IN VITRO ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF DIFFERENT SOLVENT EXTRACTS OF ONOSMA BRACTEATUM LEAVE

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ABSTRACT

Objective: To determine the antibacterial activity of n-hexane, methanol, ethyl acetate and aqueous fraction derived from *Onosma bracteatum* leave against ATCC strains *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans.*

Material and Method: Dried leavesof Onosma bracteatum were grinded into powder. Powder was subjected to extraction and fractionation procedure according to protocol and n-hexane, ethyl acetate, methanol and aqueous fractions were obtained. These four fractions were studied for selected antimicrobial activity of the Onosma bracteatum leaves.

Kirby Baur disc diffusion method at concentration of 1mg/6µl and 2mg/12µl extract per disc. Azithromycin (50µg/6µl), Ciprofloxacin (30µg/6µl) and Clotrimazol (50µg/6µl) were used as positive control against gram positive bacteria, gram negative bacteria and fungi respectively.

Results: The results revealed that all four fraction of *onosma bracteatum* leaves showed moderate antibacterial activity against tested bacteria compared to positive control. *Staph aureus* and *Pseudomonas aureuginosa* were most susceptible than *E-coli*. Antifungal activity of four fraction samples showed that methanol, n-hexane and ethyl acetate fraction were effective, while aqueous fraction showed no antifungal activity against *Candida albicans.*

Concusion: In our study significant inhibitory effect of n-hexane and ethyl acetate fraction was noted as compared to methanol and aqueous fraction of *onosma bracteatum* leave against selected microbes.

Key words: Onosma bracteatum leave, antibacterial and antifungal activity.

INTRODUCTION

Plants have great role in our lives because they maintain our health and improve the quality of life. In the developed and developing world the use of traditional medicines is increasing and gaining popularity[1]. Ethnobotany is the study of the use of indigenous plant by particular culture. In Unani, Homeopathy, Naturopathy, Sidha and Ayurveda system of medicine, medicinal plants have been used over centuries for various diseases[2]. According to World Health Organization (WHO) report more than half of population relies mainly on traditional remedies[3]. In developing country medicinal plants are used for maintenance of good health as the plants remedies are reported to have less adverse effects[4]. The medicinal plants are used for various ailments because they are economical and effective but doctors hesitate to prescribe them because of their lack of knowledge regarding safety, liability, presence of pathogens and their harmful effects on human body[5]. Globally at least 130 drugs which are extract of higher plants are currently in medical use and some of them are now being manufactured synthetically for commercial purposes[6]. Antimicrobial properties of medicinal plants are reported from different parts of the world[7]. This property of medicinal plants has enormous therapeutic potential. To determine biological activities of medicinal plant's extracts various modern techniques and procedures have been developed[8]. *Onosma Bracteatum* is an important ingredient of reputed herbal remedy Joshanda, which is used for common cold and influenza. It belongs to the family *Boraginaceae*. Locally it is known as *Gaozoban, Gojihva and Sedge*[9]. Its leaves are used as tonic, demulcent as well as diuretic and antioxidant[10]. It is also helpful for palpitations and urinary complaints. In Ayrveda it is used for asthma and bronchitis[11]. In the Unani system of medicine it is used for stress, degenerative disease like Alzheimer's disease and Parkinson's disease[12]. It also has wound healing activity[13].

OBJECTIVE

To determine the antibacterial activity of n-hexane, methanol, ethyl acetate and aqueous fraction derived from Onosma bracteatum leave against ATCC strains Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Can*dida albicans.*

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MATERIALS AND METHODS

Plant Materials

The leave of *Onosma bracteatum* was purchased from local market.The dried plants leaves were grinded with ordinary grinder into powder.

Extraction and fractionation procedure of leave of Onosma bracteatum.

Five hundred and seventy-seven grams of fine powder of *Onosma bracteatum* was soaked in menthol for seven days. Throughout that phase the mixture was stirred at regular interval. The mixture was filtered thrice by Whatmen's filter paper. The fresh methanol was again poured to the remaining plant matter and filter once more after three days. The same procedure was worked out three times. The filtrate was dried by rotary evaporator. Methanol was completely evaporated at 45□C in vacuum and the paste of 356gm crude extract was obtained for further use. The extract was re-fractionated with n-hexane, ethyl acetate methanol and aqueous by liquid partition procedure. For fractionation (re-fractionation) 100 ml distilled water was added to 300 gm extract and poured it into a separating funnel, 300 ml n-Hexane was added in separating funnel. The n-hexane soluble compounds in separated in the upper portion of separating funnel were collected and the lower aqueous phase was fractionated thrice with n-Hexane for maximum recovery. The entire N-hexane fraction were collected and then dried by rotary evaporator and water bath. The dried extract was weighed and stored into a vile. The same procedure was adopted for Ethyl acetate, methanol.

