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Antimicrobial Screening of Viscum Album L. Extracts

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***Abstract*—The leaves and twigs of Viscum album L. (Family: Loranthaceae) were extracted successively with various organic solvents and water. These crude extracts were assessed for antimicrobial activities against three Gram positive bacteria, five Gram negative bacteria, one yeast and one fungus by using disc diffusion method. The Ethylacetate, chloroform, ethanol, and methanol crude extracts of selected plant parts had significant antimicrobial activities on both Gram positive and Gram negative bacteria. The Ethylacetate and methanol crude extracts of leaves and twigs of Viscum album exhibited prominent activities against Gram positive and Gram negative bacteria used in comparison to other extracts which had moderate activity against all the tested bacteria. The antimicrobial activities of the crude extracts of the selected plant parts were more active against Gram negative bacteria than Gram positive bacteria. The standard reference antibiotics, Ciprofloxacin (100µm/ml) and Nystatin (1500u/ml) were used as positive control.**

I. Introduction

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. They have been used as a source of medicine. In fact, plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times [1]. Over 50% of all modern clinical drugs are of natural product origin [2] and natural products play an important role in drug development programs in the pharmaceutical industry [3].There has been a revival of interest in herbal medicines. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom. Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world [4,5,6]. Much work has been done on ethnomedicinal plants in India [7]. Interest in a large number of traditional natural products has increased [8]. These reports are restricted to mainly a few medicinal plant species. Most of them require a detailed study particularly with regard to the antimicrobial properties, so that in future they can effectively replace the chemically synthesized antibiotics which have a large number of side effects. The aim of the present study is to determine the chemical constituents and antimicrobial activity of *Viscum album*.

II. Materials and Methods

Viscum album belongs to family Loranthaceae commonly known, as marine are large, epiphytic profusely branching shrubby perennial, forming rounded clumps over the host trees. It is green all over: branches dichotomous or whorled [9]. The plant material was collected from Rialy, Muzaffarabad Azad Jammu and Kashmir. The whole plant was withdrawn and dried carefully under shade and then homogenized to fine powder and stored in airtight bottles.

III. Aqueous Extracts

50 grams of ground plant parts material was extracted successively with distilled water in soxhlet extraction apparatus [10]. All these extracts were collected separately and each extract was dried in vacuum rotary evaporator under reduced pressure and low temperature i.e. 70°c. The last traces of the water were evaporated at water bath, which was used as a source of heat [11].

IV. Organic Solvent Extraction

The 25grams portions of each dried powdered plant part material was soaked separately in 250ml petroleum ether, acetone, ethyl acetate, chloroform, ethanol and methanol. The extraction was carried out by maceration for 7 days in each solvent at room temperature (25±2°C). The solvents extracted material was filtered in separate flaks [11]. All extracts were then dried in a vacuum rotary evaporator, weighed and stored at 4°C until further analysis.

V. Preparation of Dilution

The dried aqueous, methanol, ethanol, petroleum ether, acetone, ethylacetate, and chloroform extracts were then dissolved in their respective solvents in a proportion of 100mg/ml. The concentration of reference antibiotics i.e. Ciprofloxacin was 100µg/ml and Nystatin 1500u/ml.

VI. Microorganisms Used

In the present study Gram positive bacteria i.e. Staphylococcus aureus, Bacillus subtilis, Enterococcus faecium, Gram negative bacteria i.e. Escherichia coli,

Bordetella bronchisiptica, Salmonella typhi,Pseudomonas aeruginosa, Pseudomonas syringae, yeast i.e. Saccharomyces cerevisiae and fungus Aspergillus flavus, were used to evaluate the antimicrobial potential of different extracts of the selected plant parts.

VII. ANTIMICROBIAL ASSAY

A 24 hours old culture of each bacterium and 72 hours old culture of fungus was used as an inoculum for the test. The slants were prepared in test tube. The nutrient agar medium was used for bacterial growth and similarly for fungus the sabouraud's dextrose agar medium was used. In vitro antimicrobial screening was performed by disc diffusion method as described by Vander and Vlientnck [12]. The sterilized nutrient agar medium when temperature reached between 40 and 45°C was poured in the petri dishes containing bacterial suspension. The sabouraud's dextrose agar medium was poured in the petri dishes containing fungal suspension. Two series of experiments were conducted. In first crude extracts were tested for their antimicrobial activity against already mentioned bacteria, yeast and fungus. In the second series of experiment, antibiotic discs were prepared from the dilution of commercially available standard reference antibiotics i.e. Ciprofloxacin and Nystatin were placed on the top of the medium in the centre of petri dishes by following the disc diffusion method [12]. The purpose of this experimental set was to compare the antimicrobial activity of the standard reference antibiotics with that of the solvent extracts of leaves and twigs of *Viscum album*. The plates containing bacterial culture were incubated at 37°C for 24 hours. On the other hand, the plates with fungal suspension were incubated as 25°C for 72 hours. After the incubation time, all the plates were examined for the presence of inhibition as a property of antimicrobial activity.

