: March 18, 2024 Keywords Antimicrobial susceptibility <i>In vitro</i> Goats Resistant genes Respiratory disease *Corresponding author Nguyen Khanh Thuan Email: nkhthuan@ctu.edu.vn	A total of 319 nasal swab samples were collected to clarify the prevalence and antimicrobial susceptibility of <i>E. coli</i> in the respiratory tract of goats in Can Tho, Vietnam. It indicated that <i>E. coli</i> was detected at a relatively minor rate (8.46%), and their prevalence in male goats and dairy goats was higher than that in others. However, the ages and health conditions of goats did not affect the presence of <i>E. coli</i> in those goats. Those <i>E. coli</i> strains were still susceptible to seven examined antibiotics, but the resistance was recorded in ampicillin (25.93%) and bactrim (25.93%) in this study. Those <i>E. coli</i> strains (33.33%) could resist one to seven antibiotics with several patterns; the pattern of ampicillin + amoxicillin/clavulanic acid (7.41%) was more common than others. Moreover, <i>E. coli</i> strains harbored antibiotic-resistance genes, and <i>blaampC</i> was detected at the highest rate (92.11%), followed by <i>sulIII</i> (43.86%), <i>tetA</i> (24.56%), and <i>qnrA</i> (5.26%). Of those <i>E. coli</i> strains, 22.81% harbored two to four examined genes with several patterns of antibiotic-resistance genes; the most detected pattern was <i>blaampC</i> + <i>sulIII</i> + <i>tetA</i> (7.89%). Thus, controlling the prevalence of antibiotic-resistant <i>E. coli</i> in the respiratory tract of goats will protect animal and public health.

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1. Introduction

Small ruminants, especially goats, have increased in the Mekong Delta, Vietnam, because of their behavior, which is suitable for raising the climate change conditions in this area. Goats

have become crucial in the economy on small-scale farms. They supply meat and milk, and their nature is to replicate and grow rapidly. However, goat diseases have been a concern; thus, keeping livestock healthy is a significant challenge. Respiratory illness affects the survival of small

ruminants because it can cause prolonged impact and depression for the rest of life (Besser et al., 2012). The diseases occur due to the interaction of infectious pathogens (bacteria, viruses, and fungi), host defense, environmental factors, and stress. Bacterial infection of the respiratory tract may be primary, occurring in healthy individuals, or secondary to several conditions that cause immunosuppression (Yesuf et al., 2012).

Escherichia coli could cause intestinal and extra-intestinal colibacillosis in humans and animals. Pathogenic *E. coli* not only had a severe impact on the breeding industry but also posed a threat to public health. Two major groups have been proposed: intestinal pathogenic *E. coli* (IPEC) and extra-intestinal pathogenic *E. coli* (ExPEC). For animals, ExPEC infects pigs, poultry, and cattle, causing septicemia, mastitis, and respiratory diseases (Russo et al., 2003; Zhang et al., 2018; Ma et al., 2021). Detecting *E. coli* in the respiratory tract of goats is essential for controlling diseases. In previous reports, *E. coli* was isolated from healthy or pneumonia goats in China and India at 9.98% to 44.16% (Puvarajan et al., 2020; Yun et al., 2022). However, there was little understanding of the characteristic prevalence of *E. coli* in the respiratory tract of goats in the Mekong Delta and Vietnam.

Furthermore, the continuous use and abuse of antibiotics in livestock have caused an increase in antibiotic-resistant bacteria, especially *E. coli*. Antimicrobial-resistant *E. coli* and multiple drug-resistant (MDR) strains have been frequently identified. These *E. coli* strains might be transmitted to humans through consuming contaminated animal products, and a risk of transmission of drug-resistant genes between different strains was also presented (Aarestrup et al., 2001; Mellata, 2013). This reveals a significant

potential hazard to the health of humans and animals. Algammal et al. (2020) showed that *E. coli* isolated from pneumonia cattle in Egypt was significantly resistant to erythromycin, gentamycin, streptomycin, and trimethoprim/sulphamethoxazole. In India, Singh et al. (2019) reported that *E. coli* strains isolated from pneumonia goats harbored antibiotic-resistance genes, such as *bla*TEM (70.60%) and *bla*SHV (1.70%). Therefore, assessing the antimicrobial resistance profile of *E. coli* in goats is necessary to contribute to the overall picture of the abuse of antibiotics in the Mekong Delta.

Therefore, this study aimed to clarify the prevalence and antimicrobial susceptibility of *E. coli* isolated from the respiratory tract of goats in Can Tho, Vietnam. These results will be a valuable reference for treating and controlling respiratory diseases caused by *E. coli* in this region and the Mekong Delta, Vietnam.

2. Materials and Methods

2.1. Sample collection

From March to September 2023, 319 nasal swab samples were collected from goats of all ages and genders in small-scale farms (< 50 heads/farm) in Can Tho, Vietnam. These goats included meat goats (n = 232) and dairy goats (n = 87). Before nasal samples were collected, these goats were examined for clinical symptoms of respiratory disease, such as coughing, nasal discharges, breath, fever, etc. The outside of the goat's nose was cleaned with sterilized cotton tissues. Then, sterilized swabs were used to collect the nasal samples and were put in the Cary Blair medium tubes (Merck, Germany). Those Cary Blair tubes were cleaned outside and put in separately sterilized zip bags with the code. They were kept in cool conditions

(cool box, 2 - 8°C with dry ice) and sent to the laboratory to isolate pathogens.

When collecting samples, this study followed the animal welfare and safety procedures and guidelines of Can Tho University, Vietnam. Samples were collected and directly sent to the laboratory for analysis within 24 h.

2.2. Isolation and identification of *E. coli* in the nasal samples

The isolation method of *E. coli* in goat nasal fluid was carried out and modified according to the guidelines of the Vietnam Standards - TCVN 8400-16:2011 (VS, 2011) and Barrow and Feltham (2003).

The nasal swabs were incubated in buffered peptone water broth (BPW, Merck, Germany) to enrich *E. coli* in samples. After incubating at 37°C for 24 h, one loop of enrichment broth of each sample was cultured on MacConkey medium (MC, Merck, Germany) for further incubation at 37°C for 24 h. Then, all suspicious colonies of *E. coli* were collected and subcultured on nutrient agar (NA, Merck, Germany) for further incubation at 37°C in 24 h to examine biochemical tests following the guidelines of Barrow and Feltham (2003).

Then, those *E. coli* strains were confirmed to subculture on trypticase soy agar (TSA, Merck, Germany) at 37°C for 24 h for other experiments.

2.3. Antimicrobial susceptibility of *E. coli* strains isolated from goats

Positive *E. coli* strains (one strain/positive sample) were examined for antimicrobial susceptibility using the disc fusion method followed by Bauer's guidelines (Bauer et al., 1966). There were seven antibiotics used in this study,

including ampicillin (Am, 10 µg), amoxicillin/clavulanic acid (Ac, 20/10 µg), ceftazidime (Cz, 30 µg), gentamycin (Ge, 10 µg), doxycycline (Dx, 30 µg), ciprofloxacin (Ci, 5 µg), trimethoprim/sulfamethoxazole (Bt, 1.25/23.75 µg). Those antibiotics were purchased by Nam Khoa Biotek Ltd. (Vietnam).

Escherichia coli ATCC 25922 was used as control quality, and the results were compared to the standards of Clinical and Laboratory Standards Institute (CLSI, 2022). Those strains, which were intermediate susceptibility, were accounted as susceptible strains.

2.4. Determination of antibiotic-resistance genes in *E. coli* isolated from goats

The DNA of *E. coli* strains was extracted using Ahmed and Dablood's heat-shock method (Ahmed & Dablood, 2017) and stored at -20°C for use in this experiment. A total of 114 *E. coli* isolates from 27 positive samples were used in this experiment.

The single PCR assay was used to detect four antibiotic-resistance genes representative of beta-lactam (*blaampC*), tetracycline (*tetA*), sulfonamide (*sulII*), and quinolone (*qnrA*). The PCR conditions and primer sequences followed the description of Randall et al. (2004), Cattoir & Nordmann (2009), and Sáenz et al. (2010). The MyTaq Mix 2X (BIO25042, Bioline, Meridian Bioscience, USA) was used in those experiments. The PCR reaction was a total of 25 µL, including Mastermix 2X (12.5 µL), forward primer (0.5 µL), reverse primer (0.5 µL), distillation water (9.5 µL), and DNA template (2 µL). Thermal cycle was modified as follows: 94°C - 5 min; 30 cycles: 94°C - 1 min, 58°C - 45 sec, 72°C - 1 min; 72°C - 10 min. The *E. coli* strains, previously isolated from cattle in the Mekong Delta, were used as a control.

2.5. Statistical analysis

Statistical analysis was used to clarify the difference in the prevalence of *E. coli* in goats and antibiotic resistance among those isolates. The Chi-square method was used at the significance rate of 95% in the Minitab 17.0 software.

3. Results and discussions

3.1. Prevalence of *E. coli* in the respiratory tract of goats in Can Tho city, Vietnam

Of 319 nasal swab samples, *E. coli* was detected in 27 samples (8.46%) (Table 1). Although *E. coli* was present at a low rate, it indicated that *E. coli* could survive and multiply in the respiratory tracts of goats. It could become an opportunistic pathogen causing respiratory diseases in goats. In

previous reports, *E. coli* belonging to the ExPEC group can infect and cause several diseases, including respiratory diseases in domestic animals (Logue et al., 2017; Zhang et al., 2018; Ma et al., 2021). De Oliveira et al. (2016) isolated *E. coli* from healthy and diseased animals with respiratory signs at a low proportion (4.22%), while another report indicated the significance of *E. coli* in bronchopneumonia (DebRoy et al., 2008). Most goat farms in this study were small and unclean; therefore, those goats could also transmit pathogens, including *E. coli*, in the husbandry environment. Pelczar et al. (1986) suggested that *E. coli*, which is usually harmless in its normal habitat, could cause pulmonary and urogenital infections. It should control this pathogen even though it is not frequently prevalent in goats.

Table 1. Prevalence of *E. coli* in the respiratory tract of goats

Factor	No. examined samples	No. of positive samples	Percentage (%)
Meat goats	232	15	6.47
Dairy goats	87	12	13.79
			(<i>P</i> < 0.05)
Male	96	13	13.54
Female	233	14	6.01
			(<i>P</i> < 0.05)
Under 6 month-age	108	13	12.04
Upper 6 month-age and under 1 year-age	109	6	5.50
Upper 1 year-age	102	8	7.84
			(<i>P</i> > 0.05)
Respiratory goats	175	16	9.14
Healthy goats	144	11	7.64
			(<i>P</i> > 0.05)
Total	319	27	8.46

This study showed that the prevalence of *E. coli* in the respiratory tract of goats did not depend on age and health conditions ($P > 0.05$). It indicated that *E. coli* could be present in goats of all ages and a common pathogen that could be dominant in the respiratory tract. However, there was a significant difference in the prevalence of *E. coli* in genders and meat or dairy goats ($P < 0.05$). This study was randomized and did not have to raise model data of meat and dairy farms. Therefore, this result could be crucial due to the difference in the number of goats examined in those farms. In previous reports, no studies have been published on the difference between raising models of meat goats and dairy goats and how this will affect the transmission of pathogens. However, Zhou et al. (2023) clarified the correlation of pathogens in the respiratory tract of cows in Northeast China. It noted that all cattle (raised for meat or milk) were equally susceptible to pathogens. Thus, other research should be conducted to clarify the exact reasons for this difference in Can Tho and the Mekong Delta.

3.2. Antimicrobial susceptibility of *E. coli* isolated from the respiratory tract of goats

Of 27 positive samples, 27 *E. coli* strains were selected from 114 identified *E. coli* strains (one strain/positive sample) to examine antibiotic resistance. The results showed that all *E. coli* strains were still susceptible to seven examined antibiotics (Table 2). Among antibiotics, those *E. coli* strains were the most sensitive to ceftazidime (96.30%), followed by doxycycline (88.89%), ciprofloxacin (88.89%), and the minor sensitive to ampicillin (74.07%). *E. coli* isolates from ruminants generally demonstrated lower resistance trends than those from poultry and swine (Lei et al., 2010; Abbassi et al., 2017). In this study, antibiotics were used less in goats because of the high natural resistance of goats to diseases, and no disease outbreaks occurred in surveyed farms in Can Tho. It indicated that those antibiotics were still effective in treating respiratory diseases in goats here.

Table 2. Antimicrobial susceptibility of *E. coli* strains isolated from goats (n = 27)

Antibiotic group	Antibiotic	Susceptibility		Resistance	
		No. of strains	Percentage (%)	No. of strains	Percentage (%)
Beta-lactam	Ampicillin	20	74.07	7	25.93
	Amoxicillin-clavulanic acid	22	81.48	5	18.52
	Ceftazidime	26	96.30	1	3.70
Aminoglycoside	Gentamycin	22	81.48	5	18.52
Tetracycline	Doxycycline	24	88.89	3	11.11
Quinolone	Ciprofloxacin	24	88.89	3	11.11
Sulfonamide	Bactrim*	20	74.07	7	25.93

*Trimethoprim/sulphamethoxazole.

However, *E. coli* strains seemed resistant to ampicillin (25.93%) and bactrim (25.93%). Those antibiotics have been usually used in treating colibacillosis in domestic animals for a long time. Additionally, according to our observations, the hygiene in those surveyed farms was not clean; therefore, *E. coli* in the husbandry environment could form and transfer resistance to those antibiotics. Pehrsson et al. (2016) reported that antibiotic-resistant *E. coli* and resistance genes might be transmitted through environments

contaminated with feces, especially in developing countries. In other research, Khalifa et al. (2021) showed that *E. coli*, isolated from the respiratory tract of diseased sheep and goats in Egypt, had very high resistance levels for different beta-lactam antibiotics, including ampicillin (78.9%), amoxicillin-clavulanic acid (39.5%), and cefoxitin (34.2%). *Escherichia coli* isolates recovered from animal sources in the USA were resistant to tetracycline, streptomycin, sulfonamide, and ampicillin (Tadesse et al., 2012).

Table 3. Antibiotic-resistance patterns of *E. coli* strains (n = 27)

No. of resistant antibiotics	Pattern	No. of strains	Percentage (%)
1	Am	1	3.70
	Ci	1	3.70
2	Am + Ac	2	7.41
	Am + Bt	1	3.70
	Ge + Bt	1	3.70
3	Am + Ge + Bt	1	3.70
6	Am + Ac + Ge + Dx + Ci + Bt	1	3.70
7	Am + Ac + Cz + Ge + Dx + Ci + Bt	1	3.70
Total		9	33.33

Am: ampicillin, Ac: amoxicillin/clavulanic acid, Cz: ceftazidime, Ge: gentamycin, Dx: doxycycline, Ci: ciprofloxacin, Bt: bactrim (trimethoprim/sulfamethoxazole).

Although those *E. coli* strains were still sensitive to all antibiotics in this study, a few exhibited multi-drug resistances (33.33%) (Table 3). Significantly, there were *E. coli* strains resistant to six or seven examined antibiotics. A more significant proportion of multi-drug resistant *E. coli* isolates was recovered from animals in the USA; it found that the pattern of tetracycline and streptomycin was the most common co-resistance phenotype (30.00%), followed by tetracycline and sulfonamide (29.00%) (Tadesse et al., 2012). In previous research, this will favor

the dissemination of the strains among animals and humans by co-selection (Belanger et al., 2011), and animal-derived *E. coli* can eventually infect humans through animal products or farm wastes, which seriously endanger public health safety (Fricke et al., 2008; Hannah et al., 2009). This study revealed that those *E. coli* strains could concern animal and human health in Can Tho. Thus, the use of antibiotics in goats should be controlled to prevent the formation and spread of multi-drug resistant *E. coli* strains and to guarantee human and animal health.

3.3. Prevalence of antibiotic-resistance genes in *E. coli* strains

Of 27 positive samples, all 114 *E. coli* strains were collected after being examined in

biochemical tests. Those strains determined the prevalence of four common antibiotic-resistance genes using PCR assay. The results were shown in Table 4.

Table 4. Prevalence of antibiotic-resistance genes in *E. coli* strains isolated from goats (n = 114)

Antibiotic group	Gene	No. of positive strains	Percentage (%)
Beta-lactam	<i>blaampC</i>	105	92.11
Tetracycline	<i>tetA</i>	28	24.56
Sulfonamide	<i>sulIII</i>	50	43.86
Quinolone	<i>qnrA</i>	6	5.26
($P < 0.001$)			

The results exhibited that *E. coli* strains could harbor most of the examined antibiotic-resistance genes at relatively high rates. Among them, gene *blaampC* was detected at the highest rate (92.11%), followed by *sulIII* (43.86%), *tetA* (24.56%), and gene *qnrA* was the lowest (5.26%). It indicated *E. coli* strains isolated from the respiratory tract of goats could remarkably resist beta-lactam antibiotics. However, in the antimicrobial susceptibility test, *E. coli* strains showed a low resistance to beta-lactam antibiotics (Table 2). It was like the resistance performance of bactrim and the prevalence of gene *sulIII*. In contrast, the less resistance of doxycycline and ciprofloxacin seemed to correspond with the infrequent presence of genes *tetA* and *qnrA* in those *E. coli* strains. Thus, the antibiotic resistance performance of those strains might be affected by different factors. Resistance genes in bacteria could be acquired through natural mutations and transferred to the next generation or due to conjugation, transduction, or mutation of resistance genes between diverse bacteria (Sommer et al., 2017). Moreover, some antibiotic-resistance genes seem like silent resistance genes that are usually not expressed or expressed at low levels, even when exposed

to antibiotics (Stasiak et al., 2021). In a previous report, *E. coli* strains isolated from pneumonia goats harbored antibiotic-resistance genes, such as *blaTEM* (70.60%) and *blaSHV* (1.70%) (Singh et al., 2019). Whereas *E. coli* isolated from goats' fecal samples in India showed multiple drugs related to a high presence of gene *blaCTXM-1*, *blaSHV*, *blaTEM*, *blaCMY-6*, *blaCITM*, *qnrB*, *qnrS*, *tetA*, *tetB*, and *sulI* (Banerjee et al., 2022).

Furthermore, those *E. coli* strains (22.81%) in this study also harbored multiple antibiotic-resistance genes (Table 5). Those strains could harbor two to four examined antibiotic-resistance genes; it showed that *E. coli* strains isolated from the respiratory tract of goats could resist many antibiotics. It could become a challenge in treating respiratory diseases in goats in Can Tho. Tenover (2006) reported that multidrug resistance often occurred with not just one but a combination of multiple resistance mechanisms. Multidrug resistance can occur in bacteria if exposed to a type of antibiotic continuously or due to the acquisition of genetic resistance elements through plasmids or transposons.

Table 5. Multi antibiotic-resistance gene patterns of *E. coli* strains (n = 114)

No. of genes	Gene patterns	No. of strains	Percentages (%)
2	<i>blaampC</i> + <i>sulIII</i>	6	5.26
	<i>blaampC</i> + <i>qnrA</i>	3	2.63
	<i>blaampC</i> + <i>tetA</i>	2	1.75
	<i>sulIII</i> + <i>tetA</i>	3	2.63
3	<i>blaampC</i> + <i>sulIII</i> + <i>tetA</i>	9	7.89
4	<i>blaampC</i> + <i>sulIII</i> + <i>tetA</i> + <i>qnrA</i>	3	2.63
Total		26	22.81

In other research on *E. coli* isolated from fecal samples, multidrug-resistant phenotypes were found in 72.7% of the examined strains (Martínez-Vázquez et al., 2021). Among those phenotypes was the presence of genes *tet* (*tetA* or *tetB*), *strA*, and *aadA*. Obaidat and Gharaibeh (2022) reported that 93.5% and 96.3% of ESBL and AmpC *E. coli* isolated from sheep and goat milk were resistant to ≥ 1 another antimicrobial class, and 44.5% and 44.4% were resistant to ≥ 3 another antimicrobial class, respectively. Antibiotic resistance to *E. coli* is an increasingly severe problem, and resistance genes can be acquired through horizontal gene transfer to make *E. coli* multi-drug resistant strains (Huddleston, 2014). Although the *E. coli* strains in this study showed a low resistance rate, a high prevalence of antibiotic-resistant genes was detected. It again revealed that the management of using antibiotics was essential to prevent multi-drug resistance *E. coli* outbreaks in animal husbandry and public health in Can Tho and the Mekong Delta, Vietnam.

4. Conclusions

Escherichia coli was present at a relatively minor rate in the respiratory tract of goats in Can Tho, Vietnam. However, that revealed a potential ability to cause diseases in the respiratory tract by *E. coli*. Further study should

be conducted to explain the high prevalence of *E. coli* in male goats and dairy goats than others. In addition, those *E. coli* strains could resist various antibiotics and harbor antibiotic-resistance genes, incredibly high to *blaampC*, with several multi-drug patterns. It indicated that those *E. coli* strains could become an emergency in treating diseases in goats and other domestic animals here. It is necessary to control the prevalence of antibiotic-resistant *E. coli* in the respiratory tract of small ruminants, including goats, to protect animals and public health in Can Tho and the Mekong Delta.

Conflict of interest

The authors have no conflicts of interest to declare.

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References

- Aarestrup, F. M., Seyfarth, A. M., Emborg, H. D., Pedersen, K., Hendriksen, R. S., & Bager, F. (2001). Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in fecal enterococci from food animals in Denmark.

