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Research Article

IN VITRO ANTIMICROBIAL POTENTIAL OF ROOT EXTRACTS OF THE MEDICINAL PLANT SPECIES, *EMILIA SONCHIFOLIA* (LINN.) DC.

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ABSTRACT

In the present investigation, evaluation of antimicrobial potential of the methanolic root extracts (25, 50, 75 and 100mg/mL) of the plant species, *Emilia sonchifolia* (Asteraceae) was carried out against certain Gram-positive bacteria (*Staphylococcus aureus, Bacillus cereus, B. subtilis* and *Streptococcus faecalis*) and Gram-negative bacteria (*Salmonella typhi, Pseudomonas aeureginosa, Shigella dysenteriae* and *Klebsiella pneumoniae*) and fungi (*Aspergillus niger, Candida albicans, Tricoderma viride, Azospirillum lipoferum and Mucor racemosus*) by detecting the zone of inhibition using disc diffusion method. The methanolic root extracts possess significant antimicrobial activity at 100mg/mL against the tested bacteria and fungi. The minimum inhibitory concentration (MIC) of the extracts ranged from 400 to1000µg/mL. Therefore, the result obtained suggests that the root extracts exhibited effective target based molecular drugs against dreadful microorganisms.

Keywords: *Emilia sonchifolia*, Asteraceae, Antimicrobial activity.

INTRODUCTION

Plants have been used for the treatment of diseases all over the world before the advent of modern clinical drugs. Natural phytochemicals are known to contain substances that can be used for therapeutic purposes or as precursor for the synthesis of novel drugs. Nearly 50% modern drugs are of natural products origin and as such these natural products play an important role in drug development in pharmaceutical industry. Plants remain the most common source of antimicrobial agents [1, 2]. Many aromatic plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeast [3]. Biologically active compounds from natural sources have always been a great interest for scientists working in infectious diseases [4]. There is an essential need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action. Therefore, search for medicinal plants with potential secondary metabolites have been extensively investigated as a source of medicinal agents.

The species, *Emilia sonchifolia* belongs of the family, Asteraceae is distributed in India, Ceylon and in most tropical and subtropical regions. Different parts of the plant have been used in the treatment of asthma, inflammation, intermittent fever, breast cancer, ophthalmia, cuts and wounds[5]. Leaf juice is used to treat eye inflammation, night blindness and sore throat. Decoction of this plant is used as a febrifuge in infantile tympanities and bowel compliant. Hydroalcoholic extract of the arial parts of *E. sonchifolia* has been reported for its antinociceptive effect [6]. Few pyrrolizidine alkaloids, senkirkine and doronine were isolated from the aerial parts of *E. sonchifolia* [7]. Despite several ethnobotanical and ethnopharmacological investigations on the therapeutic potential of this plant, laboratory data on their bioactivity is still in paucity. Therefore, the present study was carried out to investigate the possible inhibitory effect of the methanolic root extracts of *E. sonchifolia* on the growth of certain bacteria and fungi.

MATERIALS AND METHODS

Plant material

The roots of *E. sonchifolia* were procured from the Kanchikode medicinal garden, Kerala, India. The authenticity of the selected plant material was duly identified and confirmed by comparison with reference specimens preserved in the Herbarium at Botanical Survey of India, Southern Circle, Coimbatore. The plant materials were cleaned, washed with copious amounts of distilled water, shade dried, chopped into bits, and coarsely powdered in a Willy mill (Nippon Electricals, Chennai, India) to 60-mesh size for extraction.

Preparation of extracts

50g coarsely powdered plant samples were exhaustively extracted with methanol, using Soxhlet apparatus at a controlled temperature. The extracts were filtered and concentrated to dryness under reduced pressure using rotary vacuum evaporator (RE300; Yamato, Japan), lyophilized (4KBTXL-75; Vir Tis Benchtop K, New York, USA) to remove traces of water molecules and the lyophilized powders were stored at 20°C until further use directly for the assessment of antimicrobial activity.

Media used

Freshly prepared nutrient agar medium and potato dextrose agar medium were used for the culture of bacteria and fungi respectively.

