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Impact of Extraction Solvent On the Antimicrobial Potential of the Root of *Verbascum Blattaria*

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Abstract

Different solvent extracts of the root of *Verbascum blataria* were screened for the antimicrobial activity in the present study. Extracts of the root were prepared in different solvents and tested against fungal and bacterial species (*Klebsiella pneumoniae, Bacillus atrophaeus Pseudomonas aeruginosa, Citrobacter freundi, Salmonella typhi, Agrobacterium tumefaciens, Alternaria solani, Aspergillus fumigatus, Candida albicane,*). Antifungal and antibacterial assays were performed by well diffusion and disc diffusion methods. The screening of the different solvent extracted samples revealed that methanol extract was more effective against *B.subtillus, B.atrophus, A. tumefaciens, C. freundi, K. pneumoniae, A.solani,* and *A. fumigatus*. Methanol extract formed the maximum zone of inhibition of 67% ZI at 3000µg/disc against *B.atrophus* than other tested microorganism. The hexane extract produced a maximum zone of inhibition against *B.subtillus* (32% ZI), *E.coli* (54 % ZI) and *A. tumefaciens* (52% ZI) while the ethyl acetate extract reduced the growth of *P. aerugenosa* with high potential than other extracts.

Key words; *Plant extract, Microorganism, Extraction solvent, Antifungal activity*

Introduction

Medicinal flora has a potential health benefit and used in pharmaceutical and cosmetic industry worldwide (Chan et al., 2008). In addition to their use in conventional and traditional medicine, the medicinal plants have become part of modern and complementary medicine due to their good effect on health (Zanon et al., 1999). Different plant extracts are commonly used for the cure of infectious diseases (Rajbhandari et al., 2009). Verbascum is an important genus of medicinal flora and consist of 360 species which are commonly found in tropical and temperate areas (Chen et al., 2014). Verbascum species have been reported for different biological activities including anti-inflammatory, antioxidant, tumor suppressing, antiseptic, sedatives, expectorant and diuretic properties (Magiatis et al., 2001). Different bioactivities including antiviral, antifungal and antibacterial have also been reported from the different species of this genus (Rajbhandari et al., 2009; Sener & Dulger 2009; Magiatis et al., 2001). The plants included in this genus are the rich source of important bioactive compounds like alkaloid, saponin, flavonoids, phenolic acids, iridoids, steroids (Grigore et al., 2013). Among the reported species of *Verbascum*, *Verbascum blataria* is very important and used in traditional and conventional medicine (Turker & Gurel, 2005). The flowers and leaves of *Verbascum blataria* are used in folk medicine for the treatment of various respiratory disorders such as whopping cough, tuberculosis, bronchitis, whooping cough and dry cough (Magiatis, 2001). *Verbascum blataria* also described to be mild sedative and also having anti-inflammatory property (Turker & Camper, 2002). The plant extract has also been used to cure different ailments such as migraine, congestion, fever, pneumonia, and allergies as a domestic remedy (Muhammad et al., 2006). Due to excessive used of antimicrobial reagent, resulting in the development of resistance in both plants and human pathogen. This alarming situation has encouraged the researcher to seek new sources of an antimicrobial agent, and also to develop a standardized procedure for the extraction of these active agents (Cordell, 2000). In the current study, the impact of extraction solvent was investigated on antimicrobial activities of the root of *V. blataria*.

Material and Methods

Plant of *V. blataria* was collected from the different places in district Malakand, and taxonomic identification was confirmed by Prof Semen Jan at the Department of Botany, University of Peshawar. Roots were cut from the aerial parts of the plant body and washed with running tap water to remove sand and dust particles. The roots were then dried in shade and then put in the grinding machine to become powder.

Solvent, Chemicals, and Equipment

Nutrient agar, nutrient broth and Dimethylsulfoxide, (DMSO), nutrient broth, nutrient agar, potato dextrose, and potato broth media were supplied by Musaji Adam & sons. Ethanol hexane and methanol (Analytical grade) were used in extraction. The solution was dried by Rotary evaporator.

