International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-3, 2014 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Preliminary Phytochemical and Antimicrobial Studies on Stem Extracts of *Boucerosia indica* (Wight & Arn.) N.E.Br (Asclepiadaceae) - A Potent Appetite Suppressant Plant from Western Ghats of Southern India

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Manuscript No: IJPRS/V3/I3/00361, Received On: 01/08/2014, Accepted On: 10/08/2014

ABSTRACT

An ethnomedicinal plant, *Boucerosia indica* (Wight & Arn.) N.E.Br was investigated for preliminary phytochemical analysis and antimicrobial activity. Qualitative phytochemical screening of petroleum ether, acetone, methanol and aqueous extract of the aerial part revealed the presence of various classes of phytoconstituents such as carbohydrate, aminoacids, proteins, saponin, flavonoids, steroids, phenolic compounds, tannins, terpenoids, fixed oils and fats. Bioassay of antimicrobial activity of all the extracts was tested against pathogenic bacteria like gram positive (*Bacillus substilis*, *Staphylococcus aureus*, *Streptococcus pyrogenes*, *Streptococcus faecalis*), gram negative (*Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella paratyphi* A, *Salmonella typhi*) and fungal organisms like *Aspergillus flavus*, *Aspergillus niger*, and *Candida albicans*. The methanol and aqueous aerial extract showed significant antibacterial and antifungal activities respectively against the selected pathogenic microorganism when compared to the standard drug. Based on these result, the study concludes that aerial extract of *Boucerosia indica* have different phytoconstituents and possesses antimicrobial effects making it a good candidate for producing antimicrobial drugs.

KEYWORDS

Boucerosia indica, Phenolic compounds, Bioassay, Antimicrobial activity

INTRODUCTION

Boucerosia indica (Wight & Arn.) N.E.Br. (Basionym: *Caralluma indica* Wight) is an edible medicinal herb, growing in dry areas of Western Ghats, Tamil Nadu. Their shoot tip is essentially a vegetable of daily use in tribal communities of Western Ghats and also eaten for appetite suppressant[1]. The genus *Caralluma* is succulent, perennial, branched herb. They have erected, branched, 4-angled, and glabrous stem and the leaves are caduceus[2,3].

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The literature search showed documentation of *Caralluma* species medicinal properties in traditional system[4,5,6,7,8,9]. *Caralluma umbellata* has dominant medicinal properties found in Southern India and is used for indigestion[10,11] and kidney stone[12]. *Caralluma* species have been extensively used for paralysis, joint pain, fever[13], diabetics, hypertension, pain relief, anticancer[9], leprosy, antipyretic, diabetes, rheumatism, fungal diseases, snake and scorpion bite[14]. Due to the presence of pregnane glycosides in *Caralluma* it exhibits antitumor and anticancer properties. The phytochemistry of *C. tuberculata*, *C. attenuata*, *C. russeliana* and *C. negevensis* revealed the presence of

bioactive compound Luteolin flavonoid[15,16,17,18] and is characterized by megastigmane glycoside, pregnane glycoside, steroidal glycosides, triterpenoid, steroids, flavonoid glycoside and pregnane glycoside[19,20,21]. After extensive literature survey it was found that no scientific investigation has so far been conducted on the qualitative phytochemical screening and antimicrobial activity of *B. indica*. Hence the present investigation was carried to explore the phytoconstituents and antimicrobial potential of *Boucerosia indica*.

MATERIALS AND METHOD

Plant Materials

The plant materials were collected from the wild population of the Madukkarai hills (Western Ghats) of Coimbatore, Tamil Nadu. Samples of plants were identified by a plant taxonomist Binu Thomas, botany department of Bharathiar University, Tamil Nadu and binomially confirmed with authentication of Botanical Survey of India (Sothern region, Tamil Nadu, India) and voucher specimens were deposited at the herbarium section of the PG and Research Department of botany, Kongunadu Arts and Science College (Autonomous), Coimbatore-29.

Preparation of Extracts

The fresh and healthy grown aerial stems of *Boucerosia indica* were collected and washed under running tap water to remove soil particles and other dirt. The leaves were air dried in the laboratory at room temperature (30 ± 2ºC) for 30 days. The dried aerial parts were ground well into affine powder with a mixer grinder. The powder was stored in air tight plastic container at room temperature before extraction. 20g of aerial powder sample was soaked separately in 100ml of distilled water, methanol, acetone and petroleum ether for 72 hours. Each mixture was stirred at 24 hours interval using a sterile glass rod. At the end of the extraction, each extract was passed through Whatman No. 1 filter paper (Whatman, England) and the filtrate obtained was concentrated in vacuum using rotator evaporator. Then the extracts were used for further investigations.

Phytochemical Screening

The petroleum ether, acetone, methanol and aqueous extracts were subjected to qualitative phytochemical screening for the presence or absence of phenols, terpenoids, flavonoids, glycoside, saponin, tannin, steroids, reducing sugar, Fats and oils, carbohydrates, protein and aminoacids[22, 23,24].

