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Research Article

SCREENING OF ANTIDIARRHEAL PROPERTIES AND PHYTOCHEMICALS OF FOUR RARE PLANTS USED IN TRADITIONAL MEDICINE

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ABSTRACT

This paper deals with the anti-diarrheal property and phytochemical screening of four plants used in traditional medicine viz., *Syzygium laetum, Symplocos cochinchinensis* var. *laurina, Memecylon randerianum* and *Vateria indica*. Methanolic extract of four plants were tested for anti-bacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterococcus faecalis* which is associated with diarrhea. Phytochemical screening of these plants were performed for the constituents alkaloids, acidic compounds, anthraquinones, flavanoids, glycosides, carbohydrates, catacholamines, lipids, reducing sugar, resins, saponins, sterols, steroids, pholabatannins, tannins and terpenoids. Antibacterial activity revealed that all the plant extracts showed positive inhibitory activity against all tested pathogens. *Memecylon randerianum* possess highest number of phytochemicals with eleven out of sixteen. The study pointed out the importance of conservation of plant genetic resources which possess unique features in the control of awful diseases. Results of this study can be further taken up for the development of novel therapeutic compounds.

KEYWORDS: Antibacterial activity, diarrhea, phytochemical compounds, traditional medicine

INTRODUCTION

Diarrhea is considered as a dreadful disease and a leading cause of mortality second to respiratory illness among children in the developing nations. Even though diarrhea is considered as an easily preventable and manageable disease nearly 5 million children below the age five years die each year because of diarrhea[1]. As for the survivors of diarrhea the re-bouting of the disease could results significant growth shortfalls that can account for 8.2 cm up to the age of 7 which will affect the cognizance, fitness and education[2]. Unhygienic environment especially unclean water would the root cause of this disease which developing countries are still to battle with. The causative agents for the disease also differ considerably as virus is the major pathogen in developed nations and bacteria took that role in developing countries. In India the scenario is grotesque by 1000 children are dying due to diarrhea and one out of every five children die of diarrhea worldwide is an Indian [3].

There are many microorganisms belong to bacteria, virus, and protozoa are considered as the pathogens for diarrhea. Some important bacterial pathogens are *Escherichia coli*, *Salmonella* sp., *Shigella* sp., *Staphylococcus aureus*, *Vibrio* sp. Viral pathogens include Rotavirus, Enteric adenovirus, Coronavirus and protozoans of *Giardia lamblia, Entamoeba histolytica*, Cryptosporidium[4]. *Entercoccus faecalis* is considered as a low grade pathogen which commonly seen in the oral cavity, alimentary tract, genitourinary tract and skin especially in the perineal area which found to be associated with diarrhea[5]. The other common bacteria associated with are *Klebsiella pneumoniae, Proteus mirabilis* and *Pseudomonas aeruginosa*[6].

Over the year's humans have discovered many chemicals specially antibiotics for the treatment of life threatening disease which include diarrhea also. For the last several decades an important problem faced in the treatment of microbial diseases is antimicrobial resistance to a wide range of diseases. This causes the treatment of microbial disease difficult, costly, or even impossible[7]. It has been noted that there is a significant increase in the resistance to antibiotics among the enteric bacterial pathogens and this could be considered as a major problem in the treatment of infectious diarrhea[8]. Natural products from the plants can play a major role in the control of microbial diseases as many of the people in the world still depends on traditional healthcare system where plants role is dominant. In this context the present work is planned to test the effectiveness of four plants used in traditional healthcare for the control of diarrheal pathogens. Once an active biomolecule could be generated out of these plants the mass conservation as *in-situ* and *ex-situ* will be enhanced.

MATERIALS AND METHODS

The present study carried out at Community Agrobiodiversity Centre, M. S. Swaminathan Research Foundation, Kalpetta, Kerala, India. The study was conducted to observe the chemical constituents and inhibitory effect of selected four plants *Syzygium laetum, Symplocos cochinchinensis* var. *laurina, Memecylon randerianum* and *Vateria indica* against Diarrhea causing pathogens. The four plants selected on the basis of detailed review of literature, personnel communication and semi structured interview with traditional healthcare practitioners at tribal dominated areas in Wayanad district of Kerala *viz.*, *Kattunaikka* hamlet at Muthanga, *Paniya* hamlet at Puthoorvayal, *Kuruma*

hamlet at Karipur and *Adiya* hamlet at Thirunelli. The selected plants are rarely and narrowly distributed in South India.

