Ethnobotany of medicinal plants of the K'Ho people (Vietnam) and antidiarrheal activity of selected plants

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Abstract
This research documented medicinal plants traditionally utilized by K'Ho people in BiDoup-Núi Bà National Park, Vietnam and evaluated their antidiarrheal activity either in vitro or in vivo. Ethnobotanical data was gathered via semi-structured interviews and field surveys with knowledgeable informants. Information on 133 medicinal plants used to treat 63 ailments occurring in both humans and animals has been thoroughly documented. Selected medicinal plants were subjected to analyse antibacterial activity by agar diffusion method and antidiarrheal activity through a castor oil-induced mouse model. Seven medicinal plants were selected for testing antibacterial activity on 15 diarrhea-related strains.

Three species were found that they not only showed their safety at dosages up to 7500 mg.kg^{-1} in acute toxicity study but also showed their activity in fecal inhibition. Among these plants, the ethanol extract of Elephantopus mollis strongly reduced small intestine transit by 67.92% and 64.61% respectively in comparison to loperamide at a dose of 3 mg.kg^{-1}. This study contributes fundamental information for future studies on potential traditional medicinal plants which can be applied for diarrheal treatment. In addition, the results also established a scientific base for the usage of medicinal plants used by ethnic minorities.

Keywords: Antibacterial activity, Castor oil-induced diarrhea, Ethnobotany, K'Ho people, Medicinal plants.

Introduction

Humans have used medicinal plants for the treatment of various health problems for thousands of years. Day after day, the indigenous people of different localities have accumulated highly valuable knowledge and experience in using surrounding plants for medicinal purposes which led to the abundance of traditional medicine skills in many parts of the world[12]. An estimation of the prevalence of traditional medicine in low-income countries ranges from 40% to 71%[19]. Therefore, plants, especially those used in herbal remedies, have been essential for early drug discovery as well as for providing food supplements, nutraceuticals and options in complementary and alternative medicine[11]. Furthermore, traditional medicinal plants find direct application in the rapidly growing pharmaceutical industry across Europe and North America, as well as within traditional medicine programs integrated into the health care systems of several developing countries[28].

A limited proportion of the Earth's biodiversity has been investigated for drug discovery so far[24]. About 5-10% of the total existing 250,000 species of higher terrestrial plants have been investigated[28]. In Vietnam, the Institute of Medicine's incomplete survey reported 3,948 plant and mushroom species utilized for medicinal purposes by the end of 2005, over 85% of which were found in natural environments and were primarily utilized based on folk experience[14]. Moreover, traditional medicinal plants and indigenous knowledge nowadays face the risk of oblivion because it primarily passed down orally from one generation to the next. The socioeconomic changes, the overexploitation of nature and the degradation of the environment also seriously threaten medicinal plant resources[3,12,29]. It is necessary to conduct thorough studies on traditional medicinal plants not only to conserve indigenous knowledge but also to validate their value by scientific evidence.

Diarrhea, the second most common cause of mortality in children under five, claims approximately 525,000 lives annually (Factsheets of World Health Organization, last accessed 10 September 2019). Diarrhea is not a disease on its own but a symptom of various underlying conditions[27]. It is the most prevalent clinical sign of gastrointestinal issues, often caused by infections like bacteria, viruses, or parasites. Spread can occur by means of infected food, water, or direct contact between individuals due to inadequate hygiene. In Vietnam, a study of diarrheal etiology and epidemiology showed that among 587 children with diarrhea, 71.0% were less than two years old, mainly caused by group A rotavirus, diarrheagenic *Escherichia coli*, *Shigella* spp. and enterotoxigenic *Bacteroides fragilis*. Epidemiological factors involving diarrhea were identified including poor sanitation and hygiene and lack of awareness of healthcare[21].

Diarrhea treatment methods vary based on the cause and severity of the condition. Common options include oral rehydration therapy, antibiotics and pharmacological agents to regulate gut motility etc.[4] Although Western medicines like loperamide, diphenoxylate and racecadotril have been widely used, they also might lead to adverse effects such as nausea, dry mouth, abdominal pain, constipation and headache[19,27]. Due to these concerns, there is a growing interest in plant-based treatments to treat diarrhea having fewer side effects. Therefore, the present study firstly relies on the necessity of conserving and validating the folk knowledge in using medicinal plants by K'Ho people in BiDoup-Núi Bà National Park, Vietnam. The traditional knowledge concerning the use of medicinal plants was systematically documented and several potential plants were chosen for *in vitro* and *in vivo* antidiarrheal tests.

