An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

ANTIMICROBIAL ACTIVITY OF WHOLE BODY EXTRACTS OF SLUG*MARIAELLA DUSSUMIERI* (GRAY, 1855) AGAINST CLINICAL PATHOGENS

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Article Info

Key words

Antimicrobial activity, Garden slug, Pathogens, Solvent extracts

ABSTRACT

The antimicrobial activity of different solvent extracts of the whole body of garden slug *Mariaella dussumieri* was assayed using disc diffusion method. Clinically significant bacteria (*Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis, E. coli, Proteus vulgaris, Klebsiella pneumoniae*) and fungi(*Aspergillus flavus, Aspergillus niger and Pencillium* sp.) were used for the present investigation. Among eight different solvents (ethanol, methanol, acetone, ethyl acetate, petroleum ether, n-butanol, chloroform and water) used for extraction, the ethyl acetate extract of slug *Mariaella dussumieri* was effective against all the bacteria tested butexhibited very high activity against *Enterococcus faecalis* (26mm). The aqueous extract of slug failed to inhibit any of the bacteria tested. The other extracts like ethanol, petroleum ether, n- butanol showed moderate activity against the pathogens. Among the fungus tested, the solvent extracts exhibited antifungal activity except the aqueous extract. Thus the obtained results indicate the presence of antimicrobial compounds in slug *Mariaella dussumieri* and it shows the great medicinal value of slug.

INTRODUCTION

Molluscs are widely distributed throughout the world and have many representatives in the marine and estuarine ecosystem namely slugs, whelks, clams, mussels, oyster, scallops, squids and octopus [1] which vary in size, anatomical structure, behavior and in habitat [2]. There are about 200,000 living species distributed in terrestrial, freshwater and marine habitat [3]. Mucus produced by the molluscs is known to have potential medicinal property. The mucus of *Muricid* -gastropod (rock snail) defends the developing larvae against microbial infection [4]. The bioactive compounds extracted from many classes of molluscs exhibit antitumor, anti-leukemic, antibacterial and antiviral properties [5,6]. Reports on antimicrobial activity of molluscs, has shown that either single body component alone, like haemolymph and egg masses or extracts of whole bodies possess antimicrobial property[7].Many studies have reported the bioactive potential of the Molluscs like *Aphysia*sp.[8], sea hare [9], *Chromodoris*sp.[10] and *Ozhidella* [11]. The recent development in research on multi drug- resistant bacteria suggests that animals living in unsanitary and unhygienic conditions may have developed ways of protecting themselves against pathogenic microorganisms [12,13]. Bioactive lipids from mussels including

fatty acids, sphingolipids, phytosterols, diacylglycerols, diterpenes, sesquiterpenes and sapoins highly influenced the control of human diseases [14]. Hence the aim of the present study was to evaluate the antimicrobial activity of the whole body extract of the garden slug *Mariaella dussumieri* against different pathogenic bacterial and fungal strains.

Materials and Methods

Taxonomy of animal used for extraction

The animals collected were identified by Dr.R.Venkitesan, Scientist, Zoological Survey of India, Chennai-28. The taxonomy of animal used for extraction is

Phylum: Mollusca

Class:Gastropoda

Subclass:Pulmonata

Order:Stylommatophora

Super family:Helicarionoidea

Family:Helicarionidae (Ariophantidae)

Genus:*Mariaella*

Species:*dussumieri*

Collection and Extraction of Samples

Live specimens of *Mariaella dussumieri* (Gray,1855) were collected from Vellarada, southernmost part of Kerala (Latitude:8.4497°N, Longitude:77.1957°E). They were immediately brought to the laboratory. The whole body of slug was cut into small pieces and washed thoroughly with distilled water. Extraction of bioactive compounds was done with different solvents such as, ethanol, methanol, acetone, ethyl acetate, petroleum ether, n-butanol, chloroform and water. To 5g of tissues 10ml of solvent was added and grounded well with motor and pestle. The extracts were kept overnight at 4°C and then filtered with whatman No.1 filter paper. They were centrifuged at 15000 rpm for 30 minutes and supernatants were collected and evaporated. The dried crude extracts were stored at -20°C.

Test Micro Organisms

Test microorganisms, *Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis, E. coli, Proteus vulgaris, Klebsiella pneumoniae, Aspergillus flavus, Aspergillus niger*and *Pencillium* sp.were obtained from Inbiotics Research Center, Nagercoil.