- Antimicrobial Agents and Chemotherapy* 45(7), 2054-2059. <https://doi.org/10.1128/aac.45.7.2054-2059.2001>.
- Abbassi, M. S., Kilani, H., Zouari, M., Mansouri, R., Oussama, E. F., Hammami, S., & Chehida, N. B. (2017). Antimicrobial resistance in *Escherichia coli* isolates from healthy poultry, bovine and ovine in Tunisia: a real animal and human health threat. *Journal of Clinical Microbiology and Biochemical Technology* 3, 19-23. <http://dx.doi.org/10.17352/jcmbt.000021>.
- Ahmed, O. B., & Dablood, S. A. (2017). Quality Improvement of the DNA extracted by boiling method in Gram negative bacteria. *International Journal of Bioassays* 6(4), 5347-5349. <http://dx.doi.org/10.21746/ijbio.2017.04.004>.
- Algammal, A. M., El-Sayed, M. E., Youssef, F. M., Saad, S. A., Elhaig, M. M., Batiha, G. E., Hozzein, W. N., & Ghobashy, M. O. I. (2020). Prevalence, the antibiogram and the frequency of virulence genes of the most predominant bacterial pathogens incriminated in calf pneumonia. *AMB Express* 10(1), 99. <https://doi.org/10.1186/s13568-020-01037-z>.
- Banerjee, J., Bhattacharyya, D., Habib, M., Chaudhary, S., Biswas, S., Maji, C., Nanda, P. K., Das, A. K., Dandapat, P., Samanta, I., Lorenzo, J. M., Dutt, T., & Bandyopadhyay, S. (2022). Antimicrobial resistance pattern, clustering mechanisms and correlation matrix of drug-resistant *Escherichia coli* in black Bengal goats in West Bengal, India. *Antibiotics (Basel)* 11(10), 1344. <https://doi.org/10.3390/antibiotics11101344>.
- Barrow, G. I., & Faltham, R. K. A. (1993). *Cowan and Steel's manual for the identification of medical bacteria* (3rd ed.). Cambridge, UK: Cambridge University Press.
- Bauer, A. W., Kirby, W. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* 45(4), 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493.
- Belanger, L., Garenaux, A., Harel, J., Boulianne, M., Nadeau, E., & Dozois, C. M. (2011). *Escherichia coli* from animal reservoirs as a potential source of human extraintestinal pathogenic *E. coli*. *FEMS Immunology and Medical Microbiology* 62(1), 1-10. <https://doi.org/10.1111/j.1574-695x.2011.00797.x>.
- Besser, T. E., Frances, C. E., Highland, M. A., Wolff, P., Justice-Allen, A., Mansfield, K., Davis, M. A., & Foreyt, W. (2012). Bighorn sheep pneumonia: Sorting out the cause of a polymicrobial disease. *Preventive Veterinary Medicine* 108(2-3), 85-93. <https://doi.org/10.1016/j.prevetmed.2012.11.018>.
- Cattoir, V., & Nordmann, P. (2009). Plasmid-mediated quinolone resistance in gram-negative bacterial species: an update. *Current Medicinal Chemistry* 16(8), 1028-1046. <https://doi.org/10.2174/092986709787581879>.
- CLSI (Clinical and Laboratory Standards Institute). (2022). *Performance standards for antimicrobial susceptibility testing* (32nd ed.). Pennsylvania, USA: Clinical and Laboratory Standards Institute.
- DebRoy, C., Roberts, E., Jayarao, B. M., & Brooks, J. W. (2008). Bronchopneumonia associated with extraintestinal pathogenic *Escherichia coli* in a horse. *Journal of Veterinary Diagnostic Investigation* 20(5), 661-664. <https://doi.org/10.1177/104063870802000524>.
- De Oliveira, B. A. F. D., Gaeta, N. C., Ribeiro, B. L. M., Alemán, M. A. R., Marques, L. M., Timenetsky, J., Melville, P. A., Marques, J. A., Marville, V., & Gregory, L. (2016). Determination of bacterial aetiological factor on tracheobronchial lavage in relation to clinical signs of bovine respiratory disease. *Journal of Medical Microbiology* 65(10), 1137-1142. <https://doi.org/10.1099/jmm.0.000345>.
- Fricke, W. F., Wright, M. S., Lindell, A. H., Harkins, D. M., Baker-Austin, C., Ravel, J., & Stepanauskas, R. (2008). Insights into the environmental

- resistance gene pool from the genome sequence of the multidrug-resistant environmental isolate *Escherichia coli* SMS-3-5. *Journal of Bacteriology* 190(20), 6779-6794. <https://doi.org/10.1128/jb.00661-08>.
- Hannah, E. L., Johnson, J. R., Angulo, F., Haddadin, B., Williamson, J., & Matthew, M. H. (2009). Molecular analysis of antimicrobial-susceptible and -resistant *Escherichia coli* from retail meats and human stool and clinical specimens in a rural community setting. *Foodborne Pathogens and Disease* 6(3), 285-295. <https://doi.org/10.1089/fpd.2008.0176>.
- Huddleston, J. R. (2014). Horizontal gene transfer in the human gastrointestinal tract: potential spread of antibiotic resistance genes. *Infection and Drug Resistance* 7, 167-176. <https://doi.org/10.2147%2FIDR.S48820>.
- Khalifa, H. O., Oreiby, A., Abd El-Hafeez, A. A., Abd El Latif, A., Okanda, T., Kato, Y., & Matsumoto, T. (2021). High-lactam and quinolone resistance of enterobacteriaceae from the respiratory tract of sheep and goat with respiratory disease. *Animals* 11(8), 2258. <https://doi.org/10.3390%2Fani11082258>.
- Lei, T., Tian, W., He, L., Huang, X. H., Sun, Y. X., Deng, Y. T., Sun, Y., DianHong, L. V., Wu, C. M., Huang, L. Z., Shen, J. Z., & Liu J. H. (2010). Antimicrobial resistance in *Escherichia coli* isolates from food animals, animal food products and companion animals in China. *Veterinary Microbiology* 146(1-2), 85-89. <https://doi.org/10.1016/j.vetmic.2010.04.025>.
- Logue, C. M., Wannemuehler, Y., Nicholson, B. A., Doetkott, C., Barbieri, N. L., & Nolan, L. L. (2017). Comparative analysis of phylogenetic assignment of human and avian ExPEC and fecal commensal *Escherichia coli* using the (previous and revised) clermont phylogenetic typing methods and its impact on avian pathogenic *Escherichia coli* (APEC) classification. *Frontiers in Microbiology* 8, 283. <https://doi.org/10.3389/fmicb.2017.00283>.
- Ma, J. L., Cheng, Z. X., Bai, Q. K., Zhao, K. J., Pan, Z. H., & Yao, H. C. (2021). Screening virulence factors of porcine extraintestinal pathogenic *Escherichia coli* (an emerging pathotype) required for optimal growth in swine blood. *Transboundary and Emerging Diseases* 68(4), 2005-2016. <https://doi.org/10.1111/tbed.13848>.
- Martínez-Vázquez, A. V., Vázquez-Villanueva, J., Leyva-Zapata, L. M., Barrios-García, H., Rivera, G., & Bocanegra-García, V. (2021). Multidrug resistance of *Escherichia coli* strains isolated from bovine feces and carcasses in Northeast Mexico. *Frontiers in Veterinary Science* 8, 643802. <https://doi.org/10.3389/fvets.2021.643802>.
- Mellata, M. (2013). Human and avian extraintestinal pathogenic *Escherichia coli*: infections, zoonotic risks, and antibiotic resistance trends. *Foodborne Pathogens and Disease* 10(11), 916-932. <https://doi.org/10.1089/fpd.2013.1533>.
- Obaidat, M. M., & Gharaibeh, W. A. (2022). Sheep and goat milk in Jordan is a reservoir of multidrug resistant extended spectrum and AmpC beta-lactamases *Escherichia coli*. *International Journal of Food Microbiology* 377, 109834. <https://doi.org/10.1016/j.ijfoodmicro.2022.109834>.
- Pehrsson, E. C., Tsukayama, P., Patel, S., Navarrete, K. M., Calderon, M., Cabrera, L., Bertoli, M. T., Berg, D. E., Gilman, R. H., & Dantas, G. (2016). Interconnected microbiomes and resistomes in low-income human habitats. *Nature* 533(7602), 212-216. <https://doi.org/10.1038/nature17672>.
- Pelczar, M. J., Chan, E. C., & Krieg, N. R. (1986). The cultivation of bacteria. In Pelczar, M. J., Chan, E. C., & Krieg, N. R. (Eds.). *Microbiology* (5th ed., 99-114). New Dehli, India: Tata Mc-Graw Hill.
- Puvarajan, B., Lurthureetha, T., & Sivakumar, T. (2020). Isolation, characterisation and prevalence pattern of bacterial flora on pneumonic cases of goats slaughtered at Thanjavur abattoir, Cauvery Delta Zone, Tamilnadu. *Journal of Entomology and Zoology Studies* 8(6), 738-742. <https://doi.org/10.22271/j.ento.2020.v8.i6j.7932>.

- Randall, L. P., Cooles, S. W., Osborn, M. K., Piddock, L. J., & Woodward, M. J. (2004). Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of *Salmonella enterica* isolated from humans and animals in the UK. *Journal of Antimicrobial Chemotherapy* 53(2), 208-216. <https://doi.org/10.1093/jac/dkh070>.
- Russo, T. A., & Johnson, J. R. (2003). Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasingly important endemic problem. *Microbes and Infection* 5(5), 449-456. [https://doi.org/10.1016/s1286-4579\(03\)00049-2](https://doi.org/10.1016/s1286-4579(03)00049-2).
- Sáenz, Y., Briñas, L., Domínguez, E., Ruiz, J., Zarazaga, M., Vila, J., & Torres, C. (2004). Mechanisms of resistance in multiple-antibiotic-resistant *Escherichia coli* strains of human, animal, and food origins. *Antimicrobial Agents and Chemotherapy* 48(10), 3996-4001. <https://doi.org/10.1128/aac.48.10.3996-4001.2004>.
- Singh, F., Sonawane, G. G., Kumar, J., Dixit, S. K., Meena, R. K., & Tripathi, B. N. (2019). Antimicrobial resistance and phenotypic and molecular detection of extended-spectrum β -lactamases among extraintestinal *Escherichia coli* isolated from pneumonic and septicemic sheep and goats in Rajasthan, India. *Turkish Journal of Veterinary and Animal Sciences* 43(6), 754-760. <https://doi.org/10.3906/vet-1905-1>.
- Sommer, M. O. A., Munck, C., Toft-Kehler, R. V., & Andersson, D. I. (2017). Prediction of antibiotic resistance: time for a new preclinical paradigm? *Nature Reviews Microbiology* 15, 689-696. <https://doi.org/10.1038/nrmicro.2017.75>.
- Stasiak, M., Maćkiw, E., Kowalska, J., Kucharek, K., & Postupolski, J. (2021). Silent genes: antimicrobial resistance and antibiotic production. *Polish Journal of Microbiology* 70(4), 421-429. <https://doi.org/10.33073%2Fpjm-2021-040>.
- Tadesse, D. A., Zhao, S., Tong, E., Ayers, S., Singh, A., Bartholomew, M. J., & McDermott, P. F. (2012). Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950-2002. *Emerging Infectious Diseases* 18(5), 741-749. <https://doi.org/10.3201/eid1805.111153>.
- Tenover, F. C. (2006). Mechanisms of antimicrobial resistance in bacteria. *American Journal of Medicine* 34(5), 3-10. <https://doi.org/10.1016/j.ajic.2006.05.219>.
- VS (Vietnam Standards). (2011). Standard No. TCVN 8400-16:2011 dated on January 01, 2011. Animal disease - Diagnostic procedure - Part 16: Edema disease in pig. Retrieved June 20, 2023, from <https://tieuchuan.vsqi.gov.vn/tieuchuan/view?sohieu=TCVN+8400-16%3A2011>.
- Yesuf, M., Mazengia, M., & Mersha, C. (2012). Histopathological and bacterial examination of pneumonic lungs of small ruminants slaughtered at Gondar, Ethiopia. *American-Eurasian Journal of Scientific Research* 7(6), 226-231. <http://dx.doi.org/10.5829/idosi.aejsr.2012.7.6.66140>.
- Yun, J., Mao, L., Li, J., Hao, F., Yang, L., Zhang, W., Sun, M., Liu, M., Wang, S., & Li, W. (2022). Molecular characterization and antimicrobial resistance profile of pathogenic *Escherichia coli* from goats with respiratory disease in eastern China. *Microbial Pathogenesis* 166, 105501. <http://doi.org/10.1016/j.micpath.2022.105501>.
- Zhang, D. X., Zhang, Z. H., Huang, C. C., Gao, X., Wang, Z., Liu, Y. C., Tian, C. L., Hong, W., Niu, S. L., & Liu, M. C. (2018). The phylogenetic group, antimicrobial susceptibility, and virulence genes of *Escherichia coli* from clinical bovine mastitis. *Journal of Dairy Science* 101(1), 572-580. <https://doi.org/10.3168/jds.2017-13159>.
- Zhou, Y., Shao, Z., Dai, G., Li, X., Xiang, Y., Jiang, S., Zhang, Z., Ren, Y., Zhu, Z., Fan, C., & Zhang, G. (2023). Pathogenic infection characteristics and risk factors for bovine respiratory disease complex based on the detection of lung pathogens in dead cattle in Northeast China. *Journal of Dairy Science* 106(1), 589-606. <https://doi.org/10.3168/jds.2022-21929>.

Classification of pet owners based on knowledge attitude and practice about rabies and its vaccination in Duc Hue, Long An province during the period 2021 - 2023

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ABSTRACT

Rabies manifests is a zoonotic ailment affecting both humans and carnivorous animals. The administration of rabies vaccination, particularly to domesticated animals such as dogs and cats, is an efficacious prophylactic measure for safeguarding the optimal health of both animal and human populations. The conscientiousness of pet owners regarding rabies vaccination significantly contributes to the effectiveness of rabies prevention initiatives. A comprehensive survey employing the Knowledge-Attitude-Practices framework was undertaken among owners of dogs and cats to appraise the efficacy of the three-year rabies vaccination program spanning 2021 to 2023 in Duc Hue district, Long An province. The primary objectives of this inquiry were to succinctly delineate the program's outcomes and to gauge the evolution of awareness and behavioral patterns among dog and cat owners. The survey adopted a nuanced approach by categorizing participants into sub-groups, emphasizing the differentiation of outcomes across these delineated groups. Findings indicated that the vaccine coverage exceeded 80% within Duc Hue, Long An province's dog and cat population in 2023. The analytical assessment consistently identified three to four discernible population clusters annually, based on Knowledge, Attitude, and Practice scores, focusing on their alignment with the overall mean within the population. Additionally, specific attention was directed towards clusters exhibiting lower scores, with a detailed consideration of associated variables, including commune location and occupation. Identifying these clusters necessitates further exploration and warrants the development of more tailored communication and approach strategies to optimize the efficacy of the ongoing vaccination campaign.

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1. Introduction

Rabies is a viral zoonosis with potential transmission of rabid animal bites, scratches, or contact via saliva, affecting both humans and all warm-blooded animals (Tiwari et al., 2018). Nowadays, companion animals, particularly dogs and cats, are not only raised for guarding purposes but are often regarded as faithful friends and close companions of humans (Paul et al., 2010). This human-animal bond can provide significant positive benefits for both mental and physical human life (Friedmann & Son, 2009). Therefore, there is a growing inclination towards dogs and cats as pets. Nevertheless, there is generally a lack of comprehensive population data in developing countries, which elevates the risk of rabies transmission from dogs and cats (Gebremedhin et al., 2020). According to the report by WHO (2023), over 40.00% of deaths due to rabies were children under 15 years old. Globally, approximately 59,000 people die from rabies annually, with the majority (95%) being in Africa and Asia due to the shortage of post-exposure prophylaxis (PEP) services for animal-bite victims and the lack of experienced personnel and facilities for rabies surveillance (Pham et al., 2021). If the preventive measures and social awareness, especially for pet owners, are not performed appropriately, there may be several heightened likelihood of future rabies outbreaks. Rabies is entirely preventable through PEP to bite victims and is controlled through mass vaccination of domestic dogs (Vilas et al., 2017). Additionally, vaccination is a cost-effective approach compared to exposure treatment (Borse et al., 2018), and it is required to achieve at least 70.00% of vaccine coverage, sufficient to maintain herd immunity within the dog population (WHO, 2019). Maintenance of sufficient vaccination coverage in the animal

population is also an important requirement for the national plan for the prevention and control of rabies in Vietnam (GOV, 2021).

In 2021, there were 78 animals infected with rabies in 11 provinces. In 2022, there were 133 animals infected with rabies in 15 provinces. From January to September 2023, 274 animals were infected with rabies recorded in 30 provinces (DAH, 2023). In addition, the rabies vaccination coverage within the dog and cat population at the national level from January to July 2023 was not high (46.27%). This rate had a significant variation between provinces. Only 12/63 province had achieved a rate of 70%; 17/63 province had a rate varied from 50 - 69%, and 34 left had a rate of less than 50%. For public health, 378 deaths due to rabies had been recorded for the period 2017 - 2021, with an average of 76 deaths per year. Sixty-nine deaths due to rabies had been recorded for 2022. From January to September 2023, 62 death cases had been notified (DAH, 2023; NIHE, 2023).

Long An province has a relatively large land area; people raise dogs and cats for companionship or home guarantee, and a considerable proportion of these animals are raised in free-range conditions. From January 2019 to December 2022, Long An province reported seven human fatalities attributed to rabies and 9 cases of dogs and cats diagnosed with rabies or suspected rabies. The majority of human deaths resulted from dog and cat bites that were not vaccinated due to insufficient awareness and knowledge about rabies (DAH, 2023). Accordingly, Duc Hue is a high-risk locality for rabies when there were cases of rabies in humans, and the vaccination coverage rate in recent years did not reach the local requirements (80.00% of the animal target population is vaccinated annually). In order

to support the local area in controlling rabies, the Faculty of Animal Science and Veterinary Medicine of Nong Lam University, Ho Chi Minh City; Boehringer Ingelheim Vietnam Company; Long An Department of Agriculture and Rural Development; Sub Department of Livestock Production and Animal Health of Long An province; along with other agencies such as District veterinary station and communal para-veterinaries have collaboratively initiated a rabies vaccination campaign for the community during the period 2021 - 2031. The program encompassed vital activities such as vaccinating dog and cat populations in 11 communes/towns and raising awareness for pet households and elementary school students using leaflets and small performances and minigames, and conducting a Knowledge-Attitude-Practices (KAP) survey among owners. Understanding the change in knowledge, attitude, and practice is beneficial for future programs in the field, e.g., tailoring the activities according to the local needs to optimize the scarce resources and provide accurate material for teaching and learning in some related courses for students. Therefore, the study aimed to summarize and report the results achieved after three years of the campaign regarding the vaccine coverage rate, assess awareness of pet owners, and classify them based on the data collected from the KAP survey respondents

2. Materials and Methods

2.1. Time and location

This study was conducted in three stages: Stage 1 from 11 to 18 April 2021, stage 2 from 16 to 22 April 2022, and stage 3 from 14 to 22 April 2023 in 11 communes/towns belonging to Duc Hue district of Long An province (Table 1).

2.2. Sampling method

The KAP surveys were conducted collaboratively by the research team at the same time as the implementing of the vaccination campaign in Duc Hue district. The participants in this study were selected through a random sampling process, employing a sampling ratio of 1/3. This ratio dictated that one household was included in the survey for every three households that had received vaccinations. Determining the number of respondents per commune was established beforehand, considering the proportion of households in each commune and the calculated minimum sample size. Household data for each commune was obtained from the District Veterinary Station. The Statulator software, accessible at <https://statulator.com>, was utilized to compute the minimum sample size. The calculations were based on a confidence interval of 95.00%, an anticipated proportion of respondents possessing satisfactory knowledge, attitude, and practice at 75.00%, and an absolute error of 5.00%. Despite the initial calculation yielding a minimum sample size of 289, a decision was made to round it up to 300. Subsequent adjustments to the sample size were made in response to on-the-ground resource constraints, resulting in two subsequent increments.

Moreover, selecting a sampling rate was rationalized by the uncertainty surrounding the total count and annual variations in pet households. Aligned with the principle that an increased sample size augments results in representativeness, the actual number of collected samples surpassed the initially proposed quantity, as explicitly elucidated in the results section.

2.3. Methods

The study was conducted with two primary objectives including (1) summarizing the vaccination coverage rate in 2023 and (2) classification of dog and cat owners over the years 2021, 2022, and 2023 based on information regarding their knowledge, attitude, and practice.

The first objective was to synthesize vaccination information records from the local District Veterinary Stations of Long An's Sub-DAH (DAH, 2023). The second objective was achieved by implementing a Knowledge, Attitude, and Practice (KAP) survey, for which a structured questionnaire comprising four sections was devised. The first section encompassed demographic data and elucidated why owners abstained from vaccinating their pets. The second section comprised eight inquiries about knowledge, the third section entailed eleven questions related to attitude, and the final section encompassed eight questions concerning practice (see supplementary material 1). Following a thorough review and testing by a research team member, the questionnaire was digitally converted using the Kobotoolbox platform (<https://www.kobotoolbox.org>) to facilitate data collection through tablets smartphones or laptops in practice. The data collection occurred concurrently with direct interviews conducted during the vaccination process. The interviewers underwent training before field data collection. Subsequently, the acquired data was submitted and subjected to validation on the Kobo toolbox platform. The KAP survey was repeated for three years using the above-mentioned procedure. Data was encrypted and summarized automatically thanks to the available functions of Kobotoolbox.

