ISSN (O): 2581-8740 | ISSN (P): 2581-8732
Nhut and Thao, 2020
DOI: 10.21276/SSR-IIJLS.2020.6.1.1

Research Article

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Antibacterial Activity of Water Extract From Some Folk Medicine at Bidoup-Nuiba National Park, Lam Dong, Vietnam

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Received: 05 Jul 2019/ Revised: 16 Sep 2019/ Accepted: 22 Dec 2019

ABSTRACT

Background: Folk medicinal plants were used to treat some common diseases such as diarrhea, wound healing by ethnic minorities at Bidoup-Nui Ba National Park, Lam Dong province, Vietnam. However, the usage of medicinal plants was mainly based on experience and there was no scientific basis to prove the biological activity of medicinal plants. This study aims to evaluate the biological activity of some folk medicinal plants used by ethnic minorities.

Methods: In this study, 9 samples of folk medicinal plants that ethnic minorities used to treat diarrhea at Bidoup-Nui Ba National Park, Lam Dong province were water extracted and evaluated the antibacterial activity against 16 indicators bacterial sp. by well diffusion agar. The water extracts were chemically tested for the presence of different constituents, including carbohydrates, alkaloids, saponins, cardiac glycosides, anthraquinone glycosides, flavonoids, phenolic compounds, tannin, steroids and amino acids by using standard methods.

Results: The results showed that water extract from *Medinilla septentrionalis* had the highest antibacterial activity (against 16/16 indicator bacteria) with inhibition diameter zone from 9.3 mm–11.0 mm, 4/9 water extracts had weak activity and 4/9 extracts had no antibacterial activity. The preliminary phytochemical screening of the water extracts showed the presence of flavonoids and tannins.

Conclusion: Although the medicinal plants have been used widely by ethnic people in the type of water extract, this study showed that water is not a good solvent for extracting biological compounds from the medicinal plants. Further studies on theses plants should be carried out either for evaluating their antibacterial activity or investigating their antidiarrheal activity.

Key-words: Antibacterial activity, Antidiarrhea, Bidoup-Nui Ba, Folk medicine, Water extract

INTRODUCTION

Vietnam is a tropical country with an abundant and diverse medicinal plant. According to recent statistics, the flora of Vietnam has over 10,000 species. Vietnam has about 3,200 species of herbs. Although the sources of medicinal plants in our country are abundant, the use and exploitation are limited, especially the folk medicinal plants and indigenous knowledge.

How to cite this article
Nhut PM and Thao DTP. Antibacterial Activity of Water Extract From Some Folk Medicine at Bidoup-Nuiba National Park, Lam Dong, Vietnam. SSR Inst. Int. J. Life Sci., 2020; 6(1): 2449-2454.

Access this article online
https://iijls.com/

Folk medicinal plants are widely used by the ethnic people in the treatment of diseases by cooked with water until close to dry and then thoroughly drained for use [1-3].

Bidoup-Nui Ba National Park is a large biodiversity center of Vietnam with 461 species of medicinal plants. K'Ho people, the main ethnic minority live here, often use plants, leaves to treat some diseases such as diarrhea, ulcers, abrasions, etc [4]. However, there have been few studies on folk medicinal plants at Bidoup-Nui Ba National.

Diarrhea is caused by many reasons including bacterial infections such as *Salmonella enteritidis, S. typhi, Shigella flexneri, E. coli, Vibrio cholerae, Clostridium difficile* or chemicals such as castor oil or magnesium sulfate [5-7].

ISSN (O): 2581-8740 | ISSN (P): 2581-8732
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To treat diarrhea, some methods have been used such as oral rehydration therapy, antibiotics and gut motility suppressing agents or pharmacological agents, etc. The usage is depending on the cause and the severity of the disease [8-10]. However, medicine like loperamide, diphenoxylate, racecadotril might cause undesirable side effects as vomit, dry mouth, abdominal discomfort, constipation and headache [11-13]. Recently, herbal remedies are great applications of interest. Most of medicinal plants have alkaloid, steroid, tannin and phenol which are stored in some different part of the plants [14]. These metabolites are well known as antibacterial substances, which prevent bacterial infection into the plants. Although the antibacterial mechanism still was not fully reported, it is known that the metabolite inhibit bacterial DNA/RNA synthesis, interfere bacterial metabolism, and destroy bacterial cell membrane [15,16]. In this study, we evaluated the antimicrobial activity of the folk medicinal plants against some bacteria that cause diarrhea.

