International Journal of Early Childhood Special Education (INT-JECSE)
DOI:10.9756/INTJECSE/V14I5.14 ISSN: 1308-5581 Vol 14, Issue 05 2022

ANTI-MICROBIAL ACTIVITY OF REMUSATIA VIVIPARA AND THERIOPHONUM MINUTUM.

S. Anbuselvi, Punithavathy R, Roshini Esther A. and Saroja Preethy
Department of Biotechnology ,BIHER, Chennai-73.

ABSTRACT

Remusatia vivipara and *Theriophonum minutum* herbs belong to the family Araceae. The aim of this work was to analyze the anti-microbial assay of these two plants against the bacteria *Pseudomonas aeruginosa* (Gram negative), *Bacillus cereus* (Gram positive), and the fungi *Aspergillus niger*. The leaf and tuber of *Remusatia vivipara* and the leaf of *Theriophonum minutum* were selected for the experiment. Different solvents like ethanol, methanol and acetone extract of these selected parts of plants prepared for the anti-microbial activity. The ethanolic extract of *Remusatia vivipara* and *Theriophonum minutum* species reflected maximum level of zone of inhibition(mm). The minimal inhibitory concentration exhibit least value in ethanolic extract. The species *R.vivipara* have more capacity to inhibit the microbes than *T.minutum.* The minimal bactericidal inhibitory and minimal fungicidal inhibitory showed 99.9% microbes inhibition in ethanolic extract. The ethanolic extract of both species showed best result than the methanol and acetone extract.

Keywords: *Remusatia vivipara, Theriophonum minutum, pseudomonas aeruginosa, Bacillus cereus, Aspergillus niger*.

INTRODUCTION

In many decades, herbal plants have been used as traditional medicine for human diseases. The use of medicinal plants and its biproducts has named as folk medicine and through the years has been related into traditional and allopathic medicine(Cowan,1999).

The basic components in traditional medicines are extracted or use as such as medicinal plants. These medicines that have led to the discovery of natural based products that have become known pharmaceuticals and drug designing (D. Daniel, et al., 2008;Jain S.K,2006 ;Anbuselvi,2019).

According to WHO, medicinal plants said to be the good source to obtain a variety of drugs. The different medicinal plant extracts has been practiced as anti-oxidant, anti-ulcer, analgesic, anti-diabetic and they also having anti-parasitic, antimalarial activity, anti-bacterial activity and anti-fungal activity (Kelmason JE et.al.,2000).

The genus *Theriophonum* (*Araceae*), represented by dormant tuberous perennials which grown seasonally in India and Sri Lanka. Many studies illustrates that various herbs from the family Araceae exhibited anticancer property like *Colocasia esculenta* (Linn.), *Acorus calamus* resulted in moderate anticancer activities against MCF-7 and HT-29 cell lines (F.U. Afifi, et al.,2012).

Theriophonum minutum is a wild edible plant contains relatively more phytochemicals nutritive values compare to conventional foods resources. Many literature survey indicated that *Theriophonum minutum* has not that much described its phytochemical properties as well as its pharmacological action(N.P. Yadav, et al., 2008)

Remusatia vivipara is an epiphytic herb with 50m tall, arising from an underground tuber with diameter 2–4 cm and red coloured vivid . The bulbils are 5mm long, scaly and ovoid, around 0scales ending with hooked prickles. Mayo, S. J, 1985).

The bulbiliferous shoots of *R. vivipara* emerged from the tubers in top part. As they elongate upward, bulbil clusters arranged in each node of the shoot (Wang and Cronk, 2003).Bulbils has top several hooked scales which stimulate dispersal because they can easily fix to the animal's fur. *R. vivipara* flowers in spring, their pollen grains were in viable and unable to multiply in sucrose solution in-vitro. These bulbiliferous *R. vivipara* being able to reproduce sexually (Kaatz GW,1993; Ashwini Khubalkar,2018).

In the traditional systems of Ayurveda medicine implicates pharmacological and clinical studies except composite herbal drugs and plants. These plant products are said to be effective in reducing the recurrence rate of renal calculi with no side effects (S.Anbuselvi,2019).

MATERIALS AND METHODS

Collection of plants

Two plant materials were identified and authenticated from the Institute of Herbal Science Plant Anatomy Research Centre,Chennai

Collection of micro-organisms and antibiotic discs

Micro-organisms and specific antibiotic discs were collected from Royal Bio Research center, Tamil Nadu, Chennai- 42.