2.4. Statistical data analysis

The responses to each question in the Knowledge, Attitude, and Practice (KAP) survey were quantified based on correctness, assigning 1 point for correct responses and 0 points for incorrect ones or no answers. The cumulative scores obtained in each section - Knowledge, Attitude, and Practice were treated as distinct active quantitative variables in the subsequent Principal Component Analysis (PCA) to represent the participants' scores. The PCA and clustering analysis (CA) methodologies were employed to stratify pet owners longitudinally. Specifically, PCA was applied to identify primary components and eliminate less significant variables in the dataset (Cornillon et al., 2012). The analysis incorporated three primary quantitative variables ("Knowledge," "Attitude" and "Practice"), supplemented by additional quantitative variables such as "number of pets" and "number of family members," alongside qualitative variables encompassing "commune location," "educational levels," "gender of pet owners," "vaccination outcome," and "age." Subsequently, CA, contingent on the PCA and distance matrix outcomes results, was executed to categorize these variables into groups, considering their co-occurrence and distances (Cornillon et al., 2012). The computer automatically determined the optimal clusters for each dataset to streamline the process. Both PCA and CA were conducted utilizing the FactoMineR package (Le et al., 2008) in the R.4.2.1 software environment.

3. Results

3.1. Evaluation of vaccination results in 2023

The outcomes of rabies vaccination and the underlying reasons for non-vaccination within the pet populations were systematically compiled for each respective year (Table 1). Notably, the results for the years 2021 and 2022 have been documented elsewhere (Truong et al., 2023); therefore, the focus of this report centers

on the findings for the year 2023. Until April 22, 2023, the rabies vaccination coverage rate within households had reached 70.00%. Considering the entire investigated population, the overall vaccination rate for the pet populations stood at 81.00%. The commune of My Thanh Bac exhibited the highest percentage of vaccination coverage rate at 89.00%, while the lowest coverage rate was recorded in My Binh commune, amounting to 72.00%.

Table 1. Vaccination outcomes calculated based on households and total pet population, distributed by commune locations.

Order	Commune	2021				2022				2023			
		(1) ¹	(2) ²	(3) ³	(4) ⁴	(1) ¹	(2) ²	(3) ³	(4) ⁴	(1) ¹	(2) ²	(3) ³	(4) ⁴
1	Dong Thanh	270	57.40	388	71.10	270	67.80	537	66.10	280	69.30	496	81.00
2	My Thanh Dong	842	53.60	1338	83.10	829	59.80	1450	74.60	873	53.50	1332	77.00
3	Binh Hoa Nam	423	52.20	1143	39.90	396	63.60	668	76.30	423	57.20	659	83.00
4	My Quy Tay	663	64.60	702	123.60	656	71.00	1207	83.30	487	85.00	1155	86.00
5	My Quy Dong	427	58.80	712	81.60	423	65.70	770	75.20	450	61.80	831	78.00
6	My Thanh Tay	412	74.80	462	128.80	427	82.00	782	83.20	460	92.60	975	81.00
7	My Thanh Bac	254	71.70	398	89.50	251	85.70	526	80.00	417	59.70	586	89,0
8	Binh Thanh	402	30.30	399	66.20	307	60.90	463	83.80	216	126.40	698	76.00
9	Binh Hoa Hung	278	51.80	572	49.70	298	56.40	365	85.20	186	74.20	s355	79.00
10	Binh Hoa Bac	414	53.40	608	71.70	409	105.90	630	89.40	455	58.00	616	87.00
11	My Binh	280	46.40	395	64.80	214	44.40	331	74.00	201	76.60	407	72.00
	Total	4665	56.00	7117	77.10	4480	69.70	7729	79.10	4448	69.70	8110	81.00

¹Number of dog/cat-owning households; Data provided by the District Veterinary Station before the vaccination campaign (updated data on households after vaccination was not available for calculation);

²% of household vaccination coverage; data calculated by dividing the number of households that had pet vaccinated for initiative data;

³Total pet population; data generated after vaccination campaign;

⁴% of pet vaccination coverage; data calculated by dividing the number of pets vaccinated by total pet population planned for vaccinating.

3.2. Reasons for non-vaccination

The reasons for not being vaccinated among pet populations in 2023 were summarized in Table 2.

Table 2. The reasons for non-vaccination

Reasons for non-vaccination	n	%
All the animals were vaccinated	902	36.74
Non-vaccination without specific reasons	743	30.26
Previously vaccinated (under 3 months)	48	1.96
Indoor and safe dogs, do not require vaccination	4	0.16
More than 2 reasons ¹	91	3.71
Other reasons (sick dog, elderly, lactating dog, etc)	296	12.06
Total	2455	100.00

¹Reasons were mentioned in the table.

In 2023, some reasons for non-vaccination were mentioned such as all the animals previously vaccinated (36.74%), or non-vaccination without specific reasons (30.26%). Other reasons for non-vaccination such as sick dogs, elderly dogs, lactating dogs, and dogs not old enough for vaccination, were a relatively lower proportion.

3.3. Correlation between knowledge – attitude – behaviors variables and the clustering of pet owners over the years

3.3.1. Description of the demographic characteristics of interview participants in KAP surveys over the years

The demographic characteristics of participants in KAP surveys conducted over three years are briefly presented in Table 3. The

number of participants in the KAP surveys continuously increased across 2021, 2022, and 2023, with specific counts of 314, 476, and 1067, respectively. The demographic profile of dog and cat owners in the investigated area indicated a predominant occupation in farming, with the highest educational attainment, primarily secondary education. Notably, individuals aged 30 to 50 actively participated in vaccination campaigns, constituting the highest percentage (51.30%) in 2022. The average number of pets per household predominantly remained below five with most households primarily raising dogs, followed by a combination of dogs and other pets, as delineated in Table 3.

Table 3. Demographic characteristics of households participating in KAP surveys

	2021 (n = 314) %	2022 (n = 476) %	2023 (n = 1,067) %
Age			
< 30	3.80	5.60	5.00
30-50	48.40	51.30	47.90
> 50	47.80	43.10	47.10
Educational levels			
+ High school	94.60	93.30	93.00
+ Intermediate/College/University	5.40	6.30	6.90
+ Postgraduate	0.00	0.40	0.10
Occupation			
+ Farmer	85.40	77.70	76.90
+ Authority personnel	3.80	4.00	3.10
+ Soldier	0.30	0.60	0.30
+ Private enterprise/Officer	0.00	0.90	1.20
+ Business	5.10	8.40	9.20
+ Others	5.40	8.40	9.30
Number of family members			
<3	12.40	11.80	11.30
3-5	75.20	77.70	69.60
>5	12.40	10.50	19.10
Number of owned pets			
<3	53.50	54.80	52.30
3-5	39.20	35.30	37.80
>5	7.30	9.90	9.90
Number of pet species/household			
+ Only dog ownership	89.50	88.70	85.50
+ Only cat ownership	0.00	0.60	1.10
+ Other pets	0.30	0.00	0.00
+ Household with two or more species	10.20	10.50	12.70
+ Household with no species	0.00	0.20	0.70
Number of vaccinated pets / households	2.30 (0-9) ¹	2.30 (0-15) ¹	2.00 (0-16) ¹
Number of non-vaccinated pets/household	0.60 (0-5) ¹	0.70 (0-10) ¹	1.00 (0-10) ¹

¹Data presented in mean (minimum-maximum) values.

3.3.2. Correlation between knowledge – attitude – behaviors variables and the clustering of pet owners over the years

a) Pairwise correlation between variables among three years

In 2021, the correlation matrix among the active variables indicated a relatively fragile correlation between knowledge and practice (correlation coefficient [CC] = 0.19, and attitude and practice (CC = 0.12) (Table 4). The positive

correlation between knowledge and attitude was also weak (CC = 0.09) (see Table 4). In 2022, a weak correlation was observed between knowledge and attitude (CC = 0.19), knowledge and practice (CC = 0.09), and attitude and practice (CC = 0.13). In 2023, the correlation matrix elucidates a close interconnection between knowledge and practice (CC = 0.35), knowledge and attitude (CC = 0.31), and attitude and practice (CC = 0.25).

Table 4. Correlation matrix among active variables in three studies years

Pairwise Correlation	2021	2022	2023
Knowledge vs. Attitude	0.07	0.19	0.35
Knowledge vs. Practice	0.19	0.09	0.31
Attitude vs. Practice	0.12	0.13	0.25

b) Cluster classification

In 2021, the cluster analysis showed that people who raised dogs and cats in the investigated area can be divided into three distinct clusters. The average scores for the population for each variable - knowledge, attitude, and practice - were 4.80, 5.50, and 4.40, respectively. The clusters had the strongest association with the knowledge variable (CC = 0.94), a less close connection with the practice variable (CC = 0.66), and the weakest association with the attitude variable (CC = 0.02). Moreover, Cluster 1 encompassed households with an average knowledge score of 3.90 and a practice score of 4.20, which were lower than the population's mean values (Table 5). In contrast, Cluster 2 included households with average knowledge and practice scores of 5.00 and 3.80, respectively, exceeding the population's averages. Notably, the attitude variable did not serve as a categorical determinant for either

Cluster 1 or Cluster 2. Conversely, Cluster 3 consisted of households exhibiting exceptional scores in knowledge (5.00), attitude (5.70), and practice (4.90), all surpassing the overall mean of the population. The formation of distinct clusters was minimally influenced by qualitative and quantitative complementary variables, except for Cluster 2, where commune location exerted a significant impact. Specifically, the KAP scores in Cluster 2 were predominantly influenced by residents of the My Quy Tay and Binh Hoa Nam communes.

In 2022, the region's owners of dogs and cats manifested discernible distinctions that facilitated their categorization into three distinct clusters. The overall mean scores across the knowledge and attitude variables for the entire population were 5.30 and 4.80, respectively. Notably, the practice variable did not exhibit a robust statistical association and was excluded

from cluster classification. Regarding the interrelation between the three clusters and the principal quantitative variables (knowledge and attitude), the clusters exhibited a pronounced correlation with the knowledge variable ($CC = 0.91$) and a comparatively weaker association with the attitude variable ($CC = 0.61$). Cluster 1 comprised participants with average scores for attitude (4.90) and knowledge (3.90) that fell below the overall mean value. Cluster 2 encompassed households with a knowledge score (5.00) surpassing the overall average, yet the attitude score (4.20) lagged. Cluster 3 demonstrated commendable knowledge scores (5.00) and a positive attitude (6.50), with average scores exceeding the overall mean. Commune location and educational levels emerged as pivotal factors significantly impacting the formation of clusters. In particular, the KAP survey responses in cluster one predominantly originated from households in My Thanh Bac and My Quy Tay communes, with no statistically significant variance in educational levels within this cluster. Cluster 2 was principally influenced by individuals from My Thanh Tay commune, where the dominant educational level among contributors was university graduates. Cluster 3 was predominantly constituted by residents

of My Quy Dong and My Thanh Bac, with prevailing educational levels being university or college graduates.

In 2023, individuals who maintain dogs and cats in the locality exhibited distinguishable characteristics, classifying them into four discrete clusters based on the results. The mean scores for the knowledge, attitude, and practice variables were 4.60, 8.40, and 6.40, respectively. Participants in Cluster 1 displayed below-average scores in attitude (7.50), knowledge (3.60), and practice (3.90). Cluster 2 comprised individuals with below-average scores in knowledge (4.20) and attitude (7.30) but above-average scores in practice (7.20) compared to the overall population mean. Cluster 3 consisted of individuals with commendable knowledge scores (5.00) but relatively lower practice scores (4.70), with attitude scores not factoring into the classification of this cluster. Cluster 4 included individuals with scores above the population mean in all three domains, encompassing knowledge (4.90), attitude (9.20), and practice (7.50).

Table 5. Summary of cluster score and classification

	The mean score in each cluster				Overall mean score in population
	Cluster 1	Cluster 2	Cluster 3	Cluster 4	
2021					
+Knowledge	3.90	5.00	5.00	N/A	4.80
+Attitude	N/A	N/A	5.7	N/A	5.50
+Practice	4.20	3.80	4.90	N/A	4.40
+Cluster characteristics	LK ² , LP ²	HK ² , LP ²	HK ² , HA ² , HP ²		
+Significant variables ¹	Commune location (for cluster 2 only)				
2022					
+Knowledge	3.90	5.00	5.00	N/A	4.80
+Attitude	4.90	4.30	6.50	N/A	5.30
+Practice	N/A	N/A	N/A	N/A	
+Cluster characteristic	LK ² , LA ²	HK ² , LA ²	HK ² , HA ²		
+Significant variables ¹	Commune location ($P = 1.08 \times 10^{-6}$) and education levels ($P = 7.41 \times 10^{-3}$)				
2023					
+Knowledge	3.60	4.20	5.00	5.00	4.60
+Attitude	7.50	7.30	N/A	9.20	8.40
+Practice	3.90	7.20	4.70	7.50	6.40
+Cluster characteristic	LK ² , LA ² , LP ²	LK ² , LA ² , HP ²	HK ² , LP ²	HK ² , HA ² , HP ²	
+Significant variables ¹	Commune location ($P = 1.40 \times 10^{-08}$), occupation ($P = 1.04 \times 10^{-05}$) and educational levels ($P = 1.83 \times 10^{-03}$)				

¹ *p-value calculated from Chi-square test.*² *"L" low; "H" high; "K" knowledge; "A" attitude; "P" practice.*

c) Significant variables contribution to cluster

In 2021, no additional variables significantly contributed to forming the three clusters, except the contribution of the commune location variable for cluster 2. In fact, the second cluster was characterized by individuals residing predominantly in My Quy Tay (45.70%) and Binh Hoa Nam (66.60%).

In the subsequent year, 2022, location and educational level were identified as significant contributors to the formation of these clusters. The first cluster (LK, LA) in 2022 was characterized by individuals residing predominantly in My Thanh Bac (42.00%) and My Quy Tay (30.00%). The second cluster (HK, LA) in 2022 was characterized by individuals residing in My Thanh Tay (55.70%) and those who had completed their undergraduate education (5.50%). The third cluster (HK, HA) in 2022 was characterized by individuals who had undergraduate degree (83.30%) and were located in My Quy Dong (69.20%).

In 2023, three additional variables-commune location, occupation, and educational levels significantly influenced the formation of these clusters. The first cluster (LK, LA, LP) was characterized by individuals predominantly residing in My Quy Tay commune, with 48.50% of individuals from My Quy Tay commune falling into this cluster. Additionally, 16.4% of individuals engaged in farming belonged to cluster 1. Similarly, the second cluster (LK, LA, HP) was characterized by individuals mainly residing in Binh Thanh (52.70%), My Quy Dong (43.40%), and Binh Hoa Nam (42.10%) communes. Moreover, 26.40% of individuals involved in farming contributed to cluster 2. The third cluster (HK, LP) was characterized by 65% of individuals from My Thanh Bac and

33.30% from Binh Hoa Bac. The final cluster (HK, HA, HP) was characterized by 75.00% of individuals from My Thanh Tay and 79.10% from Dong Thanh commune. Furthermore, 61.60% of opinions from individuals with other occupations contributed to the latter cluster.

4. Discussion

Evaluation is a crucial step of programming implementation which helps us to be able to identify strong points, weak points as well as things that need to be improved. After 3 out of 5 years of implementing vaccination campaign in the field, there was a strong requirement for us to perform a mid-term review. Overall, the vaccination coverage in Duc Hue districts increased significantly after three years, especially in 2023 (81.00%). This result satisfied the standard requirement of a 70.00% vaccine coverage rate as stated by WHO (2019). However, the quantitative antibody testing post-vaccination on animals vaccinated was lacking due to limited resources, therefore we could not guarantee the efficacy of mass vaccination in animals to prevent rabies in animals and the human population.

Our cluster analysis highlights the role of commune-location which was notably identified as having the most significant contribution to the formation of knowledge, attitude, and practice. The cluster 2 in 2021, which was identified by high knowledge and low practice come mainly from Binh Hoa Nam where the vaccination rate in animal production was low in 2021 (39.90%). This phenomenon reflected the reality that people may have good knowledge but similar findings could not be seen in their practice (Pham et al., 2019). Binh Hoa Nam was facing the problem of human resources for doing vaccination,

therefore the vaccination rate was low. Even with the support from students but lacking the cooperation of local guides (paraveterinary, village chef), the expected vaccination rate could not be achieved. Another commune contributed to cluster 2 was My Quy Tay facing another problem of vaccination - animal statistics. The high vaccination rate reflected the fact that animal population in this area in the previous year could not be estimated with certain data. It might be because in a normal situation without the support of students for vaccinating, a high estimation of animal population could lead to in mission being uncompleted and further punishment could be followed as a consequence or a part of the population could be served as a client for their private business. In 2022, our result highlighted the significant role of educational level in influencing the levels of knowledge, with clusters 2 and 3 demonstrating elevated knowledge levels associated with individuals possessing undergraduate education. In 2023, the noteworthy contribution of individuals from My Quy Tay to Cluster 1 was observed, mirroring findings from 2021. This continuity suggests that the vaccination campaign did not effectuate a substantive change in knowledge levels for individuals in this commune. Additionally, owing to an increased sample size in 2023, farmers emerged as crucial contributors to cluster 1. Clusters characterized by lower KAP's scores often constitute individuals residing in border-area communes who identified as farmers. Consequently, future strategy in Duc Hue, Long An, should prioritize these demographics. The longitudinal findings underscored the inefficacy of conventional methods, such as the distribution of leaflets to raise awareness among animal owners. Existing studies posited that individuals with favorable

KAP attributes typically access information from at least three diverse sources (Bui et al., 2016; Nguyen & Nguyen, 2020). Providing free pet vaccination was argued to be critical and conducive to community mobilization in developing countries (Duamor et al., 2023). Nevertheless, the program exhibited a declining trend in attitude scores (data not presented in the manuscript), signaling a negative impact on community awareness. Despite this, the conviction remained that vaccination programs directly influence coverage rates and positively contribute to social awareness regarding rabies vaccination. Moreover, pet vaccination comprised only 7.00% of the estimated cost for rabies and rabies prevention (Shwiff et al., 2018), positioning proactive pet vaccination as a local objective for advancing a rabies-free status. Consequently, future initiatives should adopt diverse approaches to enhance effectiveness of awareness, considering avenues such as radio broadcasts, postcards, and awareness campaigns targeting secondary and high school students. A unique objective to point out during the awareness program is that pet owners are urged to proactively seek vaccination for their animals, underscoring their responsibility for both the health of their pets and the well-being of their communities.

5. Conclusions

Following the three-year rabies vaccination campaign from 2021 to 2023 in Duc Hue district, commendable achievements were noted in attaining high vaccination coverage rates. The analysis revealed the consistent presence of three to four distinct population clusters each year, predicated on KAP scores and their alignment with the overall mean in the

population. Additionally, particular attention was directed towards clusters exhibiting lower scores, with specific consideration given to associated variables such as commune location and occupation. These clusters warrant further exploration and demand more tailored strategies for communication and approach to optimize the efficacy of the vaccination campaign. The KAP surveys must be conducted continuously, serving as a pivotal tool for ongoing program evaluation, particularly in gauging the sustained impact of the initiative in the long term.

Conflict of interest

We certify that the article was conducted by the authoring team and there are no conflicts of interest to declare among authors.

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References

- Borse, R. H., Atkins, C. Y., Gambhir, M., Undurraga, E. A., Blanton, J. D., Kahn, E. B., Dyer, J. L., Rupprecht, C. E., & Meltzer, M. I. (2018). Cost-effectiveness of dog rabies vaccination programs in East Africa. *Plos Neglected Tropical Diseases* 12(5), e0006490. <https://doi.org/10.1371/journal.pntd.0006490>.
- Bui, U. V., Nguyen, N. T. T., & Vu, N. S. (2016). Knowledge and practice on prevention of human rabies in Son Dong and Tu Du communes, Lap Thach district, Vinh Phuc province, 2015. *Vietnam Journal of Preventive Medicine* XXVI(10), 183.
- Cornillon, P. A., Guyader, A., Husson, F., Jegou, N., Josse, J., Kloareg, M., Lober, E. M., & Rouvière, L. (2012). *R for Statistics* (1st ed.). New York, USA: Chapman and Hall/CRC. <https://doi.org/10.1201/b11828>.
- DAH (Department of Animal Health). (2023). *Report of rabies prevention and control conference 2023*. Gia Lai, Vietnam: Gia Lai Publisher.
- Duamor, C. T., Hampson, K., Lankester, F., Lugelo, A., Chagalucha, J., Lushasi, K. S., Czapryna, A., Mpolya, E., Kreppel, K., Cleaveland, S., & Wyke, S. (2023). Integrating a community-based continuous mass dog vaccination delivery strategy into the veterinary system of Tanzania: A process evaluation using normalization process theory. *One Health* 17, 100575. <https://doi.org/10.1016/j.onehlt.2023.100575>.
- Friedmann, E., & Son, H. (2009). The Human-companion animal bond: How humans benefit. *Veterinary Clinics of North America: Small Animal Practice* 39(2), 293-326. <https://doi.org/10.1016/j.cvsm.2008.10.015>.
- Gebremedhin, E. Z., Sarba, E. J., Getaneh, A. M., Tola, G. K., Endale, S. S., & Marami, L. M. (2020). Demography and determinants of dog and cat ownership in three towns of West Shoa zone, Oromia Region, Ethiopia. *BMC Veterinary Research* 16(1), 481. <https://doi.org/10.1186/s12917-020-02699-4>.
- GOV (Government of the Socialist Republic of Viet Nam). (2021). Decision No. 2151/QĐ-TTg dated on December 21, 2021. Approve the national rabies prevention and control program from 2022 to 2030. Retrieved October 25, 2023, from <https://lawnet.vn/vb/Quyet-dinh-2151-QD-TTg-2021-Chuong-trinh-quoc-gia-phong-chong-benh-Dai-2022-2030-79A0E.html>.
- Le, S., Josse, J., & Husson, F. (2008). FactoMineR: An R package for multivariate analysis. *Journal of Statistical Software* 25(1). <https://doi.org/10.18637/jss.v025.i01>.