Material and Methods

Study area: BiDoup-Núi Bà National Park (BNNP), a key biodiversity area in Vietnam, spans 70,038 hectares within Lạc Dương and Đam Rông districts, Lâm Đồng province, Central Highlands. Despite being in a tropical monsoon region, the high elevation contributes to a cooler climate with distinct seasons: rainy (May-November) and dry (December-April). The annual average temperature is about 18^0C and the average rainfall is about 1,800 mm[20] (Fig. 1).

The K'Ho, an indigenous minority of 14,585,000 in Lâm Đồng, adheres to a matriarchal system, with descent relationships traced through the female line. In previous society, after getting married, the couple still lived with their parents, which resulted in the establishment of "great" families, usually including 3-4 generations living together in a traditional big house and thus sharing the land ownership, cattle and agricultural tools. However, due to the internal development and the external impact, such great families quickly break into so-called "nuclear families" constituted by only husband/ wife and their children and therefore they possess their own properties.

Previously K'Ho people did not have their handwriting. Thereafter, French priests and K'Ho intelligentsia created the existing one based on Latin letters to facilitate missionary work. This type of handwriting is transmitted to the present but only a small proportion of people know how to use it. The K'Ho people traditionally had a nomadic farming lifestyle, but at present they have transitioned to settled agriculture, focusing on coffee cultivation as their primary crop, along with banana, vegetables, their own rice and maize variety (unlike almost other minorities). Moreover, household income is supplemented through hunting, foraging for non-timber forest products (including firewood, mushrooms, bamboo shoots and orchid flowers) as well as making handicrafts, particularly in the post-harvest period.

Currently, thanks to Government funding for infrastructure, the living conditions of the K'Ho community have greatly improved. However, the local community continues to uphold an intimate connection to the surrounding natural resources, particularly medicinal plants that are part of daily health care[23].