Preparation of Extracts

Sixty gram (60g) of root powder was weighted and poured into each extraction flask containing 600ml of the solvent. The flasks were placed in shaking incubator for five days. The solution was decanted and filter by Whatman filter paper into label bottles. Five hundred ml of the solvent was passed three times from solid residue. The solutions in bottles were then transferred into an evaporating flask of the rotary evaporator for drying. The semisolid extracts were prepared by a rotary evaporator, which was then kept in china dish for complete drying in a water bath.

Antimicrobial Activity

Potato dextrose, nutrient agar, and nutrient broth media were prepared according to manufacturer protocols. Well diffusion and disc diffusion methods were performed for testing anti-fungal and antibacterial activity of the extracts. Briefly, prepared nutrient agar plate was streaked with 50µl of standardized microbial culture (McFarland standard used). Through micropipette, three different concentration (1000µg, 2000µg, and 3000µg) were applied to each disc. For the antifungal assay, extracts of the different concentration (4000µg, 5000µg, and 6000µg/well) were poured in wells. Then a block of five days old culture was placed in the center of Petri plate containing potato dextrose agar media. The same procedures were also used for the antibiotic and antifungal solution. At the end of the experiment the zone of inhibition was measured and percent zone of inhibition was measured (Khan et al., 2017).

Statistical analysis

Mean ± standard deviation of the triplicate was calculated through Microsoft Excel 2010.

Results

Methanol extract formed 67% ZI against *B.atrophus* while the other extracts were less effective at 3000µg /disc (34% ZI by ethyl acetate and 21% ZI by hexane extracts). At 2000µg /disc, the extract reduced the growth of bacterium and produced 56% ZI shadowed by ethyl acetate extract (27% ZI) and hexane extract (9% ZI). Furthermore, the data also revealed that ethyl acetate formed 17%ZI while methanol extract produced 41% ZI at 1000µg /disc (Fig 1). Hexane extract was inactive against bacterium at 1000µg/disc and formed 0% zone of inhibition. The tested extracts also reduced the growth of *B.subtillus*. In the tested samples, the methanol and n-hexane extract formed an equal zone of inhibition (32% ZI), while ethyl acetate extract produced 35% ZI at 3000µg /disc (Fig 2). Methanol extract formed 31% ZI while n-hexane and ethyl acetate produced 27% and 34% ZI respectively at 2000µg/disc. Furthermore, 1000µg /disc of methanol extract measured 27% ZI followed by ethyl acetate (24% ZI) and hexane extracts (18% ZI). Similarly, hexane and methanol extract formed an equal zone of inhibition (52% & 50% ZI) at 3000 and 2000 µg/disc against *A. tumefaciens* while ethyl acetate produced 44% and 34% ZI at 2000 and 1000 µg/disc, respectively. Furthermore, methanol and hexane extract reduced the growth of bacterium by 37% and 44% as compared to control. Against *C. freundi*, the methanol extract was effective than other extracts (29% ZI by ethyl acetate and 12% by hexane extract) and formed 41% ZI at 3000µg /disc. Hexane extract was not effective against *C. freundi* at 2000µg /disc and 3000µg /disc while at the same concentrations ethyl acetate extracts and methanol extracts formed 28%, 18%, 38% and 28% ZI respectively (Fig 3). The growth of *P. aerugenosa* was reduced by ethyl acetate extract and formed 57% ZI shadowed by hexane (54% ZI) and methanol extract (43% ZI) at 3000µg /disc (Fig 4). The tested extracts also showed activity at 2000µg and 1000µg /disc. Ethyl acetate extract produced 53%ZI while hexane and methanol extract formed 44% and 37% ZI respectively, at 2000µg /disc. Methanol and hexane extract formed an equal zone of inhibition (37% ZI) while ethyl acetate extract produced 44% ZI at a concentration of 1000µg /disc. Furthermore, hexane extract was more effective than other extracts at 3000µg/disc and formed 54 % ZI against *E.coli* (43% ZI ethyl acetate and methanol extract) (Fig 5). Against *K. pneumonia*, methanol and ethyl acetate extracts were effective and formed 46% and 45% ZI respectively at 3000µg /disc. The tested extracts also reduced the growth of bacterium at 2000µg and 1000µg /disc. Methanol extract produced 37% and 29 % zone of inhibition while ethyl acetate and hexane extract revealed 40%, 35%, 32% and 28% at 2000µg mg/disc and 1000µg /disc respectively (Fig 6).The tested extracts were inactive against *A. fumigatus* and *A. niger* at the concentrations of 1000µg, 2000µg and 3000µg /well. However the growth of both fungi was inhibited at 4000, 5000 and 6000 µg /well by methanol and ethyl acetate extracts. N-hexane extract did not restrict fungal growth. Methanol and ethyl acetate extract formed 24% and 19% ZI respectively at 4000 µg/well against *A. fumigatus* while at the same concentration the growth of *A. niger* was reduced to 38% (ZI) by methanol and 32% (ZI) by ethyl acetate extract as compared to control (Figs 7-8). The concentration, when increased to 5000µg/well methanol extract, produced 29% ZI and ethyl acetate extract measured 21% ZI against *A. fumigatus* while at the same concentration ethyl acetate extract and methanol extract formed 33% and 40% ZI respectively, against *A.niger*. The finding of the study revealed that methanol extract formed 34% zone of inhibition against *A. fumigatus* shadowed by ethyl acetate extract (27% ZI) extract at 6000