Preparation of plant extract

Fresh leaves and tuber of *Remusatia vivipara* and leaves of *Theriophonum minutum* were shade dried and grinded in electric mixer grinder. 10g of prepared powder samples were taken in a conical flask and homogenized with 100ml of different solvents such as Ethanol, Methanol and Acetone. The crude preparation was kept in shaker for overnight. Then, the crude preparation was centrifuged at 4000rpm for 20mins. After centrifugation, the supernatant was transferred into the beaker and heat it at 60ºC until the solvents evaporate to produce a thick liquid solvent extracts. This thick solvent extracts were poured in sterile bottles and kept under refrigerated conditions for experimental use.

Preparation of culture medium

The Hi-media medium used throughout the experiment (India make) have the following composition. The media for antibacterial activities were prepared by making 28.0g of ingredients in 1 L of distilled water and sterilized in autoclave at 121ºC at 15 lbs/inch pressure for 20min.

Preparation of anti-microbial solution (Control)

The standard drugs Ampicillin, Gentamicin, Erythromycin, Norfloxacin and Penicillin G amoxicillin used as a control for the bacterial species. The standard drug Amphotericin B, Nystatin, Clotrimazole, Enilconazole and Posaconazole used as a control for fungi *Aspergillus niger*. Both the anti-microbial solution was prepared at the volume of 250mg in 10ml sterile distilled water.

Antibacterial and Antifungal activity by Agar well/ Disc diffusion method

The agar well diffusion /Disc diffusion method can be determined for antibacterial activity of prepared extracts. Well were made by cork borer in petri plates containing solid nutrient agar medium gently seeded with test organisms and well were filled with samples. After allowing diffusion of solution for 20 min, the plates were incubated at 37 ◦C for 24 hr. The zone of inhibition was measured in each plate in terms of diameter (Khandelwal KR,2010)

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) is the minimum concentration of antimicrobial compound found to stop the growth of a particular test microorganism. The nutrient broth (double strength) poured in test tubes and label them. The inoculums (three to four drops) is added to reach the final concentration of microorganisms is 10^6 cells/mL in all test tubes, test antimicrobial substance was added in the range of 0.5 to 5 ml except un-inoculated (negative control) and control (positive) tube. . Adjust the final volume (10 ml) in all test tubes by using sterile water. All plates were incubated at 37ºC for two to three days. After incubation, all test tubes were checked for the growth in the form of turbidity by nephelometry. The minimum inhibitory concentration was analysed by intrepreting all results with positive and negative control (Jenifer,2001).

Determination of Minimal Bactericidal Concentration (MBC)

The MBC was determined by the dilution representing the MIC and two of the more concentrated test product dilutions are plated using Nutrient agar or Mueller Hinton agar plate. (Kokare C,2010). In the prepared agar plate, streak the more concentrated test product dilutions. All samples kept incubation for 24hours at 37ºC. After incubation, results enumerated to determine viable CFU/ml. The colony forming unit was calculated using the formula,

CFU = Number of colonies counted / (Amount plated in ml ×dilutions).

Determination of Minimal Fungicidal Concentration (MFC)

The MFC was determined by the dilution representing the MIC and two of the more concentrated test product dilutions are plated using Potato dextrose agar (PDA) plate (Harborne JB)1973).

RESULTS AND DISCUSSION

Anti-bacterial activity by Disc diffusion method

The anti-bacterial activity by disc diffusion method was done by using five different antibiotic discs namely, Ampicillin, Gentamicin, Erythromycin, Norfloxacin and Penicillin G. Overall, erythromycin disc showed the highest level of zone of inhibition. The *R.vivipara* leaves against *B.cereus* showed maximum zone of inhibition when compared with *T.minutum* leaves. The ethanolic extract of *R.vivipara* leaves against Gram positive bacteria *B.cereus* showed highest (35mm)zone of inhibition in erythromycin disc. The minimum zone of inhibition in acetone extract of *R.vivipara* leaves against gram positive bacteria *B.cereus* (6mm) zone of inhibition in pencillin G disc. The *R.vivipara* tuber of ethanolic extract against the same bacteria showed 32mm maximum zone of inhibition in erythromycin disc. There is no zone of inhibition in methanolic and acetone extract of *R.vivipara* tuber against gram negative bacteria *P.aeruginosa*. The maximum zone of inhibition for ethanolic extract of *T.minutum* leaves against gram positive bacteria *B.cereus* showed 31mm zone of inhibition in erythromycin disc. The acetone extract of this leaves against *P.aeruginosa* showed minimum zone of inhibition in penicillin G disc was found to be 2mm (Fig 1)