Antibacterial activity

Antibacterial activities of the different extracts against *Staphylococcus aureus ATCC# 6538, Eischerichia coli ATCC# 25922, Pseudomonas aeruginosa ATCC# 9721 and Candida albicans ATCC# 10231* were determined by Kirby Baur Disc Diffusion method at concentration of 1mg/6μl and 2mg/12μl extract per disc. Azithromycin (50μg disc-1), Ciprofloxacin (30μg disc-1) and Clotrimazol (50μg disc-1) were used as positive control against gram positive bacteria, gram negative bacteria and fungi respectively. Dimethyl sulfoxide (*DMSO*) was used as negative controls.

RESULTS

The analysis of data showed that the n-hexane fraction of Onosma Bracteatum was most effective in terms of zone of inhibition against the selected microbes

Table 1: Zone of inhibition (mm) of four fractions of Onosma bracteatum against S.aureus,Pseu.aeruginosa,E-coli & Candida albicans.

Micro-organism	Zone of inhibition mm				Positive control	Negative control DMSO
	n-hexane	Ethyl acetate	Methanol	Aqueous		
S.aureus	18.83±0.23	11.83±0.24	8.83±0.62	10.67±0.41	Azithromycin 30mm	-
Pseu.aerugi	12.33±0.85	12.17±0.24	11±0.41	9.37±1.52	Ciprofloxacin 27mm	-
E-coli	11.83±0.24	11.13±0.19	8.67±0.62	8.67±0.62	27mm	-
C.albicans	10.67±1.25	9.37±1.59	8.83±0.62	0	Clotrimoxazol 30mm	-

*values are mean =±SE, SE= Stand error.

Table 2: Percent growth of inhibition of S.aureus, Pseu.aeruginosa, E-coli & Candida albicans by four fractions of onosma bracteatum compared to their respective positive control.

Micro-organism	% Growth inhibition				Positive control	Negative control DMSO
	n-hexane	Ethyl acetate	Methanol	Aqueous		
S.aureus	63%	40%	27.8%	39%	Azithromycin 30mm	-
Pseu.aerugi	46%	45%	33%	35%	Ciprofloxacin 27mm	-
E-coli	40%	37%	29%	29%	27mm	-
C.albicans	39.4%	35%	29%	0	Clotrimoxazol 30mm	-

as compared to other solvent fractions of Onosma Bracteatum as shown in table 1.

It was also shown that n.hexane had maximum Growth inhibition against the selected organisms as compared to other solvent fractions of Onosma Bracteatum as shown by table 2.

DISCUSSION

The antibacterial and antifungal activity of n-hexane, ethyl acetate, methanol and aqueous fractions of *onosma bracteatum* (leave) were investigated against selected microbes. *Staphylococcus aureus*, were selected for this study, which is the most common cause of infections in human and animals. *Pseudomonas aeruginosa* is opportunistic gram negative bacteria. It causes infection of burns, wound and respiratory tract. *E-coli* is found in normal flora of colon and cause infections of urinary tract and intestinal tract. *Candida albicans* is an opportunistic fungus and a common cause of oral thrush and genital infection.

Onosma bracteatum is locally known as Gao-Zuban[9]. It is an important ingredient of the reputed herbal medicine Jushanda.

In present study, the different fractions showed moderate activity against selected Gram Positive, Gram Negative bacterial and fungi. In study by Gautum et al. methanol fraction was most active than aqueous, acetone and petroleum ether[15]. In present study the n-hexane and ethyl acetate was most active compared to methanol and aqueous fractions. The study conducted by Walter observed that methanol fraction was active against *S.aureus* and *Pseu.aeruginosa* than *E-coli*. The order of antibacterial activity of methanol fraction were *S.ureus > P.aeruginosa > E-coli*[4] while according to Gautum's study the order of activity of methanol fraction were S*. Pneumonia >P. aeruginosa >H. infl uenzae*[15]. In the present study the activity of methanol against selected microbes were *Pseu.aeruginosa > S.aureus > Ecoli > C.albicans* which is a little different from other studies. The reasons for this difference may be different processing technique or different growth media however further studies are needed with same processing techniques and same growth media to resolve this difference.

This study also showed that the aqueous fraction was more active against *S.aureus > Pseu.aeruginosa > E-coli* which is similar to the study of Azami which also showed that aqueous fraction of Onosma Bracteatum was significantly active against *S.aureus*[11].

CONCLUSION

The current study represented the significant inhibitory role of different fraction of *Onosma Bracteatum* leaves against *S.aureus, Pseudomonas aeruginosa, E-coli & Candida albicans*. The n-hexane and ethyl acetate fractions found more potent in comparison to other solvents. *Onosma Bracteatum* is potentially a good source of antimicrobial agents which can be used in the treatment of infectious diseases caused by different microorganisms. Additional studies are needed to assess the effects of the selected plant on other microbes. The synergistic effect between antibiotics and plant extracts may lead to the new choices of treatment.

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