VIII. STATISTICAL ANALYSIS

All values were expressed as means ±standard error means. The data for each microorganism were analyzed by using one way analysis of variance (ANOVA) technique and means were compared by using LSD at 5% (0.05) probability level [13].

IX. RESULT AND DISCUSSION

Plant extracts have been studied against bacteria for years, but in a more intensified way in the last three decades. During this period, a lot of antimicrobial screening evaluations have been published based on the traditional use of Chinese, African and Asian plant drugs [14,15]. Reinsch carried out the first investigations into the chemical content of mistletoe in 1860 [16]. In recent years, although technology and medicine have developed extensively, due to the decrease in natural richness made it obligatory to use natural products for many goals. For these reasons, like in other countries, in Pakistan, *Viscum album* L. is used for the treatment of various diseases.

In this study, the antimicrobial influence of acetone, petroleum ether, ethylacetate, chloroform, ethanol, methanol and water crude extracts of *Viscum album* leaves and twigs were determined. This plant is known to have healing properties and is used for the treatment of various diseases in people. The results of the antimicrobial screening of different solvents crude extracts of leaves and twigs of *Viscum album* against 8 bacteria, 1yeast and 1fungus were presented in tables1 and 2. The statistical analyses of results have been tabulated in tables' 3, and 4. The results of spectral studies of compounds were also described.

According to investigation of Deliorman et al., [17] the samples prepared from Viscum album subspecies growing in Turkey showed antimicrobial activity against microorganisms tested. In this study acetone and petroleum ether extracts of the selected plant parts showed no activity against all the microorganisms tested (table 1 and 2).

It is evident from the results that the ethylacetate extract of leaves of Viscum album showed prominent activity against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Bordetella bronchisiptica, Pseudomonas aeruginosa, and Pseudomonas syringae, i.e. 24.33±0.7mm, 19.66±0.33mm, 24.96±0.23mm, 24.83±0.17mm, 19.66±0.33mm and 19.66±0.33mm respectively while ethyl acetate extract of leaves showed no activity against Enterococcus faecium, Salmonella typhae, Saccharomyces cerevisiae and Aspergillus flavus (table 1). The ethylacetate extract of twigs of selected plant also represented appreciable activity against Staphylococcus aureus, Bacillus subtilis, Enterococcus faecium, Escherichia coli, Bordetella bronchisiptica, Pseudomonas aeruginosa, Pseudomonas syringae, and Salmonella typhae. Which were 15.83±0.17mm, 15.06±0.06mm, 20.06±0.6mm, 24.83±0.17mm, 15.06±0.03mm, 19.66±0.33mm, 22.66±0.33mm and 15.06±0.06mm respectively but Saccharomyces cerevisiae and Aspergillus flavus showed no sensitivity (table 2). This confirms previous report by Eloff et al., [18] in which they suggested that ethyl acetate was the best extractant with an average minimum inhibitory concentration.

The chloroform extract of leaves showed activity against Escherichia coli (19.76±0.23mm), Pseudomonas aeruginosa (10.00±0.00mm) Pseudomonas syringae (15.16±0.16mm) Staphylococcus aureus, Bacillus subtilis, Bordetella bronchisiptica, Salmonella typhae, Saccharomyces cerevisiae and Aspergillus flavus were not affected by the extract (table1). The chloroform extract of twigs of Viscum album exhibited moderate activity against all the microorganisms used except Saccharomyces cerevisiae and Aspergillus flavus. The mean diameter of zones of inhibition of the extract against Staphylococcus aureus, Bacillus subtilis, Enterococcus faecium, Escherichia coli, Bordetella bronchisiptica, Pseudomonas aeruginosa, Pseudomonas syringae, and Salmonella typhae were 10.00±0.00mm, 9.66±0.33mm, 9.66±0.33mm, 20.06±0.06mm, 10.06±0.06mm, 15.1±0.1mm, 9.66±0.33mm and 9.66±0.33mm respectively (table 2). These results were also comparable with the previous studies of Farjana et al., [19] in which they explained that the chloroform extract showed comparatively more in vitro antimicrobial activity against some bacteria.