Test Microorganisms

All the microbial strains of human pathogens used in the antimicrobial bioassay were procured from Department of Microbiology, Hindustan Arts and Science College, Coimbatore. These microbes include Gram-positive bacteria such as *Bacillus substilis*, *Staphylococcus aureus*, *Streptococcus pyrogenes* and *Streptococcus faecalis*; the Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella paratyphi* A and *Salmonella typhi* and fungi such as *Aspergillus flavus*, *Aspergillus niger* and *Candida albicans* respectively.

Antibacterial Assay

Plant extracts of *B. indica* which was prepared with different solvents *viz.*, petroleum ether, acetone, methanol and aqueous were used to test their antibacterial activity by disc diffusion method[25]. Bacterial suspension is streaked on the Molten Mueller-Hinton agar medium containing the Petri plates. Discs of 6mm diameter were impregnated with stem extracts (100mg/ml). Ampicillin was used as a positive control. The plates were incubated for overnight at 37°C and the results were obtained by measuring the zone of diameter in millimeter.

Antifungal Assay

To evaluate antifungal activity, the fungal species for experiments were prepared by seeding a loopful of the respective fungus into potato dextrose broth and incubated without agitation for 48 hours at 25°C[26]. Antifungal activities of plant extracts against different species were checked by disc diffusion method[25]. Then the PDA medium was poured into the Petri plates and after solidification the

fungal species were streaked on the PDA medium separately. Circular paper discs (6 mm diameter) impregnated with known amount of each extract (100mg/ml) was placed upon the surface of inoculated plates. Tetracycline is used as positive control. The plates were kept at room temperature for 48 hours for absorption of plant extracts in the medium. Then the zone of inhibition was measured.

Statistical Analysis

The antimicrobial activities of aerial extracts of *B. indica* were indicated by clear zones of growth inhibition. All experiments were performed in triplicates and results were presented as Mean ± SD (Standard deviation). The significance in the difference of mean was determined according to Duncan's Multiple Range Test[27] significance level, $P<0.05$.

RESULTS

The qualitative phytochemical analysis in the stem extract of *B. indica* showed the presence of rich variety of phytochemical compounds (Table 1). The results showed the presence of phytochemical constituents such as carbohydrates, glycosides, saponins, proteins, aminoacids, steroids, flavonoids phenolic compounds, tannins, terpenoids, fixed oils and fats. Alkaloids, gums and mucilage and resins were absent in all the tested samples. Saponins were absent in petroleum ether and acetone extract. Glycosides were absent only in petroleum ether extract but other extract exhibited positive result. Terpenoids were absent in acetone and aqueous extract. The results of the study clearly indicated that methanol extract contained maximum phytoconstituents followed by aqueous extract.

The results regarding *in vitro* antibacterial and antifungal screening of stem extracts of *B. indica* had prominent antimicrobial activity against the human pathogenic bacteria and fungi as summarized in Table 2. The effect of various alcoholic and aqueous solvent extracts of *B. indica* for the antibacterial activity was determined to be varied across the bacterial species tested. Methanol extract showed significant activity against gram positive bacterial strains particularly *Bacillus subtilis* (21 mm diameter). Among the solvent tired, highest degree of zone of inhibition by aqueous aerial extract against the bacterium *Escherichia coli* (23 mm diameter). For antifungal activity (Table 2) the highest zone of inhibition was observed against the fungi, *Candida albicans* by aqueous stem extract (15 mm diameter). Lower inhibitory activity was ranging between 5 to 8 mm diameter were observed in acetone extract against tested pathogens.

DISCUSSION

Tremendous use of antibiotics has developed drug resistance in many bacterial and fungal pathogens. Similarly, synthetic drugs are harmful for human health and have many side effects. Medicinal plants are the reservoirs of valuable phytochemicals which act as key compound in new natural drugs and their use as an alternative medicine for treatment of microbial diseases has been increased in the last few decades[28]. Therefore, this study has been carried out to evaluate the phytochemical and antimicrobial activities of petroleum ether, acetone, methanol and aqueous extracts of stems of *Boucerosia indica*. In the present study, the qualitative phytochemical screening of petroleum ether, acetone, methanol and aqueous extracts of *B. indica* revealed the presence of maximum degree of secondary metabolites. Asclepiadaceae members are the main source of bioactive compounds which possessed very high levels of saponin, pregnane glycosides, polyphenols and flavonoids, and are employed in medicinal uses[29,30]. The results of zone of inhibition among the four tested extracts varied, which is based on colonial growth and susceptibility. Methanol extract showed better inhibition against gram positive bacteria. The differences in the sensitivity of the bacteria may be attributed to the fact that the cell wall layers differ for different bacteria. Gram positive bacteria consist of a single layer whereas the gram negative bacteria cell wall is multilayered. The walls act as a diffusional barrier making gram negative bacteria less susceptible to the

Table 1: Preliminary phytochemical screening of various extracts of the aerial parts of *Boucerosia indica*