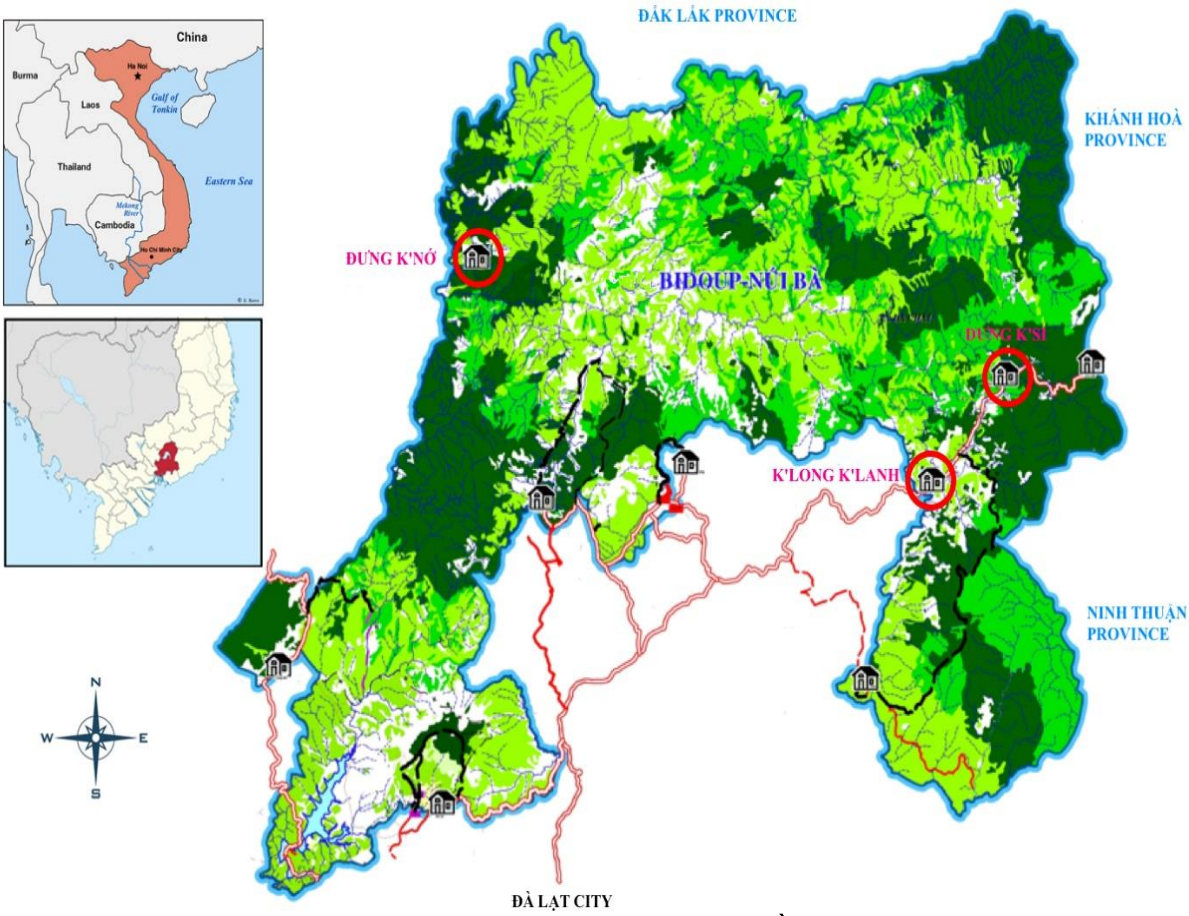

Fig. 1: Map of BiDoup-Núi Bà National Park, Lâm Đồng Province, Vietnam

Ethnobotanical data collection: Three K'Ho villages, namely K'Long K'Lanh (K), Đưng K'Si (DS) and Đưng K'Nớ (DN), were selected for the ethnobotanical survey conducted from 2013 to 2015 based on the following criteria: proximity to forests and the diversity of nearby forest habitats. BNNP officials facilitated initial discussions with local officials and village leaders to explain the study's goals and secure verbal agreements for documenting their traditional medicinal knowledge.

The information collection followed two successive phases:

First phase (May 2013-March 2014): First phase consisted of documenting traditional medicinal plants, with results presented in this manuscript. Twelve key informants (10 males, 2 females), skilled in utilizing herbal medicine, were chosen based on the recommendation of local authorities, residents and BNNP's officials. Accompanied by researchers, they went on field trips to collect the referenced medicinal plants along the pathways, using semi-structured interviews based on Martin's methodology[16].

Second stage (July-August 2015): Second phase consisted of analyzing the relationship between medicinal plants and local people. The results obtained in this process were presented elsewhere. Local participants were mostly familiar with the Kinh language, the official language of Vietnam. If necessary, the translation was handled by the village head or key informants who were with the researchers.

Collection and identification of plants: Voucher specimens were created for all plants mentioned by key informants during field trips and were deposited at the Herbarium of the University of Science, Vietnam National University-Ho Chi Minh City (HCMUS). The plant identification was carried out by Dr. Nguyen Xuan Minh Ai, a botanist from the Laboratory of Botany, with the help of standard literature[13,26]. The nomenclature of species and the taxonomy system adhered to The World Flora Online (https://www.worldfloraonline.org) last accessed on 12 August 2022).

Preparation of plant extract: Seven plants from BNNP including *Calamus palustris* (bud), *Cinnamomum burmannii* (leaves), *Chromolaena odorata* (stem with leaves), *Dacrycarpus imbricatus* (leaves), *Elephantopus mollis* (whole plant), *Euodia lepta* (leaves) and *Polygala paniculata* (whole plant), were dried, finely ground and processed following a revised protocol by Milosevic et al[18]. Plant extraction was carried out with 70% ethanol at room temperature for 48 hours, followed by filtration, evaporation at 40^0C under reduced pressure and stored in brown bottles. Before pharmacological experiments, the extract was mixed with 1% dimethyl sulfoxide (DMSO) and stored in sterilized containers at 4^0C.

Antibacterial testing using well diffusion agar method: Fifteen bacteria species (3 strains of *Escherichia coli*, 4 strains of *Salmonella* spp., 3 strains of *Vibrio* spp. and 5 strains of other pathogenic bacteria) were used to evaluate the antibacterial activity of plant extracts prepared above by well diffusion agar method[8]. Bacteria strains were grown at 37^0C for 24 hours. A 100 µL solution of diluted bacteria (10^6 cfu.mL^{-1}) was applied on agar medium and allowed to dry. Using sterile metal cylinders, 6 mm-diameter wells were made in each plate and 100 µL of each extract (concentration of 100 mg.mL^{-1}) was added. DMSO 1% was used as a negative control. The plates were held at 37.0 ± 0.1°C for 24 hours of incubation. The diameter of the zone of inhibition (in mm) was assessed. Each experiment was replicated three times and the data obtained were analyzed statistically.

Acute toxicity study: Albino mice (25-30 g) were employed to assess the acute toxicity and anti-diarrheal activity of plant extracts. Animals were housed in laboratory conditions in glass cages for a minimum of 1 week before the experiment. The acute toxicity of the herbal extracts was determined following established protocols[10]. Following an 18-hour fast, the mice were randomly separated into five groups of six. A range of dosages of plant extracts were orally administered (2 mL.kg^{-1} body) to the animals, with the control group receiving DMSO 1% (2 mL.kg^{-1} body). Mice were observed for indications of acute toxicity and mortality over 5 days.

