INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 22, No. 5, p. 160-167, 2023

RESEARCH PAPER OPEN ACCESS

In vitro antibacterial effect of the extracts of *Maranta arundinacea* rhizomes against selected pathogens

Balan Aswini, Sivagurunathan Paramasivam, Sivaprakasam Sowndarya, Uma Chinnaiyan*

Department of Microbiology, Faculty of Science, Annamalai University, Chidambaram, Tamil Nadu, India

Key words: Test pathogens, Urinary tract infection, Antibacterial, *Maranta arundinacea,* Arrowroot

http://dx.doi.org/10.12692/ijb/22.5.160-167 Article published on May 18, 2023

Abstract

Plants are known to synthesize an array of secondary metabolites referred to as phytochemicals that have disease-prevention properties. Potential efficacy and minimum to no side effects are key advantages of plant-derived products, making them sustainable choices for medical treatments. The aim is to investigate the antibacterial activity and phytochemical screening of methanolic, ethanolic, ethyl acetate and chloroform extracts of *Maranta arundinacea* (arrowroot) rhizomes. New antimicrobial agents need to be developed to battle the rapidly evolving pathogens.

* **Corresponding Author:** Uma Chinnaiyan ✉ umasaravanan1@gmail.com

Introduction

The emergence and spread of antibiotic resistance, as well as the evolution of new strains of disease-causing agents, are of great concern to the global health community. Effective treatment of a disease entails the development of new pharmaceuticals or some potential source of novel drugs. Commonly used medicinal plants of our community could be an excellent source of drugs to fight off this problem (Manandhar *et al.,* 2019). The vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds as an alternative that can potentially be effective in the treatment of these problematic bacterial infections (Iwu *et al.,* 1999). According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs (WHO, 2002).

UTIs are contagious infection, affecting both the male and female population and can infect various parts of the urinary tract system (UTS), such as urethra, ureter, urinary bladder and kidneys mainly focused on the lower UTS like urethra and ureter.

Escherichia coli and *Klebsiella sp* are the main causative agents, although other Gram-positive bacteria and even fungi have also been isolated in numerous infected population (Flores-Mireles *et al.,* 2015).

Herbal formulation involves the use of fresh or dried plant parts. The exact mechanisms of medicinal herbs and their phytochemical constituents that are responsible for the effect on UTI are still to be investigated. Further research is needed to elucidate clearly the mode of action of these phytochemicals. Additional studies are needed to confirm the phytoconstituents that are responsible for the treatment of UTI (Aswini *et al.,* 2022).

The arrowroot plant *M. arundinacea* L. is identified to possess phytochemicals that make them medically important in exhibiting antidiarrheal, probiotic, antiulcer, antioxidant, antimicrobial, vibriocidal and immunostimulatory effects (Firoskhan and Muthuswamy, 2021). Considering the vast potentiality of plants as sources for antimicrobial drugs, this study aimed to investigate *invitro* antibacterial activity of extracts from some selected medicinal plants from Nepal against the most common microbial pathogens including MDR (Multi-Drug Resistant) bacteria (Manandhar *et al.,* 2019).

The aim of this research is to study different extracts for its antimicrobial activity and phytochemical screening. It is a preliminary step for the identification of medicinal value of the plant.

Materials and methods

Collection of leaves

Disease free, fresh, young, and green leaves were collected from the papaya plants. The leaves were washed thoroughly 3–10 times in sterile distilled water. Then, they were air-dried under shade at room temperature for 8 days and finely powdered using a blender.

Test organisms

The test pathogens were procured from Rajah Muthiah Medical College and Hospital, Chidambaram and then, they were further reconfirmed by morphological, cultural, and biochemical characteristics. The cultures were emulsified in 5ml of Nutrient Broth (NA) and incubated for 24 hrs. The test pathogens used in the study were tabulated in Table-1.

Culture media and inoculums preparation

Fresh cultures were employed to assess the antibacterial activity of the papaya leaf-extracts. Antimicrobial susceptibility test was performed for all microbial isolates by modified Kirby Bauer disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guideline.

Crude Extract Preparation

Shade-dried leaves (200 g) were coarsely powdered and subjected to successive solvent extraction by continuous Soxhlet extraction. The extraction was done with different solvents in their increasing order of polarities such as methanol, ethanol, ethyl acetate

and chloroform. Each time the marc was air-dried and later extracted with other solvents. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The dried extracts were dissolved in dimethyl sulphoxide (DMSO) and subjected to antibacterial activity (Elumalai *et al.,* 2011).

