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ANTIMICROBIAL EFFICACY OF *BOUCEROSIA PROCUMBENS*, (GRAVELY & MAYUR.) AN ENDEMIC RED LISTED MEDICINAL PLANT

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ABSTRACT

Boucerosia procumbens (Gravely & Mayur.) an endemic and endangered medicinal plant belonging to the family Apocynaceae was analyzed for antibacterial and antifungal activity. It was carried out with ethanol, aqueous, benzene, acetone and chloroform extracts. The zone of inhibition possessed by various extracts was compared. The ethanol extract showed the highest inhibitory effect against the selected pathogenic microorganisms when compared with the control. The results proved that this plant can be used as an antimicrobial agent.

KEYWORDS: ***Boucerosia procumbens*, antibacterial activity, antifungal activity.**

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INTRODUCTION

Nature has provided a complete store house of remedies to cure ailments of mankind. The knowledge of drugs has accumulated over thousands of years as results of man's inquisitive nature so that we possess many effective means of ensuring health care[1]. In developing countries, medicinal plants have been the most accessible source of medicaments and in rural areas, traditional medicine is part of the first line treatment for common pathologies[2]. The medicinal plant used as a therapeutic agent in addition to being used as food in many countries[3]. Presently there is an increasing interest worldwide in herbal medicines accompanied by increased laboratory investigations into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases. Various drugs have entered into the international market through exploration of ethnopharmacology and traditional medicine. Although scientific studies are carried out on a large number of plants but smaller number of marketable drugs or phytochemical entities have entered the evidence based there apeutics[4]. This has led to intensified efforts on the documentation of medicinal plants[5.] *Boucerosia* that are found in the dry regions of the world have medicinal values and have significant anti-bacterial and anti-fungal, anti-ulcer and antitumor activities.[6, 7, 8, 9.] *Boucerosia* include succulent plants with leaf less erect, trailing or decumbent stems, with ephemeral vestigial leaves. This genus is different from the genus *caralluma* mainly due to the presence of umbellate terminal cymes. The genus *Boucerosia* is represented by 8 species in India[10.] Among the them endemic. *B.procumbens* is a critically endangered species[11,12] and found in Maruthuvamalai and Aramboli rocky hills in Kanyakumari district in Tamil Nadu.[13,14]. It has long trailing stems, sometimes exceeding up to 3. It has no prominent leaf scars on the stem and the edges of the stems were sharp with square angles. The species grows mainly near seasonal streamlets in rocky crevices on the dry hills[9]. Locally *B.procumbens* is known as"Paaraikalli". Since it grows in the rocky substratum and looks like cactus. Traditionally the species is used for bowel complaints and also medicated oil prepared from the plant was used for rheumatism and to relieve pain. So, far there was no report on biological activity of this species. In the present study antimicrobial efficacy of the species is studied.

MATERIALS AND METHODS

Boucerosia procumbens, selected for this study was collected from Marunthuval malai hills lying at latitude 8 9' N and longitude 77 33' E of Kanayakumari District of Tamilnadu state in India. The plant material was thoroughly washed with running tap water and washed with sterile distilled water, followed by drying in room temperature. Then it was grounded in to powder and stored in air tight sterile containers .The weighed quantity 20g of dry powder was subjected to successive solvent extraction method by using Benzene, Ethanol, Acetone, Chloroform and Distilled water. The extracts were passed through double – layered muslin cloth and filtered through filter paper. The filtrates were pooled and evaporated in the air at room temperature to obtain a final residue. The extracts were dissolved in Dimethyl – sulphoxide to make the final concentrations and stored in refrigerator for further use.

MICROBIAL BIOASSAYS

Antibacterial activity

The extract in Benzene, Ethanol, Acetone, Chloroform and Distilled water extracted from the selected plant tissue were screened against eight bacterial strains. The test organisms used are *Bacillus substilis, Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeroginosa, Shigella flexneri, Salmonella typhi* and *Staphylococcus aureus.*

ANTIMICROBIAL ASSAY METHOD – Well diffusion method

The modified agar well diffusion method was employed to determine the antibacterial activity[15]. The test organism in nutrient agar (NA) was spread into sterile plates. Amikacin was used as a positive control and Acetone was used as a negative control. These plates were incubated at 37ºc for 48hrs. At the end of the inhibition period, inhibition zones formed on the agar were evaluated in millimeter (mm).

The diameters of zone of inhibition were measured in millimeter.[16,17,18.]