Castor oil-induced diarrhea in mice: Castor oil-induced diarrhea was carried out according to Akuodor et al[2]. After fasting for 18 hours, the mice were randomly separated into six groups of six. Experimental mice of positive and negative control groups were orally administered with loperamide (3 mg.kg^{-1} body) and DMSO 1% (2 mL.kg^{-1} body) respectively while treated groups were administered with plant extract at different doses. After 30 minutes of treatment, each group of animals received 0.4 mL of castor oil orally.

They were then housed separately in cages with clean filter paper and diarrhea episodes were recorded over 4 hours. The first defecation time, the number of animals having wet feces and the cumulative wet fecal mass in each group were recorded. Diarrheal inhibition (PI) was expressed as a percentage and defined by the formula:

$$PI\ (\%) = \frac{\text{Mean of defecation (control group- treated group)}}{\text{Mean of defecation of control group}} \times 100$$

Castor oil-induced enteropooling test: Mice received castor oil and plant extract treatments as mentioned earlier. One hour after the administration of castor oil, all animals were sacrificed. The small intestine (from the pylorus to the caecum) was carefully dissected and weighed. The contents were collected and measured for volume. The empty intestine was then weighed again to determine the percentage reduction in intestinal secretion volume as previously described[25].

Small intestine transit test: Mice were subjected to fasting and treated with the plant extract as previously described. Thirty minutes after administration, the mice received 0.4 mL of castor oil, followed by 0.2 mL of a charcoal meal consisting of a 3% charcoal suspension in 0.5% (w/v) carboxymethyl cellulose. Each animal was then placed in an individual cage. One hour later, all animals were sacrificed and the small intestine was extracted. The distance covered by the charcoal meal was measured and expressed as a percentage of the distance from the pylorus to the cecum[25].

Preliminary phytochemical analysis: Plant extracts were subjected to preliminary screening to identify the presence of major components including carbohydrates, amino acids, steroids, saponins, cardiac glycosides, anthraquinone glycosides, phenolic compounds, flavonoids, tannins and alkaloids using standard methods[9].

Results and Discussion

Plant diversity: A total of 133 medicinal plants cited by 12 key informants were recorded. These plants were distributed in 98 genera, 55 families, 35 orders and 5 subclasses. Among them, 100 taxa were identified to species while 120 taxa were identified to genera and families. The most dominant family was Fabaceae with 12 taxa used for therapeutic purposes (9.02% of total taxa), followed by Asteraceae (9 taxa, 6.76%), Rubiaceae (8 taxa, 6.01%) and Zingiberaceae (7 taxa, 5.26%). The popularity of fabaceous and asteraceous plants was also found in previous study[17]. The remaining taxa were distributed in families containing from 1 to 6 ones. It was interesting that over half of the families were represented by only 1 taxon (32 families) indicating a high diversity and there might be specificity in species used for medicinal purposes of the K'Ho people.

Local health problems: Concerning traditional uses, 133 taxa were used for the treatment of 63 health problems which were categorized into 14 groups of diseases based on the International Statistical Classification of Diseases (ICD, version 11) (Table 1). Out of which, nearly fifty percent of medicinal plants (65/133 taxa) were used for treatments of the digestive system. At the second position were health problems involving infections with 30 taxa cited, followed by the group "skin/ subcutaneous cellular tissue" with 20 taxa. Diarrhea, cough, toothache and scabies were the four most common diseases in the study area with 31, 15, 10 and 10 taxa reported. These ailments seem to be related to living conditions and hygienic state.

Together with diseases which were caused by dysfunction of a specified bodily organ or system, there were also two ailments involving cultural belief including enuresis in children and severe headaches due to the effect of someone's spell. The term "culture-bound syndromes" was coined to describe illnesses that exist beyond the scope of contemporary biomedicine and are believed to manifest within specific cultural contexts or among particular ethnic groups[7]. To treat these specific ailments, local people did not use conventional preparation and administration of herbal remedies. Instead, health problems were healed based on their belief in supernatural powers. For example, when a child was unable to control urination during sleep, the inflorescent of *Zingiber* sp. was used by tapping gently on his/her head 10 times. Regardless of the effect of this treatment, this is a cultural characteristic of each ethnic group.

Plants specially used for the treatment of diarrhea: Out of 133 medicinal plants reported for treating various health issues, 37 were specifically identified for diarrhea/dysentery treatment constituting approximately one-third of the total species. This aligns with a UNICEF report which identifies diarrhea as one of the most prevalent illnesses globally.