Phytoconstituents	Phytochemical test	Petroleum ether	Acetone	Methanol	Aqueous
Alkaloids	Mayer's test	-	-	-	-
	Hager's test	-	-	-	-
Carbohydrates	Molish's test	+	+	+	+
	Fehlimg's test	+	+	+	+
Glycosides	Keller-Killiani test	-	+	+	+
	Borntrager's test	-	+	+	+
Saponins	Frothing test	-	-	+	+
Proteins	Millon's test	+	+	+	+
	Biuret test	+	+	+	+
Aminoacids	Ninhydrin test	+	+	+	+
Steroids	Libermann-Burchard's test	+	+	+	+
Flavonoids	Lead acetate test	+	+	+	+
	Alkaline reagent test	+	+	+	+
Phenolic compounds and Tannins	Ferric chloride test	+	-	+	+
	Lead acetate test	+	-	+	+
Terpenoids	Salkowski test	+	-	+	-
	Libermann-Burchard's test	+	-	+	-
Fixed oils and Fats	Spot test	+	-	+	-
Gums and Mucilage	-	-	-	-	-
Resin	-	-	-	-	-

Table 2: Antibacterial and antifungal activities of different extracts of *Boucerosia indica*

Microorganism tested	Diameter zone (mean ± SD) of inhibition (mm).					
	Petroleum ether	Acetone	Methanol	Aqueous	Reference drug	
					Ampicillin	Tetracycline
Gram positive						
Bacillus substilis	-	12.08 ± 1.12**	21.08 ± 2.10**	17.04 ± 1.62**	24.05 ± 1.55**	NT
Staphylococcus aureus	13.05 ± 1.51**	9.00 ± 1.25*	10.04 ± 1.70*	13.00 ± 1.74**	20.08 ± 1.40**	NT
Streptococcus pyrogenes	6.02 ± 1.53	13.08 ± 1.76**	17.04 ± 1.84**	10.08 ± 1.90*	25.06 ± 1.95**	NT
Streptococcus faecalis	-	14.06 ± 1.35**	18.07 ± 2.15**	11.02 ± 1.35*	25.00 ± 1.60**	NT
Gram negative						
Escherichia coli	17.6 ± 1.75**	7.05 ± 2.10	13.05 ± 1.76**	23.00 ± 1.30**	28.01 ± 1.13**	NT
Klebsiella pneumoniae	9.04 ± 1.60*	19.08 ± 1.73**	14.03 ± 1.70**	16.06 ± 2.25**	23.05 ± 1.65**	NT
Salmonella paratyphi A	16.5 ± 1.32**	5.00 ± 1.40	7.04 ± 1.25	10.0 ± 1.73*	24.06 ± 1.75**	NT
Salmonella typhi	15.08 ± 2.30**	6.03 ± 1.35	8.08 ± 1.70	9.06 ± 1.43*	24.05 ± 1.65**	NT
Fungi						
Aspergillus flavus	-	5.08 ± 1.51	12.04 ± 1.74**	14.08 ± 1.12**	NT	20.04 ± 1..30**
Aspergillus niger	-	6.03 ± 1.69	10.05 ± 1.63*	13.03 ± 1.15**	NT	20.00 ± 1.55**
Candida albicans	12.05 ± 1.62**	8.06 ± 1.76	14.07 ± 1.44**	15.08 ± 2.60**	NT	24.05 ± 1.35**

Values were performed in triplicates and represented as mean ± SD *P <0.05;**P <0.01.

-, Indicates no zone of inhibition

antimicrobial agents than the gram positive bacteria[31]. Various workers have supported that the Asclepiadaceae members had wide spectrum of antimicrobial activity like *Caralluma adscendens*[32], *Caralluma speciosa*[33] and *Gymnema sylvestre*[34]. Among the four extracts studied aqueous extract had moderate activity against the colonial growth of bacteria and potential activity against the mycelial growth of fungi. Similar trend of results were obtained by Patil and Saini (2012)[35] who observed that the aqueous extract of *Calotropis gigantea* flower moderately inhibited the growth, spore formation and biomass production of the fungus, *Candida albicans* and *Tinea capitis.* The antimicrobial properties exhibited by the stem extract of *B. indica* may be associated with presence of more variety of phytochemicals like saponins, glycosides, steroids, flavonoids, phenolic compounds, tannins and fixed oils and fats. *Caralluma* and other members of the Asclepiadaceae are also ideal for pregnane glycosides or their esters[36] which might be associated with antimicrobial activities[37] and thus have used in the treatment of obesity, rheumatism, diabetes, leprosy and as antiseptic as well as disinfectants. The present study confirms that stem extracts of *B. indica* have significant antibacterial and antifungal activity along with valuable phytochemicals.

CONCLUSION

In conclusion, it is to be stated that *B. indica* stem extracts have exhibited broad spectrum of activity against certain pathogenic bacteria and fungi. However in view of this, more study is needed in the areas of isolation, purification and identification of specific constituent with the antimicrobial property as this will help curb the menace of microbial resistance in chemotherapy and to enhance the exploration of medicinal properties of ethnobotanicals.

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