Microorganisms

In vitro antimicrobial activity was examined for the methanolic root extracts of the species, *E. sonchifolia* against eight bacterial species which include the gram positive strains viz., *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (ATCC 10987), *B. subtilis* (ATCC 6633), and *Streptococcus faecalis* (ATCC 11700) and gram negative strains viz., *Salmonella typhi* (ATCC 13311), *Pseudomonas aeruginosa* (ATCC 27853), *Shigella dysenteriae* (ATCC 13313) and *Klebsiella pneumoniae* (ATCC 700603), and five fungal species viz., *Aspergillus niger* (ATCC 1015), *Candida albicans* (ATCC 10261), *Tricoderma viride* (ATCC 28020), *Azospirillum lipoferum* (ATCC 29709) and *Mucor racemosus* (ATCC 13604). All these organisms were obtained from the Department of Microbiology, Tamil Nadu Agricultural University, Coimbatore. All the bacteria and fungi were maintained at 4°C on nutrient agar slants and PDA slants respectively until further use.

Sterility Proofing of the Extracts

The extract was tested for sterility after Millipore filtration by introducing 2mL of this sterile extract into 10mL of sterile nutrient broth and Potato dextrose broth. Incubation was done at 37°C for 24hours. A sterile extract was indicated by the absence of turbidity or clearness of the broth after the incubation period [8].

Standardization of the microbial Cell Suspension

Each test organism was picked into sterile test tubes containing sterile nutrient broth and PDA broth and incubated at 37°C for 24 hours. The turbidity produced by this organism was adjusted and used to match the turbidity (opacity) standard prepared as described by Monica [9].

Antimicrobial assay

The methanolic extracts were tested for their effect against the growth of pathogenic bacteria and fungus by disc diffusion method [10]. The root extract of *E. sonchifolia* at four different concentrations viz., 25, 50, 75 and 100mg/mL were employed for antimicrobial activity. The antibiotic discs, oxacillin (10µg) and tetracycline (10µg) served as positive control for bacteria and fungi respectively. The bacteria and fungi tested were inoculated into nutrient agar and PDA medium respectively. After the incubation period of 24 hours at a temperature of 35°C, three or four colonies isolated from these media were inoculated on 4ml of nutrient broth and incubated for 2 hrs at 35°C. The cultures were adjusted with sterile saline solution to obtain turbidity. Petri dishes containing Muller-Hinton agar medium were streaked separately with these microbial suspensions of bacteria and fungi. Sterile filter paper discs impregnated with 25, 50, 75 and 100mg/mL extracts and control discs were applied over the culture plates. After equilibrium at 4°C, the plates were incubated overnight at 37°C and the diameter of any resulting zones of inhibition was measured. Triplicates were maintained for all these experiments.

Determination of Minimum Inhibitory Concentration (MIC) of the extracts on the test organisms

The MIC of the plant extracts was determined on broth (nutrient agar and potato dextrose broth) using the method of Ibekwe *et al*. [11]. The range of concentration used was 50-500mg/mL.

Statistical analysis

The results were expressed as mean±SD. The data were subjected to one way analysis of variance (ANOVA) and the significance between mean was determined by Duncan's Multiple Range test with significance level, P<0.05. ANOVA was performed using the statistical software SPSS (SPSS Inc. Chicago, USA).

RESULTS AND DISCUSSION

In the present investigation, the antimicrobial activities of *E. sonchifolia* root extracts against the test microorganisms were qualitatively assessed by zone of inhibition and minimum inhibitory concentration and the results were compared with the standard drugs, oxacillin and tetracycline for bacteria and fungi respectively (Tables 1 & 2). The extracts of *E. sonchifolia* exhibited strong antimicrobial effects against the tested microorganisms with the inhibition zones ranged from 7-21mm. It was found that 100mg/mL root extract exhibited significant activity against the bacteria, *Bacillus cereus* (21mm), *Staphylococcus aureus* (14mm), *Salmonella typhi* (13mm) and *Streptococcus faecalis* (12mm) and the fungal species, *Aspergillus niger* (14mm) and *Mucor racemosus* (12mm). 75 mg/mL root extract have moderate activity against *Salmonella typhi* (12mm), *Pseudomonas aeruginosa* (13mm), *Aspergillus niger* (12mm) and *Mucor racemosus* (12mm). This finding is similar to that of Nwadinigwe and Alfreda [12], who also recorded that 100mg/mL of stem extract of *Bryophyllum pinnatum* has significantly the most inhibitory effect against these bacteria. However, the inhibitory activity of the methanol extract of root part of *E. sonchifolia* was also considerably higher against the other bacteria and fungi tested. Many studies are supporting that methanol extracts of several medicinal plant species are having higher antimicrobial activities than that of any other alcoholic solvents [13-16].