Pokhrel et al., [20] described that the alcoholic extract of Bauhinia variegate was found to have antimicrobial activity against Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhae, Shiegella dysenteriae, Staphylococcus aureus, and Vibrio cholerae. The largest zone of inhibition was found to be exhibited against B. subtilis. The extract was found to be more effective against Gram positive than Gram negative bacteria. In present study it was observed that the ethanolic extracts of leaves and twigs of Viscum album exhibited moderate activity against all the bacteria tested. The Saccharomyces cerevisiae and Aspergillus flavus were resistant to the extracts (table1 and2).

Pokhrel et al., [21] described that antimicrobial activity of methanolic extracts of different parts of Tribulus terrestris was evaluated against four bacteria by broth dilution assay and agar diffusion assay. The methanolic extracts of all parts of the plant showed considerable activity against all bacteria but in this study methanolic extracts of leaves and twigs also exhibited considerable activity against all the bacteria used. The largest zones of inhibition produced by the leaves extract against Enterococcus faecium, Pseudomonas aeruginosa and Pseudomonas syringae were 19.83±0.17, 19.83±0.17, and 20.93±0.06 respectively. The largest zones of inhibition produced by methanolic extract of twigs against Staphylococcus aureus and Escherichia coli were 19.83±0.16 and 24.83±0.17 respectively. Saccharomyces cerevisiae and Aspergillus flavus were not influenced by the extracts of leaves and twigs (table1and 2).

The water extract of leaves represented less activity against Staphylococcus aureus, Bacillus subtilis, Enterococcus faecium, Escherichia coli, Bordetella bronchisiptica, Pseudomonas aeruginosa, Pseudomonas syringae, and Salmonella typhae i.e. 9.66±0.13mm, 10.66±0.33mm, 9.83±0.17mm, 9.16±0.17mm, 10.00±0.00mm, 9.9±0.1mm, 9.83±0.17mm and 9.00±0.00mm respectively but Saccharomyces cerevisiae and Aspergillus flavus were resistant to the extract. The water extract of twigs had no activity against all the microorganisms tested (table 1, 2).

The antibiotic ciprofloxacin showed high activity against all the microorganisms used except Saccharomyces cerevisiae and Aspergillus flavus, which were resistant to the ciprofloxacin. The mean diameter of zones of inhibition against Staphylococcus aureus, Bacillus subtilis, Enterococcus faecium, Escherichia coli, Bordetella bronchisiptica, Pseudomonas aeruginosa, Pseudomonas syringae, Salmonella typhae were mentioned in table no. 1. The Nystatin exhibited activity against yeast and fungus tested while all the bacteria were resistant to Nystatin (table 1). Avato et al., [21] reported that extracts from Bellis perennis have a high antimicrobial activity against bacteria than fungus. The results of Zavala et al., [21] were similar to ours. They showed that extracts from some plants have high activity against bacteria than yeast and fungus. On the other hand the antimicrobial activity against Gram negative bacteria was more effective than Gram positive bacteria (table1 and 2).

Antimicrobial activity of leaves of *Viscum album* L.

TABLE I. CONCENTRATION OF CRUDE EXTRACTS 100MG/ML, CIPROFLOXACIN 100µG/ML AND NYSTATIN 1500U/ML

Mean diameter of zones of inhibition (mm) ± standard error of mean (S. E. M.)										
S.No.	Microorganisms	AC	*1)* E	EA	CH	ET	MT	WT	CF	NS
1	S. aureus	---	---	24.33±0.17	---	15.9±0.35	15.16±0.08	9.66±0.13	32.13±0.13	---
2	B. subtilis	---	---	19.66±0.33	---	15.83±0.16	15.83±0.17	10.66±0.33	31.93±0.06	---
3	E. faecium	---	---	---	---	15.9±0.1	19.83±0.17	9.83±0.17	30.03±0.03	---
4	E.coli	---	---	24.96±0.23	19.76±0.23	16.66±0.33	16.93±0.06	9.16±0.17	31.83±0.17	---
5	B. bronchisiptica	---	---	24.83±0.17	---	16.9±0.2	16.13±0.06	10.00±0.00	31.5±0.1	---
6	P. aeruginosa	---	---	19.66±0.33	10.00±0.00	13.83±0.16	19.83±0.17	9.9±0.1	29.83±0.17	---
7	P. syringae	---	---	19.66±0.33	15.16±0.16	13.66±0.33	20.93±0.06	9.83±0.17	30.83±0.17	---
8	S. typhae	---	---	---	---	15.83±0.17	15.26±0.13	9.00±0.00	29.93±0.06	---
9	S. cerevisiae	---	---	---	---	---	---	---	---	23.00
10	A. flavus	---	---	---	---	---	---	---	---	15.00