MATERIALS AND METHODS

Folk medicinal plants and extraction- Medicinal plants *(Elephantopus mollis Kunth, Lantana camara* L., *Euodia lepta (Spreng.) Merr., Acorus tatarinowii Schott., Calamus* sp., *Medinilla septentrionalis* (W.W.Sm) *M.P. Nayar., Polygala paniculata* L., *Podocarpus imbricatus Blume., Eupatorium odoratum* L.*)* (Table 1) were collected from Bidoup-NuiBa National Park, Lam Dong province, Vietnam and identified at Department of Ecology and Evolutionary Biology, Faculty of Biology, University of Science, VNU-HCMC from August 2016 to September 2017. These samples were extracted according to Akinyemi *et al.* [4] with modification. Samples were dried, powdered and extracted with water by immersion method [1:10 (w/v)] at room temperature for 4 hours. The extracts were filtered and evaporated at 40^0C using a rotary evaporator to constant weight. The extracts were then dissolved in sterile distilled water and stored in a sterilized bottle in 4^0C temp.

Table 1: Medicinal plants from Bidoup-Nui Ba National Park, Lam Dong province, Vietnam

Plants name	Vietnamese name	Used plant parts
Elephantopus mollis Kunth	Chan voi mem	Bud
Lantana camara L.	Bong oi	Bud
Euodia lepta (Spreng.) Merr.	Ba chạc	Bud and leaf
Acorus tatarinowii Schott	Thachxuongbo	Base
Calamus sp.	May rung	Bud
Medinilla septentrionalis (W.W.Sm) M.P.Nayar	Xidranguom	Bud and leaf
Polygala paniculata L.	Kichnhuthom	Whole tree
Podocarpus imbricatus Blume	Bach tung	Stem core, leaf
Eupatorium odoratum L.	Co hoi	Root

Indicator bacterial species- Indicator bacteria were provided by the Department of Molecular and Environmental Biotechnology, Faculty of Biology, University of Science, VNU including 3 strains of *Shigella* sp. (*S. sonnei, S. flexneri,* and *S. boydii*), 4 strains of *Salmonella* sp. (*S. typhimurium, S. typhii, S. dublin, S. enteritidis*), 3 strains of *E. coli* (*Enterotoxigenic E. coli (*ETEC), *E. coli* O157:H7, *E. coli)*, 3 strains of *Vibrio* sp. (*V. cholerae, V. parahaemolyticus*), 2 strains of *Listeria* sp. (*L. monocytogenes, L. innocua*) and *S. aureus.*

Antibacterial activity- Antibacterial activity of 9 water extracts was evaluated by well diffusion agar method [17,18]. Indicator bacterial sp. was enriched in the suitable medium at temperature 37^0C at 24 hours. 100µl of diluted bacteria (10^6 cfu/ml^{-1}) solution was spread on agar medium until drying. Then, wells (6 mm diameter) were made in each plate by using sterile metal cylinders. 100 µl of every water extract (100 mg/ml^{-1}) were dropped into the wells. Sterile distilled water was used as a control. Plates were incubated at 37.0±0.1^{0}C at 24 hours. The diameter of the inhibition zone (mm) was

ISSN (O): 2581-8740 | ISSN (P): 2581-8732
Nhut and Thao, 2020
DOI: 10.21276/SSR-IIJLS.2020.6.1.1

measured. Each experiment was triplicated and collected data were subjected to statistical analysis.

Preliminary phytochemical analysis- Total 9 water extracts were identified [17] the presence of constituents, including carbohydrate (Molisch, Fehling and Barfoed test), alkaloid (Mayer, Dragendorff, Hager and Wagner test), saponin (foam test), cardiac glycoside (Legal and Keller-Killiani test), anthraquinone glycoside (Bontrager test), flavonoid (alkaline and ferric chloride test), tannin (ferric chloride and lead acetate test), steroid (Salkowski and Libermann Burchard test), amino acid (ninhydrin test).

Statistical Analysis- Statistical analysis values were expressed as mean±standard deviation. Mean values were evaluated by Analysis of Variance. Duncan test was used to determine the statistical significance (P< 0.05).