These plants were chosen for further ethnobotanical surveys using questionnaire interviews with non-specialist informants (data was published elsewhere). Fidelity level (FL) was calculated, representing the percentage of informants citing a plant for diarrhea. The 37 antidiarrheal plants were categorized into high FL, medium FL and low FL groups. Seven representative plants from these categories were selected for bacterial-related-diarrheal tests (Table 2).

Table 1
Local health problems treated by medicinal plants

Category of ailments	Health problem	Number of plants used
I. Circulatory system	1. Tonic for anemic people	2
	2. Heart attack	1
II. Culture-bound syndromes	3. Enuresis	1
	4. Intense headache (due to the influence of a spell)	1
III. Digestive system	*5. Diarrhea*	*31*
	6. Toothache	10
	7. Dysentery	*8*
	8. Stomachache	7
	9. Parasitic worm	*4*
	10. Cirrhosis	2

	11. Constipation	1
	12. Intestinal occlusion (children)	1
	13. Promote function of liver	1
IV. Eye, ear, nose and throat	14. Nosebleed	2
	15. Runny nose/ Stuffy nose	2
	16. Eye fatigue	1
V. Genitourinary system	17. Dark-yellow urine	1
	18. Diabetes	1
	19. Irregular menstruation	1
	20. Kidney pain	1
VI. Infections/ infestations	21. Malaria	7
	22. Typhoid	7
	23. Cold	4
	24. Fever	4
	25. Dandruff	3
	26. Chickenpox	1
	27. Measles	1
	28. Pulmonary tuberculosis	1
	29. Shingles	1
	30. Thrush (children)	1
VII. External injuries	31. Stop bleeding	7
	32. Wounds	6
	33. Burn (due to fire or hot water)	2
	34. Painful injuries after a slip and fall	2
	35. Dislocation	1
VIII. Skeleto-muscular	36. Backache	5
	37. Arms and legs pain	3
	38. Spinal column pain	3
	39. Arthralgia	1
	40. Shoulder pain	1
	41. Waist pain	1
IX. Nervous system	42. Insomnia	1
	43. Vertigo	1
X. Poisoning	44. Insect bites	3
	45. Snake bites	1
XI. Pregnancy/ Birth puerperium	46. Giving birth easily	2
	47. [Abortion]	1
	48. Galactagogue	1
	49. Stop bleeding after parturition	1
XII. Respiratory system	50. Cough	15
XIII. Skin/ Subcutaneous cellular tissue	51. Scabies	10
	52. Furuncle	3
	53. Ringworm	2
	54. Itching	2
	55. Scabies resulting in hair losing (dogs)	*1*
	56. Sunburn	1
	57. Vesicles	1
XIV. Other/ Whole body	58. Headache	5
	59. Aches and pains (Body pain)	2
	60. Foster health	2
	61. Tiredness	2
	62. Arms and legs swelling	1
	63. General bad conditions in animals	*1*

The words in brackets [ ] represent induced ailments.
The words in *italic* represent the health problems also manifest in animal.
Ailments in each group are sorted by the number of plants used, then by alphabetical order in case of equal values

***In vitro* antibacterial activity of selected medicinal plants:** The antibacterial activity of seven traditional plants was presented in table 2, showing that 6/7 plants exhibited antibacterial effects, except for *C. burmannii*.

Notably, *D. imbricatus*, *C. odorata*, *E. mollis* and *P. paniculata* demonstrated significant inhibitory activity against various strains of bacteria associated with diarrhea including *E. coli*, *Salmonella* spp., *Vibrio* spp and *Shigella flexneri*. Based on this result, four extracts exhibiting strong antibacterial activity were selected for subsequent toxicity (Table 3) and *in vivo* antidiarrheal tests.

Table 2
Antibacterial activity of medicinal plant extracts against diarrheal-related bacterial strains