µg/well. On the other hand, methanol and ethyl acetate extracts reduced the growth of *A.niger* by 42% and 34% respectively at 6000 µg/well. The tested extract of the root did not affect the growth of *C.albicans* and *T. rubrum*.

Discussion

Different and numerous bioactive compounds are present in plant species at different concentration showing the bioactive nature of the natural flora (Skrovankova et al., 2015). The isolation of compounds from the plant is based on the mode of extraction and solvent extraction system (Altemimi et al., 2017), (Khan et al., 2018). The current study demonstrates the effect of extraction solvent on the antimicrobial activity of the root of *V. blataria*. Hexane, methanol and ethyl acetate solvents extracts were tested against the bacterial and fungal pathogens. Results of the study demonstrated that the root extracts showed variation in the antimicrobial property against *K.pneumoniae, B.atrophaeus P.aeruginosa, C.freundi, S.typhi, A.tumefaciens, A. solani, A.fumigatus, C.albicane,*. Our finding supports the result of the previous studies which stated that *Verbascum* species are rich of bio active compounds with antimicrobial potential such as saponins, flavonoids, neolignan glycosides, iridoid glycosides and phenylethanoid (Tatli and Akdemir, 2006), (Alper and Basaran, 2009). The screening of the different solvent extracted samples revealed that methanol extract was more effective against *B.subtillus, B.atrophus, A. tumefaciens, C. freundi, K. pneumoniae, A.solani,* A. *fumigatus*. Ghasemi et al. (2015) also reported that the methanol extract of *V. thapsus* flower oil was effective against the *P. aeruginosa*, and *E. coli*. Similarly, the significant antimicrobial potential was also reported in the methanol extract of leaves against *Y.pestis, E coli, S aureus* and *B. cereus* (Prakash et al., 2016). Furthermore, antifungal activity against *A. niger* and *A. fumigatus* was also found in the methanol extract of the leaf (Khan et al., 2011). On the other hand, the hexane extract produced a maximum zone of inhibition against *B.subtillus*. *E.coli* and *A. tumefaciens* while the ethyl acetate extract reduced the growth of *P. aerugenosa* with high potential than other extracts. These findings suggest that methanol is the best solvent for the optimized extraction and recovery of antimicrobial substances from the root of *V. blataria*. Similar results were also reported in the previous studies (Lin et al., 1999; Ahmad et al., 1998; Eloff, 1998). *C.albicans, T. rubrum* and *S.typhi* were resistant to the tested extracts. The difference in susceptibility among the microbes against the bioactive agents present in plant extract may be attributed to the difference in composition of the cell wall and/or the presence of antimicrobial resistance genes in their plasmids.

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Statement of conflict of interest.
All the authors of manuscript are unanimous in submitting the research paper to journal and there is no conflict of interest.

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