Preliminary phytochemical screening

The phytochemical study includes the identification of the presence and absence of flavonoids, alkaloids, tannins, glycosides, steroids, phenols, cardiac glycosides, and saponins. All the extracts were subjected to preliminary phytochemical qualitative screening for the presence or absence of various primary or secondary metabolites (Snathi *et al.,* 2011).

Antimicrobial Assay of Plant Extracts: Well Diffusion method

Antimicrobial assay of extracts of different plants was performed by agar well diffusion method in Mueller Hinton Agar (MHA) plates. The test organisms were inoculated in Nutrient broth and incubated overnight at 37°C to adjust the turbidity to 0.5McFarland standards giving a final inoculum of 1.5×10^8CFU/ml. MHA plate was lawn cultured with standardized microbial culture broth. Plant extracts of 50 mg/ml concentration were prepared in Dimethyl Sulfoxide (DMSO). Six wells of 6 mm were bored in the inoculated media with the help of a sterile cork-borer (6 mm). Each well was filled with 50 μl extracts from different plants: positive control (amikacin 30 mcg and nitrofurantoin 300 mcg) for bacteria and 1 mg/ml of cyclohexylamine for fungal isolates and negative/solvent control (DMSO), respectively. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37°C.

After incubation, plates were observed for the formation of a clear zone around the well, which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. The plates were then incubated according to the growth requirement of each organism. Each sample was tested in triplicates and antibacterial activity was evaluated by measuring and recording the zones of inhibition in mm (including the 6 mm disk).

Results

The present research work was carried out in the Department of Microbiology, Faculty of Science, Annamalai University. The studies were done to find the antimicrobial activity of *M. arundinacea L.* on common pathogens.

Table 1. Test pathogens.

Sl No:	Test pathogens
	Gram-positive
1	*Staphylococcus aureus*
2	*Bacillus sublitis*
3	*Enterococcus sp.*
	Gram-negative
4	*Salmonella paratyphi*
5	*Psudomonas aeroginosa*
6	*Proteus mirabilis*
7	*Escherichia coli*
8	*Klebsiella pneumoniae*
9	*Salmonella typhi*
10	*Enterobacter sp.*

The crude extracts of of *M. arundinacea L.*. rhizomes were investigated for their phytochemical screening and *in vitro* antibacterial activity. The dried rhizomes of of *M. arundinacea L.* were extracted with four common solvents used in plant extraction, namely methanol, ethanol, ethyl acetate and chloroform.

Phytochemical Screening

The presence of different phytoconstituents was seen during the screening of arrowroot crude extracts. The strongest presence of phytocompounds was seen in the methanolic extraction, followed by ethanol, then ethyl acetate, followed by chloroform. The degree of presence of phytoconstituents will actively affect the antibacterial activity. The antibacterial assay shows the correlation (Table 2).

Table 2. Screening of phytoconstituents of various extracts of *M.arundinacea*.

Phytoconstituents	Biochemical assay	*M.arundinaceae* rhizome extracts			
		Methanol	Ethanol	Ethyl acetate	chloroform
Alkaloids	Hager's Test	++	+	-	-
Carbohydrate	Benedict's Test	+	+	-	+
Glycosides	Keller Killiani Test	++	+	+	+
Flavonoids	Alkaline reagent Test	+	+	+	-
Terpenes	Salkowski's Test	+	+	-	-
Saponins	Froth Test	++	+	+	+
Phenol	Lead acetate Test & Ferric chloride Test	+	+	+	-
Tannins	Lead acetate Test	++	++	+	-
Proteins & amino acid	Xanthoproteic Test	+	+	-	-
steroids		+	+	+	-

(++ strong presence, + Presence, - absence).

Table 3. *M.arundinacea* methanolic extract inhibitory zone of measurement.

Sl no:	Test pathogens	Zone of inhibition (mm) of methanolic extract				
	Gram-positive	Positive Ciprofloxacin 10 µg/mL	Negative	60 µg/ml	80 µg/ml	100 µg/ml
1.	*Staphylococcus aureus*	20	-	16	19	20
2.	*Bacillus sublitis*	18	-	10	12	15
3.	*Enterococcus sp.*	16	-	08	14	16
	Gram-negative					
4.	*Salmonella paratyphi*	16	-	13	16	20
5.	*Psudomonas aeroginosa*	22	-	12	15	19
6.	*Proteus mirabilis*	16	-	14	16	18
7.	*Escherichia coli*	21	-	12	17	19
8.	*Klebsiella pneumonia*	14	-	14	17	20
9.	*Salmonella typhi*	18	-	14	19	20
10.	*Enterobacter sps.*	18	-	19	20	22

Antimicrobial Assay of Plant Extracts: Well diffusion method

The well was prepared for three concentrations- 60 µg/ml, 80 µg/ml and 100 µg/ml of plant extracts against different Gram-positive and Gram-negative bacteria. The well diffusion assay showed that methanolic extract gives the highest zone of inhibition, followed by ethanol, then ethyl acetate, followed by chloroform. All the crude extracts show some activity; methanol has the highest antibacterial activity. Methanol extract shows a zone between 10 – 22 mm. The ethanolic extract shows zone range of 7-18mm, ethyl acetate extract between 6-15 mm and chloroform between 6-16mm.