Antimicrobial Assay

Antibacterial activity of different solvent extracts of whole body of slug

Mariaella dussumieri were tested using standard disc diffusion method of Bauer [15]. The bacterial strains were inoculated in sterile nutrient broth and incubated at 37°C for 24 h. Pathogens were spread on the surface of Muller Hinton agar plates and sterile disc of 6mm (Himedia) were loaded with 50µl of crude extract of slug. The crude extract loaded discs as well as positive and negative control discs for comparison were also placed in the plates. For positive control streptomycin disc (25µg disc) and negative control, sterile discs were used. *In vitro* antifungal activity of slug *Mariaella dussumieri* crude extract was also determined. The 48hour old culture of fungal strains was distributed

uniformly on the surface of potato dextrose agar plate with the help of sterile cotton swab and Flucanozale (100µg / disc) was used as positive control. The bacterial plates were incubated at 37°C for 24hour and fungal plates were incubated at 27°C for 48hour and the antimicrobial activity was measured based on the diameter zone of inhibition using millimeter scale.

RESULTS AND DISCUSSION

Antimicrobial activity

Slug, *Mariaella dussumieri* crude extracts were tested for inhibition against six pathogenic bacteria and three fungal pathogens. The inhibition zone of eight different solvent extracts against test organisms are given in table:1. The maximum inhibitory zone (26mm) was observed against *Enterococcus faecalis* by ethyl acetate extract of slug *Mariaella dussumieri* and it also showed anti microbial activity against all pathogens tested. Ethanol extract and n-butanol extract of slug exhibited good antimicrobial activity. Petroleum ether showed moderate activity against the bacteria and fungus. Methanol and acetone extracts recorded poor activity against the pathogens. Methanol extract of slug showed 10 mm inhibition zone against *Streptococcus mutans and Enterococcus faecalis, Proteus vulgaris* (9 mm), *Aspergillus niger*(12 mm) and *Pencillium sp*.(11 mm). The acetone extracts failed to inhibit all the bacteria tested whereas 15 mm inhibition zone was recorded in *Pencillium sp*. and 9 mm against *Aspergillus niger*. Maximum zone of inhibition was recorded with chloroform extract (21mm) against *Aspergillus flavus* .The aqueous extracts were not effective in eluting the bioactive compounds and showed negative results on all the bacterial and fungal pathogens tested. Antibacterial activity varies with the bioactive compounds of different solvent extracts and bacterial strains. Overall study revealed that ethyl acetate extract of slug *Mariaella dussumieri* has anti microbial properties against all pathogens. In traditional Indian medicine, especially Siddha medical preparations, the opercula, of gastropods are used as an ingredient to combat different diseases[16]. Antibacterial activity has previously been described in a wide range of molluscan species [17, 18, 19, and 20]. Potent antibacterial activity has been detected in *B.spirata* [21],*Crassostreavirginica* (oyster), *Mytilusedulis* and *Geukensiademissa* (mussel) [22], *Dicathaisorbita* (muricid molluscs) [23] and *Dolabellaauricularia* (sea hare) [24]. Molluscs rely predominantly on cellular defense reactions in which invading microorganisms are encapsulated by blood cells or phagocytosed [25]. Defensive mechanism is one such adaptation which is well studied in most of invertebrates. As invertebrates, molluscs lack adaptive immune system, but have evolved sophisticated strategies and rely exclusively on their innate immunity to defend themselves against a variety of pathogens [26]. According to [27,28, 29], the antibacterial activity of mucin found in the mucous secretion of *Achatinafulica* is related to antibacterial factors found in its protein moiety rather than to its activity on the cell surface of bacteria. The antibacterial protein in the mucus of the giant African snail referred to as achacin, is known to bind both Gram positive and Gram negative bacteria [30, 31].Antimicrobial compounds are found in various solvents of oyster*Pteriachinersis* and bivalve *Pernaviridis* [32] and in marine molluscs [33]. Antifungal activity were also reported from the extracts of various bivalve molluscs [34] and two edible bivalve species of *Pernaviridis* and *Meretrixcasta* showed antifungal activities[35,36]. Methanolic extract of *Murex virgineus*showed significant antifungal activity against all the tested strains. [37].