The varying degree of inhibitory effect of methanolic extract of root of the study species may be due to specificity of bacterial and fungal strains [17]. It may also be explained that the activity of antibiotics in plant extracts against bacterial growth may be due to their mechanism of action, chemical structure or spectrum of activity [16, 18]. These antimicrobial activities against bacteria and fungi may be due to the presence of broad spectrum of antibiotic compounds in this species which has been reported earlier [19]. Thus, it is anticipated that phytochemicals with adequate antimicrobial efficacy are used for the treatment of microbial infections [20]. In the same family, Asteraceae, various species are reported to have better inhibitory effect against the bacteria and fungi even in low concentrations [21]. When comparing the antimicrobial activity of the tested samples to that of reference antibiotics, the inhibitory potency of tested extracts could mostly be considered as important.

The results of the Minimum Inhibitory Concentration (MIC) showed that the methanolic extracts of *E. sonchifolia* had MIC value of 400µg/mL for *Bacillus cereus* and 600µg/mL for *Staphylococcus aureus* and *Salmonella typhi*. However, *Aspergillus niger* and *Mucor racemosus* recorded moderate MIC value of 700 and 800µg/mL respectively. The variations in the Minimum Inhibitory Concentrations reported in the species might be due to differences in phytochemical composition and sensitivity of microorganisms tested [22]. Further the presence of some antimicrobial secondary metabolites such as alkaloids, beta-sitosterol, stigmasterol in *E. sonchifolia* may also be explained as a factor for sup processing the colonial growth of tested microorganism.

In conclusion the results of this study have shown that the root extracts of *Emilia sonchifolia* have great potential as antimicrobial agents in the treatment of infectious diseases. Thus, the study ascertains the value of plants used in Ayurveda which could be of considerable interest in the development of new drugs.

Table 1: Antimicrobial activity of methanolic root extract of *Emilia sonchifolia*.

S. No	Microrganisms	Extract concentrations (zone of inhibition in mm)				
		Control	25	50	75	100
Bacteria						
Gram positive						
1.	*Staphylococcus aureus*	13±0.21**	7±0.061	8±0.07	10±0.64	14±0.36**
2.	*Bacillus cereus*	20±0.37**	8±0.34	8±0.46	9±0.24	21±0.72**
3.	*B. subtilis*	12±0.17*	-	-	8±0.051	10±0.06
4.	*Streptococcus faecalis*	15±0.64**	6±0.42	7±0.25	7±0.08	12±0.83*
Gram negative						
5.	*Salmonella typhi*	13±0.70*	5±0.36	8±0.86	12±0.86*	13±0.57*
6.	*Pseudomonas aeruginosa*	15±0.43**	13±0.56	7±0.16	13±0.68*	-
7.	*Shigella dysenteriae*	13±0.75*	-	-	-	10±0.07
8.	*Klebsiella pneumoniae*	12±0.34*	-	8±0.78	9±0.72	12±0.46
Fungi						
1.	*Aspergillus niger*	15±0.73**	6±0.75	8±0.48	12±0.68*	14±0.87**
2.	*Candida albicans*	16±0.89**	-	-	7±0.012	10±0.30
3.	*Tricoderma viride*	13±0.65*	6±0.82	7±0.12	10±0.46	11±0.48
4.	*Azospirillum lipoferum*	15±0.03**	5±0.19	6±0.87	8±0.072	10±0.74
5.	*Mucor racemosus*	18±0.44**	-	5±0.57	12±0.97*	12±0.53*

Data represent mean ±SD of three replicates per treatment * *P* <0.05; ***P* <0.01.

Table 2: Determination of Minimum Inhibitory Concentration (MIC) of the methanolic extracts of *E. sonchifolia* root on the test organisms.

S. No	Organism	Concentrations of the plant extract (µg/mL)									
		100	200	300	400	500	600	700	800	900	1000
1.	*Bacillus cereus*	-	-	-	+	+	+	+	+	+	+
2.	*Staphylococcus aureus*	-		-	-	-	+	+	+	+	+
3.	*Salmonella typhii*	-	-	-	-	-	+	+	+	+	+
4.	*Aspergillus niger*	-	-	-	-	-	-	+	+	+	+
5.	*Mucor racemosus*	-	-	-	-	-	-	-	+	+	+

-, No inhibition at the concentrations tested

+, inhibition at the concentrations tested

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