The least showing for *Enterococcus* sp. and the highest for *Enterobacter sp.* In the methanolic extract, the least zone is shown by *Enterococcus* with 8, 14, 16 mm at the concentration of 60, 80, 100 µg/ml, respectively and highest for *Enterobacter* sps. In ethanolic extract, the least zone was shown by Enterococcus with 08, 10, 12 mm zone and highest in Enterobacter with 10, 16, 18 mm., in ethyl acetate the least was shown by Enterococcus with 06, 10, 14 mm and highest by *Proteus mirabilis* with zone size of 12, 13, 15 mm and chloroform showing least in *Enterobacter* -, 06,09 mm and highest in *Pseudomonas aeruginosa* with 10, 11, 14 mm at the concentration of 60, 80, 100 µg/ml respectively. As the concentration increases, the zone of inhibition also increases. All the experiments were done in triplets confirming the antibacterial activity. The results are tabulated. The presence of antimicrobial activity is proven because of its phytoconstituents, mainly phenols, flavonoids, tannins, alkaloids, steroids, and terpenoids (Rahman *et al.,* 2015).

Table 4. *M.arundinacea* ethanolic extract inhibitory zone of measurement.

Sl no:	Test pathogens	Zone of inhibition (mm) of ethanolic extract				
	Gram-positive	Positive Ciprofloxacin 10 µg/mL	Negative	60 µg/ml	80 µg/ml	100 µg/ml
1.	*Staphylococcus aureus*	20	-	12	14	18
2.	*Bacillus sublitis*	18	-	09	10	12
3.	*Enterococcus sp.*	16	-	08	10	12
	Gram-negative					
4.	*Salmonella paratyphi*	16	-	08	12	15
5.	*Psudomonas aeroginosa*	22	-	09	10	14
6.	*Proteus mirabilis*	16	-	12	14	16
7.	*Escherichia coli*	21	-	09	14	16
8.	*Klebsiella pneumonia*	14	-	07	14	17
9.	*Salmonella typhi*	18	-	08	15	16
10.	*Enterobacter sps.*	18	-	10	16	18

Discussion

The different chemical components that make up a medicinal plant are what give it its therapeutic significance. Plant bioactivity is related to its phytochemical components (Elumalai *et al.,* 2011).

For instance, plants with high tannin content are capable of killing bacteria by directly harming their cell membranes because of their inherent ability to react with proteins to generate stable water-soluble chemicals (Mohamed *et al.,* 2010). A significant class of phenolic chemicals is known for its antiviral (Mehrangiz *et al.,* 2011), antimicrobial (Maria *et al.,* 2009) and spasmolytic (Julianeli., 2011) properties. Alkaloids isolated from plants are commonly found to have antimicrobial properties (Ahmed *et al.,* 2010).

Table 5. *M.arundinacea* ethyl acetate extract inhibitory zone of measurement.

Sl no:	Test pathogens	Zone of inhibition (mm) of ethyl acetate extract				
	Gram-positive	Positive Ciprofloxacin 10 µg/mL	Negative	60 µg/ml	80 µg/ml	100 µg/ml
1.	*Staphylococcus aureus*	20	-	12	14	16
2.	*Bacillus sublitis*	18	-	09	10	14
3.	*Enterococcus sp.*	16	-	06	10	14
	Gram-negative					
4.	*Salmonella paratyphi*	16	-	08	10	16
5.	*Psudomonas aeroginosa*	22	-	09	12	13
6.	*Proteus mirabilis*	16	-	12	13	15
7.	*Escherichia coli*	21	-	10	13	14
8.	*Klebsiella pneumonia*	14	-	08	09	11
9.	*Salmonella typhi*	18	-	08	10	12
10.	*Enterobacter sps.*	18	-	10	12	14

Steroids, alkaloids, flavonoids, tannins, phenols, and other aromatic compounds, which are secondary plant metabolites, serve as protective macromolecules against microbes. Phytochemicals have many methods by which they are antibacterial. As secondary metabolites, bioactive compounds frequently accumulate in all plant cells. Secondary metabolites exert their antibacterial effects in several ways. Positive correlations exist between total phenolic content and antibacterial activity (Kudo *et al.,* 2004; Wu *et al.,* 2006; Shan *et al.*, 2007). The substances having phenolic structures had a

significant level of antimicrobial activity. Depending on the concentration utilised, members of this class are known to be either bactericidal or bacteriostatic agents (Rasooli *et al.,* 2002). Tannins have been demonstrated to stably attach to proline-rich proteins, preventing cells from producing new proteins. Herbs with a high tannin content have an astringent effect and are used to treat gastrointestinal conditions like diarrhoea and dysentery. The phytochemicals stop the manufacture of peptidoglycans for the cell walls of the bacteria as well as the formation of proteins.