ANTI FUNGAL ACTIVITY

To evaluate antifungal activity, the fungal species for the experiment were prepared by seeding a loopful of the respective fungus into potato dextrose broth and incubated without agitation for 72 hours at 25^0C[19]. Antifungal activities of plant extracts against different species were checked by disk diffusion method[20]. The culture was maintained on Sabouraud Dextrose Agar [SAD]. Then the SAD medium was poured into the sterile petri plates and after solidification the fungal species were streaked on the SAD medium separately. Fluconazole is used as a positive and negative control. The plates were incubated at room temperature for 72 hours for observation of plant extracts in the medium. Then the zone of inhibition was measured.

RESULTS AND DISCUSSION

In the present investigation, *in vitro* antibacterial and antifungal efficacy of the crude extract of *B. procumbens* was quantitatively assessed on the basis of zone of inhibition. The stock crude extracts prepared from the plant of *B. procumbens* by using Distilled water, Benzene, Ethanol, Acetone and Chloroform were subjected to antimicrobial activity against *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Shigella flexneri, Salmonella typhi, Proteus vulgaris, Staphylococcus aureus* and *Bacillus substilis* and the results were recorded (Table 1). In the acetone extract of the plant, the growth of *Shigella flexneri* was inhibited to the maximum (10 mm). While low levels of inhibition was seen in the growth of *Proteus vulgaris* (8mm). The chloroform extract of *B. procumbens* showed significant antibacterial activity against *Shigella flexneri* with an inhibition zone of (8 mm). In general the chloroform extract of *B. procumbens,* failed to inhibit the growth of other microorganisms. The ethanol extract of the *B. procumbens* showed the highest inhibition of 18 mm against the growth of; while medium levels of inhibition was seen in the growth of *Salmonella typhi, Proteus vulgaris, and Staphylococcus aureus.* Growth of *Klebsiella pneumonia* exhibited very low inhibition zone of 13mm. Low level of inhibition was also seen in the *Bacillus substilis* 9mm. The benzene extract of *B. procumbens* showed significant antibacterial activity against *Shigella flexneri* with the inhibition zone of 9 mm. In general the benzene extract of *B. procumbens* failed to inhibit the growth of *Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Salmonella typhi, and Pseudomonas aeruginosa.* (Fig.1). The aqueous extract of *B. procumbens* reported no significant antibacterial activity. Various workers have supported that the Asclepiadaceae members had a wide spectrum of antimicrobial activity like *Caralluma adscendens*[21], *Caralluma speciosa.*[22] The result of antifungal assay tested against *Aspergillus flaver, Aspergillus oryzae, Trcohoderma gamsil*, and *Actinomyces* using the crude extract obtained from the whole plant of *B. procumbens* by using Distilled water, Benzene, Ethanol, Acetone and Chloroform are shown [Table 2]. The ethanol extract of *B. procumbens* showed significant antifungal activity with 15 mm inhibition. The highest antifungal activity found has shown zone of inhibition 15 mm, against *Trcohoderma gamsil*, and low activity observed the zone of inhibition at 14 mm against *Aspergillus flaver.* In general the ethanol extract of *B. procumbens,* failed to inhibit the growth of *Aspergillus oryzae, Actinomyces, Trcohoderma gamsil*. The results of zone of inhibition among the five tested extracts varied. This is based on colonial growth and susceptibility. Ethanol extract showed better inhibition. *Caralluma* and other members of the Asclepiadaceae are also ideal for pregnane glycosides or their esters[23,] which might be associated with antimicrobial activities[24.]

Figure 1

a. Habit of Boucerosia procumbens Antibacterial effect of b,c,d pseudomonas aeruginosa , Proteus vulgaris and shigella flexneri