- Nguyen, T. T., & Nguyen, S. M. (2020). Status of knowledge, attitudes, and practices regarding rabies prevention in Tuy An district, Phu Yen province, 2019. *Journal of Medical Research* 128(4), 189-198.
- NIHE (National Institute of Hygiene and Epidemiology). (2023). *Image of rabies in Vietnam and national program of control and eradication of rabies 2022 - 2030*. Gia Lai, Vietnam: Gia Lai Publisher.
- Paul, M., King, L., & Carlin, E. P. (2010). Zoonoses of people and their pets: A US perspective on significant pet-associated parasitic diseases. *Trends in Parasitology* 26(4), 153-154. <https://doi.org/10.1016/j.pt.2010.01.008>.
- Pham, D. Q., Phan, T. L., Nguyen, T. P. T., Doan, Q. N. M., Nguyen, H. D., Luong, Q. C., & Nguyen, T. V. (2021). An evaluation of the rabies surveillance in Southern Vietnam. *Frontiers in Public Health* 9, 610905. <https://doi.org/10.3389/fpubh.2021.610905>.
- Pham, P. D., Cook, M. A., Cong, H. H., Nguyen, H. T., Padungtod, P., Nguyen, H. T., & Dang, S. X. (2019). Knowledge, attitudes and practices of livestock and aquaculture producers regarding antimicrobial use and resistance in Vietnam. *Plos One* 14(9), e0223115. <https://doi.org/10.1371/journal.pone.0223115>.
- Shwiff, S. A., Brown, V. R., Dao, T. T., Elser, J., Hoang, T. X., Nguyen, T. N., Nguyen, H. T., Nguyen, H. T. T., Riewpaiboon, A., Ernst, K., Shwiff, S., & Payne, D. (2018). Estimating the economic impact of canine rabies to Viet Nam 2005 - 2014. *Plos Neglected Tropical Diseases* 12(10), e0006866. <https://doi.org/10.1371/journal.pntd.0006866>.
- Tiwari, H. K., Vanak, A. T., O'Dea, M., & Robertson, I. D. (2018). Knowledge, attitudes and practices towards dog-bite related rabies in para-medical staff at rural primary health centers in Baramati, western India. *Plos One* 13(11), e0207025. <https://doi.org/10.1371/journal.pone.0207025>.
- Truong, B. D., Ly, S. H., Nguyen, D. T. T., Le, T. Q., Duong, M. T., Nguyen, P. T., T., Vo, N. T., Nguyen, D. D., Tran, V. T., Vo, T. K. O., Le, T. M. K., & Vo, M. D. (2023). Evaluation rabies vaccination and changing in knowledge - attitude and practice of rabies prevention in animal in Duc Hue district - Long An province, 2021- 2022. *Journal of Veterinary Science and Technology* XXX(4), 76-79.
- Vilas, V. J. D. R., Carvalho, M. J. F. D., Vigilato, M. A. N., Rocha, F., Vokaty, A., Pompei, J. A., Flores, B. M., Fenelon, N., & Cosivi, O. (2017). Tribulations of the last mile: Sides from a regional program. *Frontiers in Veterinary Science* 4. <https://doi.org/10.3389/fvets.2017.00004>.
- WHO (World Health Organisation). (2023). Rabies. Retrieved October 25, 2023, from <https://www.who.int/news-room/fact-sheets/detail/rabies>.
- WHO (World Health Organization). (2019). Zero by 30: the global strategic plan to end human deaths from dog-mediated rabies by 2030: United Against Rabies Collaboration: first annual progress report: global strategic plan to end human deaths from dog-mediated rabies by 2030. Retrieved October 25, 2023, from <https://iris.who.int/handle/10665/328053>.

Optimization of alkali-catalyzed organosolv treatment of spent coffee grounds for obtaining polysaccharides

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ABSTRACT

The coffee industry is growing rapidly and generating increasing amounts of spent coffee grounds annually. Spent coffee grounds contain high levels of polysaccharide, which needs in-depth research to obtain and transform into value-added products. This study was carried out to optimize the alkali-catalyzed organosolv treatment of spent coffee grounds to enrich the polysaccharide content. A three-factor central composite design of the response surface model was used to optimize the treatment variables including reflux time, NaOH, and acetone concentration to yield the highest polysaccharide level. As a result, the maximum polysaccharide content was 73.13% obtained at a reflux time of 4.5 h, 62% acetone with 0.91% NaOH. The polysaccharide-rich material from spent coffee ground was composed of 39.37% mannan, 10.40% glucan, and 9.33% galactan. Partial removal of lignin and protein was observed during the treatment. Enzymatic hydrolysis of the spent coffee polysaccharides released the highest reducing sugars of 5583 mg/L using an enzyme cocktail containing 4% of cellulase and 1% of mannanase after 96 h. The enzymatic hydrolysate contained 3190 mg/L mannose and 1790 mg/L glucose, showing a feasible transformation of spent coffee polysaccharides.

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1. Introduction

Spent coffee grounds (SCGs) have no commercial value and are usually discarded as solid waste or sent to compost facilities. In recent years, the increasing need for waste reduction and environmental protection has stimulated the search for possible methods of valorizing

this waste. SCGs contain large amounts of organic compounds including lipid, cellulose, hemicellulose, and lignin. Currently, SCGs have been investigated for the production of biodiesel, activated carbon, and a source of sugars (Preethu et al., 2007; Mussatto et al., 2011; Caetano et al., 2012){Mussatto, 2011 #1; Caetano, 2012 #20}.

Being a porous and carbon-rich material, SCGs have been studied to become an inexpensive heavy metal adsorbent to replace traditional materials (Fiol et al., 2008).

Polysaccharides account for about 50% of total SCGs (Trinh et al., 2022). Arabinogalactan, galactomannan and cellulose are major polysaccharides in the SCGs, in which galactomannan is the most abundant component. Galactomannan has been noted as an excellent stiffener and stabilizer of emulsions, which shows potential in the food, pharmaceutical, and cosmetic industries (Ballesteros et al., 2015). Some findings have shown the antimicrobial and antioxidant capacities of polysaccharides extracted from SCGs (Ballesteros et al., 2015) and the immunostimulatory properties provided by coffee galactomannans and arabinogalactans (Simões et al., 2009). The prebiotic potential of spent coffee mannan has been also proved since it improves the health of human intestinal microflora (Asano et al., 2003; Gniechwitz et al., 2007). Besides being applied in the food and biomedical sector, spent coffee polysaccharides have been performed with potential uses in the manufacture of biodegradable materials.

The application of spent coffee polysaccharides is limited due to the presence of other components such as lignin, phenolic compounds, protein, and lipids attached to its structure. In our previous study, SCGs were treated using alkali and solvent to remove these components and make changes in SCG's structure. As a result of this, we obtained polysaccharide-rich materials that were effectively hydrolyzed into soluble sugars (Trinh et al., 2022). Combining alkali and solvent significantly improved the delignification of SCGs because both agents can cleave the ester linkages between polysaccharides and lignin. From these important findings, the

present study was conducted to optimize the alkali-catalyzed organosolv treatment of SCGs to achieve the highest polysaccharide level. Polysaccharides derived from SCGs can be applied in various sectors such as foods, medicals, and biomaterials. The conversion of SCGs into value-added products not only provides benefits for the coffee industry, improves income for farmers, but also reduces environmental impacts.

2. Materials and Methods

2.1. Materials

The SCGs were obtained from branched coffee shops in Ho Chi Minh City from October 2022 to December 2022. The collected samples were mixed and dried at 50°C until their moisture content reached below 10% before storing at room temperature.

Cellulase enzymes (700 EGU/g) and Mannanase enzymes (500 U/g) were provided by Novozyme and LeafCleanTech, respectively. 3,5-Dinitrosalicylic acid (DNS) was purchased from Duksan (Korea). Citric acid monohydrate and sodium sulfite were purchased from Merck. Sodium citrate trihydrate, Rochelle salt, sodium hydroxide, phenol, and n-hexane were supplied by Xilong (China).

2.2. Methods

2.2.1. Defatting

The SCGs were defatted using the process described previously with some modifications (Trinh et al., 2022). Briefly, 100 g of SCGs were immersed in n-hexane overnight with a ratio of material to solvent of 1:5 (w/v). The lipid extraction was conducted by sonication for 30 min twice. The defatted SCGs were separated by filtration and dried in an oven at 60°C. Its moisture content was measured before further experiments.

2.2.2. Alkali-catalyzed organosolv treatment of spent coffee grounds

Polysaccharide-rich materials were obtained by removing lipid, lignin, protein, and some phenolic compounds in the SCGs matrix. Defatted SCGs were treated by refluxing (0.5 - 6.5 h) using aqueous acetone (10 - 90%) with a ratio of material to solvent of 1:10. Alkali (NaOH) functioned as a catalyst with a concentration range of 0.1 - 1.3% (w/v). After processing, the solid was washed intensively with distilled water to remove residual alkali and other degradation products until the pH value reached 7.0. The solid was dried at 70°C and stored at 4°C before determining the polysaccharide content.

2.2.3. Experimental design

Optimization of polysaccharide content in SCGs was conducted using a central composite

design (CCD) of response surface methodology (RSM). Three independent variables were reflux time (X_1 , 0.5 - 6.5 h), NaOH concentration (X_2 , 0.1 - 1.3%), and acetone concentration (X_3 , 10 - 90%). There were 21 experimental runs including eight factorial points, six axial points, and seven center points. The significance level of the response surface model and the equation terms were studied by analysis of variance (ANOVA) with 95% confidence on Minitab 16 software. The quality of fit of the regression model was evaluated through the coefficient of determination (R^2), adjusted correlation coefficient ($\text{adj-}R^2$), and predictive correlation coefficient ($\text{pred-}R^2$). Independent variables and their levels used in the response surface design are presented in Table 1.

Table 1. Independent variables and their levels used in the response surface design

Independent variables	Unit	Coded	Coded factor level				
			- α	-1	0	+1	+ α
Reflux time	h	X_1	0.5	2	3.5	5	6.5
NaOH concentration	%	X_2	0.1	0.4	0.7	1	1.3
Acetone concentration	%	X_3	10	30	50	70	90

2.2.4. Determination of polysaccharide content

Polysaccharide content in the treated SCGs was determined by measuring the total reducing sugars released during the complete hydrolysis process using sulfuric acid reported previously. Polysaccharide-rich materials were hydrolyzed with 72% aqueous sulfuric acid for 60 min at 30°C, then distilled water was added to the mixture to dilute the acid to a 4% concentration, which was autoclaved for 60 min at 121°C. The acid hydrolysate was made up to a volume of 100

mL. Total reducing sugar content was quantified using 3,5-dinitrosalicylic acid (DNS) assay (Miller, 1959). Briefly, 1 mL of sample or glucose standard was mixed with 1 mL of DNS reagent. The mixture was kept in a boiling water bath for 5 min, then cooled down by immersing in a cold water bath before adding 3 mL of water. The absorbance of the mixture was measured using a spectrophotometer at 515 nm. Glucose was used as the standard with the range of 50 - 300 mg/L (Trinh et al., 2022).

2.2.5. Chemical compositions of spent coffee grounds

The moisture content of SCGs was measured by gravimetric analysis after drying in an oven at 105°C until obtaining constant weight mass. The nitrogen content of SCGs was estimated by the Kjeldahl method according to TCVN 10791:2015 (VS, 2015) and the protein content was measured using an appropriate Nitrogen Factor of 6.25. Ash is the inorganic residue remaining after dry oxidation at 550°C. The ash content of SCGs was quantified by gravimetric analysis according to TCVN 8124:2009/ISO 2171:2007 (VS, 2009). Acid-insoluble lignin or Klason lignin is the residual fraction after removing the ash by the complete acid hydrolysis of SCGs (Sluiter et al., 2008). Acid insoluble lignin was estimated by gravimetric analysis of the dry solid residue on the crucibles subtracted from the ash.

2.2.6. Enzymatic hydrolysis of SCGs

Polysaccharides obtained from SCGs can be used as a source of sugars through enzymatic hydrolysis. The treated SCGs were hydrolyzed using a combination of commercial polysaccharide-degrading enzymes including cellulase (Cellulast, 0 - 5%, enzyme to substrate) and mannanase (0 - 5%) at different ratios. The control experiment was conducted without adding an enzyme. Enzymatic hydrolysis experiments were performed in 0.05 M citrate buffer at pH 4.8 with a ratio of material to buffer of 1:25 (w/v). The mixture was incubated at 50°C

in a shaker at 120 rpm. The hydrolysate was collected every 24 h and then filtered through a nylon membrane of 0.22 µm before determining the total reducing sugars and monosaccharides (Trinh et al., 2022).

2.2.7. Analysis of monosaccharides

Monosaccharides including mannose, glucose, and galactose in the acid and enzymatic hydrolysate were determined by high-performance liquid chromatography (HPLC Agilent 1200 Infinity II) (Sluiter et al., 2008). The Rezex RPM-Monosaccharide Pb+2 (8%) column (100 x 7.8 mm) was stabilized at 40°C. The refractive index (RI) detector was set up at 80°C. The mobile phase was deionized water with a flow rate of 0.2 mL/min. Monosaccharide standards were prepared in the range of 100 - 1000 mg/L (Trinh et al., 2022).

3. Results and discussion

3.1. Optimization of treatment conditions of SCGs for obtaining polysaccharides

The experimental matrix, actual values, and predicted values are shown in Table 2. In the optimized model, the significance level $P < 0.05$ was used to test the effect of independent variables and their interaction with the response variable. The effect of reflux time (X_1), NaOH concentration (X_2), and acetone concentration (X_3) on polysaccharide content (Y) was expressed by a regression equation as follows:

$$\text{Polysaccharides (\%)} = 44.94 + 1.19 X_1 + 62.70 X_2 - 0.058 X_3 - 1.731 X_1^2 - 63.66 X_2^2 - 0.010327 X_3^2 + 3.82 X_1 \times X_2 + 0.1782 X_1 \times X_3 + 0.5809 X_2 \times X_3$$

Table 2. Response surface central composite design for polysaccharides

Run	Time (h)	Treatment conditions		Polysaccharide content	
		NaOH concentration (%)	Acetone concentration (%)	Actual value (%)	Predicted value (%)
1	2.0	0.4	30	63.73	64.96
2	5.0	0.4	30	53.75	52.80
3	2.0	1.0	30	66.06	64.15
4	5.0	1.0	30	58.19	58.86
5	2.0	0.4	70	46.07	44.88
6	5.0	0.4	70	52.73	54.10
7	2.0	1.0	70	57.59	58.01
8	5.0	1.0	70	75.84	74.10
9	0.5	0.7	50	54.72	55.19
10	6.5	0.7	50	59.06	59.12
11	3.5	0.1	50	40.70	40.22
12	3.5	1.3	50	58.41	59.42
13	3.5	0.7	10	58.41	58.63
14	3.5	0.7	90	53.49	53.79
15	3.5	0.7	50	72.81	72.74
16	3.5	0.7	50	71.55	72.74
17	3.5	0.7	50	72.50	72.74
18	3.5	0.7	50	73.98	72.74
19	3.5	0.7	50	72.54	72.74
20	3.5	0.7	50	72.54	72.74
21	3.5	0.7	50	72.54	72.74

Table 3 shows an analysis of variance (ANOVA) for the response surface quadratic model. The regression model was statistically significant ($P < 0.05$) and the reflux time, NaOH concentration, and acetone concentration significantly affected the polysaccharide content. The coefficient of determination $R^2 = 0.9909$ demonstrated experimental data were fitted well to the predicted model. The value of the predicted

determination coefficient of 0.9437 (pred- R^2) was high indicating the reliability of predicted values. In addition, the predicted- R^2 was in reasonable agreement with the adjusted- R^2 (the difference < 0.2). Furthermore, the p-value of lack of fit was not statistically significant ($P > 0.05$) demonstrating the regression model was compatible with the actual experiments.

Table 3. ANOVA for response surface quadratic model

Factor	Df	Adj Sum of Squares	Adj Mean Square	F	P
X_1	1	15.47	15.467	8.76	0.0143
X_2	1	368.35	368.353	208.51	0.0000
X_3	1	23.42	23.421	13.26	0.0045
$X_1 * X_1$	1	381.49	381.491	215.95	0.0000
$X_2 * X_2$	1	825.31	825.305	467.17	0.0000
$X_3 * X_3$	1	429.02	429.024	242.85	0.0000
$X_1 * X_2$	1	23.64	23.635	13.38	0.0044
$X_1 * X_3$	1	228.72	228.724	129.47	0.0000
$X_2 * X_3$	1	97.18	97.181	55.01	0.0000
Residual					
Lack of fit	5	14.62	2.29	4,79	0,0553
Model Summary					
S	R-sq		R-sq (adj)	R-sq (pred)	
1.32913	99.09 %		98.28 %	94.37 %	

The effect of reflux time, NaOH concentration, and acetone concentration on polysaccharide content is illustrated in Figure 1. As seen in the RSM plot, polysaccharide content increased as reflux time, NaOH and acetone concentration increased. The highest polysaccharide content was achieved for a 4.5 h reaction in 62% aqueous acetone in the presence of 0.91% NaOH. However, polysaccharide content gradually decreased by prolonging residence time and increasing acetone and NaOH concentration. Lignocellulosic biomass is composed of cellulose, hemicellulose, and lignin, which are linked together to form a complex and rigid structure. The treatment of lignocellulose with alkali generally breaks linkages between lignin - polysaccharides matrix, thus lignin was partially removed during the processing, leading to increased polysaccharide content in the treated solids (Chen et al., 2008; Garlock et al., 2012). In addition, organic solvents have a prominent effect on disrupting hydrogen and ether bonds, thereby a large amount of lignin was eliminated during organosolv treatment (Yin et al., 2021).

However, hemicellulose was easily degraded under harsh treatment conditions such as long reaction time and high alkali concentrations (Harun & Geok, 2016), resulting in a decrease in the polysaccharide content in the treated sample as clearly shown on the RSM plot.

The maximized polysaccharide content was predicted as 74.41% using the optimized model. The treatment of SCGs was conducted for 4.5 h refluxing in 62% aqueous acetone and 0.91% NaOH, thereby polysaccharide content in the treated materials was found to be 73.13%. The t-test at a 5% significance level showed no significant difference between the actual value and the predicted value. Therefore, the optimized conditions in this experiment were suitable for the treatment of SCGs to obtain the highest polysaccharide content. Polysaccharide content in the treated SCG significantly increased by 1.6 times compared to that in the defatted sample (45.24%). SCG polysaccharides can be used to produce biodegradable films, coatings, adsorbents, biopolymers, or additives in food and feed industry (Simões et al., 2009).

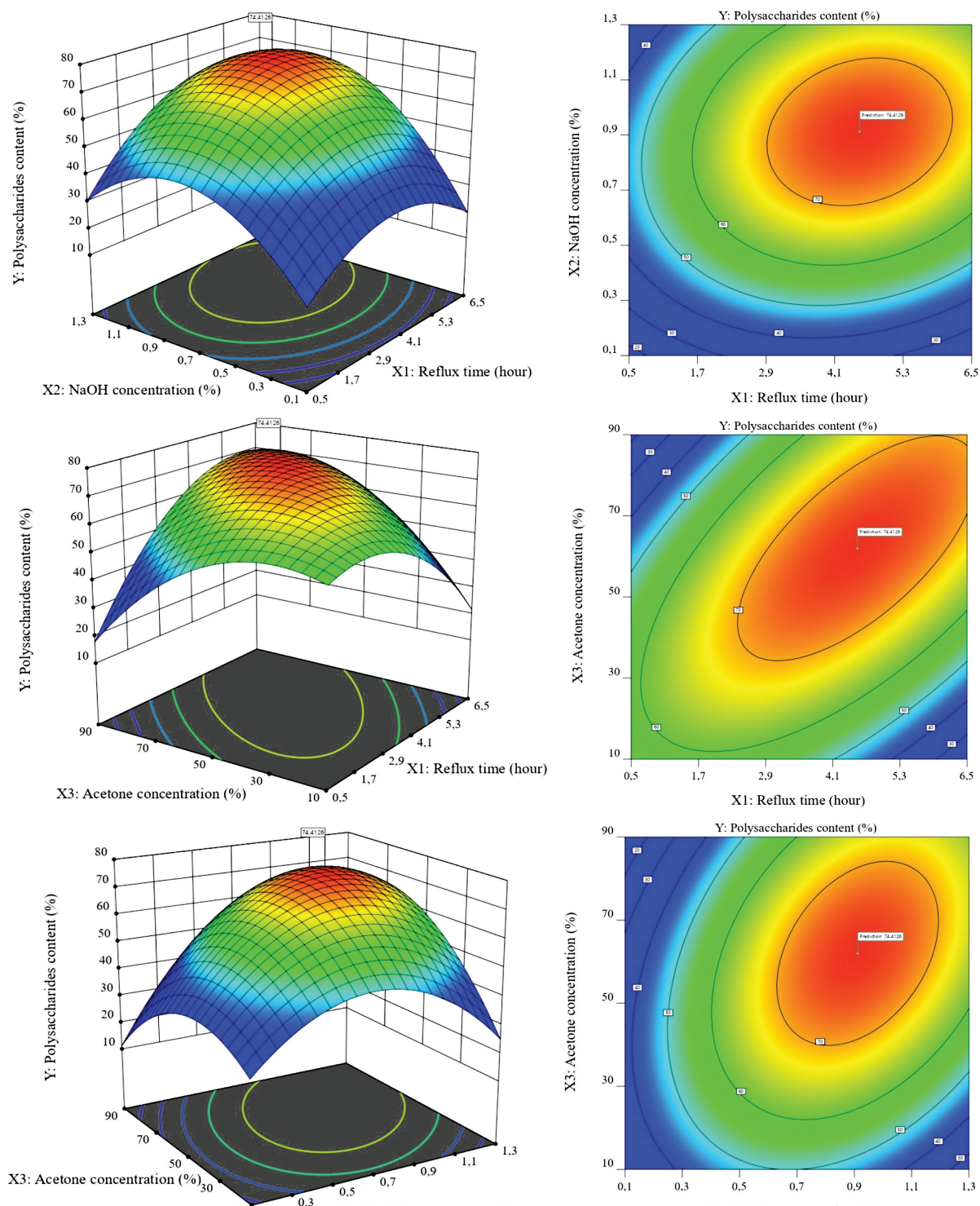


Figure 1. Response surface and contour plots showing the effect of the factors on polysaccharide content.