Table 6. *M.arundinacea* chloroform extract inhibitory zone of measurement.

Sl no:	Test pathogens	Zone of inhibition (mm) of chloroform extract				
	Gram-positive	Positive Ciprofloxacin 10 µg/mL	Negative	60 µg/ml	80 µg/ml	100 µg/ml
1.	*Staphylococcus aureus*	20	-	09	10	12
2.	*Bacillus sublitis*	18	-	08	10	11
3.	*Enterococcus sp.*	16	-	06	09	12
	Gram-negative					
4.	*Salmonella paratyphi*	16	-	06	08	14
5.	*Psudomonas aeroginosa*	22	-	10	11	14
6.	*Proteus mirabilis*	16	-	07	10	12
7.	*Escherichia coli*	21	-	06	08	10
8.	*Klebsiella pneumonia*	14	-	08	09	11
9.	*Salmonella typhi*	18	-	06	09	10
10.	*Enterobacter sps.*	18	-	-	06	09

Additionally, they disrupt peptide bonds act as chelating agents during the synthesis of nucleic acids, obstruct metabolic pathways, and prevent germs from utilising available nutrients. Some compounds cause bacterial cell lysis. *Maranta arundinacea* L. was used to treat a variety of diarrheal illnesses and was mostly popular as a starchy food. The chemical makeup of elements in plant extract can be revealed through phytochemical screening, which is also utilised to look for bioactive molecules that could be used to create very beneficial medications. The result showed that there is a positive correlation between phytochemical and antibacterial activity. By experiment, it is shown as methanol shows the highest percentage of phytoconstituents. Similarly, the highest ZOI is shown in methanolic extract showing. The extract is effective against all the test organisms with maximum ZOI. Arrowroot contains a variety of biologically active plant chemicals, including flavonoids and terpenoids, which in some studies, have antibacterial effects (Jayakumar and Suganthi, 2017).

Fig. 1. Matured rhizomes of *M. arundinacea* with and without skin.

Flavonoids decrease the formation of nucleic acids, cytoplasmic membrane function, and energy

metabolism, among other bacterial processes. The development of covalent bonds and protein complexes by generic mechanisms like hydrogen bonding and hydrophobic effects is another tactic (Ren *et al.,* 2014). Terpenoids can harm a bacterium's membrane function and physical makeup. Additionally, they can stop ion transport and bacterial respiration. The plant extract exhibits antibacterial action against the examined species, it is concluded. The zone of inhibition changed, indicating that the herb's phytochemical composition and level of effectiveness on the target organism fluctuated. The presence of numerous active components in the plants' rhizomes may be the cause of their antibacterial action. The zone of inhibition changed, indicating that the herb's phytochemical composition and level of effectiveness on the target organism fluctuated.

Conclusion

The presence of numerous active components in the plants' rhizome may be the cause of their antibacterial action. Steroids, alkaloids, flavonoids, tannins, phenols and other phytocomponents helps in establishing the antimicrobial activity of the plant which is being studied in the plant. It is the primary step for identifying the medicinal importance of a plant. Through the studies, the plant extracts shows immense potential as a medicinal plant. To isolate and describe the bioactive components and create new antibacterial medications, more research is required.

References

Ahmed el-HM, Nour BY, Mohammed YG, Khalid HS. 2010. Antiplasmodial activity of some medicinal plants used in Sudanese folk-medicine. Environmental Health Insights **4(4),** 1-6.

Aswini B, Sivagurunathan P, Sowndarya S, Sumathi V, Vivekanandhan S, Uma C. 2022. A Study On The Effect Of Methanolic Extracts Of Maranta Arundinacea Leaves Against Urinary Tract Infection Causing Pathogens. Uttar Pradesh Journal of Zoology **43(22),** 71–77. https://doi.org/10.56557/UPJOZ/2022/v43i223235

Elumalai EK, Ramachandran M, Thirumalai T, Vinothkumar P. 2011. Antibacterial activity of various leaf extracts of Merremia emarginata. Asian Pacific Journal of Tropical Biomedicine **1(5),** 406–408. https://doi.org/10.1016/S2221-1691(11)60089-0

Firoskhan N, Muthuswamy R. 2021. Review on Maranta arundinacea L. (Marantacea) International Journal of Pharmacognosy and Pharmaceutical Research. International Journal of Pharmacognosy and Pharmaceutical Research Genus **3(1),** 1–4.