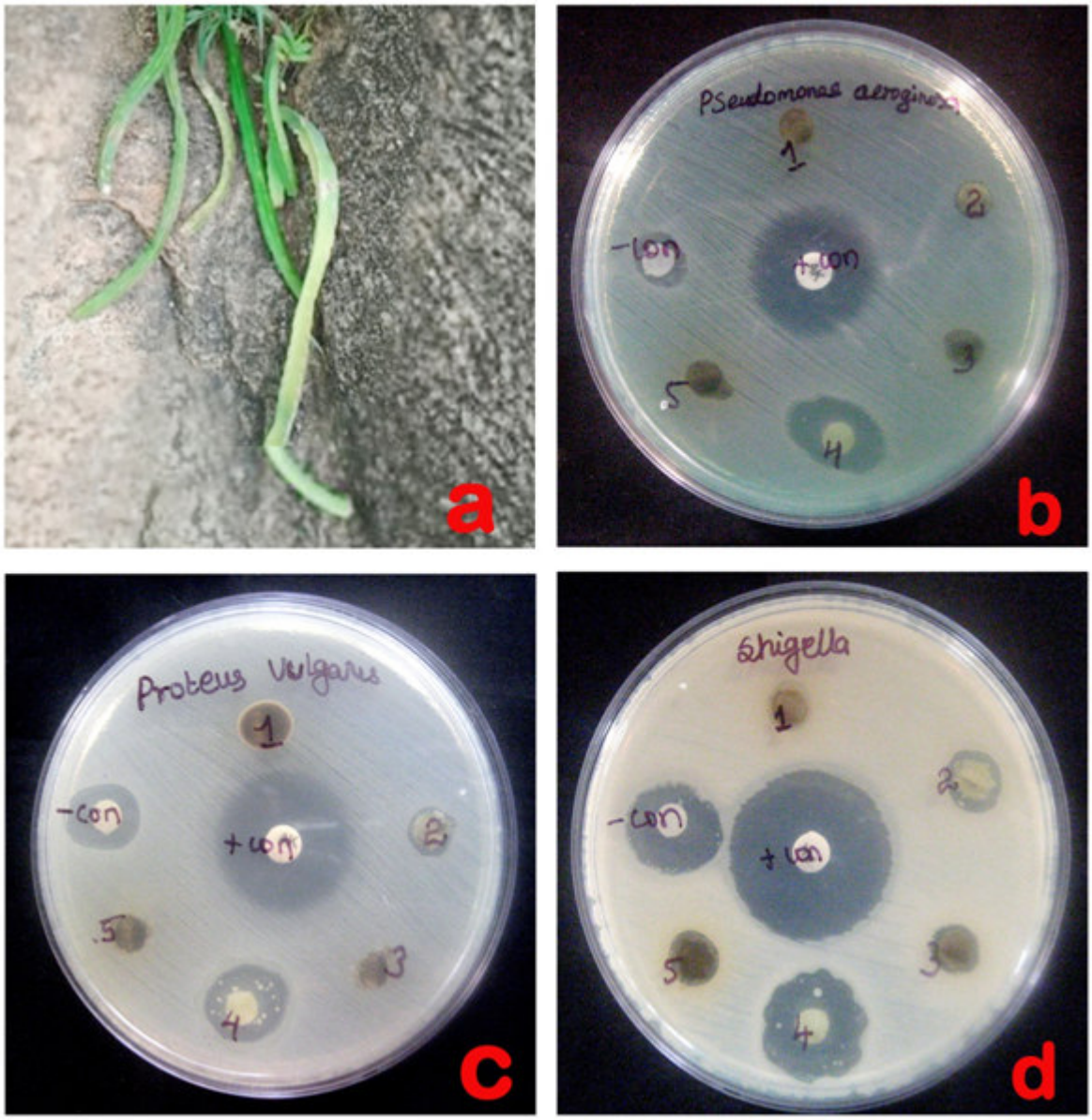

Table 1

Antibacterial assay of Boucerosia procumbens. Using different solvents

S.No	Test Microorganisms	Zone of inhibition (mm)						
		Water	Acetone	Chloroform	Ethanol	Benzene	-Control (Acetone)	+Control (Amikacin
1	*Escherichia coli*	-	-	-	-	-	18	26
2	*Klebsiella pneumonia*	-	-	-	13	-	14	11
3	*Pseudomonas aeruginosa*	-	-	-	16	-	9	19
4	*Shigella flexneri*	-	10	8	18	9	17	28
5	*Salmonella typhi*	-	-	-	14	-	13	35
6	*Proteus vulgaris*	-	8	-	14	-	14	16
7	*Staphylococcus aureus*	-	-	-	14	-	6	20
8	*Bacillus substilis*	-	-	-	9	-	-	23

Table 2
Antifungal assay of Boucerosia procumbens. Using different solvents

S.No	Fungi	Zone of inhibition (mm)						
		Water	Acetone	Chloroform	Ethanol	Benzene	+Control Flucanazole)	-Control Flucanazole)
1	*Aspergillus flavus*	-	-	-	14	-	15	17
2	*Aspergillus oryzae*	-	-	-	-	-	-	NR
3	*Talaromyces flavus*	-	-	-	-	-	10	NR
4	*Trcohoderma gamsil*	-	-	-	15	-	-	23
5	*Actinomyces*	-	-	-	-	-	15	NR

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