3.2. Chemical composition of polysaccharide-rich materials

The SCG polysaccharides were obtained by treating SCG with alkali and acetone at optimized conditions. Polysaccharides including glucan, galactan, and mannan in the untreated (defatted sample) and treated solids were analyzed by measuring glucose, galactose, and mannose in the acid hydrolysate of SCGs using HPLC. As shown in Figure 2, polysaccharide content in the treated solids significantly improved as compared to untreated SCGs. Mannan was the most abundant polysaccharide identified in the treated material, accounting for 39.37% of the solids and 1.5-fold higher than that in the untreated sample. Similarly, glucan and galactan were enriched in the obtained solid with values of 10.40%

and 9.33%, respectively. The enhancement of mannan, glucan, and galactan content in the treated samples was attributed to the effective removal of some components during the alkali-catalyzed organosolv treatment such as lignin and protein as shown in Figure 3. The findings were in agreement with our earlier studies (Trinh et al., 2022). The mannan level obtained by the integrated alkali-catalyzed organosolv method in this study was higher than in the SCG treated with individual ethanol (Nguyen et al., 2017) or alkaline (Trinh et al., 2022). Polysaccharide-rich materials derived from SCGs can be used as a potential source of prebiotics, sugars, and adsorbents, which would be applied in medical, food and feed industry, or biomaterial sectors (Simões et al., 2009).

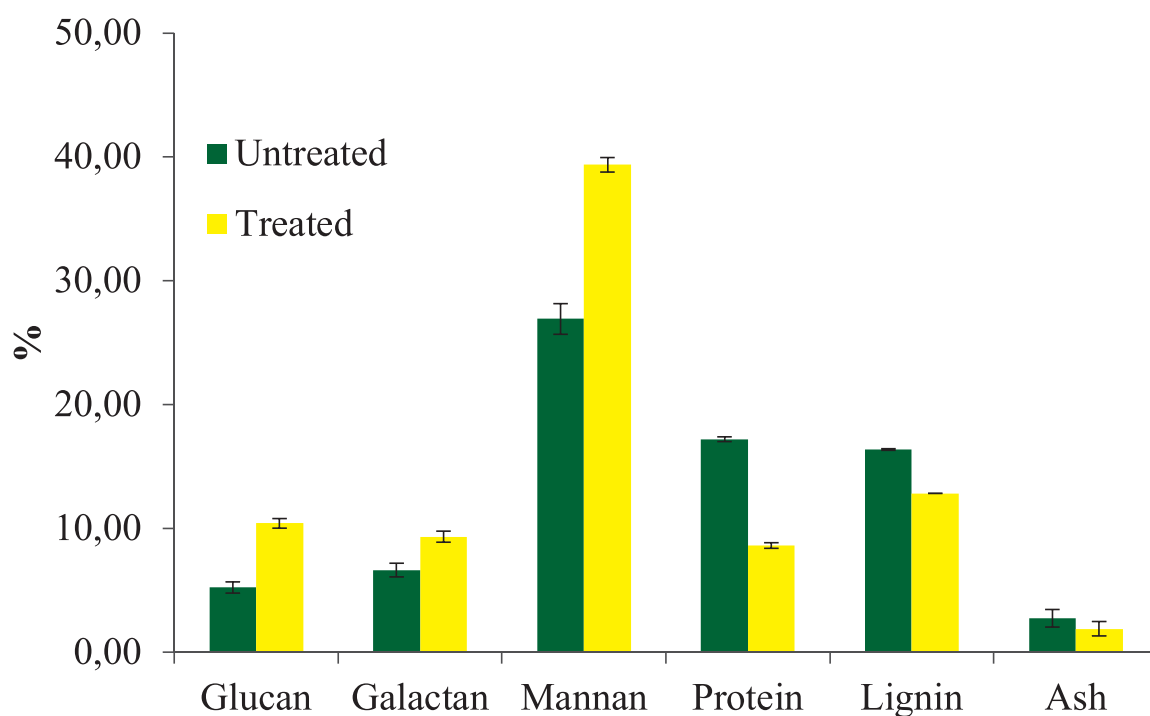


Figure 2. Chemical components of polysaccharides derived from spent coffee grounds.

3.3. Enzymatic hydrolysis of SCG polysaccharides

The SCGs-derived polysaccharides were rich in mannan, glucan, and galatan, which make them to be promising to produce soluble sugars. Enzymatic hydrolysis was an effective method to achieve high sugar yields from various lignocellulosic biomass (Trinh et al., 2018). Generally, enzymatic hydrolysis of biomass containing cellulose and hemicellulose requires a mixture of cellulase and hemicellulase to increase hydrolysis efficiency (Tang et al., 2020). Cellulast is a commercial cellulase that has been widely

used in the treatment of lignocellulosic materials because it can break β -1,4-glycosidic bonds in the structure of both cellulose and hemicellulose (Roy et al., 2020). Mannanase enzymes belonging to the hemicellulase group can catalyze the hydrolysis of the β -1,4-mannosidic bonds in the main chain of mannans that are found as the major polysaccharide in SCGs (Liao et al., 2014). To improve the release of sugar during the hydrolysis of SCG polysaccharides, our study performed a combination of cellulase and mannanase enzymes with different proportions at 5% of enzyme loading (enzyme to the substrate).

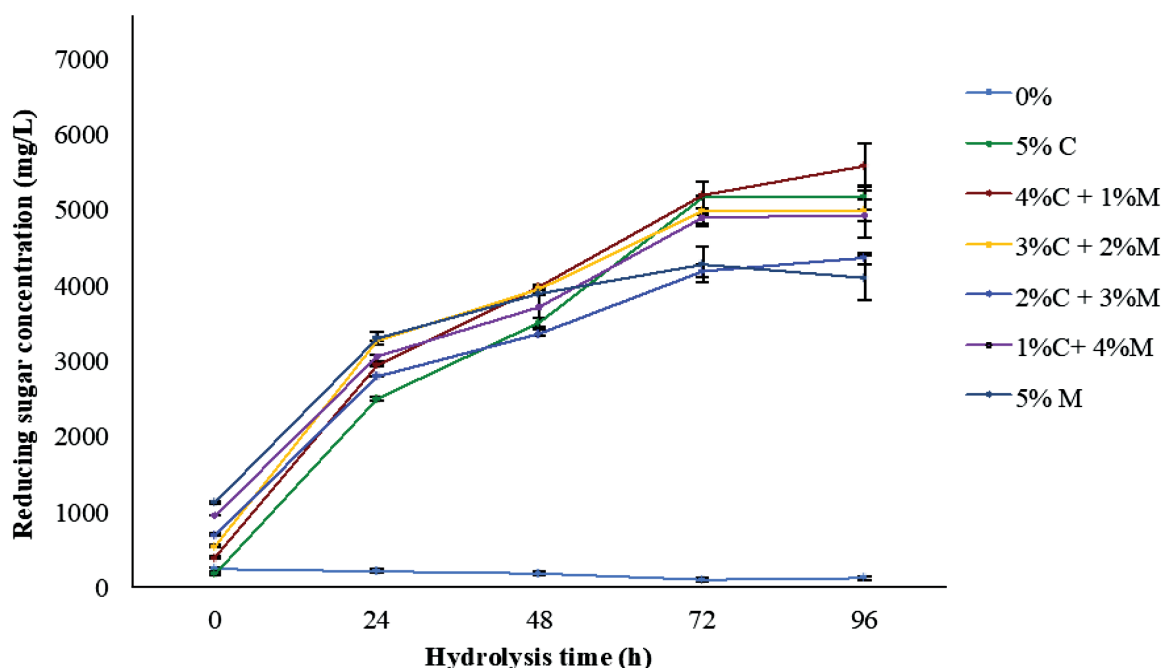


Figure 3. Enzymatic hydrolysis of spent coffee ground polysaccharides. C: Cellulase; M: mannanase.

Figure 3 shows the release of total reducing sugars during enzymatic hydrolysis of SCG polysaccharides. Reducing sugar concentrations reached 5141 mg/L and 4074 mg/L when using individual cellulase and mananase after 96 h, respectively. The findings indicated cellulase is more effective than mananase to hydrolyze SCG polysaccharides. The treatment using 4% of cellulase and 1% of mannanase (enzyme to the substrate) showed the highest reducing sugar yield of 5583 mg/L and it was statistically different from the other treatments ($P < 0.05$). The use of an enzyme combination is regarded as a promising strategy to enhance the hydrolysis efficiency of SCGs because of its synergistic effects. Additional mannanase has been reported to give better sugar yield than using only cellulase or xylanase for the hydrolysis of SCGs (Jooste et al., 2013). However,

increasing mannanase loading from 2 - 4% didn't have a positive effect on sugar yields as compared to using cellulase alone.

The data revealed the major role of cellulase with a suitable amount of mannanase required to enhance the hydrolysis efficiency of SCGs. Enzymatic hydrolysate obtained at the optimal treatment after 96 h contained 3190 mg/L mannose and 1790 mg/L glucose. Enzymatic conversion yield from polysaccharides to soluble sugars was estimated about 431.4 mg/g which were higher than our previously reported results using Viscozyme (Trinh et al., 2022). Additionally, alkali-catalyzed organosolv treatment made changes in SCG's structures and compositions, facilitating the enzyme accessibility to its substrate. However, galactose was not found in the hydrolysate (Figure 4).

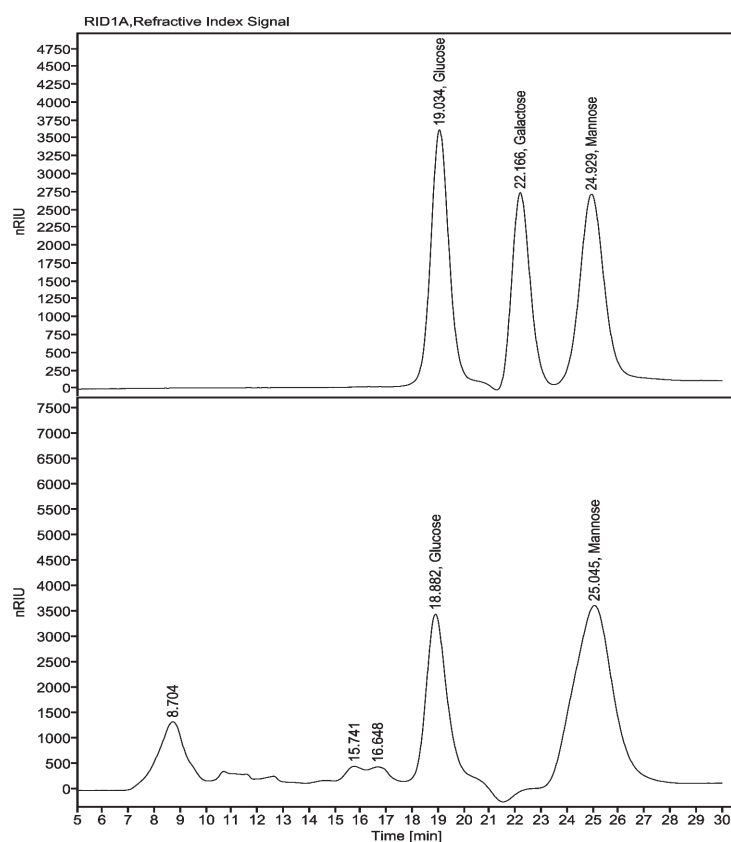


Figure 4. Chromatogram of sugar standards (above) and spent coffee hydrolysate (below).

5. Conclusions

In this study, the highest polysaccharide content reached 73.13% at optimized conditions including a reflux time of 4.5 h, 62% acetone with 0.91% NaOH. The polysaccharides from SCGs were composed of 39.37% mannan, 10.40% glucan, and 9.33% galactan. Lignin and protein were partially removed during the processing. The use of the enzyme mixture containing 4% of cellulase and 1% of mannanase gave the highest sugar production of 5583 mg/L through 96 h of hydrolysis. Enzymatic hydrolysis of the SCG polysaccharides released 3190 mg/L mannose and 1790 mg/L glucose.

Conflict of interest

The authors have no conflicts of interest to declare.

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References

- Asano, I., Hamaguchi, K., Fujii, S., & Iino, H. (2003). In vitro digestibility and fermentation of mannoooligosaccharides from coffee mannan. *Food Science and Technology Research* 9(1), 62-66. <https://doi.org/10.3136/fstr.9.62>.
- Ballesteros, L. F., Cerqueira, M. A., Teixeira, J. A., & Mussatto, S. I. (2015). Characterization of polysaccharides extracted from spent coffee grounds by alkali pretreatment. *Carbohydrate Polymers* 127, 347-354. <https://doi.org/10.1016/j.carbpol.2015.03.047>.
- Caetano, N. S., Silva, V. F., & Mata, T. M. (2012). Valorization of coffee grounds for biodiesel production. *Chemical Engineering Transactions* 26. <http://dx.doi.org/10.3303/CET1226045>.
- Chen, H. T., O'hara, J. F., Azad, A. K., Taylor, A. J., Averitt, R. D., Shrekenhamer, D. B., & Padilla, W. J. (2008). Experimental demonstration of frequency-agile terahertz metamaterials. *Nature Photonics* 2(5), 295-298. <http://dx.doi.org/10.1038/nphoton.2008.52>.
- Fiol, N., Escudero, C., & Villaescusa, I. (2008). Chromium sorption and Cr(VI) reduction to Cr(III) by grape stalks and yohimbe bark. *Bioresource Technology* 99(11), 5030-5036. <https://doi.org/10.1016/j.biortech.2007.09.007>.
- Garlock, R. J., Balan, V., & Dale, B. E. (2012). Optimization of AFEX™ pretreatment conditions and enzyme mixtures to maximize sugar release from upland and lowland switchgrass. *Bioresource Technology* 104, 757-768. <https://doi.org/10.1016/j.biortech.2011.11.034>.
- Gniechwitz, D., Reichardt, N., Blaut, M., Steinhart, H., & Bunzel, M. (2007). Dietary fiber from coffee beverage: degradation by human fecal microbiota. *Journal of Agricultural and Food Chemistry* 55(17), 6989-6996. <https://doi.org/10.1021/jf070646b>.
- Harun, S., & Geok, S. K. (2016). Effect of sodium hydroxide pretreatment on rice straw composition. *Indian Journal of Science and Technology* 9(21), 1-9. <https://dx.doi.org/10.17485/ijst/2016/v9i21/95245>.
- Jooste, T., García-Aparicio, M. P., Brienzo, M., van Zyl, W. H., & Görgens, J. F. (2013). Enzymatic hydrolysis of spent coffee ground. *Applied Biochemistry and Biotechnology* 169(8), 2248-2262. <https://doi.org/10.1007/s12010-013-0134-1>.
- Liao, H., Li, S., Zheng, H., Wei, Z., Liu, D., Raza, W., Shen, Q., & Xu, Y. (2014). A new acidophilic thermostable endo-1,4-β-mannanase from *Penicillium oxalicum* GZ-2: Cloning, characterization and functional expression in *Pichia pastoris*. *BMC Biotechnology* 14, 90. <http://dx.doi.org/10.1186/s12896-014-0090-z>.

- Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry* 31(3), 426-428. <https://doi.org/10.1021/ac60147a030>.
- Mussatto, S. I., Machado, E. M. S., Martins, S., & Teixeira, J. A. (2011). Production, composition, and application of coffee and its industrial residues. *Food and Bioprocess Technology* 4(5), 661-672. <http://dx.doi.org/10.1007/s11947-011-0565-z>.
- Nguyen, A. Q., Cho E. J., Trinh T. P. L., Jeong J. S., & Bae H. J. (2017). Development of an integrated process to produce d-mannose and bioethanol from coffee residue waste. *Bioresource Technology* 244 (Part 1), 1039-1048. <https://doi.org/10.1016/j.biortech.2017.07.169>.
- Preethu, D., Bhanu Prakash, B., Srinivasamurthy, C., & Vasanthi, B. (2007). Composting and co composting of coffee husk and pulp with source-separated municipal solid waste: a breakthrough in valorization of coffee waste. In Joseph, K., Nagendran, R., & Thanasekaran, K. (Eds.), *Proceedings of International Conference on Sustainable Solid Waste Management* (270-275). Chennai, India: Centre for Environmental Studies, Department of Civil Engineering, Anna University.
- Roy, R., Rahman, M. S., & Raynie, D. E. (2020). Recent advances of greener pretreatment technologies of lignocellulose. *Current Research in Green and Sustainable Chemistry* 3, 100035. <https://doi.org/10.1016/j.crgsc.2020.100035>.
- Simões, J., Madureira, P., Nunes, F. M., do Rosário Domingues, M., Vilanova, M., & Coimbra, M. A. (2009). Immunostimulatory properties of coffee mannans. *Molecular Nutrition and Food Research* 53(8), 1036-1043. <https://doi.org/10.1002/mnfr.200800385>.
- Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., & Crocker, D. (2008). Determination of structural carbohydrates and lignin in biomass. *Laboratory Analytical Procedure* 1617, 1-16.
- Tang, S., Cao, Y., Xu, C., Wu, Y., Li, L., Ye, P., Luo, Y., Gao, Y., Liao, Y., Yan, Q., & Cheng, X. (2020). One-step or two-step acid/alkaline pretreatments to improve enzymatic hydrolysis and sugar recovery from *Arundo Donax* L. *Energies* 13(4), 948. <https://doi.org/10.3390/en13040948>.
- Trinh, L. T. P., Nguyen, T. V., Nguyen, A. T. V., & Nguyen, A. Q. (2022). Two-step pretreatment for improving enzymatic hydrolysis of spent coffee grounds. *The Journal of Agriculture and Development* 21(3), 44-52. <https://doi.org/10.52997/jad.6.03.2022>.
- Trinh, L. T. P., Choi, Y.-S., & Bae, H. J. (2018). Production of phenolic compounds and biosugars from flower resources via several extraction processes. *Industrial Crops and Products* 125, 261-268. <https://doi.org/10.1016/j.indcrop.2018.09.008>.
- VS (Vietnam Standards). (2015). Standard No. TCVN 10791:2015 dated on June 17, 2015. Malt - Determination of the nitrogen content and calculation of the crude protein content - Kjeldahl method. Retrieved May 20, 2023, from <https://tieuchuan.vsqi.gov.vn/tieuchuan/view?sohieu=TCVN+10791%3A2015>.
- VS (Vietnam Standards). (2009). Standard No. TCVN 8124:2009 (ISO 2171:2007) dated on January 01, 2009. Cereals, pulses and by-products - Determination of ash yield by incineration. Retrieved July 15, 2023, from <https://tieuchuan.vsqi.gov.vn/tieuchuan/view?sohieu=TCVN+8124%3A2009>.
- Yin, X., Wei, L., Pan, X., Liu, C., Jiang, J., & Wang, K. (2021). The pretreatment of Lignocelluloses with green solvent as biorefinery preprocess: A minor review. *Frontiers in Plant Science* 12, 670061. <https://doi.org/10.3389/fpls.2021.670061>.

Aerogel materials from sugarcane bagasse: the effect of additives

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ABSTRACT

Cellulose nano fibers (CNF) are being considered as potential material for producing aerogel, an advanced materials for filtration technology. In this study, CNF were extracted from the sugarcane bagasse (a common agricultural waste) using ultrasonic technology and then fabricated with different additives including polyvinyl alcohol (PVA), polyethylene glycol (PEG) and graphene oxide (GO) to formulate aerogels. Various advanced techniques including polarized and scanning electron microscopy, fourier transform infrared spectroscopy (FTIR) and texture analyzer were applied to elaborate on the physicochemical properties of the products. The obtained results revealed that aerogel had low bulk density (0.032 kg/m^3 ; 0.035 kg/m^3 & 0.041 kg/m^3) and a high porosity (96.89%; 97.06% & 98.61%) when combined with PVA, PEG and GO, respectively. Among investigated samples, aerogel fabricated with GO had the highest mechanical resistance as well as the best elasticity.

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1. Introduction

Sugarcane is a common agricultural product of most tropical countries. While this plant plays an important role in improving the income of farmer, the sugar industry also releases a high amount of sugarcane bagasse (approximately 30% of raw material). According to research many studies (Somerville et al., 2010; Mahmud et al., 2021), this waste currently creates pressure on landfills and pollution on incineration.

Although sugarcane bagasse could be utilized for bioconversion purposes, such as bioethanol, xylitol, and specialty enzymes but these approaches still have not yet been widely applied due to the limit of technology and economics constraints (Chandel et al., 2012).