Group	**High FL**						**Medium FL**			**Low FL**			
Plant species	**Di**		**Co**		**Em**		**Cb**	**Cp**		**Pp**		**El**	
	MIC	**IZ**	**MIC**	**IZ**	**MIC**	**IZ**	**IZ**	**MIC**	**IZ**	**MIC**	**IZ**	**MIC**	**IZ**
E. coli O157:H7	50	14.33 ± 1.15	50	8.67 ± 0.58	50	11.17 ± 0.76	-	100	9.50 ± 0.50		-	50	12.33 ± 0.29
E. coli 0208	-	-	50	8.83 ± 0.29	50	10.67 ± 0.29	-	-	-	-	-	100	8.67 ±0.29
E. coli	100	10.67 ± 0.29	50	9.33 ± 0.58	50	13.33 ± 0.58	-	-	-	100	9.67 ± 0.29	-	-
Salmonella dublin	100	10.33 ± 1.15	50	9.50 ± 0.87	-	-	-	-	-	75	10.00 ± 0.50	-	-
Salmonella typhii	50	11.33 ± 0.29	50	9.50 ± 0.00	50	11.33 ± 0.58	-	-	-	100	11.83 ± 0.29	-	-
Salmonella typhimurium	-	-	25	13.17 ± 0.29	25	13.33 ± 0.58	-	100	9.17 ± 0.76	87.5	10.67 ± 0.76	100	10.17 ± 0.29
Salmonella boydii	-	-	-	-	25	12.33 ± 0.29	-	-	-	100	10.17 ± 0.29	-	-
Vibrio alginolyticus	50	11.00 ± 0.00	-	-	25	14.83 ± 0.29	-	-	-	-	-	-	-
Vibrio cholerae	50	10.50 ± 0.00	-	-	100	11.83 ± 0.76	-	-	-	87.5	10.67 ± 1.16	-	-
Vibrio parahaemolyticus		11.33 ± 0.58	-	-	-	-	-	-	-	62.5	8.50 ± 0.00	-	-
Shigella flexneri	25	11.33 ± 0.58	100	9.17 ± 1.15	25	10.67 ± 0.58	-	-	-	87.5	8.83 ± 0.29	100	9.50 ± 0.50
Listeria monocytogenes	50	10.33 ± 0.58	25	11.67 ± 0.58	25	12.50 ± 0.87	-	-	-	100	11.83 ± 0.76	-	-
Pseudomonas aeruginosa	50	11.00 ± 0.00	-	-	100	13.00 ± 0.50	-	-	-	75	8.67 ± 0.29	-	-
Staphylococcus aureus	-	-	100	9,5 ± 0,00	-	-	-	-	-	75	-	-	-
Enterococcus faecalis	-	-	25	11.83 ± 0.29	-	-	-		-	-	-	-	-

FL: Fidelity level, MIC: Minimum Inhibitory Concentration ($mg.mL^{-1}$), IZ: Inhibition Zone (mm), Di: *Dacrycarpus imbricatus*, Co: *Chromolaena odorata*, Em: *Elephantopus mollis*, Cb: *Cinnamomum burmannii*, Cp: *Calamus palustris*, Pp: *Polygala paniculate*, El: *Euodia lepta*, (-): Not applicable.

Anti-diarrheal activity of plant extract on castor oil–induced diarrheal mice model: Three non-toxic plants, exhibiting a 100% survival rate at doses of 5000 $mg.kg^{-1}$ or higher, were assessed for their anti-diarrheal activity in mice (Table 3). At the concentration of 1000 $mg.kg^{-1}$, the inhibition rates of defecation of *C. odorata*, *E. mollis* and *P. paniculata* were 19.23%, 74.47% and 25.64% respectively while loperamide inhibited 80.85% of the frequency of defecation at 3 $mg.kg^{-1}$. *E. mollis* showed dose-dependent fecal inhibitory activity, with higher concentrations yielding greater antidiarrheal effectiveness. The extracts from the plants also exhibited a dose-dependent reduction in intestinal weight, particularly at a low dose of 63 mg. kg^{-1}, resulting in nearly 50% inhibitory activity (Table 5). Evaluating gastrointestinal motility (Table 6) revealed that *M. septentrionalis* significantly reduced charcoal meal transit in castor oil-induced mice (64.61% at 1000 $mg.kg^{-1}$ compared to 65.09% for loperamide at 3 $mg.kg^{-1}$).

Preliminary phytochemical screening of plant extract: Phytochemical screening of the ethanolic extract from seven plants revealed a composition rich in bioactive constituents including alkaloids, flavonoids and tannins, with the exception of the *C. burmannii* extract (Table 7). It is widely recognized that flavonoids and tannins possess antibacterial properties and have the ability to precipitate electrolyte proteins, thereby reducing small intestine transit and intestinal secretion.

Discussion

This study has comprehensively documented information on 133 medicinal plants utilized by the K'Ho people residing in BNNP. Another ethnobotanical study on K'Ho people living in Chư Yang Sin National Park (CYSNP) was conducted by Nguyen[22]. Several similar aspects of using medicinal plants between the two adjacent communities were found.