Sample collection

The leaves of *Syzygium laetum, Symplocos cochinchinensis* var. *laurina, Memecylon randerianum* and *Vateria indica* were collected from the Botanic garden of Community Agro biodiversity Centre, M. S. Swaminathan Research Foundation, Puthoorvayal, Wayanad, Kerala.

Preparation of plant Extracts

The fresh leaves of *Syzygium laetum, Symplocos cochinchinensis* var. *laurina, Memecylon randerianum* and *Vateria indica* were properly washed with tap water, rinsed with sterile water, shade dried and pulverized with an electric blender. 5g of powdered sample of each plant was extracted with 20 ml of ethanol. Extraction was allowed to proceed for 72 h. Extract filtered using Whatman No.1 filter paper and evaporated to dryness on a boiling water bath. The extracts were stored in an air tight sample bottles and kept in a refrigerator until use[9].

Test organisms and culture maintenance

The test organisms used in this study were diarrheal pathogens gram positive *Staphylococcus aureus*, *Pseudomonas aeruginosa* and Gram negative *Escherichia coli, Enterococcus faecalis* were obtained from District Hospital, Wayanad. The bacterial cultures were first sub cultured in nutrient broth and incubated at 37^0C for 24 h.

Antibacterial activity assay

The antibacterial activity of methanolic extracts of *Syzygium laetum, Symplocos cochinchinensis* var. *laurina, Memecylon randerianum* and *Vateria indica* were evaluated against diarrheal pathogens *E.coli, E. faecalis, P.aeruginosa* and *S. aureus* by well diffusion method. Nutrient agar plates were prepared for all plant extracts, 50 µl of inoculums of each selected bacterium was seeded on agar plates, after solidification approximately 7mm diameter wells was bored with the help of borer. Well was filled with 100µl of different concentrations (100 µg, 200µg, 300µg, 400µg and 500µg/ml) of methanolic extract of each plant. All the plates were kept in a refrigerator at 2 to 8 ^{0}C for a period of two hours for effective diffusion of test and standard compounds. Ampicillin standard was used in comparison with anti-bacterial activity of other organism. Later they were incubated at 37^0 C for 24 h. Three replicates for each of the concentrations were maintained. Presence of zone around the well was measured and recorded[10].

Phytochemical screening

The methanolic extracts of *Syzygium laetum, Symplocos cochinchinensis* var. *laurina, Memecylon randerianum* and *Vateria indica* were subjected to various tests for the identification of various active chemical constituents like alkaloids, acidic compounds, anthraquinones, flavanoids, glycosides, carbohydrates, catacholamines, lipids, reducing sugar, resins, saponins, sterols, steroids, pholabatannins, tannins and terpenoids using different methods[11,12].

RESULTS AND DISCUSSION

A detailed review of literature, personnel communication and semi structured interview were conducted with traditional healthcare practitioners at tribal dominated areas in Wayanad district of Kerala to document some rare plants used to treat diarrhea. Scientific and vernacular names of the plants, family, brief method of administration against diarrhea of the selected plants are tabulated (Table 1).

Table 1. Details of plants selected for the study with brief method of administration

Scientific name	Family	Local name	Brief Method of administration to treat diarrhea
Memecylon randerianum SM & MR Almeida	Melastomataceae	Kasaavu	Five drops of leaf infusion twice in a day for three days
Symplocos cochinchinensis var. *laurina* (Retz.) not.	Symplocaceae	Parala, Pachotti	One table spoon of leaf extract or bark infusion twice in a day for two days
Syzygium laetum (Buch.-Ham.) Gandhi	Myrtaceae	Kattuchamba	One table spoon of leaf juice twice in a day for three days
Vateria indica L.	Dipterocarpaceae	Vellapandham	A pinch of powder of dried resin add in a glass of water and given twice in a day till cure

The inhibitory effect of *Syzigium leatum* extracts against four diarrheal pathogens are shown in Table2. The results indicates that while increasing the concentration of the plant extract the inhibition of microorganism also increasing and is found to be statistically significant ($P<0.01$, $P<0.05$). Highest inhibition was observed with 500µg of plant extract where the maximum sensitivity shown by *Pseudomonas aeruginosa*with2.3±0.08cm of inhibition followed by *Staphylococcus aureus* with 2.1±0.08 cm, *E.coli*2.06±0.12cm and *Enterococcus faecalis* exhibits 1.8±0.05cm. All the other tested quantity of plant extract was shown lesser inhibitory percentage. The results also pointed out that plant extract do not have any specificity for its inhibitory effect.