Indeed, sugarcane bagasse is a fibrous material containing cellulose as its main component and therefore, it is a potential raw material for producing aerogels. These advanced gels are

an outstanding class of porous materials, with an extremely low bulk density, and very high porosity, and a low thermal conductivity (Nguyen et al., 2021). Aerogels are being considered as the most potential material for water treatment and desalination. Cellulose based-waste materials including sugarcane bagasse are ideal sources for developing aerogels due to their renewable, and biodegradable properties incineration (Thai et al., 2020). Our study, therefore, aimed to transform sugarcane bagasse into cellulose nano fibers (CNF) and applied them for producing aerogels. Besides, various additives including polyvinyl alcohol (PVA), polyethylene glycol (PEG) and graphene oxide (GO) was fabricated with CNF to strengthen the structure of the aerogels.

2. Materials and Methods

2.1. Materials

Sugarcane bagasse was collected from sugarcane juice carts. Chemical substances including polyvinyl alcohol (PVA), polyethylene glycol (PEG), sulfuric acid (H_2SO_4), hydrochloric acid (HCl), hydrogen peroxide (H_2O_2), sodium hydroxide (NaOH), graphite powder (94%), sodium nitrate (NaNO_3) and potassium permanganate (KMnO_4) were provided by Xilong Scientific (China). All chemical substances were in the analytical grade.

2.2. Preparation of cellulose nanofibers (CNF)

Cellulose nanofibers were prepared based on the combination of ultrasound homogenization with chemical pretreatments (Chen et al., 2011b; Xu et al., 2017). Briefly, raw materials were cut into 1 - 2 cm pieces and washed several times with water to remove impurities. Then, the sugarcane fibers were dried in a laboratory oven (Memmert, Germany) at 90°C for 72 h to the moisture content of 65% and finely ground into powder. The size of dried powder was controlled using a laboratory shaking sieve (BK-TS200, Biobase, China). Sugarcane fiber powders with a

size range of 0.25 mm were reacted with NaOH solution (3% wt) with a ratio of 1:5 at 90°C for 3 h to break lignocellulosic linkages. Then, the sample was bleached by H_2O_2 (10% wt) with a ratio 1:5 at 60°C for 10 h. The mixture was rinsed with distilled water to neutral pH and dried at 70°C for 24 h to obtain cellulose fibers. These fibers, then, were grinded finely and soaked into distilled water at 80°C for 1 h before ultrasound dispersing for 15 min to obtain the CNF suspension. CNF was refrigerated at 4°C for use in later experiments.

2.3. Preparation of graphene oxide (GO)

Graphene oxide was prepared according to the modified Hummer's method (Li et al., 2019). Firstly, the mixture including graphite (2 g), NaNO_3 (1 g) and KMnO_4 (6 g) was slowly added into H_2SO_4 (98% wt). Reaction was conducted at 5°C for 90 min and hold at 40°C for 30 following minutes. Then, 92 mL of deionized water was added dropwise to the solution, and the temperature of the reaction was maintained at 95°C until the color of mixture turns from dark green to light brown. H_2O_2 (30% wt) was added to the reaction mixture until no more visible bubbles were produced, and the mixture was allowed to stand. The solution was filtered with HCl (2% wt) to completely remove the remaining Mn ions. After adjusting to pH of 5 - 6 (using distilled water), the solution was dried at 70°C for 24 h to obtain GO.

2.4. Preparation of CNF/PVA & CNF/PEG & CNF/GO

The PVA and PEG solutions (1.5% wt) were prepared by dissolving these polymers in deionized water at 75°C for 12 h. Hydrogels were formed by mixing CNF suspension (2.5% wt) with PVA/PEG/GO solution (1.5% wt) and then ultrasound homogenizing the mixture for 15 min. Aerogels were formed by freeze-drying hydrogels for 72 h using a laboratory lyophilizer (BK-FD10S, Biobase, China).

2.5. Physicochemical properties characterization of sugarcane bagasse aerogels

The density (ρ) and porosity (P%) of aerogels were determined based on their dimensions (20 x 40 mm) and weight. The specific surface area of the aerogel was determined through the N_2 adsorption–desorption isotherm using the Brunauer–Emmett–Teller (BET) method. Besides, mechanical properties of products were determined based on texture profile analysis (TPA) and stress relaxation (SR) methods using a texture analyzer (TA.XT Plus, Stablemicrosystem,

UK) equipped with a cylinder geometry (diameter of 40 mm). The strain deformations of TPA and SR methods were 50 and 10%, respectively while the relaxation time was 300 sec.

The morphology of aerogels was observed using a polarized light microscope (DM2500P, Leica, Germany) and a scanning electron microscope (JSM-IT500, Japan). Fourier Transform Infrared (FTIR) spectroscopy of samples was performed in the range of 400 - 4000 cm, with a 4 cm resolution at room temperature (Tensor 27, Bruker, Germany).

3. Results and Discussion

3.1. Morphology and microstructure of sugarcane bagasse aerogels

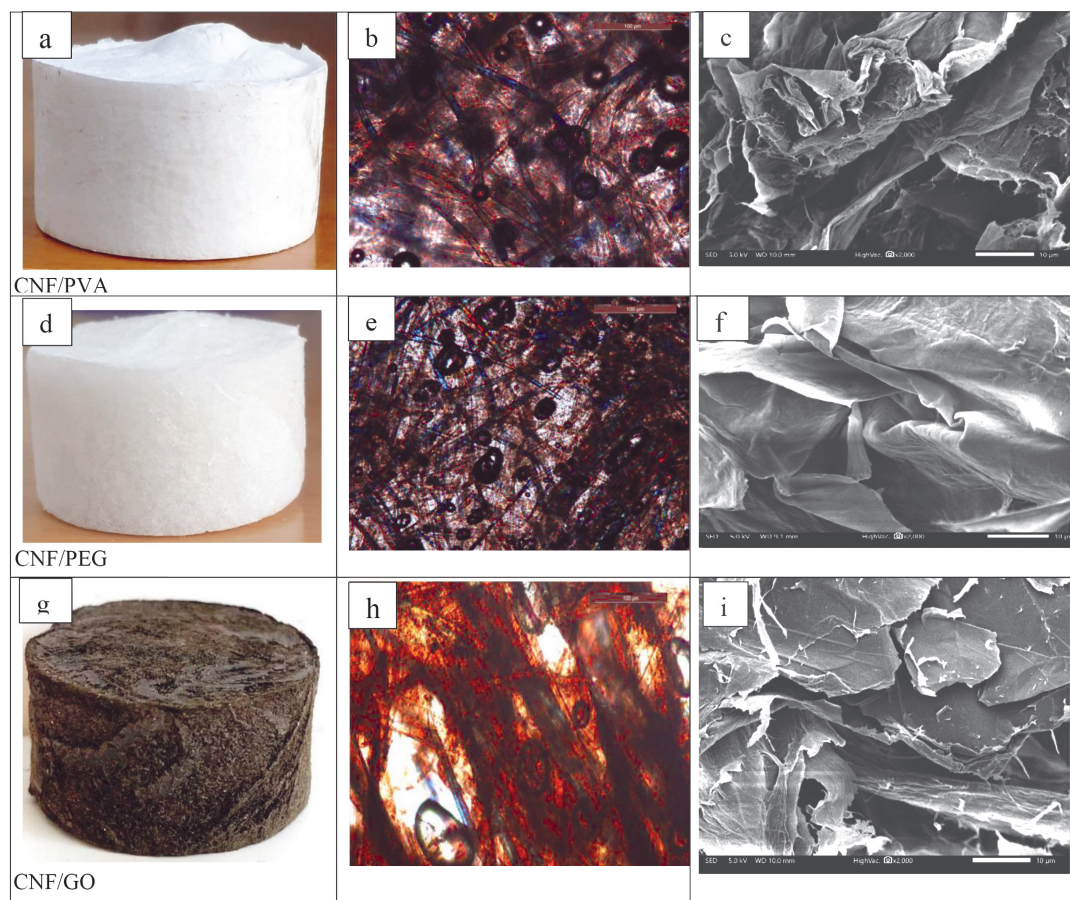


Figure 1. Morphology and microstructure of aerogels observed by naked eye (a, d, and g), polarized light microscopy (b, e, and h) and scanning electron microscope (c, f and i). CNF: cellulose nano fibers; PVA: polyvinyl alcohol; PEG: polyethylene glycol; GO: graphene oxide.

The effect of additives on the morphologies and microstructure of aerogels was displayed in Figure 1 while their physical properties were summarized in Table 1. As expected, polarized light microscope (PLM) and scanning electron microscope (SEM) observations revealed that polymer fibers from sugarcane bagasse were CNF with a diameter in a range of 50 - 500 nm. Similar to the findings from previous studies (Chen et al., 2011a, 2011b & 2011c), sugarcane bagasse-based aerogels also had a characteristic porous structure (with a pore size diameter of 10 - 50 μm) and a high porosity degree (97 - 98%). In comparison between PVA and PEG (Figure 1c & 1f), the latter showed a better compatibility with cellulose particles resulting in

a better homogenous crystal network for aerogel as well as a lower surface area (12.97 and 2.70 m^2/g for CNF/PVA and CNF/PEG, respectively). However, the difference of porosity degree between these samples was not significant. For sample fabricated with GO (Figure 1i), a more highly ordered structure was observed. Instead of the heterogenous aggregation of CNF, GO nanosheets tended to parallel arrange during the drying and therefore helped to orientate the CNF in the gel structure (Nguyen et al., 2021). Besides, the fabrication of GO on sugarcane bagasse CNF also significantly increased the density of aerogel ($P < 0.05$) in compared with samples adding PVA or PEG (Table 1).

Table 1. The density and porosity of sugarcane bagasse aerogels with different additives¹

Sample	Density (g/cm^3)	Porosity (%)
CNF/PVA	0.032 ± 0.003^a	96.89 ± 0.002^a
CNF/PEG	0.035 ± 0.005^a	97.06 ± 0.001^a
CNF/GO	0.041 ± 0.002^b	98.61 ± 0.001^a

¹Data expressed as mean $\pm$ standard deviation ($n = 3$).

^{a,b}Values within the same column having different letters are significantly different ($P < 0.05$) between means of samples.

CNF: cellulose nano fibers; PVA: polyvinyl alcohol; PEG: polyethylene glycol; GO: graphene oxide.

3.2. FTIR

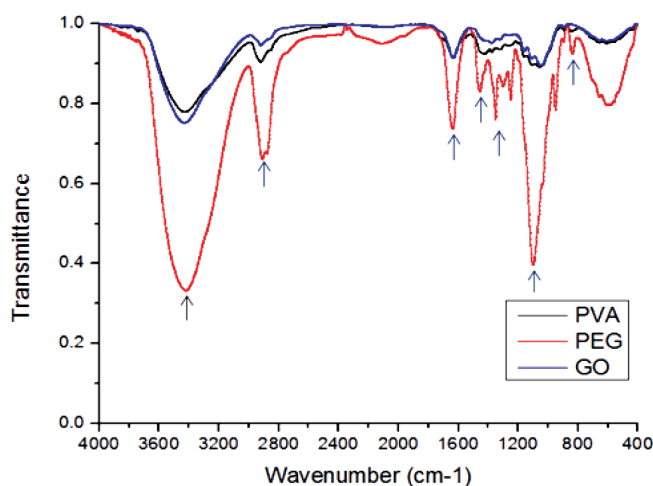


Figure 2. Fourier transform infrared spectroscopy spectra of sugarcane bagasse aerogels with different additives. PVA: polyvinyl alcohol; PEG: polyethylene glycol; GO: graphene oxide.

To elaborate further the effect of additive on sugarcane bagasse-based aerogels, FTIR spectroscopy was used to analyze functional groups of samples and the results was depicted on Figure 2. As hypothesized, lignin lignocellulose fibers have been significantly removed after alkaline treatment and bleaching step. Therefore, their characterized peak at 1730 cm was not recognized in the FTIR spectrum of investigated aerogels (Thai et al., 2020). For CNF, strong adsorption peaks were observed at 1606 cm and

1010 cm characterizing for the presence of C=O and C-O-H bonds of their backbone (Dao et al., 2022) while other peaks at 3300, 2900 and 1000 cm were contributed by O-H, C-H and C-H₂ linkages of glucose units. Besides, the addition of PVA and GO seem not to affect the chemical properties of CNF. For sample added PEG, some extra adsorption peaks were recognized at 1452 cm and 1245 cm. They should be associated with the bending and stretching vibrations of C-H and C-O groups, respectively (Sahu et al., 2022).

3.3. Mechanical properties of aerogels

Table 2. Mechanical properties of sugarcane bagasse aerogels with different additives¹

Sample	Texture profile analysis		Stress relaxation (SR)	
	Hardness (N)	Cohesiveness	Firmness (N)	SR (%)
CNF/GO	55,593 ± 1,15 ^a	0,942 ± 0,001 ^a	31,096 ± 2,93 ^a	40,08 ^a
CNF/PVA	48,86 ± 1,296 ^a	0,877 ± 0,012 ^b	28,75 ± 0,8 ^a	45,51 ^a
CNF/PEG	27,261 ± 1,098 ^c	0,775 ± 0,015 ^c	18,22 ± 0.11 ^b	65,65 ^b

¹Data expressed as mean ± standard deviation (n = 3).

^{a-c}Values within the same column having different letters are significantly different ($P < 0.05$) between means of samples.

CNF: cellulose nano fibers; PVA: polyvinyl alcohol; PEG: polyethylene glycol; GO: graphene oxide.

The mechanical properties of aerogels play an important role for their applications as well as their shelf-life. Two different testing methods including TPA and SR were applied to compare the texture properties between samples and the results were summarized in Figure 3 and Table 2. As expected, aerogels adding GO had a highest hardness and mechanical resistance. The presence of GO particles contributed to improving the strength for the crystal network of sugarcane bagasse-based aerogel (Figure 1i). In line with the findings from FTIR analysis, mechanical parameters between CNF/PVA and CNF/GO aerogels had a high similarity, and the

difference was mainly recognized for CNF/PEG sample. The hardness and firmness of the latter was approximately 40% of CNF/GO aerogel. All samples showed a well recovery property with a high cohesiveness (0.8 - 0.9) but their SR degrees showed a significant difference. While CNF/PVA and CNF/GO aerogels showed a well balance between the elastic and viscous components (SR in a range of 40 - 50), the addition of PEG to aerogels significantly increased the loss modulus of sample. It could be assumed that the presence of hydrophilic groups in PEG affected the linkages between hydrophobic groups of polymers in CNF aerogels.

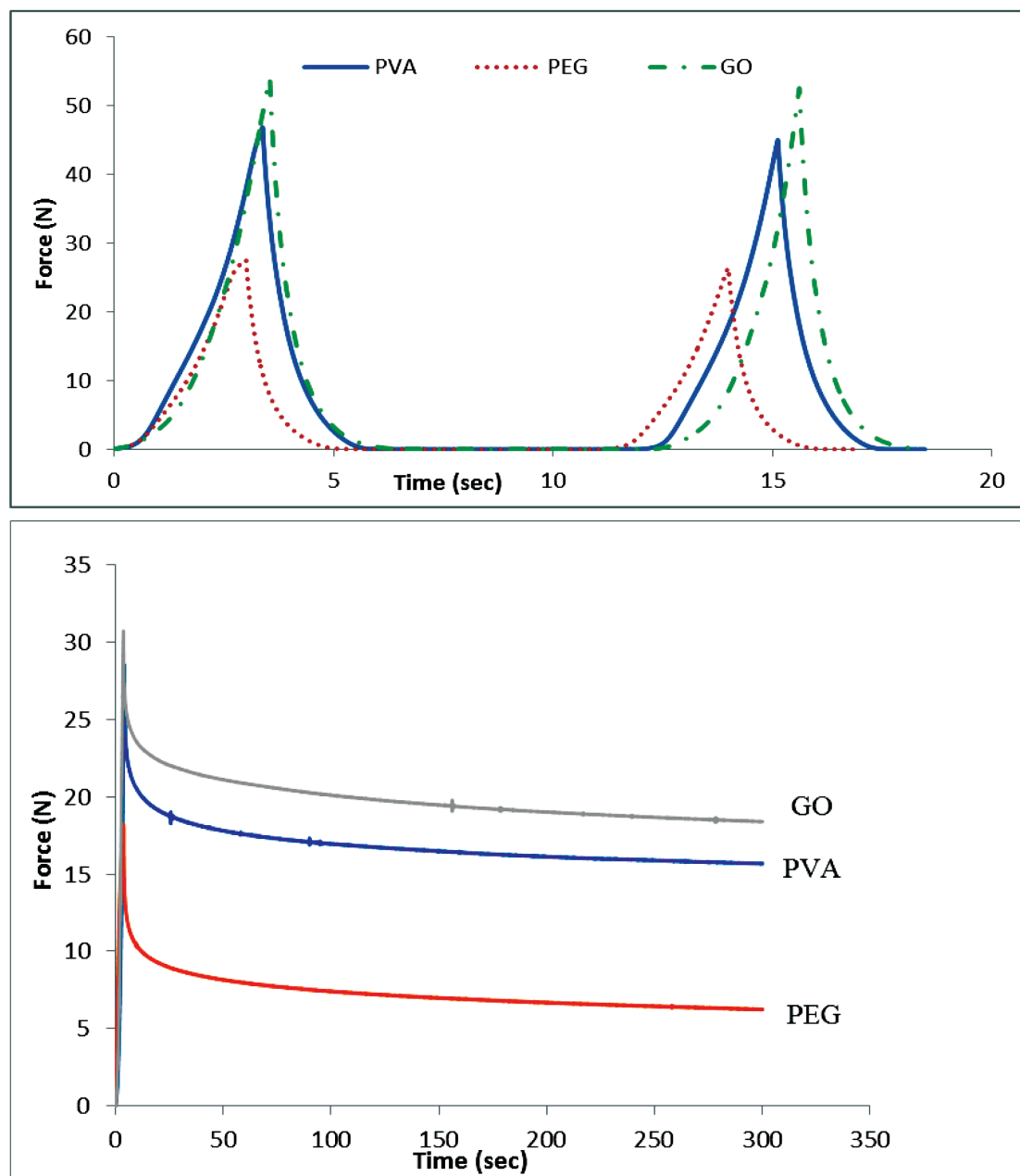


Figure 3. Texture profile analysis and stress relaxation of sugarcane bagasse aerogels. PVA: polyvinyl alcohol; PEG: polyethylene glycol; GO: graphene oxide.

4. Conclusions

This study successfully formulated aerogels from cellulose nano fiber of sugarcane bagasse. As expected, the microstructure of these aerogels was modified when using different additives

(PEG/PVA/GO). In general, GO and PVA aerogels had a high similarity of physicochemical and mechanical properties while PEG aerogels had a weak crystal network. Further studies could apply these aerogels for filtration technology or water treatment.

Conflict of interest

The authors have no conflicts of interest to declare.

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References

- Chandel, A. K., Silva, S. S. D., Carvalho, W., & Singh, O. V. (2012). Sugarcane bagasse and leaves: Foreseeable biomass of biofuel and bio-products. *Journal of Chemical Technology and Biotechnology* 87(1), 11-20. <https://doi.org/10.1002/jctb.2742>.
- Chen, W., Yu, H., Li, Q., Liu, Y., & Li, J. (2011a). Ultralight and highly flexible aerogels with long cellulose I nanofibers. *Soft Matter* 7(21), 10360-10368. <https://doi.org/10.1039/C1SM06179H>.
- Chen, W., Yu, H., & Liu, Y. (2011b). Preparation of millimeter-long cellulose I nanofibers with diameters of 30-80 nm from bamboo fibers. *Carbohydrate Polymers* 86(2), 453-461. <https://doi.org/10.1016/j.carbpol.2011.04.061>.
- Chen, W., Yu, H., Liu, Y., Chen, P., Zhang, M., & Hai, Y. (2011c). Individualization of cellulose nanofibers from wood using high-intensity ultrasonication combined with chemical pretreatments. *Carbohydrate Polymers* 83(4), 1804-1811. <https://doi.org/10.1016/j.carbpol.2010.10.040>.
- Dao, D. N., Le, P. H., Do, D. X., Dang, T. M. Q., Nguyen, S. K., & Nguyen, V. (2022). Pectin and cellulose extracted from coffee pulps and their potential in formulating biopolymer films. *Biomass Conversion and Biorefinery* 13, 13117-13125. <https://doi.org/10.1007/s13399-022-02339-x>.
- Li, M., Miao, Y., Zhai, X., Yin, Y., Zhang, Y., Jian, Z., Wang, X., Sun, L., & Liu, Z. (2019). Preparation of and research on bioinspired graphene oxide/nanocellulose/polydopamine ternary artificial nacre. *Materials and Design* 181, 107961. <https://doi.org/10.1016/j.matdes.2019.107961>.
- Mahmud, M. A., & Anannya, F. R. (2021). Sugarcane bagasse - A source of cellulosic fiber for diverse applications. *Heliyon* 7(8), e07771. <https://doi.org/10.1016/j.heliyon.2021.e07771>.
- Nguyen, V. T., Ha, L. Q., Nguyen, T. D. L., Ly, P. H., Nguyen, D. M., & Hoang, D. Q. (2021). Nanocellulose and graphene oxide aerogels for adsorption and removal methylene blue from an aqueous environment. *ACS Omega* 7(1), 1003-1013. <https://doi.org/10.1021/acsomega.1c05586>.
- Sahu, M., Reddy, V. R. M., Kim, B., Patro, B., Park, C., Kim, W. K., & Sharma, P. (2022). Fabrication of Cu₂ZnSnS₄ light absorber using a cost-effective mechanochemical method for photovoltaic applications. *Materials* 15(5), 1708. <https://doi.org/10.3390/ma15051708>.
- Somerville, C., Youngs, H., Taylor, C., Davis, S. C., & Long, S. P. (2010). Feedstocks for lignocellulosic biofuels. *Science* 329(5993), 790-792. <https://doi.org/10.1126/science.1189268>.
- Thai, Q. B., Nguyen, S. T., Ho, D. K., Tran, T. D., Huynh, D. M., Do, N. H. N., Luu, T. P., Le, P. K., Le, D. K., Thien, N. P., & Duong, H. M. (2020). Cellulose-based aerogels from sugarcane bagasse for oil spill-cleaning and heat insulation applications. *Carbohydrate Polymers* 228, 115365. <https://doi.org/10.1016/j.carbpol.2019.115365>.
- Xu, Z., Zhou, H., Jiang, X., Li, J., & Huang, F. (2017). Facile synthesis of reduced graphene oxide/trimethyl chlorosilane-coated cellulose nanofibres aerogel for oil absorption. *IET Nanobiotechnology* 11(8), 929-934. <https://doi.org/10.1049/iet-nbt.2017.0063>.

