International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (2): 1062-1066
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

In Vitro Analysis of Antibacterial Activities in Selected Medicinal Plant Species from Rubiaceae

M. Kalusalingam and V. Balakrishnan*
PG and Research Department of Botany, Arignar Anna Government Arts College, Namakkal - 637 002, Tamil Nadu, India.

Received: 20 Jan 2019 / Accepted: 22 Mar 2019 / Published online: 1 Apr 2019
Corresponding Author Email: palanivbalu@gmail.com

Abstract
Benckara malabarica (Lam.), Tarenna asiatica (Linn.) and Pavetta zeylanica (Hook. F.) belong to the family Rubiaceae. The plant species collected from Sathuragiri hills of Virudhunagar District, Tamil Nadu State. The plant samples such as stem, leaf and root were selected for the present investigation. The plant extracts were treated against microorganisms such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Bacillus subtilis. Based on the ethanomedicinal report obtained from Paliyar tribals Sathuragiri hills, Virudhunagar district, Tamil Nadu state. The present study reveals the antimicrobial activity of selected medicinal plants against microbes.

Keywords
Antibacterial, Paliyar tribal, Rubiaceae, Sathuragiri.

*****

INTRODUCTION
Microbial diseases have parallel history with the civilization of human beings. The usage of medicinal plant parts and its products and in some cases the whole plant were in the practice for curing many microbial diseases prior to the knowledge of microorganism. The knowledge of herbal wealth possessed by Indians is dating back to prehistorical times. Indian medicinal systems like Siddha and Ayurvedha are as old as human civilization. The unrecorded knowledge of such pioneers faded away with passing of time. But even now many nomadic tribal and rural Indians are using the herbal wealth for the treatment of various ailments. Since the range of microorganism is very large, the variety of disease caused by them is also vast. Though we use potent antimicrobial agents at present, multi resistant strains appear and it forces us to incessant search for discovery of new drugs (1). Exploiting the knowledge of rural Indian medicinal practitioners and systematic screening of it may lead to the discovery of potent novel phytochemicals from medicinal plants.

Most traditional medicine relies heavily on medicinal plant resources (2). *Staphylococcus aureus* has become a global threat to antimicrobial chemotherapy. This seems to pose an outlook for the use of many antimicrobial drugs in future to be uncertain (3). Medicinal plant extracts and their active principles have an enormous therapeutic potential (4). Secondary metabolites have led to an important breakthrough in pharmacology and has helped in various ways in the development of modern pharmacotherapeutics (5).

Medicinal plant contains a wide variety of active chemical compounds that exhibit antimicrobial activities (6). The presence of natural products from medicinal plants such as terpenes, alkaloids, Flavonoids and coumarins support the wide applications and uses of plants in medicines (7).

According to WHO report in 2014, antimicrobial resistance overcoming the antibiotic resistance in the issue to the WHO for the next millennium. Screening and investigations of medicinal plants for antimicrobial agents and reached the goal and promoting in the development and ultimate user of medicinal plant resources in the traditional medicinal system (8).

The present study deals with, medicinal plants survey has been conducted in the Paliyar hamlet of Sathuragiri hills, Tamil Nadu State, India, which is considered as the heart land of Siddha medicinal practice. The Hindu religion supreme god Shiva's temple is situated on the top of the hill. It is still believed that Siddhas, are visiting the shrine during moonless and full moon days. Till the mid of the twentieth century, the Paliyar tribal were leading nomadic life in the dense forest. Their knowledge about medicinal plant is unrivalled. The usage of 83 plants species from 37 families to treat various ailments by this exotic tribal was recorded (9).

Among the recorded 37 families, three plant species belong to the family Rubiaceae, *viz Benckara malabarica* (Lam.). D. D. Tirvengadum, *Tarenna asiatica* (Linn.) Ktze. Ex Schum. and *Pavetta zeylanica* (Hook. F.) have been consciously selected for further antimicrobial studies. The selected plant species have been used by the Paliyar tribal for the treatment of various ailments (Table 1). Diversity is rationale behind the selection of plant species from Rubiaceae. Rubiaceae is one of the largest family in the angiosperm with around 13,000 species. The most economically and medicinally important plant species from the family are *Coffea arabica* and *Cinchona* respectively. An important alkaloid quinine is used to cure malaria, is obtained from *Cinchona*. Plant species of Rubiaceae family are continuously tested in the laboratories for their medicinal values reported (10). Twenty-six medicinal plants from Rubiaceae family in eastern Uttar Pradesh alone (11). According to (12) 40 medicinal plants from family Rubiaceae in India. Twenty-six species medicinal plants from family Rubiaceae in Kerala forests (13).