Table 2: Inhibitory diameter of *Syzygium laetum* plant extracts against pathogens

Pathogens	Inhibition Diameter (cm) of *Syzygium laetum* (Mean± Standard error)				
	100µg	200 µg	300 µg	400 µg	500 µg
E.coli	0.4±0.05	0.86±0.033	1.2±0.14	1.73±0.08	2.06±0.12
E. faecalics	0.3±0.05	0.7±0.03	0.86±0.03	1.3±0.08	1.8±0.05
P. aeruginosa	0.6±06	0.9±0.05	1.3±0.08	1.5±0.14	2.3±0.08
S. aureus	0.8±0.05	1.1±0.08	1.5±0.11	1.9±0.05	2.1±0.08

Antimicrobial effects of *Symplocos cochinchinensis* var. *laurina* are provided in Table 3. It is obvious that the highest tested quantity of plant extract (500µg) has the maximum antimicrobial property against the pathogens. Gram positive cocci *Enterococcus faecalis* has shown maximum inhibitory activity with 1.8cm ensued by *E. coli* with 15cm, *Pseudomonas aeruginosa*14cm and *Staphylococcus aureus* exhibits 1.3cm.

Table 3: Inhibitory diameter of *Symplocos cochinchinensis* var. *laurina* plant extracts against pathogens

Pathogens	Inhibition Diameter (cm) of *Symplocos cochinchinensis* var. *laurina* (Mean± Standard error)				
	100µg	200 µg	300 µg	400 µg	500 µg
E.coli	0.56±0.06	0.76±0.06	0.93±0.03	1.03±0.08	1.53±0.08
E. faecalics	0.4±0.03	0.8±0.11	1.1±0.08	1.3±0.14	1.8±0.11
P. aeruginosa	0.63±0.08	0.73±0.08	1.13±0.14	1.3±0.08	1.43±0.8
S. aureus	0.53±0.08	0.9±0.05	1.13±0.08	1.13±0.14	1.3±0.11

(Mean± Standard error)

The antimicrobial properties of the plant extracts *Memecylon randerianum* and *Vateria indica* are given in Table 4 and 5respectively. Dependency of the concentration of plant extracts against the inhibition of microbial growth was visible with the results. *Memecylon randerianum* restricted the growth of *E. coli* maximum with 2.1±0.11cm followed by *Enterococcus faecalis*1.9±0.05cm, *Staphylococcus aureus* with 1.7±0.11 and *Pseudomonas aeruginosa*1.53±0.08cm. All the other tested amount of plant extracts was lesser in controlling the growth of diarrheal pathogens as illustrated in the table. *Vateria indica* showed positive results to all of the tested diarrheal pathogens. From the Table 4 it is clear that the extracts can control *Staphylococcus aureus* for the maximum with 2.03±0.08cm proceeded by *E.coli* 1.9±0.05cm, *Enterococcus faecalis* 1.9±0.05 cm and the lowest shown by the gram negative *Pseudomonas aeruginosa* 1.3±0.11cm. The lowest quantity of plant extract (100µg) also exhibited considerable antimicrobial abilities

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Table 4: Inhibitory diameter of *Memecylon randerianum* extract against pathogens

Pathogens	Inhibition Diameter (cm) of *Memecylon randerianum* (Mean± Standard error)				
	100µg	200 µg	300 µg	400 µg	500 µg
E.coli	0.76±0.12	0.9±0.05	1.53±0.08	1.8±0.11	2.1±0.11
E. faecalics	0.4±0.03	0.73±0.08	1.03±0.45	1.53±0.08	1.9±0.05
P. aeruginosa	0.4±0.03	0.73±0.08	0.83±0.08	1.13±0.08	1.53±0.08
S. aureus	0.53±0.03	0.83±0.03	1.43±0.08	1.53±0.08	1.7±0.11

Table 5: Inhibitory diameter of *Vateria indica* plant extracts against pathogens

Pathogens	Inhibition Diameter (cm) of *Vateria indica* (Mean± Standard error)				
	100µg	200 µg	300 µg	400 µg	500 µg
E.coli	0.53±0.03	1±0.11	1.23±0.08	1.7±0.11	1.9±0.05
E. faecalics	0.76±0.06	0.9±0.05	1.4±0.05	1.5±0.05	1.63±0.08
P. aeruginosa	0.16±0.03	0.53±0.03	0.73±0.08	1.03±0.08	1.3±0.11
S. aureus	0.46±0.03	0.73±0.08	1.23±0.08	1.8±0.11	2.03±0.08

The phytochemical compositions of the methanolic extract of all the selected plants have been analyzed and are given in the Table 6. The presence of sixteen different phytochemicals have been screened and found that all the plants showed positive results for seven phytochemicals and negative results for 3 phytochemicals *viz*. alkaloids, anthraquinones and pholoabatannins. The maximum number of tested phytochemicals was shown by *Memecylon randerianum* (11) followed by *Syzygium laetum*and *Vateria indica* (9) and lowest was exhibited by *Symplocos cochinchinensis* var. *laurina* (8).