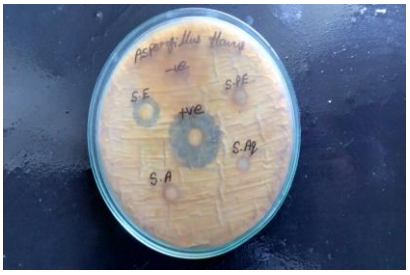
Table 1: Antibacterial activity of various solvent extracts from *Mariaella dussumieri* against various bacterial pathogens

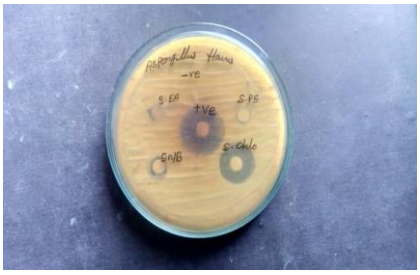
Whole body extract	*Streptococcus mutans*	*Staphylococcus aureus*	*Enterococcus faecalis*	*E. coli*	*Proteus vulgaris*	*Klebsiella pneumoniae*
E	-	24	10	-	-	9
M	10	-	-	-	9	10
A	-	-	-	-	-	-
AQ	-	-	-	-	-	-
EA	16	20	26	18	23	15
PE	13	-	-	-	-	13
NB	10	15	11	13	14	-
CHO	-	-	-	-	10	-
PC	20	15	15	26	25	15

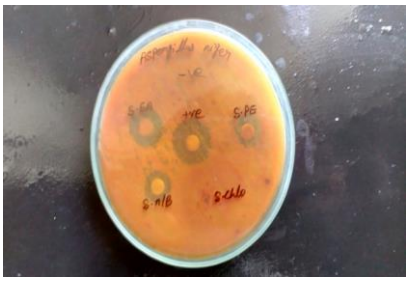
Table 2: Antifungal activity of various solvent extracts from *Mariaella dussumieri* against various fungal pathogens

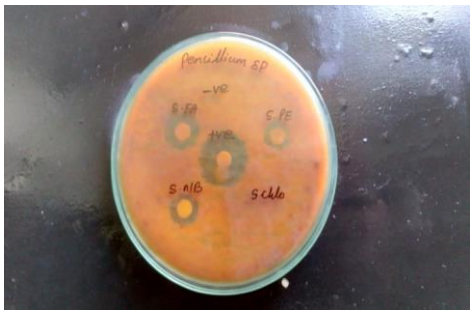
Whole body extract	*Aspergillus flavus*	*Aspergillus niger*	*Pencillium* sp
E	13	13	13
M	-	12	11
A	-	9	15
AQ	-	-	-
EA	7	15	15
PE	7	13	12
NB	9	13	13
CHO	21	-	-
PC	26	22	23

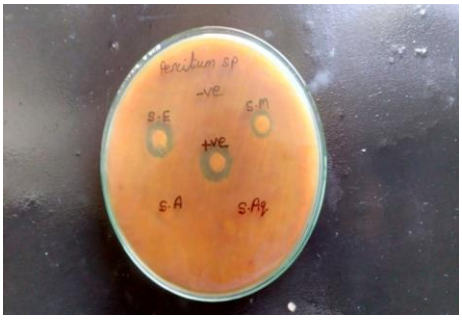
*E- Ethanol extract, M- Methanol, A- Acetone, AQ – Aqueous, EA –Ethyl acetate, NB- n- Butanol, PE- Petroleum ether, CHO –chloroform, PC- Positive control.