Table 1. Ethnomedicinal values of Rubiaceae family members, Sathuragiri hills, Tamil Nadu State, India.

S.No	Botanical Name	Tamil Vernacular Name	Mode of Preparation and medicinal Usage
1.	*Benkara malabarica* (Lam.). D. D. Tirvengadum	Sirukarai/ Pidathi	* Decoction of leaves is given for cough, phlegm and throat infection. * Leaf paste is applied for skin diseases. * Dried leaf powder is macerated with sesame oil and applied as pain reliever
2.	*Tarenna asiatica* (Linn.) Ktze. Ex Schum.	Velichi	* Leaf paste is used for skin diseases. * Burned dry leaves and stem is mixed with tooth powder.
3.	*Pavetta zeylanica* (Hook. F.)	Naru manchanathi	* Leaf paste is applied for skin disorders. * Bark is used for preparation of tooth powder. * Decoction of leaf is given for stomach pain.

The ethanomedicinal values of report (Table. 1) reveals that the medicinal usage of selected medicinal plants, belongs to the family Rubiaceae. The plants were not only used as a medicine after the occurrence of diseases but also used as precautionary measure to arrest the microbial infection. Proper phytochemical analysis and testing of plant extracts against microorganisms can lead to the discovery of new phytochemicals, which can serve the human race to fight against multi drug resistant microorganisms. The aim of the present investigation is to carryout antimicrobial activity against selected microorganisms by using three medicinal plant species from Sathuragiri hills, belong to the family Rubiaceae.

MATERIALS AND METHODS

Location of the Sathuragiri hill

Sathuragiri hills has lot of herbal plants and identified by those who are experienced in knowledge about value medicine with spirituality.

Collection of plant samples

Plant samples were collected from the study area Sathuragiri hill region after the north east monsoon season in November 2018. The plant species were healthy and green at the time of collection. Identities

of the collected samples were properly checked with the herbaria. The samples were segregated into leaf, stem and root and dried under shadow for 20 days.

Preparation of methanolic extracts

The dried plant samples were powdered with mixer grinder. The powders were sieved through 40 mesh sized sieve. The sieved powders were used for the extraction of phytochemicals. Crude plant extract from the plant parts such as stem, leaves and root were obtained individually from selected medicinal plant species using Soxhlet apparatus. Methanol was used as solvent.

Preparation of disc

The crude extracts were obtained and bottled in conical flask and concentrated on hot water bath. The concentrated crude extracts were tested for the presence of phytochemicals and antimicrobial activities though disc diffusion method.

Inoculum preparation

Four bacterial strains were used for the antimicrobial studies. The following bacterial strains such as *Staphylococcus aureus* (MTTC 3160)*, Escherichia coli* (MTTC 46)*, Pseudomonas aeruginosa* (MTTC 1688) and *Bacillus subtilis* (MTTC 441) were selected for the investigation. The cultures were obtained from MTTC, Chandigarh. Loop full of stock cultures were taken and mixed with nutrient broth in sterile condition. The cultures were allowed to grow for 24 hours in shaker at the speed of 110 rpm. From the nutrient broth culture, strains were taken and spread on the prefilled petriplate, which contained solid Muller Hinton Agrar medium. The dried test extracts at the concentration of 2mg were loaded on the sterile disc. The sterile discs were placed on surface the petriplate and incubated for 24 hours at 37°C. Ciproflaxin 5µg per disc was used as control. The zone of inhibition was measured at the end of experiment. The tests were conducted three times to calculate the standard error of the result.

RESULTS

Phytochemical analysis of crude plant extracts with different methods revealed that the presence of secondary metabolites. The methanol dissolved crude extract shows that the presence of alkaloid, flavonoids, phenol and glycosides in the plant samples. Name of the methods, used for the detection of secondary metabolites and their presence in plant samples are furnished in table 2.

Table. 2 – Phytochemical analysis of selected medicinal plant species from Rubiaceae

S. No	Phytochemicals	Name of the test	*Pavetta zeylanica*			*Beckara malabarica*			*Tarenna asiatica*		
			S	L	R	S	L	R	S	L	R
1.	Alkaloids	Mayer's	-	+	-	-	+	-	-	+	-
2.	Flavonoids	Alkaline Reagent	-	-	-	-	+	-	-	-	-
3.	Phenol	Ferric Chloride	-	+	-	+	-	-	-	+	+
4.	Glycosides	Legal's	-	-	-	+	+	-	+	+	-