Table 3
Acute toxicity of plant extracts on mice model

Treatment	Dose ($mg.kg^{-1}$)	Survival rate (%)
Di	2000	33
Co	7500	100
Em	7500	100
Pp	7500	100

Di: *Dacrycarpus imbricatus*, Co: *Chromolaena odorata*, Em: *Elephantopus mollis*, Pp: *Polygala paniculata*

Table 4
The activity of fecal inhibition of plant extracts

Treatment	Onset of diarrhea (min)	Animals with diarrhea	No. of feces within 4h (g)	% fecal inhibition
CT + DMSO 1% (2 $mL.kg^{-1}$)	85.33 ± 11.54	6/6	0.78 ± 0.12	-
CT + Loperamide (3 $mg.kg^{-1}$)	220.50 ± 7.78	2/6	0.15 ± 0.07	80.85
CT + CoEE (1000 $mg.kg^{-1}$)	120.50 ± 17.84	6/6	0.63 ± 0.16	19.23
CT + EmEE (1000 $mg.kg^{-1}$)	195.00 ± 8.49	2/6	0.20 ± 0.07	74.47
CT + EmEE (500 $mg.kg^{-1}$)	161.00 ± 9.27	4/6	0.24 ± 0.04	69.68
CT + EmEE (250 $mg.kg^{-1}$)	148.75 ± 4.57	4/6	0.30 ± 0.07	61.70
CT + EmEE (125 $mg.kg^{-1}$)	139.40 ± 4.87	5/6	0.33 ± 0.07	57.87
CT + EmEE (62.5 $mg.kg^{-1}$)	113.60 ± 10.92	5/6	0.43 ± 0.09	45.74
CT + PpEE (1000 $mg.kg^{-1}$)	115.75 ± 3.87	6/6	0.58 ± 0.12	25.64

CT: castor oil, CoEE: ethanolic extract of *Chromolaena odorata*, EmEE: ethanolic extract of *Elephantopus mollis*, PpEE: ethanolic extract of *Polygala paniculata*, (-): not applicable

Table 5
Enteropooling reduced activity of medicinal plants

Treatment	Weight intestinal content (g)	% Inhibition of fluid
CT + DMSO 1% (2 $mg.kg^{-1}$)	1.12 ± 0.18	-
CT + Loperamide (3 $mg.kg^{-1}$)	0.25 ± 0.09	76.87
CT + EmEE (1000 $mg.kg^{-1}$)	0.32 ± 0.08	71.34
CT + EmEE (500 $mg.kg^{-1}$)	0.40 ± 0.06	64.18
CT + EmEE (250 $mg.kg^{-1}$)	0.48 ± 0.12	57.01
CT + EmEE (125 $mg.kg^{-1}$)	0.54 ± 0.14	51.64
CT + EmEE (63 $mg.kg^{-1}$)	0.64 ± 0.13	42.69

CT: castor oil, EmEE: ethanolic extract of *Elephantopus mollis*

Table 6
Small intestine transit reduced the activity of plant extracts

Treatment	Length of intestine (cm)	Distance traveled by charcoal (cm)	% Movement	% Inhibition of intestinal transit
CT + DMSO 1% (2 ml.kg^{-1})	43.58 ± 5.04	35.08 ± 1.39	81.28 ± 8.63	18.72 ± 8.63
CT + Loperamide (3 mg.kg^{-1})	39.25 ± 6.21	13.50 ± 2.12	34.91 ± 4.99	65.09 ± 4.99
CT + EmEE (1000 mg.kg^{-1})	45.50 ± 2.51	14.62 ± 3.67	32.08 ± 7.70	67.92 ± 7.70
CT + EmEE (500 mg.kg^{-1})	46.25 ± 2.46	16.12 ± 2.79	34.88 ± 6.25	65.12 ± 6.25
CT + EmEE (250 mg.kg^{-1})	42.02 ± 2.09	19.62 ± 2.99	46.57 ± 5.39	53.43 ± 5.39
CT + EmEE (125 mg.kg^{-1})	47.67 ± 2.40	25.42 ± 2.48	53.27 ± 6.64	46.73 ± 6.64
CT + EmEE (62.5 mg.kg^{-1})	46.92 ± 2.33	28.33 ± 2.80	60.43 ± 5.65	39.57 ± 5.65

CT: castor oil, EmEE: ethanolic extract of *Elephantopus mollis*

Table 7
Preliminary phytochemical analysis of medicinal plant ethanolic extracts

Phytochemical constituents	Test	Di	Co	Em	Cb	Ca	Pp	El
Carbohydrate	Molisch	+	+	+	+	+	+	+
	Fehling	+	+	+	+	+	+	+
	Barfoed	+	+	+	+	+	+	+
Amino acid	Ninhydrin	–	–	–	–	–	–	–
Steroid	Salkowski	+	+	+	+	+	+	+
	Libermann-Burchard	+	+	+	+	+	+	+
Saponin	Foam	+	+	+	+	+	+	+
Cardiac glycoside	Legal	+	+	+	+	+	+	+
	Keller-Killiani	+	+	+	+	+	+	+
Anthraquinone glycoside	Borntrager	+	+	+	–	–	+	–
Flavonoid	Alkaline	+	+	+	–	+	+	+
	Ferric chloride	+	+	+	–	+	+	+
Tannin	Ferric chloride	+	+	+	–	+	+	+
	Lead acetate	+	+	+	–	+	+	+
Alkaloid	Mayer	–	–	–	–	–	+	+
	Dragendorff	–	–	–	–	–	+	+
	Hager	–	–	–	–	–	+	+
	Wagner	–	–	–	–	–	+	+