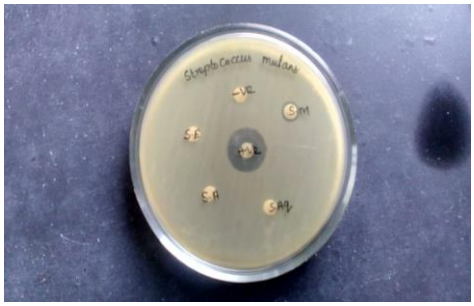







Penicillium sp
-ve
S.EA
S.PE
+ve
S.nlB
S.chlo


Penicillium sp
-ve
S.E
S.M
+ve
S.A
S.Ag


Streptococcus mutans
-ve
S.M
S.E
+ve
S.A
S.Ag


Streptococcus mutans
-ve
S.EA
S.PE
+ve
S.nlB
S.chlo

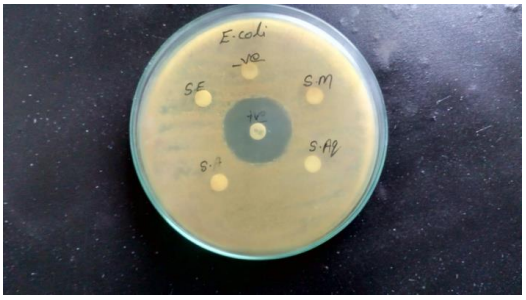

Staphylococcus aureus
-ve
S.E
S.M
+ve
S.A
S.Ag

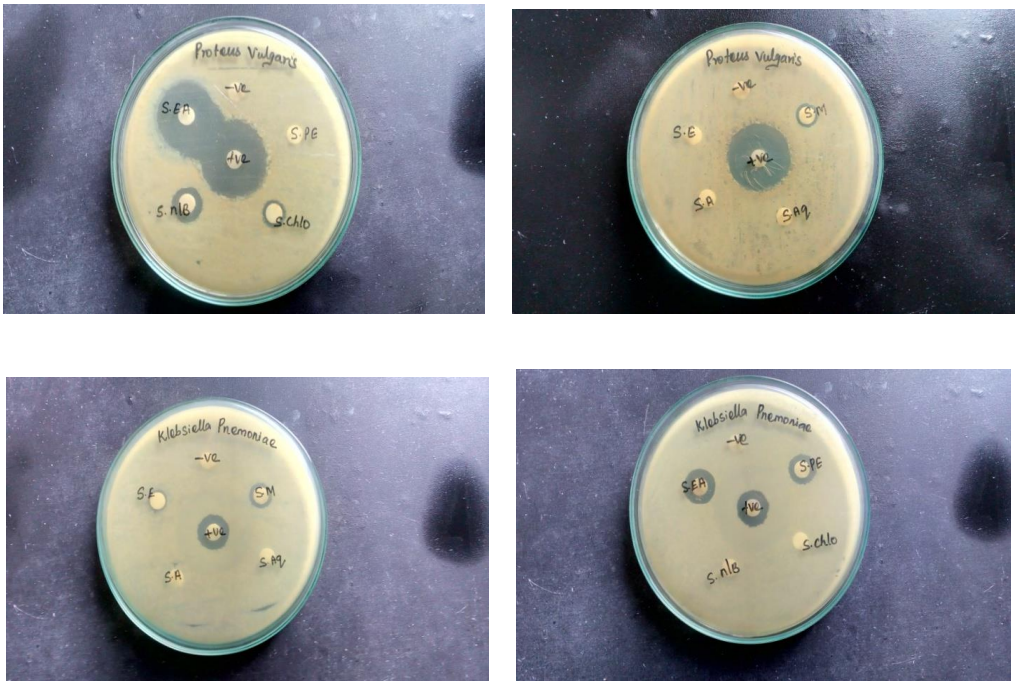

Staphylococcus aureus
-ve
S.EA
S.PE
+ve
S.nlB
S.chlo


Enterococcus faecalis
-ve
S.E
S.M
+ve
S.A
S.Ag


Enterococcus faecalis
-ve
S.EA
S.PE
+ve
S.nlB
S.chlo


E.coli
-ve
S.EA
S.PE
+ve
S.nlB
S.chlo


E.coli
-ve
S.E
S.M
+ve
S.Ag



A Study by [38] did not report a significant difference in antibacterial activity between mucous secretion of *Achatinafulica* and metronidazole. Snails have special proteins that aid their survival in the environment and also limit bacterial contamination. Disc-diffusion assays have the ability to rapidly identify active metabolites and therefore are particularly useful in the initial screening for antimicrobial activity and as the means for following activity during chemical purification [39]. The flesh of *Meretrixmeretrix* was used widely in India and China as a fisher folk medicine to treat several liver diseases like jaundice, hepatitis-A and B [40].Anti microbial activity of slug *M.dussumieri* extracts were compare with bacterial antibiotics showed that the ethyl acetate extracts have significant activity more than antibacterial antibiotics. Slugs are damaged pests in moist shady gardens. Controlling the slugs in garden is a great problem for farmers. Thus the present study indicates*Mariaella dussumieri*as a potentialantimicrobial agent and the garden slug which is pestcan be converted as a potential biomedical compound. Further studies will be carried out on the antibacterial factors in slug *M.dussumieri* and purification of bioactive compounds.

Acknowledgements

We are thankful to Dr. K.P. Srinivasakumar, Chief Scientific Officer and staff members of M/S Inbiotics, Nagercoil-629001 and Department of Zoology, Holy Cross College, Nagercoil for providing laboratory facilities.

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