(+): Present (-): Not detected / S – Stem, L – Leaf and R - Root

Phytochemical analysis of plant samples indicates that the presence of alkaloid in all the three leaf samples. Flavonoids is present only in the leaf sample of *B. malabarica.* Presence of phenol is recorded in the leaves of *P. Zeylanica* and *T. asiatica*. Stem of *B. malabarica* and root of *T. Asiatica* also showed the presence of phenol. Glycosides are present only in the stem and leaf samples of *B. malabarica* and *T. Asiatica.*

There are no clear zones recorded in stem and root extracts of *P. zeylanica*. *P. zeylanica* leaf alone showed antimicrobial activity, that is also only against *B. subtilis* with 8 mm of clear zone. The stem extract of *B. malabarica* also showed antimicrobial activities against *E. coli* and *S. aureus* with 8 mm of clear zone. The leaves extract showed moderate antimicrobial activities, against all the four tested microorganisms. In *E. coli S. aureus* and *P. aeruginosa*, the clear zone width is 12mm. In case of *B. subtilis* the width of clear zone is 13mm. Stem extract of *T. asiatica* showed activity against *P. aeruinosa* with 8mm clear zone. Leaf extract of *T. asiatica* registered moderate activity against all the four microorganisms. In *S. aureus* and *P. aeruginosa*, the diameter of clear zone is 11 mm (Table 3). In case of *E. coli* and *B. subtilis* clear zone width was 12 mm.

Table 3. Antimicrobial activities of plant samples selected from Rubiaceae

Plant Name	Plant Parts	Name of microorganism & Diameter of inhibition zone (mm)			
		E. coli	*S. aureus*	*P. aeruginosa*	*B. subtilis*
	Stem	-	-	-	-
Pavetta zeylanica	Leaf	-	-	-	8 ± 0.3
	Root	-	-	-	-
	Stem	8 ± 0.5	8 ± 0.4	-	-
Benckara malabarica	Leaf	12 ± 0.6	12 ± 0.7	12 ± 0.4	13 ± 0.5
	Root	-	-	-	-
	Stem	-	-	8 ± 0.3	-
Tarenna asiatica	Leaf	12 ± 0.7	11 ± 0.6	11 ± 0.4	12 ± 0.5
	Root	-	-	-	-
Positive Control (Ciprofloxacin (5µg/disc))		25 ± 0.2	28 ± 0.1	18 ± 0.2	27 ± 0.2

(-): Not detected / Results were expressed as mean ± SD, N=3

DISCUSSION

Preliminary phytochemical analysis showed that the presence of secondary metabolites in plant samples. The leaves of *B. malabarica* and *T. asiatica* contain more than two secondary metabolites. In case of stem and root *B. malabarica* and *T. asiatica* plant parts only showed positive results for phenol and glycosides. According to (14) phytochemicals such as terpenes and phenols are responsible to exhibit antimicrobial properties.

Tamil Nadu is one of the popular botanized areas of South India. A wide knowledge regarding how to use the plants against different diseases and cure (15). The identification of new antimicrobial metabolites from medicinal plants is an important alternative to overcome the increasing levels of drug resistance (8). Antimicrobial activity of medicinal plant resources and its phytocompounds have been deliberated in the late 19th century (16). However, antibiotics abuse has become the main factor for the emergence and dissemination of multidrug resistant strains of several microorganisms (17).

When antimicrobial activities are concerned, leaves of *Benckara malabarica* and *Tarenna asiatica* were showing good results. The stem and root extracts were showing negligible activities in all three plants. In case of *Pavetta zeylanica* the leaf sample alone shows that antimicrobial activity with 8 mm clear zone. The significant antimicrobial properties observed in *J.neesii* screening may be due to the presence of one of these phytochemicals in the ethanolic extract (18).

Some recent studies reported antimicrobial activity, *in vitro* assay and ethnobotanical studies correlate with our findings (19 - 21).

The presence of phytochemicals and antimicrobial activity in the plant samples is proving their medicinal values. Paliyar tribal were using the selected plants for various ailments caused by microorganisms. Worthiness and correctness of tribal knowledge could be understood from the present investigation.

CONCLUSION

The results of the present research also correlate the presence of secondary metabolites with antimicrobial activity. *B. malabarica* and *T. asiatica* leaves contain more than two phytochemicals, which can be interpreted as a reason for their antimicrobial activity. Thorough investigation of the leaf samples can lead to the discovery of novel phytochemicals, which can serve as good antibiotics for human beings.

ACKNOWLEDGEMENT

The authors are thankful to The Head, Department of Botany, and the Principal, Arignar Anna Government Arts College for providing necessary facilities to carry out the work successfully.

REFERENCES

(1). Uddin B., Taslima, N., Ibrahim Khalil, M., Shahdat, H. *In vitro* antibacterial activity of the ethanol extract of *Paederia foetida* L. (Rubiaceae) leaves. Bangladesh J. Life Sci, 19 (2): 141-143, (2007).

