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ANTI-BACTERIAL ACTIVITIES OF *ZATORIAMULTIFLORA* AND *VALERIANAWALLICHII* CRUDE EXTRACTS AND ESSENTIAL OIL

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Abstract:
Zatoriamultiflora (Family) and Valerianawallichii (Family)both are wild herb and used in Unani system of medicine.Phytochemicals present in medicinal plant increase their usage so, that's why widely used all over the world. The present study aimed to determine minimum and maximum reduction in colony growth of the crude extract and essential oil of aerial parts of Zatoria multiflora and roots of Valerianawallichiito control Shigellaboydii, Escherichia coli and Bacillus Subtilis. The crude extract and essential oil was tested in vitro with twelve concentrations: 0.5. 1.0, 1.5, 2.0, 2.5, and 3.0gm for crude extract and 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6ml for essential oil plus control using potato dextrose agar medium.Crude extract and essential oil of both plants showed significant antibacterial activity. The result showed with these evaluations, reduction in colonial growth was calculated by CFU method.The maximum reduction in colonial growth was up to 0.00mm at 0.6ml/LPDA dose of Zatoria multiflora essential oil against Shigellaboydii. While minimum reduction in colony growth noticed was 23mm at 0.5gm/PDA dose of Valerianawallichii against Shigellaboydii.

Keywords: *Zatoriamultiflora, Valerianawallichii, Crude extract, Essential oil, antibacterial activity.*

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INTRODUCTION:

Plants are the vital source of medicines for hundreds of years. Per WHO, more than 80% of peoples still depends on herbs for medicinal purpose [01].Naturally occurring antioxidants and antimicrobials paid attention to explore now-a-days because synthetic chemical additives has great demand for foodstuffs.

Zatoria multifora" Boiss belonging to the family "*Lamiaceae"* to which many plants belong, known as Avishan-e-Shirazi (in Iran), and SAATAR, AZGHIND (in Pakistan) is an expedient plant [02,03,04,05].*Zatoria multiflora* is wooded, leathery roots leave of *Z. multiflora* are tiny, fine, oval and greenish grey in color [06]. It is first described in 1876 [07]The height of this plant is about 40-80cm with thin, several branched stems. *ZM* contains four stamens and the corolla which is little higher than calyx that is white in color [08].*Zatoria multiflora* is mostly found in warm region of southern and central part of Iran. In Pakistan, it is widely distributed in southwestern part (specially in Baluchistan province) [09].The major chemical constituents of *ZM* are thymol and carvacrol [10]. The present studies the methanolic extract and essential oil of the plant were examined forits activity against bacteria.

Valeriana wallichii is such an important plant contains medicinal properties. This plant is a small 14-45cm in height and is perennial herb [11].Valeriana wallichii is cultivated like an ornamental plant [12,13]. The rhizome of the plant is short and bitter in taste, stems are 30-150 cm, leaves are usually pinnate with 3-25 leaflets that are linear and the flower asexual and white and pink and 2-7 cm long. The fruit is 2-5 mm long; hairy Leaves are 2.5-3000 cm long and thick horizontal root stick with 40-50cm height of herb of this plant[14]. The chemicals which are present in Valeriana jatamansi are, Chlorogenic Acid, Valerenic Acid (Sesquiterpenoids), Caffeic Acid, Valerosidatum, Baldrinal, Valepotriates (Iridiod Esters) [15].Different type of skin diseases, liver protection, snake poisoning, wound healing, obesity, sleep improvement and antispasmodic are some traditional uses of *Valeriana wallichii* [16].It was also used in First World War due to its tranquillizer action for the treatment of the soldiers [17].The present studies of plant included to examine antibacterial activity. Further, crude extract and essential oil of the plant showed antibacterial activity due to the presence of volatile oils.

MATERIALS AND METHODS:

The aerial parts of the plant *Z. multiflora*were collected at flowering stage from HazargangiNational Park Quetta and the roots of *V. wallichii* was collected from the local market. These plants were identified by the help of local people and then taxonomic identity was confirmed by Department of Botany University of Baluchistan Quetta. Both plants material was washed with tape water and then dried to protect these from direct exposure of sunlight. Dried under shad to protect the form dirt ex Bothplants were grinded to make powder. Thendehydrated powdered materials were kept in air tight bottles.

Preparation of extracts

100 grams of plant dehydrated powder was dissolved in 80% methanol for 10 days at room temperature 25 ±30.after that extract were filtered by using what man (No. 2) filter paper. The filtrates were kept on rotary evaporator for 2 days. For analyzing and testing, then methanolic extracts were kept in glass tubes [18].