Figure 1: Disc diffusion method for leaves and tuber of *R.vivipara* and leaves of *T.minutum* species against *B.cereus*

Standardization of anti-bacterial activity by Agar-well diffusion

Agar-well diffusion helps to find out the exact concentration of plant extract against the microbe (Prasad KVSRG,2007).s. The species *R.vivipara* leaves having more capacity to inhibit the microbes than the species *T.minutum* leaves. The plant species *R.vivipara* leaves and tuber in ethanolic extract against the Gram positive bacteria *B.cereus* at 100µg concentration found to be highest significant level of zone of inhibition 32mm and 24mm respectively (Table 1). The ethanolic extract of *T.minutum* leaves at 100µg concentration showed higher antibacterial activity against both *B.cereus* and *P.aeruginosa* exhibited 26mm.The minimum level of zone of inhibition for *T.minutum* leaves against the Gram negative bacteria *P.aeruginosa* showed 6mm only(Figure 2).

Table 1: Standardization of anti-bacterial activity by agar-well diffusion method of *R.vivipara* and *T.minutum* species against the Gram positive bacteria *B.cereus*

S.NO	Plant extrcts	Zone of inhibition (mm)								
		R.vivipara against *B.cereus*						*T.minutum* against *B.cereus*		
		Leaves			Tuber			Leaves		
		40 (µg)	100 (µg)	C	40 (µg)	100 (µg)	C	40 (µg)	100 (µg)	C
1.	Ethanolic extract	20	32	42	10	24	30	18	26	39
2.	Methanolic extract	16	28	28	9	21	20	17	23	26
3.	Acetone extract	19	25	21	11	20	17	13	21	24

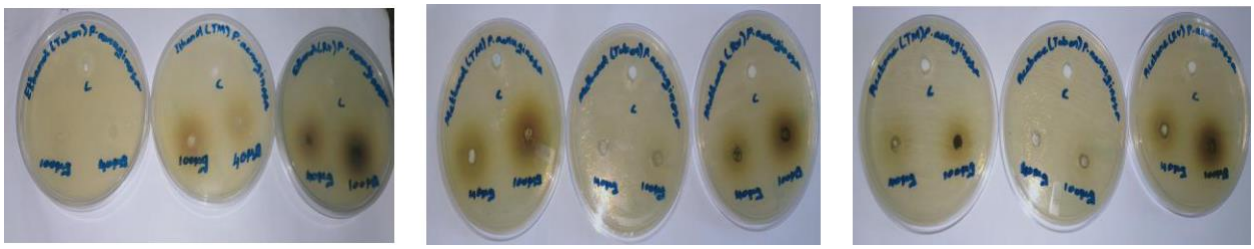

Figure 2: Agar-well diffusion method for leaves and tuber of *R.vivipara* and leaves of *T.minutum* species against *P.aeruginosa*

Anti-fungal activity by disc diffusion method

The anti-fungal activity by disc diffusion method was done by five different anti-fungal disc namely, Amphotericin B, Nystatin, Clotrimazole, Enilconazole and Posaconazole (Kamali HH EL, Amir MYEL (2010. The highest level zone of inhibition for both the extract showed in clotrimazole disc. The ethanolic extract of *R.vivipara* leaves and tuber against A.niger showed maximum zone of inhibition at 100µg/ml in clotrimazole was found to be 37mm and 26mm. The maximum level of ethanolic extract of *T.minutum* leaves was found to be 29 mm in clotrimazole disc.