Jayakumar A, dan Suganthi A. 2017 "Biochemical and phytochemical analysis of *Maranta arundinacea* (L.) Rhizome," International Journal of Research in Pharmacy and Pharmaceutical Sciences, **2(3),** hal. 26–30.

Julianeli TDL, Jackson RGS, Kelly S, Ana Sílvia SC. 2011. Selective spasmolytic effect of a new furanoflavoquinone derivative from diplotropin on guinea-pig trachea. Journal of Chemical and Pharmaceutical Research **3(1),** 249-258.

Kudo YH, Kobayashi A, Konishi YS, Kondo K. 2004. Antibacterial activity of plants used in cooking aroma and taste. Journal of Food Protocol **67,** 2820-2824.

Iwu MW Duncan AR, Okunji CO. 1999. "New antimicrobials of plant origin in. Perspectives on new crops and new uses," in *Plant Breeding Reviews*, J. Janick, Ed., ASHS Press, Alexandria, Virginia.

Maria Lysete AB, Maria Raquel FL. 2009. Studies on the antimicrobial activity and brine shrimp toxicity of Z. tuberculosa extracts and their main constituents. Annals of Clil Microb Antimic **8,** 16.

Manandhar S, Luitel S, Dahal RK. 2019. In Vitro Antimicrobial Activity of Some Medicinal Plants against Human Pathogenic Bacteria. Journal of Tropical Medicine. https://doi.org/10.1155/2019/1895340

Mehrangiz KK, Seyed AE, Masoud SG, Esmaeel AS, Amirhossein S. 2011. Antiviral activities of aerial subsets of Artemisia species against Herpes Simplex virus type 1 (HSV1) in vitro. Asian Biomed **5(1),** 63-68.

Mohamed Sham Shihabudeen H, Hansi Priscilla D, Kavitha T. 2010. Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. International Journal of Pharmaceutical Sciences and Research 1(10), 430-434.

Pérez E, Lares M. 2005. Plant Foods for Human Nutrition **60(3),** 113–116. https://doi.org/10.1007/s11130-005-6838-9

Rahman MK, Chowdhury MAU, Islam MT, Chowdhury MA, Uddin ME, Sumi CD. 2015. Evaluation of Antidiarrheal Activity of Methanolic Extract of Maranta arundinacea Linn. Leaves. Advances in Pharmacological Sciences 1–6.

Rahman MK, Chowdhury MAU, Islam MT, Chowdhury MA, Uddin ME, Sumi CD. 2015. Evaluation of Antidiarrheal Activity of Methanolic Extract of Maranta arundinacea Linn. Leaves. Advances in Pharmacological Sciences. https://doi.org/10.1155/2015/257057

Rajashekhara N, Ashok B, Sharma P, Ravishankar B. 2014. (An International Quarterly Journal of Research in Ayurveda) **35(4),** 433. https://doi.org10.4103/0974-8520.159013

Ren L. 2014. "Antibacterial Activities of Flavonoids: Structure Activity Relationship and Mechanism," Current Medicinal Chemistry **22(1),** hal. 132–149. https://doi.org/10.2174/092986732166614091611343

Shan B, Cai YZ, Brooks JD, Corke H. 2007. The *in vitro* antibacterial activity of dietery spices and medicinal herbs extracts. International Journal of Microbiology **117,** 112-119.

Snathi R, Lakshmi G, Priyadharshini AM, Anandraraj C. 2011. Phytoscreening of Nerium ollender leaves and M. charatia leaves. International research journal of pharmacy **2(1),** 131-135.

Sholichah E, Deswina P, Sarifudin A, Andriansyah CE, Rahman N. 2019. Physicochemical, structural and morphological properties of some arrowroot (*Maranta arundinacea*) accessions growth in Indonesia. AIP Conference Proceedings 2175. https://doi.org/10.1063/1.5134572

World Health Organization (WHO). 2002. Traditional Medicine Strategy, Geneva.

Wu C, Chen F, Wang X, Kim HJ, He GQ, Haley-Zitlin V, Huang G. 2006. Antioxidant constituents in feverfew (*Tanacetum parthenium*) extract and their chromatographic quantification. Food chemistry **96,** 220-227.