RESULTS

Identification of the extractive efficiency of water extracts- Water is a solvent that used to extract the compound from medicinal plants by K'Ho people at Bidoup-Nui Ba National Park. We, therefore utilized it for preparation of the plant extracts. The extractive efficiency of 9 medicinal plants with water was a statistically significant difference (p <0.05) (Table 2). The extraction efficiency of *E. lepta* was highest (29.52%), while the extraction efficiency from *E. mollis, P. imbricatus, L. camara* was low (<20%).

Table 2: The extractive efficiency of water extract

Medicinal plants	Extractive efficiency of water extract (%)
L. camara	12.36 ± 0.43^{ef}
A. tatarinowii	25.74 ± 0.52^{ab}
E. lepta	29.52 ± 1.04^{a}
Calamus sp.	21.69 ± 0.44^{d}
M. septentrionalis	20.18 ± 1.19^{de}
P. paniculata	20.53 ± 1.09^{d}
E. mollis	15.15 ± 0.46^{ef}
P. imbricatus	11.99 ± 0.43^{f}
E. odoratum	25.46 ± 2.04^{bc}

Antibacterial activity of water extracts- The results of the antimicrobial activity of water extracts against indicator bacteria showed that 4/9 plant extracts did not inhibit indicator bacteria, while another 5/9 water extracts showed different antibacterial activity (Table 3). In particular, *M. septentrionalis* water extract showed the strongest antibacterial activity (inhibition to 16/16 indicator bacteria), *P. paniculata*, *P. imbricatus, E. odoratum,* and *E. mollis* water extract inhibited 6/16, 2/16, 1/16 and 1/16 indicator bacteria at 100 mg/ml concentration, respectively.

Table 3: Antibacterial inhibition zone of water extracts against indicator bacteria (mm)

Bacterial sp.	LC	AT	EL	CA	MS	PP	EM	PI	EO
***E. coli* O157:H7**	NA	NA	NA	NA	11.00±0.50	NA	NA	NA	NA
E. coli	NA	NA	NA	NA	10.17±1.15	8.17±0.29	NA	NA	NA
ETEC	NA	NA	NA	NA	9.83±0.29	8.83±0.39	NA	NA	NA

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L. innocua	NA	NA	NA	NA	10.17±0.58	NA	NA	NA	NA
L. monocytogenes	NA	NA	NA	NA	12.83± 0.29	10.00±0.00	NA	NA	NA
S. dublin	NA	NA	NA	NA	11.00±0.50	NA	NA	NA	NA
S. enteritidis	NA	NA	NA	NA	10.75±0.35	NA	NA	NA	NA
S. typhii	NA	NA	NA	NA	9.67±0.29	9.00 ± 1.00	NA	9.83±0.29	NA
S. typhimurium	NA	NA	NA	NA	10.83±0.58	NA	NA	NA	NA
S. boydii	NA	NA	NA	NA	11.75±0.40	NA	NA	NA	13.17±0.29
S. flexneri	NA	NA	NA	NA	9.83±0.29	NA	12.17±0.29	NA	NA
S. sonnei	NA	NA	NA	NA	9.33±0.60	NA	NA	11.5±0.00	NA
V. cholerae	NA	NA	NA	NA	10.00±1.00	NA	NA	NA	NA
V. parahaemolyticus	NA	NA	NA	NA	9.83±0.76	8.33±0.59	NA	NA	NA
S. aureus	NA	NA	NA	NA	9.83±0.29	NA	NA	10.00±0.00	9.5±0.00
E. feacalis	NA	NA	NA	NA	9.50±0.50	11.50±1.50	NA	NA	NA

LC: ***Lantana camara;*** **MS:** ***Medinilla septentrionalis;*** **PP:** ***Polygala paniculata;*** **AT:** ***Acorustatarinowii;*** **EM:** ***Elephantopusmollis;*** **PI:** ***Podocarpusimbricatus;*** **EL:** ***Euodialepta*****; EO:** ***Eupatorium odoratum;*** **CA:** ***Calamus*** **sp.**

Phytochemical identification of the water extracts- The results of the chemical composition of water extracts from 9 medicinal plants showed that carbohydrates presented at all water extracts. In addition, there was the presence of several biologically active compounds, including alkaloids, tannins, and flavonoids (Table 4).