Preparation of essential oils:

Plant samples were used for extraction of essential oil to steam distillation by using Clevenger type apparatus about 4 hours. For testing and analyzing, the obtained essential oil was stored at about 4 ℃.

Test microorganisms

The three bacterial strains and three fungal strains were used in this study. And these strains obtained from Quetta lab. The bacterial strain used were *Shigellaboydii, Escherichia coli and Bacillus Subtilis.*

Antibacterial activity of plant samples

Per APHA (American Public Health Association) different bacterial strains were analyzed by using Potato Dextrose Agar (PDA).

For crude extract, 0.5gm/LPDA, 1.0gm/LPDA, 1.5gm/LPDA, 2.0gm/LPDA,2.5gm/LPDA, 3.0gm/LPDA and Control.For essential oil,0.1ml/LPDA,0.2ml/LPDA, 3ml/LPDA, 0.4ml/LPDA, 0.5ml/L PDA, 0.6ml/LPDA and Control, different concentrations were prepared.

All the microbial strains were inoculated on PDA slants with the help of sterilized inoculating loop at 35 ^{0}C for 72 hours. All the process was done in a Laminar air flow cabinet to avoid contamination. These petri dishes were labeled. The plates were left in an incubator (for the better growth of microbes).Antibacterial activity of both plant extract and essential oil was documented based on linear colony growth. And linear colony forming growth of microbes was determined by CFU (colony forming unit) measurements.

RESULTS:

EO's and crude extracts of *Z. multiflora*and *V. wallichii* showed significant effect against bacteria at doses 0.5gm/LPD, 1.0gm/LPDA, 1.5gm/LPDA, 2.0gm/LPDA, 2.5gm/LPDA and 3.0gm/LPDA for crude extract; and 0.1ml/LPDA, 0.2ml/LPDA, 0.3ml/LPDA, 0.4ml/LPDA and 0.5ml/LPDA for essential oil. As 0.6ml/Liter Potato Dextrose Agar of Z. multifloraessentional oil (table no; 03) showed excellent reductionthan those of other doses of essential oil,0.1ml/LPDA, 0.2ml/LPDA, 0.3ml/LPDA, 0.4ml/LPDA and0.5ml/LPDA has showed closest anti-bacterial efficacy to 0.6ml/LPDA. All dosages have presented antibacterial efficacy as related to control. However maximum reduction of colonial growth was shown by essential oil of *Z. multiflora* against bacteria where CFU calculation recorded up to 0.00mm at 0.6 ml/Liter Potato Dextrose Agar dose as showed in table no. 03. Moreover, the PDA control showed about 25mm against bacteria.

Table 1: Efficacy of *Z. multiflora* crude extract against bacteria

Dose/lit PDA	*Shigellaboydii*	*E. coli*	*Bacillus subtillis*
	C/growth mm	C/growth mm	C/growth mm
0.5gms	22_{b}	22_{aa}	18_{b}
1gms	16_{b}	22_{ab}	12_{c}
1.5gms	10_{c}	14_{b}	8_{d}
2gms	8_{c}	9_{c}	4_{e}
2.5gms	2_{da}	3_{da}	0_{fa}
3gms	0_{db}	3_{db}	0_{fb}
Control	25_{a}	24_{a}	25_{a}
LSD	**2.06**	**1.92**	**1.7**

Table 1 shows the efficacy of Z. multiflora crude extract against three different strains of bacteria such as Shigella*boydii, E. coli and Bacillus* subtillis by using PDA medium with different doeses. All the doses showed antibacterial activity. While, CFU calculations estimates that miximum reduction in colonial growth was observed in *Bacillus subtillis*that is up to 0.00mm at dose 2.5gm/LPDA and 3.0gm/LPDA. And minimum reduction in colonial growth was observed in *E. coli* that was 22mm at dose 0.5gm/LPDA.

Table 2 shows the efficacy of *V. wallichii*crude extract against three different strains of bacteria such as Shigella*boydii, E. coli and Bacillus* subtillis by using PDA medium with different doses. All the doses showed antibacterial activity. While, CFU calculations estimates that maximum reduction in colonial growth was observed in *Bacillus subtillis*that is up to 0.00mm at dose 3.0gm/LPDA and 3.0gm/LPDA. And minimum reduction in colonial growth was observed in *Shigellaboydii* that was 23mm at dose 0.5gm/LPDA.