Table 6: Result of phytochemical screening of methanolic extract of plant extracts

Phyto constituents	*S. laetum*	*S.cochinchinensis* var. *laurina*	*M.randerianum*	*V. indica*
Acidic compounds	-	+	+	+
Alkaloids	-	-	-	-
Anthraquinones	-	-	-	-
Carbohydrates	+	+	+	+
Catacholamines	-	-	+	-
Flavanoids	-	-	+	+
Glycosides	+	+	+	+
Lipids	+	+	+	+
Phenols	+	+	+	+
Pholabatannins	-	-	-	-
Reducing sugar	+	+	+	+
Resins	-	-	+	-
Saponins	+	+	+	+
Sterols	+	-	-	-
Tannins	+	+	+	+
Terpenoids	+	-	-	-

+: present; - : absent

DISCUSSION

World population especially in developing countries heavily depends on traditional practitioners and medicinal plants to meet their healthcare needs [13]. This shows the importance of medicinal plants in the daily life of people even though modern medicines are available. Plants still remain as an untapped source of active biomolecules which have immense potential in the control of many dreadful diseases. In India *Ayurveda* is a system of treatment practiced since 5000years where medicinal plants playing a major role in healing of the diseases which also gives emphasize on the body, mind and spirit [14]. Of all the plant species known on earth only 6% of them have biologically screened and 15% of the plants have phytochemically analyzed and the active biomolecules were identified [15]. There is a vast gap exists on the information on the plant species with reference to its chemical composition both in qualitatively and quantitatively. The Western Ghats remained as a mystery with its depth of plant genetic resources (ca.5000 flowering plants) it is high time to study on these plant resources for its bioactive components.

In the present study out of the four plant species *Syzygium laetum* showed maximum inhibitory activity against two pathogens viz. *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The studies related to the antimicrobial properties and phytochemical contents about this plant are scarce or limited because of its distribution. This work is unique and first of its kind in attempting the screening of chemical composition and antibacterial properties. Out of the sixteen different phytochemicals tested nine of them showed positive results. Presence of phenolic content along with saponins, tannins and terpenoids could be considered as the agents responsible for the antimicrobial properties for this plant [16,17]. Several other studies pointed out the sensitivity of plant extracts on the inhibition of *Staphylococcus aureus*[18, 19, 20].

The plant demonstrated high inhibition against *Enterococcus faecalis* and *E. coli* was *Memecylon randerianum* with the inhibition percentage of 1.9cm and 2.1cm respectively. Suresh *et al.,* [21] described the antimicrobial properties of *Aegle marmelos* leaf extracts and high lightened the inhibition against *E. coli.* Odunayo *et al*.,[22] reported that the palm wine extract of *Kalanchoe crenata* was highly active against the *E. coli* organisms studied. The results of the current study revealing that *Memecylon randerianum* also have the potential to inhibit the growth of microorganisms. Chemical constitution of its leaf extract revealed that it contains eleven phytochemicals and ranks highest in the selected four plants. These secondary metabolites would be the reason for antimicrobial properties. The plants such as *Symplocos cochinchinensis* var. *laurina* and *Vateria indica* displayed positive inhibitory activity against all the tested microbes. Phytochemical determination revealing *Symplocos cochinchinensis* var. *laurina* possess 8 and *Vateria indica* owned 9 constituents out of the sixteen chemicals tested.

CONCLUSION

The present study pointed out that the importance of conservation of plant genetic resources which have immense potential as therapeutic agents in the control of various dreadful diseases. The aqueous leaf extracts of all the tested plantsof *Syzygium laetum, Symplocos cochinchinensis* var. *laurina, Memecylon randerianum* and *Vateria indica* showed positive results in the control of diarrheal disease causing pathogens. Results have significance in the current scenario of emergence of antibiotic resistant microorganisms where the commonly used antibiotics are futile in disease control. All the tested plants are the store house of novel biochemical constituents which can be commercially exploited for the production novel bioactive components. Economic importance of plants will boost the efforts of sustainable conservation in on farm and nonfarm approaches. This study necessitates detailed investigation into the quantification, identification and structure elucidation of various chemical components present in these plants.

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