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Research Article

ANTIMICROBIAL EFFICACY OF METHANOLIC EXTRACT OF ROOT, CALLUS AND FRUIT EXTRACTS OF MYXOPYRUM SMILACIFOLIUM BLUME

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Abstract

The aim of the present study was to compare the antimicrobial efficacy of methanolic extract of root, callus and fruit of *Myxopyrum smilacifolium* Blume. Antimicrobial activity was tested using agar well diffusion with four bacterial strains viz: *Escherichia coli*, *Enterococcus faecalis*, *Bacillus subtilis* and *Staphylococcus aureus* of which *E. coli* alone was gram negative. The fungal strain employed was *Candida albicans*. Root extracts shown to be effective only against *B. subtilis*. Fruit extracts showed the maximum antimicrobial activity against all the microbial species considered for the current study except against *S. aureus*. Highlight of the present study was the antimicrobial activity of callus extracts.

Keywords: Callus; Antibacterial; Antifungal; zone of inhibition.

Introduction

Since ancient times, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases (Verpoorte; 1998). Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts (Sandhya *et al.*, 2006). Plant derived chemicals are a promise for it to be used as the active ingredients of modern medicine or as the lead compounds for new drug discovery.

Myxopyrum smilacifolium is a large woody climbing shrub belonging to the family Oleaceae. Its root, stem, leaves are of much medicinally active and is employed in many traditional systems of medicine. The roots are used to treat various diseases like scabies, cough, rheumatism, fever, cuts and wounds. The leaves are astringent, acrid, sweet, thermogenic, anodyne, febrifuge and tonic. They are useful in vitiated conditions of kapha and vata, cough, asthma, rheumatism, cephalalgia, nostalgia, fever, otopathy, neuropathy and cuts and wounds (Warrier; 1996). pharmacokinetic evaluation has been made for the plant and reported for the presence of terpenoids, flavones, anthraquinones, sugars, alkaloids, phenols, tannins, and saponins, antimicrobial study has been carried out in leaves (Gopalakrishnan *et al*; 2012). Previous studies have shown the presence of triterpenoid ursolic acid in leaves (Sudharmini and Ashalatha; 2008) and the iridoid glycoside myxopyroside (Franzyk; 2001).

Medicinal plants are also a rich source of many antimicrobial agents. They can be used as a substitute for many synthetic antimicrobial agents. An antimicrobial is a compound that kills or inhibits the growth of microbes such as bacteria (antibacterial activity), fungi (antifungal activity).

The present study was carried out to in order to have a comparative study on the antimicrobial activity between the methanolic root, callus and fruit extracts. In order to evaluate the antibacterial efficacy Agar well diffusion method was conducted against four strains viz: *E coli*, *Enterococcus faecalis*, *Bacillus subtilis* and *Staphylococcus aureus*. *Candida albicans* was the only fungus used for testing antifungal activity.

Materials and Methods

Collection and processing of plant material

Fresh plant roots of *M. smilacifolium* were collected from Botanical garden, Dept. of Botany, University of Kerala, Kariavattom. The roots were washed thoroughly with tap water followed by sterile distilled water. Shade dried roots were then crushed to coarse powder and were stored at room temperature in air tight containers.

For callus induction surface sterilized internode explants were first inoculated in MS medium supplemented with 0.1% 2, 4 Dichlorophenoxy acetic acid (2,4-D) and then sub-cultured on to same medium supplemented with a combination of 0.1% 2,4-D and 1.0 % Benzyl amino purine (BAP). Four week old callus was collected and dried in hot

air oven at 50°C. Dried callus then powdered using mortar and pestle and stored in refrigerator.

Fresh matured fruits collected were washed thoroughly under running tap water, chopped into pieces and dried in shade. It was then crushed with mortar and pestle and stored in refrigerator.

Extraction

Powdered root, callus and fruit of *M.smilacifolium* were extracted using methanol in soxhlet apparatus for 12 hrs. The extracts were then filtered through Whatmann No.1 filter paper and concentrated using vacuum evaporator. The extract value calculated and then stored in refrigerator for further use.

Antimicrobial activity

Antimicrobial efficacy of the samples were tested against four bacterial strains and one fungal strain. Agar well diffusion method was employed for both antibacterial and antimicrobial studies.

Agar- well diffusion method

The principle of this method is that antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zone of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition was measured in centimeters. The reagents required and their preparations are as given below.

Muller Hinton Agar Medium

The medium was prepared by dissolving 33.9 g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100 mm petriplates (25-30ml/plate) while still molten.

Nutrient broth

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HiMedia) in 1000 ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Procedure

Petriplates containing 20 ml Muller Hinton medium were seeded with 24h culture of bacterial strains *E coli*, *Enterococcus faecalis*, *Bacillus subtilis* and *Staphylococcus aureus*. Wells of approximately 10mm was bored using a well cutter and 25 µl, 50 µl and 100 µl of sample was added to the well. The plates were then incubated at 37°C for 24h. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Gentamycin, a standard antibacterial agent at a concentration of 20 mg/ml was used as the positive control.