Table 2: Efficacy of *V. wallichii* crude extract against bacteria

Dose/lit PDA	*Shigellaboydii*	*E. coli*	*Bacillus subtillis*
	C/growth mm	C/growth mm	C/growth mm
0.5gm	23_{ab}	21_{aa}	22_{ab}
1gm	18_{b}	19_{ab}	17_{b}
1.5gm	14_{ca}	17_{ac}	7_{ca}
2gms	12_{cb}	15_{ad}	6_{cb}
2.5gms	5_{da}	9_{ba}	4_{cd}
3gms	4_{db}	7_{bb}	2_{ce}
Control	25_{aa}	24_{a}	23_{aa}
LSD	3.50	2.67	2.78

Table 3: Antibacterial activity of *Z. multiflora* essential oil

Dose/lit PDA	*Shigellaboydii*	*E. coli*	*Bacillus subtillis*
	C/growth mm	C/growth mm	C/growth mm
0.1ml	7_{b}	6_{ba}	5_{ba}
0.2ml	2_{ca}	7_{bb}	4_{bb}
0.3ml	1_{cb}	3_{c}	2_{ca}
0.4ml	1_{cc}	1_{da}	1_{cb}
0.5ml	1_{cd}	0_{db}	0_{cc}
0.6ml	0_{ce}	0_{dc}	0_{cd}
Control	26_{a}	25_{a}	23_{a}
LSD	**4.8**	**1.66**	**2.21**

Table 4: Antibacterial activity of *V. wallichii* essential oil

Dose/lit PDA	*Shigellaboydii*	*E. coli*	*Bacillus subtillis*
	C/growth mm	C/growth mm	C/growth mm
0.1ml	6_{ba}	13_{ba}	10_{ba}
0.2ml	6_{bb}	11_{bb}	9_{bb}
0.3ml	2_{ca}	7_{c}	7_{bc}
0.4ml	1_{cb}	4_{da}	4_{c}
0.5ml	0_{cc}	2_{db}	1_{da}
0.6ml	0_{cd}	1_{dc}	0_{db}
Control	25_{a}	25_{a}	24_{a}
LSD	**1.92**	**2.75**	**2.23**

Table 3 shows the efficacy of *Z. multiflora* essential oil against three different strains of bacteria such as Shigella*boydii, E. coli and Bacillus* subtillis by using PDA medium with different doeses. All the doses showed antibacterial activity. While, CFU calculations estimates that miximum reduction in colonial growth was observed in *Bacillus subtillis*that is up to 0.00mm at dose 0.5ml/LPDA and 0.6ml/LPDA. And minimum reduction in colonial growth was observed in *Shigellaboydii*that was 07mm at dose 0.1ml/LPDA

Table 4 shows the efficacy of *V. wallichii*essential oil against three different strains of bacteria such as Shigella*boydii, E. coli and Bacillus* subtillis by using PDA medium with different doses. All the doses showed antibacterial activity. While, CFU calculations estimates that maximum reduction in colonial growth was observed in *Shigellaboydii* that is up to 0.00mm at dose 0.5ml/LPDA and 0.6ml/LPDA. And minimum reduction in colonial growth was observed in *E. coli* that was 13mm at dose 0.1ml/LPDA.

Statistical analysis

The experiment was carried in triplicate; the result was in mean ± standard deviation. all experiment data was statically analyzed by using standard ANOVA with significance > 0.05 probability level by using RCBD model.

DISCUSSION:

In our study, crude extracts and essential oils of Zataria multiflora and Valeriana wallichii at a dose showed antibacterial activity.

Zataria multiflora essential oil chemical composition has reported. Essential oils composition may depend on geographic region from which they have been collected [19,20].

Several pharmacological and biological properties have been shown by Zataria multifora. It also acts as an antimicrobial agent in food industry [21,22] and acts as anesthetic and antispasmodic agent 14. Different studies shown that Thymol present in Zataria multiflora, acts on microbial cell membrane [23,24].

Motevasel et al., showed that the growth of Gram

positive and Gram negative bacteria could be inhibited by alcoholic extracts of Zataria multiflora [25]. Further, Eftekhar also reported that standard cultures of bacteria can be inhibited by in vitro study of Zataria multiflora essential oil, and this activity was recorded due to the presence of Carvacrol and Thymol [26].

Further studies of Valerian wallichii presented that different extracts of this plant presentedthoroughgoing inhibition zone against all microbial strains in cup plate method [27]. Katoch reported that anti-leishmanial effect was shown by different root extracts of Valeriana wallichii [11].Moreover, in our study all the extracts and essential oilsof both plants showed activity against different bacterial strains. But essential oil of Zataria multiflora essential oil showed excellent reduction up to0.00mm colony growth at 0.6ml/Liter Potato Dextrose Agar dose.

CONCLUSION:

The antibacterial activity of *Zataria multiflora* and *Valeriana wallichii* showed significant results as compared with control. Therefore, it has been concluded from above mentioned results that the *Zataria multiflora* and *Valeriana wallichii* exhibits antibacterial activity.

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