Community-based ecotourism: A case study in Thanh Phu district, Ben Tre province, Vietnam

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ABSTRACT

This study explored the potential and challenges of community-based ecotourism in Thanh Phu district, Ben Tre province, Vietnam. By investigating 52 households involved in community-based ecotourism through the Participatory Rural Appraisal method and semi-structured interviews, it outlined the transformative impact of community-based ecotourism on the local community. The research underscored positive outcomes in economic growth, social equity, and cultural preservation while highlighting the imperative need for prudent management to mitigate environmental concerns and prevent unsustainable tourism growth. It advocated for a balanced approach that integrated economic development with environmental and cultural preservation strategies. The findings emphasize the importance of tailored solutions to navigate challenges and foster sustainable community-based ecotourism, ensuring long-term prosperity while safeguarding the district's natural and cultural treasures.

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1. Introduction

Technological advancements and media progress have propelled tourism into one of the globe's most prominent and fastest-growing economic sectors. In 2022, there was a robust recovery in international tourism earnings, reaching USD 1.0 trillion, marking a 50% surge

from 2021 (adjusted for inflation) (UNWTO, 2023). The expansion of tourism infrastructure like resorts, jetties, walkways, artificial lagoons, beaches, and groins has led to habitat loss, wildlife disturbances, reduced coral growth (Gladstone et al., 2013), and interference with wild animals in national parks (Buckley, 2001). Moreover, tourism has not consistently catered

to local interests, resulting in misrepresented experiences (Nguyen et al., 2019) and cultural detachment among ethnic minorities (Vu, 2020), leading to conflicts between hosts and tourists. Addressing these challenges requires a shift in tourism paradigms, focusing not just on economic and technical aspects but also on preserving the environment (Fennell, 2005) and prioritizing the needs and welfare of local communities (Scheyvens, 1999). Community-based ecotourism emerged as a viable alternative.

Unlike conventional mass tourism, community-based tourism empowers local communities by granting them control over development and management, ensuring that benefits predominantly stay within the community (Denman, 2001). Scheyvens (2002) highlights community-based tourism's goal of holistic empowerment at economic, psychological, social, and political levels. Evolving over three decades, community-based tourism integrates social, environmental, and economic aspects to meet local community needs, particularly around protected areas (Goodwin & Santilli, 2009). As a subset, community-based ecotourism serves as a tool for biodiversity conservation while economically benefiting the local community, emphasizing active community involvement and sometimes ownership of ecotourism enterprises (Wunder, 2000). Aligned with community-based natural resource management, community-based ecotourism has become crucial to conservation strategies since the 70s, recognizing the vital role of local community support in protected area conservation (Goodwin & Santilli, 2009). Sustainable community-based ecotourism development often involves private community partnerships and joint ventures, leveraging the strengths of private sector partners, community expertise, and external support for infrastructure and capacity-building (Kiss, 2004). Community-based ecotourism also fosters policy coordination,

minimizes stakeholder conflicts, and fosters synergies through knowledge exchange among community members (Kibicho, 2008).

In Vietnam, since the late 1990s, there has been an integration of community-based ecotourism programs into the country's tourism development and poverty alleviation initiatives (Nguyen et al., 2022). The introduction of terms such as "eco," "green," or "community-based" began in a 1999 workshop focused on establishing an ecotourism development strategy. This approach emphasizes tourism rooted in nature and local culture, highlighting environmental education, contribution to conservation efforts, and sustainable development, actively involving the local community (Pham, 2002). These terms have gradually surfaced in legislative documents like the 2017 Tourism Law and the most recent National Strategy for Sustainable Tourism Development up to 2020 and the vision for 2030. Given Vietnam's diverse natural resources and culturally rich heritage alongside concerns about the impact of commercialized and mass tourism activities, the Vietnamese government anticipates promising prospects for advancing community-based ecotourism (Tran, 2002).

Thanh Phu district in Ben Tre province, Vietnam, boasts immense potential for tourism development. The area is endowed with the Thanh Phu Nature Reserve, aligning with the contemporary trend of fostering tourism enterprises within buffer zones and national parks/nature reserves. This strategy interweaves conservation efforts with tourism development, fostering sustainable financial support for conservation initiatives. Additionally, Thanh Phu presents a wealth of diverse cultural tourism resources, deeply rooted in the Mekong Delta's heritage, further enriching its tourism prospects. However, akin to the broader Vietnamese context, community-based ecotourism initiatives flourish primarily in specific areas with distinct natural

resources, favorable transportation, or superior communication advantages, as highlighted by Eja et al. (2017). Other areas remain limited, failing to harness the full potential of the region's tourism resources. Against this backdrop and following context, policy analysis, and field surveys conducted in Thanh Hai commune and Binh Thanh commune, this article aims to (1) Assess the potential of community-based ecotourism in Thanh Phu; (2) Evaluate community involvement and the efficacy of existing community-based ecotourism models; and (3) Analyze the challenges faced by community-based ecotourism initiatives in Thanh Phu.

2. Materials and Methods

2.1. Study site

Thanh Phu, situated in the southeastern part of Ben Tre Province, Vietnam, spans an area of approximately 411 km² and lies about 45 km from

the coastline (Nguyen et al., 2019). The district was formed over centuries through the confluence of the Ham Luong and Co Chien rivers, shaping its coastal landscape, characterized by expanses of paddy fields, sand dunes, and thriving mangrove forests. Renowned as a pivotal hub within the third economic region, notably the “salty region”, Thanh Phu grapples with salinity intrusion across its terrain, fostering a suitable environment for brackish farming systems. These systems include rice-shrimp rotation, extensive-intensive shrimp cultivation, and clam harvesting along the coastal tidal mudflats (Le et al., 2013; Nguyen et al., 2019). This study focuses on two community-based ecotourism sites in Thanh Phu: the Chin Song tourist area in Binh Thanh Commune and Con Bung Beach in Thanh Hai Commune (Figure 1).

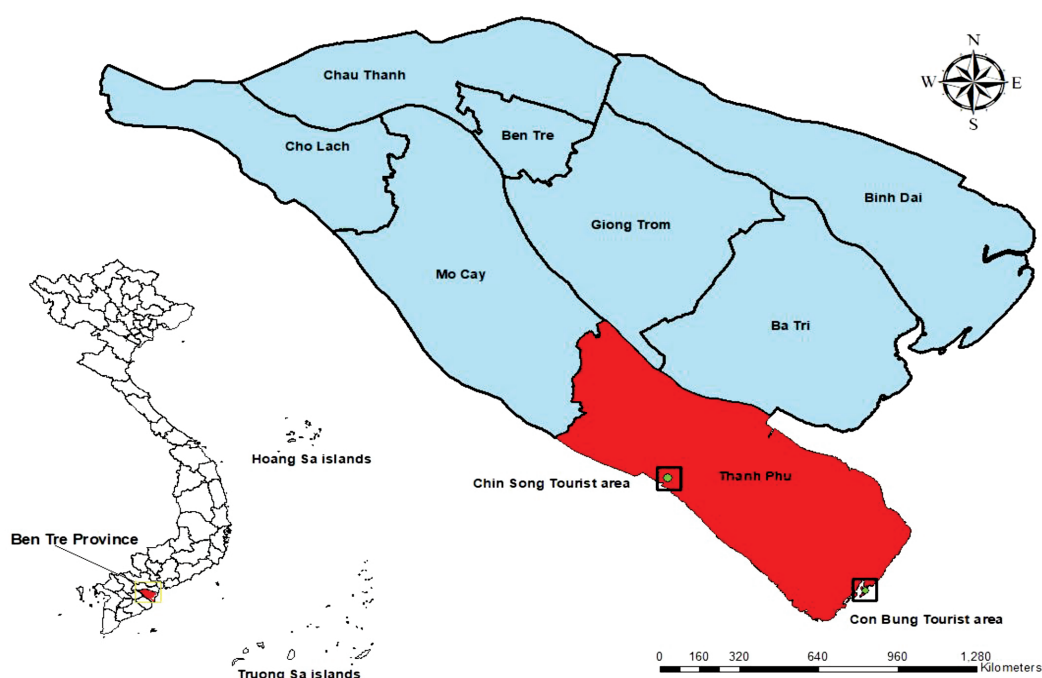


Figure 1. The map of the study site.

2.2. Data collection

Various methods were employed in this study, including secondary data analysis, Participatory Rural Appraisal (PRA), and interviews. Initially, secondary data collection involved reports, documents, policies, and resolutions about Thanh Phu District's economic and tourism development. Concurrently, primary data was acquired through PRA and semi-structured interviews conducted during fieldwork.

The PRA method documents local experiences and daily life, engaging local participants or key informants (Silverman, 2009). In this research, PRA was instrumental in assessing the recent ecotourism developments in Thanh Phu. The study involved observing daily activities and specialized professional endeavors from March to May 2022, aiming to comprehend local perspectives while maintaining the researcher's outsider viewpoint. Throughout the research, detailed field notes were meticulously recorded, capturing essential information, conversations, interactions, and noteworthy events (Stake, 2010).

Semi-structured interviews were conducted to gather insights from various participants in Thanh Phu. This method allowed for an in-depth understanding of their perspectives on community-based tourism development (Creswell et al., 2007; Stake, 2010). The sample size was calculated according to Yamane's formula (1967):

$$n = \frac{N}{1 + N(e)^2}$$

In this equation, N represents the number of households participating in community-based tourism (which was 110 in 2019), and e signifies the desired level of precision (set at 0.1). Consequently, a total of 52 respondents involved in community-based ecotourism were interviewed. They were encouraged to express

their perceptions and attitudes based on their experiences freely (Creswell et al., 2007). Each interview, lasting 20 - 30 min, was conducted at convenient locations such as participants' homestays, restaurants, boats, homes, or public cafes. To ensure accuracy, smartphone recordings were used during the interviews, supplemented by detailed notes taken during and after the discussions to facilitate data triangulation (Creswell et al., 2007; Denzin, 2008; Stake, 2010).

2.3. Data analysis

A Likert-type scale employing a 5-point rating system (1 = strongly disagree, 2 = disagree, 3 = neutral, 4 = agree, 5 = strongly agree) served as the evaluation tool for assessing the impact of community-based ecotourism models. Mean values were computed for each item. To stratify the impact based on this rating scale, overall mean values for respective subscales were determined and categorized into five levels. The divisions were delineated as follows: 0 - 0.8, 'no impact'; 0.81 - 1.6, 'low impact'; 1.61 - 2.4, 'moderate impact'; 2.41 - 3.2, 'significant impact'; and 3.21 - 4.0, 'very high impact' (Pham & Nguyen, 2020).

All data were statistically analyzed using one-way analysis of variance (ANOVA) and Tukey's post hoc ANOVA test for individual comparisons ($P < 0.05$, level of significance). SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

3. Results and Discussion

3.1. Conditions and potential for community-based ecotourism development in Thanh Phu district

Thanh Phu is a region with immense potential and notable advantages for developing community-based tourism. The strength of tourism in Ben Tre Province, particularly in

Thanh Phu District, lies in its rich and diverse natural landscape. Vast green coconut gardens and lush fruit orchards characterize the area, which benefits from year-round illuvial deposits. Notably, Thanh Phu boasts a pristine ecological environment, maintaining its rural essence with interlaced rivers and canals. It is abundant in seafood resources, embodies a simple lifestyle, and reflects the closeness and harmony of its people with nature.

Thanh Phu Nature Reserve was established on August 9, 1997, under Decision No. 1928/NNKH/QĐ by the Ministry of Agriculture and Rural Development. It spans a total area of 8,825 ha, comprising a buffer zone of 4,315 ha, a strictly protected area of 1,788 ha, a 949 ha endangered coastal protection area, and a 1,773 ha scientific research area. Positioned between the Co Chien and Ham Luong rivers' estuaries, the reserve serves as an ideal breeding ground for various aquatic species, including shrimp (*Penaeus* spp.), diverse fish species (Notopteridae, Toxotidae, Nandidae, and Anguillidae), and a multitude of squid species like *Loligo formosana*, *Sepioteuthis lessoniana*, and *Sepia pharaonis* (Hughes et al., 2001). These estuaries also host a diverse array of invertebrates such as crabs, clams, and mussels. Coastal mangrove forests in Thanh Phu primarily feature dominant plant species like *Rhizophora apiculata* (occupying the largest area), alongside *Avicennia marina*, *Avicennia officinalis*, *Excoecaria agallocha*, and *Sonneratia* spp. (Hughes et al., 2001). The area is also a habitat for waterbird species, including commonly found and widely distributed birds like grey plover (*Pluvialis squatarola*), common greenshank (*Tringa nebularia*), and sanderling (*Calidris alba*) (Hughes et al., 2001). The Thanh Phu Nature Reserve sustains a population of 1,470 individuals within its boundaries,

with over 6,935 people residing in the buffer zone. The primary economic activities revolve around agriculture, fishing, and forestry. The local community heavily relies on the natural resources within the reserve for their livelihoods.

Beyond its stunning natural scenery, Thanh Phu District is home to numerous traditional craft villages. These include the My An broom-making craft village, My Hung hat-making craft village, Hoa Loi jar-molding craft village, and others producing coconut candy, rice paper, puff pastry, handicrafts from coconuts, seedlings, and ornamental flowers. The region also resonates with the folk songs typical of the Vietnamese Mekong Delta: *đờn ca tài tử* (a chamber music genre recognized as UNESCO's intangible cultural heritage in 2013), *sắc bùa* (a blend of singing, music, and dancing typically performed during Lunar New Year celebrations), *hát bội* (a form of Vietnamese theatre akin to classical opera, influenced by Chinese opera), and *bóng rỗi* (a ritual practice closely linked to the worship of goddesses in Southern Vietnam).

Some prominent tourist attractions in Thanh Phu include:

- (1). Huynh Phu Ancient House (Figure 2a): Constructed between 1890 and 1904, this 133-year-old house underwent restoration from 2013 to 2015, maintaining its original appearance. The house boasts 48 large, round ironwood pillars (*Erythrophleum fordii* and *Xylia xylocarpa*) and intricate carvings. Preserved within are unique iron window frames, ancient furniture, and even a garden lamppost from France, over a century old. Recognized as a national monument, it is a top check-in point for tourists.

(2). Ho Chi Minh Sea Trail Relic Area at Con Bung beach: This historic site served as a transfer point for weapons during the Vietnam war. Covering 635 ha, it features a monumental memorial and stunning coastal poplar rows. Recognized as a National Historic Site, it is in close proximity to hotels and beaches.

(3). Thanh Phong Wind Power Area: Located on Thanh Phong beach, this picturesque area hosts a series of wind power plants and wharves, aiding in the modernization of a previously war-affected coastal commune.

(4). Place of Departure of Battalion 307 (Figure 2b): Giong Luong base in Dai Dien Commune is historically significant, marking the departure point of Battalion 307, a renowned force during the Indochina War. Known through the famous song “Battalion 307” by musician Nguyen Huu Tri, this site bears a testament to Vietnam’s heroic historical memory during its resistance wars.

(5). Giong Luong Coconut Cake Ovens: Nearby the departure place of Battalion 307, these ovens produce the famous coconut cakes - a specialty of Thanh Phu and Ben Tre. Visitors can relish and purchase these hand-wrapped delicacies at reasonable prices, tied traditionally with rattan instead of nylon string.

(6). My An Broom Making Craft Village: Home to about 168 broom-making households, this village creates brooms using nipa palm leaf stems and coconut leaves sourced from the Mo Cay Bac and Mo Cay Nam districts. Predominantly managed by women, these broom makers craft during their leisure, supplementing their income and attracting tourists.

In addition, Thanh Phu boasts numerous high-tech shrimp farms, Sau Dau Street adorned with beautiful shade trees, and roads lined with mangrove trees along the canal, all serving as attractive check-in spots for tourists.



Figure 2. a) Huynh Phu Ancient House and b) Place of Departure of Battalion 307.

Thanh Phu has the potential to collaborate with neighboring districts, provinces, or even international destinations to develop joint ecotourism packages or itineraries. The extension of Con Bung Beach to Ba Tri and Binh Dai beaches, centrally located between Ba Ria-Vung Tau and Ba Dong tourist beaches (Tra Vinh), provides a strategic advantage for connectivity. Thanh Phu's geographical position is also conducive to tourism development, connecting the Ho Chi Minh City - Thanh Phu - Con Dao route. This connectivity allows visitors to explore a diverse range of attractions and activities, encouraging longer stays and contributing to the overall tourism economy.

After several years of implementing Plan No. 4573/KH-UBND, dated October 9, 2017, by the People's Committee of Ben Tre Province, which aimed to execute Action Program No. 22-CTr/TU from July 20, 2017, by the Provincial Party Committee, the tourism industry in Ben Tre has undergone significant positive changes. Gradually, it has assumed a pivotal role within the Mekong Delta region and the country as a whole. Within Ben Tre Province's tourism development plan up to 2020 and the envisioned trajectory towards 2030, tourism stands out as the focal provincial economic sector. The plan anticipates a 20% annual growth in tourism revenue, and an expected annual increase of 12% in the number of tourists visiting Ben Tre Province. The 2022 resolution of the Thanh Phu District Party Committee outlines several critical determinations: (1) Emphasizing the promotion and advertising of tourism; (2) Enhancing the quality of tourism services and diversifying tourism products, linked explicitly to the exploration and promotion of local cultural, historical, and spiritual landmarks; (3) Establishing efficient connections with tours and tourist routes in neighboring districts to maximize effectiveness; (4) Persisting in inviting infrastructure investment projects geared towards serving tourism needs; (5) Prioritizing

investment in the development of community-based ecotourism interconnected with marine tourism; (6) Crafting distinctive tourism products unique to the district's offerings.

3.2. Level and type of community participation in ecotourism

In terms of age, survey participants engaged in local ecotourism predominantly fell within the 31 - 40 age bracket, constituting the highest proportion at 34.6% (Table 1). This age group typically holds stable employment, manages households, and possesses good health, primarily contributing to their families' livelihood activities. Following closely are individuals aged 18 - 30 and those above 60, comprising 23.1% and 25% of participants, respectively (Table 1). Conversely, the age group from 41 - 60 constitutes the lowest percentage at 17.3% (Table 1). Concerning gender distribution, males accounted for more than half of the total survey respondents, making up 53.8% (Table 1). All surveyed individuals belonged to the Kinh ethnic group. Regarding education, participants involved in community-based ecotourism activities generally exhibit an average educational attainment. Individuals with primary school education represented 9.6% (Table 1), typically individuals engaged in farming due to limited access to schooling. The educational spectrum of locals participating in ecotourism primarily focuses on secondary and high school groups, comprising 42.3% and 30.8%, respectively (Table 1). Despite the relatively modest educational levels, the training, coaching, and skill enhancement policies in Thanh Phu District have facilitated more accessible participation in local ecotourism activities for these individuals. A majority of respondents have resided in the locality for over 20 years, accounting for 65.4% (Table 1). Following this, 17.3% have lived in the area for 6 - 10 years, while 9.6% and 5.8% have lived there for 1 - 5 years and 11 - 15 years, respectively (Table 1).

Table 1. Demographic features of respondents

Variables	Number	Percentage (%)
Age		
18 - 30	12	23.1
31 - 40	18	34.6
41-50	9	17.3
> 50	13	25.0
Gender		
Male	28	53.8
Female	24	46.2
Education		
Primary School	5	9.6
Secondary School	22	42.3
High School	16	30.8
Intermediate college	4	7.7
University	5	9.6
Commune		
Thanh Hai	32	61.5
Binh Thanh	20	38.5
Local living duration		
1 - 5 years	5	9.6
6 - 10 years	9	17.3
11 - 15 years	3	5.8
16 - 20 years	1	1.9
> 20 years	34	65.4

With favorable traffic conditions (approximately 150 km from Ho Chi Minh City, Vietnam's biggest city), Thanh Phu's tourism sector has experienced significant growth, transforming this rural area into a thriving destination. Formerly reliant on agricultural production, most farmers have diversified into the tourism industry. Our fieldwork, aimed at exploring the extent of local community involvement in various tourism services, reveals diverse sectors and forms of participation.

Community engagement in tourism is pivotal to the success or failure of a sustainable community-based tourism model (Pearce et al., 1996; Pham, 2012). The findings show that the Thanh Phu community has progressively provided tourism services like catering, sightseeing, souvenir sales, artistic performances, and homestay businesses (Table 2). They partake in the tourism value chain, actively contributing as both 'tourism resources' and 'developers' in their homeland. Table 2 outlines the primary tourism service activities in which locals participate.