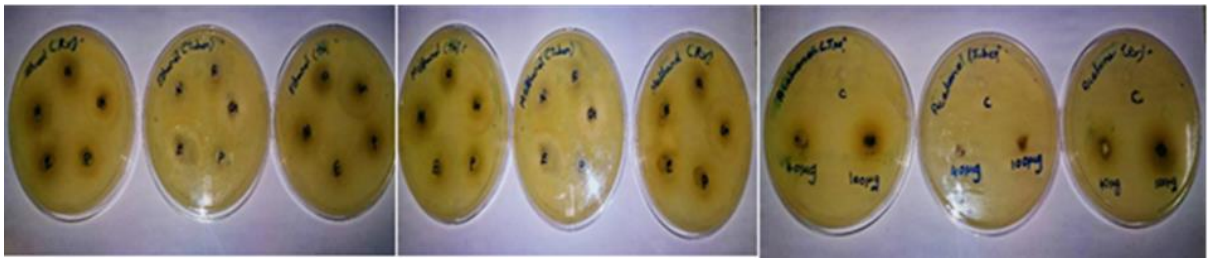

Figure: 3 Disc diffusion method for leaves and tuber of *R.vivipara* and leaves of *T.minutum* species against *A.niger*

The ethanolic extract of *R.vivipara* leaves against A.niger showed minimum level of zone of inhibition was 8mm in posaconazole disc and the tuber showed 3mm in posaconazole disc of methanolic extract. The acetone extract of *T.minutum* showed minimum level in posaconazole disc was found to be 9mm.

Standardization of anti-fungal activity by agar-well diffusion

The leaves and tuber of ethanolic extract of *R.vivipara* species at 100µg concentration against the fungi *A.niger* showed greater level of zone of inhibition was 26mm and 19 mm. The minimum level of zone of inhibition of *R.vivipara* leaves at 40µg against *A.niger* was found to be 9mm in acetone extract. The highest level of zone of inhibition of *T.minutum* leaves against *A.niger* showed 17mm at 100µg concentration in ethanolic extract. The lowest lev9el of zone of inhibition of *T.minutum* leaves at 40µg was found to be 7mm in acetone extract.

Minimal inhibitory concentration by macro-broth dilution method

The ethanolic extract of both the plant species showed least MIC value compared to other extracts. The gram positive bacteria *B.cereus* showed the least MIC value than gram negative bacteria *P.aeruginosa.* (Ashwini Khubalkar,2018)

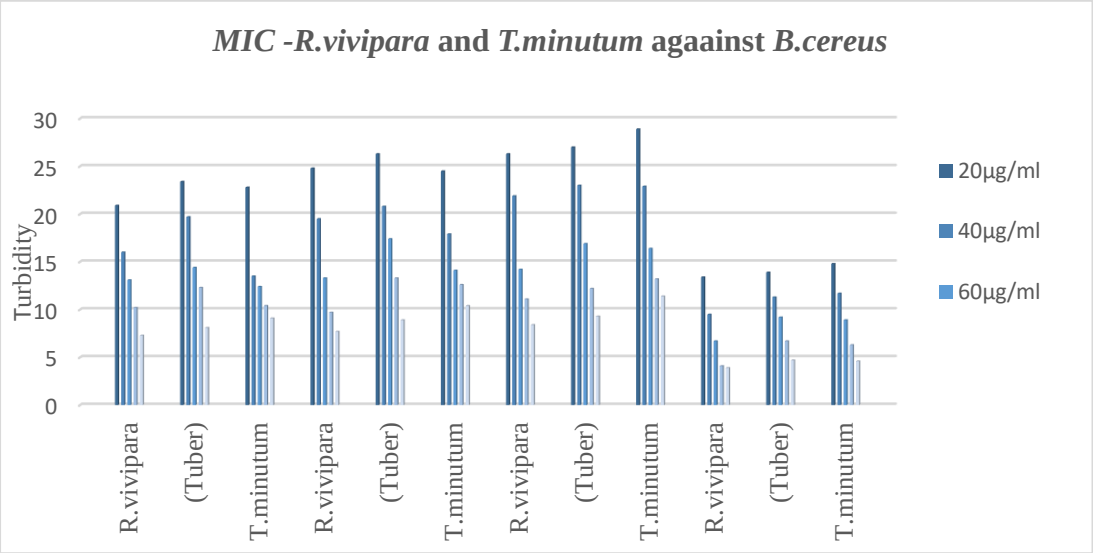

Figure 4: Minimal Inhibitory Concentration of *R.vivipara* and *T.minutum* species against *B.cereus*