Table 4: Phytochemical constituents of water extracts

Constituents	Test	LC	AT	EL	CA	MS	PP	EM	PI	EO
Carbohydrate	Molisch	+	+	+	+	+	+	+	+	+
	Fehling	+	+	+	+	+	+	+	+	+
	Barfoed	+	+	+	+	+	+	+	+	+
Alkaloid	Mayer	+	–	+	–	–	+	–	–	–
	Dragendorff	+	–	+	–	–	+	–	–	–
	Hager	+	–	+	–	–	+	–	–	–
	Wagner	+	–	+	–	–	+	–	–	–
Saponin	Foam	+	+	+	+	+	+	+	+	+
Cardiace glycoside	Legal	+	+	+	+	+	+	+	+	+
	Keller-Killiani	+	+	+	+	+	+	+	+	+
Anthraquinone glycoside	Borntrager	–	–	–	–	+	+	+	+	+
Flavonoid	Alkaline	+	+	+	+	+	+	+	+	+
	Ferric chloride	+	+	+	+	+	+	+	+	+
Tannin	Ferric chloride	+	+	+	+	+	+	+	+	+
	Lead acetate	+	+	+	+	+	+	+	+	+

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Steroid	Salkowski	+	+	+	+	+	+	+	+	+
	Libermann-Burchard	+	+	+	+	+	+	+	+	+
Amino acid	Ninhydrin	–	–	–	–	–	–	–	–	–

(+): positive; (–): negative
LC: ***Lantana camara;*** **MS:** ***Medinilla septentrionalis;*** **PP:** ***Polygala paniculata;*** **AT:** ***Acorustatarinowii;*** **EM:** ***Elephantopusmollis;***
PI: ***Podocarpusimbricatus;*** **EL:** ***Euodialepta*****; EO:** ***Eupatorium odoratum;*** **CA:** ***Calamus*** **sp.**

DISCUSSION

Understanding the effectiveness of traditional plants, which used by ethnic people are important in order to sustainable mining folk medicinal plants and develop the usage, this study aimed to evaluate the antibacterial effectiveness of folk medicinal water extract that used by the K'Ho ethnic group in the treatment of some common diseases including diarrhea.

To elucidate the antibacterial activity of the plants and the indicator bacterial sp. belonged to the gastroenterogenic bacteria including *S. typhii* caused typhoid; *S. enteritidis, S. flexneri* and *E. coli* caused diarrhea; *S. sonnei, S. boydii* caused dysentery; *Vibrio* sp. caused many dangerous intestinal diseases such as cholera (*V. cholerae*) [17,18]. The results showed that the antimicrobial activity of water extracts from 9 folk medicinal plants was very low, except *M. Septentrionalis* water extract had high antibacterial activity. This study showed that water was not a good solvent to extract antimicrobial compounds for those plants in this study. However, in the daily file, ethnic people, mostly boiled the medicinal plants in water and used to treat diarrhea. Our study results suggested that the usage even based on people experiences but may not high efficiency in treatment as the antibacterial activity is low in 8/9 plants except *M. septentrionalis*.

Besides, our results of the preliminary phytochemical screening also provided the scientific evidences that the plants contain some well known antibacterial compounds such as flavonoid phenolic compound. The plants also contain some compounds that can precipitate protein, which functions on the electrolyte and reduce small intestine transit and intestinal secretion such as tannin and flavonoid [19,20]. Taken together, the study suggested that the folk medicinal plants play antidiarrheal activity via their antibacterial potential and their metabolite activity on electrolyte, intestine. However, the traditional usages should be improved by using different solvent for plant extracts.

CONCLUSIONS

In 9 folk medicinal plant samples at Bidoup-Nui Ba National Park, only 5/9 water extracts had antibacterial activity against diarrhea relating indicator bacteria and *M. septentrionalis* water extract had the strongest antibacterial activity (19/19 indicator bacteria) at 100 mg/ml concentration.

This result showed that water is not a good solvent for extracting biological compounds from all medicinal plants; therefore, it is important to investigate before using water to extract from medicinal plants.

ACKNOWLEDGMENTS

We thank Vietnam National University in Ho Chi Minh City for funding this study. We thank Directors of Bidoup-Nui Ba National Park, Lam Dong Province for facilitating to collect samples. We thank BSc. Pham Thi Thao, Lam Pham Hue Tam at Ho Chi Minh City University of Technology has supported the experiment.

CONTRIBUTION OF AUTHORS

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ISSN (O): 2581-8740 | ISSN (P): 2581-8732
Nhut and Thao, 2020
DOI: 10.21276/SSR-IIJLS.2020.6.1.1

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