Di: *Dacrycarpus imbricatus*; Co: *Chromolaena odorata*; Em: *Elephantopus mollis*; Cb: *Cinnamomum burmannii*; Ca: *Calamus palustris;* Pp: *Polygala paniculata*; El: *Euodia lepta.*

For example, Asteraceae was the most common family having the greatest number of species used in healthcare or most of plant resources were harvested from nature. Similarities were also observed in plant part preferences, with leaves being favored for home remedies due to their physical vulnerability, potentially resulting in higher chemical defense compound production[5].

However, Nguyen[22] did not include the list of a total of 66 traditional plants with relevant information so that a quantitative comparison of ethnobotany between two populations could be drawn. Some plants used by local people in CYSNP with the same usage as those living in BNNP were observed. For instance, *E. mollis* and *A. conyzoides* were used for the treatment of dysentery or to stop bleeding. These similarities in traditional knowledge of medicinal plants found here might be the result of sharing a common ancestor. However, it is believed that there still exist some divergences of indigenous knowledge because it is affected by various factors which have been proved in previous studies[1,30].

Based on medicinal plant usage frequency for treating diarrhea, seven plants representing high, medium and low fidelity level (FL) groups were assessed for anti-diarrheal bioactivity through *in vitro* and *in vivo* tests. The results demonstrated that those medicinal plants play their function in anti-diarrhea via their potential against diarrheal-related bacteria. Notably, all three high-FL group plants (*D. imbricatus*, *C. odorata* and *E. mollis*) showed their antibacterial activity against a wide range of diarrhea-related bacteria (10, 10 and 11/15 strains tested respectively).

However, plants from the medium-FL group (*C. burmannii* and *C. palustris*) did not exhibit the expected effects.

The acute toxicity assessment indicated the safety of three out of four tested plants up to 7500 mg/kg, with no observed acute signs, except for *D. imbricatus.* The plant, despite exhibiting a high FL value and antibacterial activity, demonstrated toxicity at 2000 $mg.kg^{-1}$. Hence, additional research is warranted to investigate its potential applications. Ethanolic extracts from the remaining three plants, *C. odorata* (CoEE), *E. mollis* (EmEE) and *P. paniculata* (PpEE), were tested in a castor oil-induced mice model, all exhibiting fecal inhibition activity. The EmEE exhibited superior antidiarrheal activity despite all three medicinal plants demonstrating high antibacterial activity.

Diarrhea is denoted by an increased secretion of water and electrolytes into the intestinal lumen, along with the leakage of protein and fluid from the mucosal surface. This causes changes in intestinal motility, leading to faster transit times and an increase in watery stools[6].

Castor oil causes diarrhea due to its hydrolysis by lipase to ricinoleic acid in the intestinal lumen. This acid triggers peristaltic activity in the small intestine, leading to modifications in the electrolyte permeability of the intestinal mucosa and a rise in intestinal transit. Additionally, castor oil also produces prostaglandin, leading to stomach cramps and diarrhea due to the effect on the smooth muscle and secretion[4].

Therefore, the antidiarrheal activity of herbal extracts against castor oil-induced diarrhea may be ascribed to their action in reducing electrolyte permeability. In this study, the ethanolic extract of *E. mollis* significantly reduced diarrhea rate, wet feces, secretion fluid and gastrointestinal propulsion in castor oil-induced mice. Given the widespread use of these medicinal plants in some Vietnamese minorities, these findings offer a robust foundation for their utilization and safety.

Conclusion

The knowledge of 133 medicinal plants utilized by K'Ho people in BNNP was recorded systematically. Despite their long-standing use in the community, scientific validation of their activities is lacking. For this reason, eight plants traditionally used for digestive infections were chosen for *in vitro* and *in vivo* tests in mice. Among these, ethanol extract of *Elephantopus mollis* not only strongly reduced small intestine transit in comparison to the standard drug loperamide, but showed their safety in acute toxicity study.

Additionally, phytochemical screening revealed the presence of flavonoids and tannins, known for their ability to precipitate protein and to decrease small intestine transit and intestinal secretion. This study provides fundamental information for future research on potential traditional medicinal plants applicable in diarrheal treatment.

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