The ethanolic extract of *R.vivipara* leaves at 100µg/ml showed least MIC against gram positive bacteria *B.cereus* was found to be 7.3% (Figure4). The same extract of *R.vivipara* tuber at 100µg/ml against *B.cereus* showed 8.1%. The R.vivipara leaves and tuber against gram negative bacteria P.aeruginosa showed least MIC value in ethanolic extract at 100µg/ml showed 7.9% and 9.6%.The gram positive bacteria *B.cereus* showed least MIC value for *T.minutum* species.(Bashir,et.al , 2010)The ethanolic extract of *T.minutum* leaves against gram positive bacteria *B.cereus* at 100µg/ml showed least MIC value was 9.1%. The ethanolic extract of same leaves against gram negative bacteria at 100µg/ml showed least MIC value was 9.8%.

Determination of Minimal Bactericidal Concentration(MBC)

Based on MIC, the MBC was calculated. The 80µg/ml and 100µg/ml of all three extracts of both species showed least value (which inhibit large amount of microbial growth) in MIC ethanolic extract. The ethanolic extract of *R.vivipara* showed maximum result was found to be 5.5 CFU/ml against B.cereus and 8 CFU/ml against *P.aeruginosa* and the tuber showed 8.7CFU/ml against B.cereus and 8.5CFU/ml against *P.aeruginosa* (Figure 5) The ethanolic extract of *T.minutum* showed maximum result was found to be 10.5 CFU/ml against *P.aeruginosa and B.cereus.* The fungi *A.niger* also showed in ethanolic extract was found to be 8.1 CFU/ml for species *R.vivipara* and the tuber showed 8.9CFU/ml in same extract. The species T.minutum showed 8.7CFU/ml in ethanolic extract.

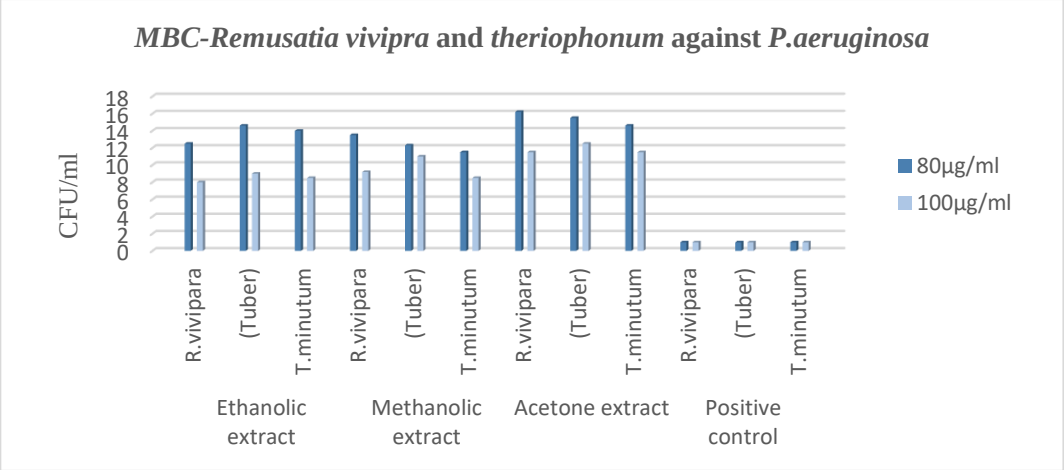

Figure 5: Minimal Bactericidal Concentration of *R.vivipara* and *T.minutum* species against *P.aeruginosa*

CONCLUSION

Remusatia vivipara and *Theriophonum minutum* is an herb having various medicinal properties. The species *R.vivipara* leaves are used as folk medicine for treating inflammation and arthritis and the species *T.minutum* is a unique ethanomedicinal plant. The present study justified the claimed uses of leaves in the traditional system of medicine to treat various disease caused by microbes. Ancient people as well as our ancestors were mainly dependent on pants for their recovery against disease. But, the recent tendency to avoid natural sources rather than artificial sources against disease is frustrating. Based on this view, the pathogenic microbes were tested against both the plant species of different extracts. Both the different plant extracts showed anti-microbial activity. The ethanolic extract of both *R.vivipara* and *T.minutum* exhibited highest level of anti-microbial activity and minimal inhibitory concentration. The species *R.vivipara* species having more capacity to inhibit the microbes than the species *T.minutum.* The phytoconstituents need to be isolated from the extracts and further screen for antimicrobial activity.

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