(2). Eloff, J. N, Which Extract Should be used for screening and isolation of antimicrobial components from plants? J. Ethnopharmacol, 60: 1-8, (1998).

(3). Nascimento G. G. F., Locatelli, J., Freitas, P. C., Silva, G. L. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. Bras, J. Microbiol, 31: 247 – 256, (2000).

(4). Iwu M. M., Duncan, A. R., Okunji C. O., New antimicrobials of plant origin, in: Janick J (ed.) Perspectives of on new crops and uses. ASHS Press, Alexandria, VA. Pp 457 - 462. (1999).

(5). Nwaogu L. A, Alis C. S., Ibegbulem, C. O., Igwe C. V. Phytochemical and antimicrobial activity of ethanolic extract of *Landolphia owariensis* leaf, Afr. J. Biotech, 6(7), 890 – 893, (2007).
(6). Sillma R., and P. Daneshwar. *In vitro* antimicrobial and larvicidal properties of wild *Ricinus communis* L. In Mauritius, Asian Pac J Tropical Biomed, 6 (2); 100 - 107, (2016).
(7). Mohamed A. A., Ali, S. I., El Baz, F. K. Antioxidant and antibacterial activity of crude extracts and essential oils of *Syzygium cumini* leaves, Plus One, 8(4): e 60269 (2013).
(8). Suresh M., Mohammead, A. A., Pradipta, K. R., Pannerselvam, A., Thajuddin, N. Screening and antibacterial efficiency of selected Indian Medicinal Plants, Asian Pac. J. Trop. Biomed, 6(3); 185 -191 (2016).
(9). Kalusalingam M and V. Balakrishnan. Ethnomedicinal survey in the Paliyar Hamlet of Sathragiri hills in Virudhunagar District, Tamil Nadu, India. Research and Review Journal of Botany, Vol 7 (2); 1- 10, (2018).
(10). Karou SD., Tchacondo, T., Ilboudo, D. P., Simpore, J. Sub-Saharan Rubiaceae: A Review of Their Traditional Uses, Phytochemistry and Biological Activities, *Pakistan Journal of Biological Sciences,* 14: 149-169 (2011).
(11). Singh P., S. J. Ali. Ethanomedicinal plants of family Rubiaceae of Eastern UP, Indian J. L. Sci, 1(2): 83-86 (2012).
(12). Khare C. P, Indian Medicinal Plants an Illustrated Dictionary, ISBN. 978-0-387-70637-5, Springer Publications, New Delhi, (2007).
(13). Nambiar Krishnan V. P., Sasidaran, N., Renuka, C., Balagopalan, M. Studies on the medicinal plants of Kerala, Research Report No. 42 of Kerala Forest Research Institute, Trichur, Kerala, India, (1985).
(14). Shabir G, Anwar, F., Sultana, B., Khalid, Z. M., Afzal, M., Khan, Q. M., Ashrafuzzaman, M. Antioxidant and antimicrobial attributes and phenolics of different solvent extracts from leaves, flowers and bark of gold mohar [*Delonix regia* (Bojer ex Hook.) Raf]. Molecules, 16: 7302-19 (2011).
(15). Hemalatha M., Thirunavukkarasu, T., Rajamani, S., Elumalai, E. K., David, E. A review on antimicrobial efficiency of home traditional medicinal plants in Tamil Nadu, Journal of Acute Diseases: 99 – 105 (2013).
(16). Zaika L. L, Species and herbs: their antimicrobial activity and determination. J. Food. Stat, 9: 97 -118 (1988).
(17). Al-Talib H., Al-Khateeb, A., Hassan, H. Antimicrobial resistance of *Staphylococcus aureus* isolates in Malaysian Tertiary Hospital, Int. Med, 22(1): 1-3 (2015).
(18). Sridhar N, Suguna Lakshmi, D., Goverdhan, P. Antimicrobial activity of ethanolic extracts of *Justicia neesii*, Bangladesh J Pharmacol, 9: 624-27 (2014).
(19). Dorin D., and G. Marilena. Taste of phytocompounds; A better predictor for ethnopharmacological activity of medicinal plants than the phytochemical class? Journal of Ethnopharmacology, 220: 129 -146 (2018).
(20). Akhalwaya S., Van, S., Patel, M. An *invitro* investigation of South African medicinal plants used to treat oral infections, Journal of Ethnopharmacology, 210: 359-371 (2018).
(21). Razafintsalama, V. E., Ralambonirinia, S. T., Randria-Petit, T., Sarter, S. Antibacterial activity of fourteen medicinal plants from endemic plant diversity of Madagascar, South African J. of Botany, 112: 303-306 (2017).