Table 2. Descriptions of participation type in ecotourism in Thanh Phu

No.	Type of participation	Households	Share
1	Providing catering services	26	50.0%
2	Tour guiding	16	30.8%
3	Transporting tourists	10	19.2%
4	Producing and selling souvenir	8	15.4%
5	Tourism management	8	15.4%
6	Supplying agricultural and aquatic products	7	13.5%
7	Participating in community meetings, contributing opinions on issues related to local ecotourism development	7	13.5%
8	Participating in art performances	7	13.5%
9	Homestay business	6	11.5%
10	Participating in community cultural exchanges	6	11.5%
11	Participating in improving the local landscape	6	11.5%
12	Participating in the analysis, planning, and decision-making process related to local ecotourism development	6	11.5%
13	Portering and transporting goods	3	5.8%

Furthermore, this research reveals that: (1) Most households are involved in food and beverage businesses, ranging from small eateries to catering services; (2) Some families engage in transporting tourists and serve as tour guides; (3) Many households produce, supply, and sell handicrafts, agricultural, and aquatic products not only for tourists but also for restaurants and travel agencies; (4) Some locals participate in local tourism management activities; (5) Other activities include homestay operations, artistic and cultural performances, or landscape enhancement, albeit with fewer participants.

However, despite these findings, it is evident that the Thanh Phu community's involvement in decision-making and formulating implementation plans for community-based tourism remains limited. Moreover, tourism activities and services lack an essential element:

a learning component, integral to community-based tourism (Suansri, 2003). There are few initiatives aimed at fostering a shared learning process between hosts and guests, or raising awareness about natural and cultural conservation among tourists and the local community. Consequently, the core values of community-based tourism have yet to be fully realized.

3.3. Community-based ecotourism effectiveness

During the period spanning 2017 to 2022, the community-based ecotourism models of Con Bung Beach and Chin Song collectively hosted over 2 million tourists (Table 3). From 2017 to 2019, there was a consistent rise in visitor numbers, culminating in 515,200 visitors in 2019. However, the emergence of the COVID-19 pandemic significantly disrupted tourism development in Thanh Phu district, causing a

near-halt in activities for two consecutive years (2020 - 2021). During this period, the district could not organize the Culture-Tourism and Sea Cuisine Week. Tourism experienced a severe decline in visitor numbers and revenue, leading to temporary suspensions of tourism operations due to necessary social distancing measures. In 2020, the district only welcomed 241,820 visitors, marking a more than 50% reduction compared to the previous year. By 2021, the number of visitors further declined to approximately 138,500. The

district's tourism sector encountered substantial setbacks due to the COVID-19 pandemic, similar to other regions in Vietnam (Huynh et al., 2021). By 2022, a gradual recovery was observed in the community-based ecotourism models within Thanh Phu District, with an influx of 356,000 visitors. This recovery signifies a positive trajectory toward rejuvenating the tourism industry within the district after the challenging period caused by the pandemic.

Table 3. Number of visitors to community-based ecotourism models in Thanh Phu district

Year	Con Bung beach, Thanh Hai commune	Chin Song ecotourism area, Binh Thanh commune	Total
2017	390,600	10,950	401,550
2018	405,900	11,275	417,175
2019	501,900	13,300	515,200
2020	234,520	7,300	241,820
2021	135,000	3,500	138,500
2022	346,000	10,000	356,000
Total	2,013,920	56,325	2,070,245

The impact assessment of community-based ecotourism, derived from Likert scale ratings, illuminates its diverse effects on various aspects of community life (Table 4). The data underscores the considerable consensus regarding the positive implications of ecotourism initiatives. Specifically, the notably high ratings for job creation and income generation (4.33 - very high impact) emphasize ecotourism's pivotal role in fortifying the community's employment opportunities and economic prosperity. It is notable that 90.4% of individuals engaged in community-based ecotourism report an income exceeding 2 million VND/month from tourism, with 36.5% stating that this income aids in covering daily expenses, and an impressive 44.2%

able to accumulate surplus. Research by Karta et al. (2016) aligns with these findings, highlighting that the involvement of local communities in tourism activities in Kintamani (Bali, Indonesia) generates income, creates employment, amplifies production volumes, expands marketing networks, and elevates the destination's visibility globally. Additionally, the ratings indicating stimulated growth across various business sectors (3.92 - significant impact) underscore ecotourism's capacity to foster local economic diversification. These outcomes collectively underscore the substantial contribution of community-based ecotourism to the economic upliftment of local communities.

Social equity within community-based ecotourism embodies the principle of providing all participating user groups with equitable access to resources, fair revenue sharing, and the right to partake in decision-making and administrative processes related to tourism activities (Masud et al., 2017). Ensuring that every member of the community involved in community-based ecotourism benefits from the project is imperative. The consensus on improved entertainment opportunities (3.98 - significant impact), facilitated equal opportunities (3.81 - significant impact), and investments in essential infrastructure (3.79 - significant impact) (Table 4) highlights ecotourism's role in enhancing leisure activities, positively influencing residents' well-being, and fostering fairness and inclusivity among community members in Thanh Phu. In tourism development, local authorities display keen interest in investing in infrastructure to attract more tourists. Through the capital from the national target program on culture, the district has seen substantial investments sanctioned by the Provincial People's Committee. Projects such as the renovation and enhancement of Huynh Phu Ancient House Relics and grave sites in Dai Dien and Phu Khanh communes, with an approximate budget of nearly 26 billion VND, stand as prime examples. Additionally, efforts to preserve, enhance, and promote the historical value of the Ho Chi Minh Sea Trail Historical Relic in Thanh Hai and Thanh Phong communes, with an initial investment exceeding 80 billion VND (from 2013 to 2017), illustrate these commitments. The district has also undertaken endeavors such as the construction of memorials and upgrades to numerous relics like Dong Khoi Battle Memorial House, Military Event Relics on October 30, An

Linh Pagoda, and Lang Ong through socialization sources. Transport infrastructure developments, including the upgrade and paving of Highway 57 from Van Bridge to Con Chim intersection and the introduction of roads connecting various communes, especially the Van Bridge linking upper communes with coastal areas, manifest these efforts to provide favorable conditions for locals and tourists, notably when visiting Con Bung Beach. These collective initiatives substantiate the comprehensive impact of community-based ecotourism on various social facets in Thanh Phu.

Moreover, the consensus on heightened awareness and knowledge about ecotourism (3.67 - significant impact), promoted conservation efforts (3.65 - significant impact), and cultural promotion and preservation (3.56 - significant impact) underscores community-based ecotourism's pivotal educational role (Table 4). It actively fosters environmental consciousness, safeguards biodiversity, and fosters the preservation of cultural heritage within communities. Research by Tuyen et al. (2023) corroborates these findings, emphasizing the commitment of the Nhon Ly coastal community in Binh Dinh, Vietnam, including local business households, in safeguarding natural resources and the environment for sustained local ecotourism development. Moreover, community-based tourism effectively facilitates the preservation of cultural heritage, creating a cohesive link between conservation, livelihoods, and cultural integrity (Tran & Walter, 2014). Additionally, Eshun & Tonto (2014) contend that ecotourism plays a significant role in safeguarding and promoting the survival of

unique cultures within local communities. It assists communities in preserving their distinct cultural identity and historical heritage while fostering respect for their traditions.

Ecotourism, while generating positive impacts, can also negatively affect the natural environment and local socio-cultural values (Butarbutar & Soemarno, 2013). The findings reveal perceived negative consequences linked to community-based ecotourism. Heightened environmental pollution (3.90 - significant impact) in Thanh Phu (Table 4) raises concerns about potential ecological repercussions. Previous research acknowledges that any permanent environmental alterations (e.g., construction of lodges or viewing platforms) unavoidably involve site modification and ongoing waste production (Weaver & Lawton, 2002). Ecotourism might involve indirect environmental costs tied to induced restructuring, like constructing new residences by migrating individuals lured by ecotourism-generated job opportunities. This phenomenon is evident in locales such as the Monteverde Cloud Forest Preserve, where tourism-induced economic growth led to one-sixth of the residents migrating from other parts of Costa Rica by 1992 (Honey, 1999). Another indirect cost lies in exposing a destination to less sustainable tourism forms. Ecotourists' discovery of a destination can spur local involvement in servicing these visitors, making the area more accessible and prone to increased visitation, aligning with the Butler (1980) resort cycle model. However, without remedial

measures, ecotourism may overload the local capacity and transition into unsustainable mass tourism. At Con Bung Beach, an excessive influx of tourists during national holidays, like April 30 - May 1, resulted in adverse impacts on the marine environment, particularly notable with plastic waste accumulation due to the influx of tourists. Additionally, there is a noted rise in social issues (3.52 - significant impact) (Table 4), including imitative behavior within the local community, materialistic attitudes, unregulated peddling, moral disorders, and a declining interest in the agricultural sector, as supported by Butarbutar & Soemarno (2013). According to Nguyen et al. (2022), social security and safety is also an important factor affecting ecotourism development. Moreover, the data indicates a moderate impact on the escalation of commodity prices (3.13 - moderate impact), while there is no impact observed regarding difficulties in finding peaceful living spaces, negative impacts on local cultural values, degradation of community moral standards, and disruption of local daily life (2.42 to 2.21 - no impact).

In conclusion, while community-based ecotourism showcases significant positive impacts in economic, educational, and cultural aspects, concerns regarding environmental sustainability, social harmony, and community well-being emerge. Efficient management strategies and sustainable practices are imperative to maximize positive outcomes while mitigating the negative impacts of ecotourism within communities.

Table 4. Impact of community-based ecotourism

Impact	Mean	Level of impact
Positive		
Job creation and income generation	4.33	Very high impact
Improved entertainment opportunities lead to enhanced quality of life	3.98	Significant impact
Stimulated growth across various business sectors	3.92	Significant impact
Facilitated equal opportunities within the community	3.81	Significant impact
Fostered investments in transportation, electricity, and water infrastructure	3.79	Significant impact
Raised awareness and knowledge about ecotourism	3.67	Significant impact
Promoted conservation efforts for landscapes, flora, and fauna	3.65	Significant impact
Access to incentives provided by local authorities	3.56	Significant impact
Local culture promotion	3.56	Significant impact
Encouraged localities to preserve their culture	3.46	Significant impact
Negative		
Heightened environmental pollution	3.90	Significant impact
Rise in social issues	3.52	Significant impact
Escalation of commodity prices	3.13	Moderate impact
Difficulty in finding peaceful living spaces	2.42	Low impact
Negative impact on local cultural values	2.40	Low impact
Degradation of community moral standards	2.29	Low impact
Disruption of local daily life	2.21	Low impact

3.4. Challenges to the development of sustainable community-based ecotourism

In the realm of managing community-based ecotourism, specific pressing issues arise, notably revolving around the concept of carrying capacity and the local advantages derived from ecotourism sites. While ecotourism ideally caters to a limited number of visitors, monitoring and controlling the capacities of these targeted areas often present challenges. Striking a balance in managing tourists in Thanh Phu necessitates considering environmental and cultural preservation, political interests, and ensuring equitable benefits for the local community.

The environmental impact of tourism encompasses the depletion of natural resources, pollution, soil erosion, loss of natural habitats, increased strain on endangered species, and heightened susceptibility to forest fires. Adverse effects arise when visitor levels exceed the environment's capacity to withstand this influx within acceptable change limits. Discussions around tourist surges, economic gains, and infrastructure development suggest that improper management could transform ecotourism into conventional mass tourism. Presently, minor negative effects exist, but these might escalate over time if left unmanaged. All stakeholders, including the local populace, have limited experience in handling ecotourism and its multifaceted objectives.

4. Conclusions

The study illuminates community-based ecotourism's immense potential and challenges in Thanh Phu district. It emphasizes the district's rich natural reserves and cultural heritage, pointing to the transformative impact on the local community. While emphasizing the positive economic impact, particularly in job creation and income generation (rated at 4.33), social equity, and cultural preservation, it also emphasizes the importance of careful management to address environmental concerns (notably heightened environmental pollution, rated at 3.90) and prevent the transition of ecotourism into unsustainable mass tourism. The findings call for balanced strategies harmonizing economic growth with environmental and cultural preservation. By navigating these challenges with tailored approaches, Thanh Phu can foster a sustainable community-based ecotourism model, ensuring long-term prosperity while safeguarding its natural and cultural treasures. To tackle the challenge of Thanh Phu tourism resources facing either overload or underutilization, future research needs to employ a suitable method for assessing the tourism resource carrying capacity.

Conflict on interest

The authors declare no conflict of interest.

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References

- Buckley, R. (2001). The environmental impacts of ecotourism. In Weaver, D. B. (Ed.). *The encyclopedia of ecotourism* (379-394). Wallingford, UK: CABI Publishing.
- Butarbutar, R., & Soemarno, S. (2013). Environmental effects of ecotourism in Indonesia. *Journal of Indonesian Tourism and Development Studies* 1(3), 97-107. <https://doi.org/10.21776/ub.jitode.2013.001.03.01>.
- Butler, R. (1980). The concept of a tourist area cycle of evolution: implications for management of resources. *Canadian Geographies* 24(1), 5-12. <https://doi.org/10.1111/j.1541-0064.1980.tb00970.x>.
- Creswell, J. W., Hanson, W. E., Plano, V. L. C., & Morales, A. (2007). Qualitative research designs: Selection and implementation. *The Counseling Psychologist* 35(2), 236-264. <https://doi.org/10.1177/0011000006287390>.
- Denman, R. (2001). *Guidelines for community based ecotourism development*. Gland, Switzerland: WWF International.
- Denzin, N., Lincoln, Y., & Smith, L. T. (2008). *Handbook of critical and indigenous methodologies*. California, USA: Sage Publishing.
- Eja, E. I., Out, J. E., Ewa, E. E., & Bernadette, N. N. (2011). The role of private sector participation in sustainable tourism development in Cross River State, Nigeria. *International Journal of Business and Social Science* 2(2), 153-160.
- Eshun, G., & Tonto, J. N. P. (2014). Community-based ecotourism: its socio-economic impacts at Boabeng-fiemma monkey Sanctuary, Ghana. *Bulletin of Geography. Socio-economic Series* 26, 67-81. <http://dx.doi.org/10.2478/bog-2014-0045>.
- Fennell, D. A. (2005). *Ecotourism* (2nd ed.). London, UK: Routledge.
- Gladstone, W., Curley, B., & Shokri, M. R. (2013). Environmental impacts of tourism in the Gulf

- and the Red Sea. *Marine Pollution Bulletin* 72(2), 375-388. <https://doi.org/10.1016/j.marpolbul.2012.09.017>.
- Goodwin, H., & Santilli, R. (2009). Community-based tourism: A success? *ICRT Occasional Paper* 11, 1-37.
- Honey, M. (1999). *Ecotourism and sustainable development: Who owns paradise?* Washington DC, USA: Island Press.
- Hughes, R., Trai, L. T., Andrew, W., & Dung, V. V. (2001). *Sourcebook of existing and proposed protected areas in Vietnam: Vol. 2, Southern Vietnam*. Ha Noi, Vietnam: BirdLife International.
- Huynh, D. V., Truong, T. T. K., Duong, L. H., Nguyen, N. T., Dao, G. V. H., & Dao, C. N. (2021). The COVID-19 pandemic and its impacts on tourism business in a developing city: Insight from Vietnam. *Economies* 9(4), 172. <https://doi.org/10.3390/economies9040172>.
- Karta, N. L. P. A., Sukarsa, I. M., Hardini, W., & Suartha, I. K. P. (2016). Local community participation in community based tourism in Kintamani and their impact to the economy. *The 12th Biennial Conference of Hospitality and Tourism Industry in Asia*. Bali, Indonesia: Atlantis Press.
- Kibicho, W. (2008). Community-based tourism: A factor - Cluster segmentation approach. *Journal of Sustainable Tourism* 16(2), 211-231. <https://doi.org/10.2167/jost623.0>.
- Kiss, A. (2004). Is community-based ecotourism a good use of biodiversity conservation funds? *Trends in Ecology and Evolution* 19(5), 232-237. <https://doi.org/10.1016/j.tree.2004.03.010>.
- Le, K. V., Vo, M. Q., Pham, V. T., & Nguyen, S. T. C. (2013). Agroecological zoning according to hydrology, pedology and present land use for coastal districts in Ben Tre province. *Can Tho University Journal of Science* 26, 227-236.
- Masud, M. M., Aldakhil, A. M., Nassani, A. A., & Azam, M. N. (2017). Community-based ecotourism management for sustainable development of marine protected areas in Malaysia. *Ocean and Coastal Management* 136, 104-112. <https://doi.org/10.1016/j.ocecoaman.2016.11.023>.
- Nguyen, D. T. H., Korsem, T., Nguyen, C. T., Phonphan, W., & Vo, M. Q. (2019). Determination of aquaculture distribution by using remote sensing technology in Thanh Phu district, Ben Tre province, Vietnam. *Vietnam Journal of Science, Technology and Engineering* 61(2), 35-41. [https://doi.org/10.31276/VJSTE.61\(2\).35-41](https://doi.org/10.31276/VJSTE.61(2).35-41).
- Nguyen, H. N., Doan, H. T., Le, N. T., Trinh, T. T., Le, T. Q., & Nguyen, T. V. D. (2022). Factors influencing the ecotourism development in Thanh An island, Can Gio district, Ho Chi Minh City, Vietnam. *The Journal of Agriculture and Development* 21(5), 56-65. <https://doi.org/10.52997/jad.7.05.2022>.
- Nguyen, T. D., Hoang, H. D., Nguyen, T. Q., Fumikazu, U., Vo, T. P. T., & Nguyen, C. V. (2022). A multicriteria approach to assessing the sustainability of community-based ecotourism in Central Vietnam. *APN Science Bulletin* 12(1), 123-140. <https://doi.org/10.30852/sb.2022.1938>.
- Nguyen, T. Q., Ubukata, F., & Nguyen, D. C. (2019). Development process of the community-based tourism: From two case studies in Central Vietnam. In *Proceedings of Vietnam-Japan Science and Technology Symposium* (124-128). Ha Noi, Vietnam: VNU Vietnam Japan University. https://www.researchgate.net/publication/343306146_Development_Process_of_the_Community-based_Tourism_From_Two_Case_Studies_in_Central_Vietnam.
- Pearce P. L., Moscardo G., & Ross G. F. (1996). *Tourism community relationships*. New York, USA: Emerald Publishing Limited.
- Pham, G. X., & Nguyen, T. T. P. (2020). Accuracy of a concept in quantitative research. *Journal of Science and Technology* 46(4), 158-161. [https://doi.org/10.31276/VJSTE.46\(4\).158-161](https://doi.org/10.31276/VJSTE.46(4).158-161).

- doi.org/10.46242/jst-iiuh.v46i04.657.
- Pham, L. H. (2012). Tourism impacts and support for tourism development in Ha Long Bay, Vietnam: An examination of residents' perceptions. *Asian Social Science* 8(8), 28-39. <https://doi.org/10.5539/ass.v8n8p28>.
- Pham, L. T. (2002). *Ecotourism: Theoretical and practical issues of development in Vietnam*. Ha Noi, Vietnam: Vietnam Education Publishing House.
- Scheyvens, R. (2002). *Tourism for development: Empowering communities*. London, UK: Prentice Hall.
- Scheyvens, R. (1999). Ecotourism and the empowerment of local communities. *Tourism Management* 20(2), 245-249. [https://doi.org/10.1016/s0261-5177\(98\)00069-7](https://doi.org/10.1016/s0261-5177(98)00069-7).
- Silverman, D. (2009). *Doing qualitative research*. California, USA: Sage Publishing.
- Stake, R. E. (2010). *Qualitative research: Studying how things work*. New York, USA: Guilford Press.
- Suansri, P. (2003). *Community based tourism handbook*. Bangkok, Thailand: Responsible Ecological Social Tour-REST.
- Tran, L., & Walter, P. (2014). Ecotourism, gender and development in Northern Vietnam. *Annals of Tourism Research* 44, 116-130. <https://doi.org/10.1016/j.annals.2013.09.005>.
- Tran, L. D. (2002). Strategic planning and standards for ecotourism development in Vietnam. *International Relations*, 1-19.
- Tuyen, Q. D., Nguyen, Q. X. T., Nguyen, H. V., Dang, V. Q., & Tang, N. T. (2023). Toward sustainable community-based tourism development: Perspectives from local people in Nhon Ly coastal community, Binh Dinh province, Vietnam. *Plos One* 18(10), e0287522. <https://doi.org/10.1371/journal.pone.0287522>.
- UNWTO (United Nations World Tourism Organization). (2023). *International tourism highlights, 2023 Edition – The impact of COVID-19 on tourism (2020 - 2022)*. Madrid, Spain: UNWTO. <https://doi.org/10.18111/978928442450-4>.
- Vu, C. M. (2020). Alienation of ethnic minorities in community-based tourism. *Current Issues in Tourism* 23(21), 2649-2665. <https://doi.org/10.1080/13683500.2020.1733942>.
- Weaver, D., & Lawton, L. (2002). Overnight ecotourist market segmentation in the gold coast hinterland of Australia. *Journal of Travel Research* 40(3), 270-280. <https://doi.org/10.1177/004728750204000305>.
- Wunder, S. (2000). Ecotourism and economic incentives: An empirical approach. *Ecological Economics* 32(3), 465-479. [http://dx.doi.org/10.1016/S0921-8009\(99\)00119-6](http://dx.doi.org/10.1016/S0921-8009(99)00119-6).
- Yamane, T. (1967). *Statistics: An introductory analysis*. New York, USA: Harper and Row.