In order to access the antifungal efficacy of the extracts the minimal inhibitory activity was determined by Agar well diffusion method. Potato Dextrose agar plates were prepared and overnight grown species of fungus such as *Candida albicans* was swabbed. Wells of approximately 10 mm was bored using a well cutter and samples of 50 µl and 100 µl concentration were added, the zone of inhibition was measured after overnight incubation and compared with that of standard antibiotic clotrimazole.

Results

Antimicrobial activity

The result of the antimicrobial activity of the extracts are as shown in the Table 1 and 2. Table 1 shows antibacterial and Table 2 shows antifungal activity.

Table 1: Antibacterial activity of root, callus and fruit extracts on various bacteria

Method	Methanolic Extracts	Volume (µl)	Microorganism/zone of inhibition (cm)			
			EC	EF	BS	SA
Agar-well Diffusion method	Root	25	-	-	-	-
		50	-	-	1.1±0.13	-
		100	-	-	1.3±0.05	-
	Callus	25	-	-	-	-
		50	-	1.2±0.19	-	-
		100	-	1.4±0.09	1.2±0.09	1.0±0.06
	Fruit	25	-	2.7±0.03	1.5±0.15	-
		50	1.1±0.12	3.5±0.07	1.7±0.04	-
		100	1.2±0.06	3.9±0.05	1.8±0.11	-
	Gentamycin standard	20 mg/ml	3.2	3.3	3.2	2.9

Each value represents the mean ± SD; EC- *Escherichia coli*; EF- *Enterococcus faecalis*; BS- *Bacillus subtilis*; SA-*Staphylococcus aureus*

Table 2: Antifungal activity of root, callus and fruit extracts on fungus, *Candida albicans*

Method	Methanolic Extracts	Concentration (μ l)	zone of inhibition (cm)
Agar-well Diffusion method	Root	50	-
		100	-
	Callus	50	-
		100	1.1 \pm 0.07
	Fruit	50	-
		100	1.3 \pm 0.09
	Clotrimazole (Standard antifungal agent)	20 mg/ml	2.6

Each value represents the mean \pm SD

Antibacterial activity

Of the four bacterial strains employed fruit extract was comparatively more effective against all the three strains except *S. aureus*. All the extracts shown almost similar inhibitory effect against *E. faecalis* and *B. subtilis*. Fruit extract showed much higher zone of inhibition against *E. faecalis* than the standard antibacterial Gentamycin. Only callus extract showed some inhibitory against *S. aureus* that too at higher concentration. Root and callus extract has no effect on *E.coli*.

Antifungal activity

It is clear from the Table 2 that root has no inhibitory effect on *C. albicans*. On increasing concentration callus and fruit extracts shown inhibitory activity on comparison with the antifungal standard.

Discussion

Medicinal plants used in traditional medicines are potentially effective antimicrobial agents. Agar well diffusion method was employed in order to compare the antimicrobial efficacy of extracts. Both antibacterial and antifungal activity was assayed by measuring the diameter of zone of inhibition. The antimicrobial and antifungal activities of the extracts increased linearly with increase in concentration of extracts. Except *E. coli* all other bacterial strains were gram positive. The extracts were effective at least at higher concentrations against all bacterial strains except against *S. aureus* for which only callus extract in its higher concentration (100 μ l) showed a positive result. Only the fruit extract exhibited activity against *E. coli*. It was quite interesting that against *E. faecalis* fruit extracts inhibiting activity exceeded that of the standard antibiotic Gentamycin. Root extract showed no activity against *E. faecalis*, but callus extracts showed activity on increasing concentration. Although there is considerable variation in the concentration range and diameter of zone of inhibition, it was against *B. subtilis* all extracts showed activity of which fruit extracts showed the maximum. Root extracts were not effective against any of the bacterial strains except against *B. subtilis*. Coming to antifungal activity which was tested against *C. albicans* that too by Agar well diffusion assay showed that root extract showed no activity while both callus and fruit extract showed significant activity on

increasing concentration with reference to the standard antifungal Clotrimazole used.

Present study clearly indicates that all the extracts possess antimicrobial activity. Study must be extended further to other bacterial and fungal species and on increasing concentration of samples. The remarkable point of the present study is the antimicrobial efficiency of callus extracts. It is clear that *M. smilacifolium* is much promising as a medicinal plant which could serve as a good candidate in pharmaceutical industry. Higher antimicrobial activity of fruit extract against *E. faecalis* strictly proves its efficiency and presence of some specific phytoconstituent responsible which should be traced out and characterized. But the major limitation of this plant is its low flowering and fruiting habit which must be traced out. The present study surely forms a basic for further studies to investigate the bioactive compounds, their isolation and structure elucidation.

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