Key:AC. Acetone, PE. Petroleum ether, EA. Ethylacetate, CH. Chloroform, ET. Ethanol, MT. Methanol, WT. Water, CP. Ciprofloxacin, NS. Nystatin

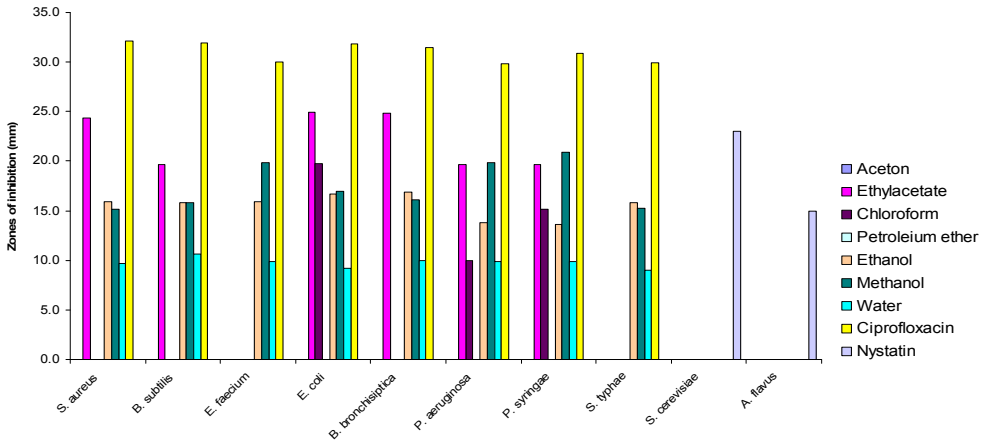

Fig.2. Antimicrobial activity of leaves of *Viscum album* L. (Zones of inhition mm)

TABLE II. ANTIMICROBIAL ACTIVITY OF TWIGS OF *VISCUM ALBUM* L.CONCENTRATION OF CRUDE EXTRACTS 100MG/ML

Mean diameter of zones of inhibition (mm) ± standard error of mean (S. E. M.)								
S.No.	Microorganisms	Acetone	*2)* Petro leum ether	Ethyl acetate	Chloroform	Ethanol	Methanol	Water
1	S. aureus	---	---	15.83±0.17	10.00±0.00	11.67±0.33	19.83±0.16	---
2	B. subtilis	---	---	15.06±0.06	9.66±0.33	11.83±0.17	19.9±0.1	---
3	E. faecium	---	---	20.06±0.06	9.66±0.33	12.00±0.00	15.03±0.3	---
4	E.coli	---	---	24.83±0.17	20.06±0.06	14.83±0.17	24.83±0.17	---
5	B. bronchisiptica	---	---	15.06±0.03	10.06±0.06	10.66±0.33	14.83±0.17	---
6	P. aeruginosa	---	---	19.66±0.33	15.1±01	12.76±0.23	17.03±0.03	---
7	P. syringae	---	---	22.66±0.33	9.66±0.33	11.66±0.33	15.83±0.17	---
8	S. typhae	---	---	15.06±0.06	9.66±0.33	9.06±0.06	10.06±0.06	---
9	S. cerevisiae	---	---	---	---	---	---	---
10	A. flavus	---	---	---	---	---	---	---

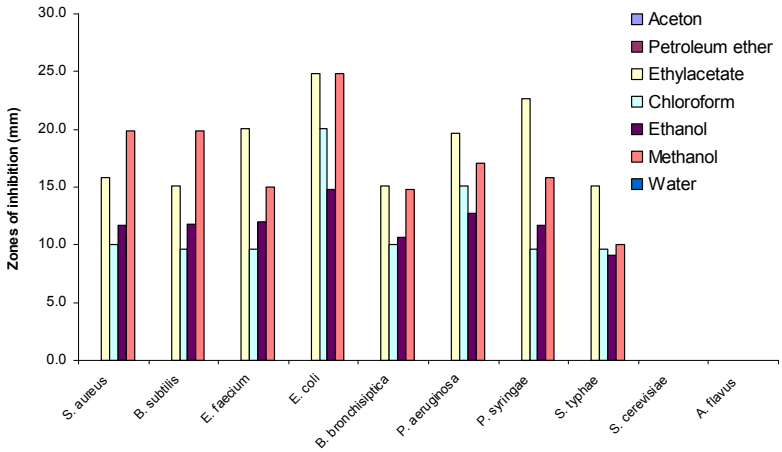

Fig.1. Antimicrobial activity of twigs of *Viscum album* L. (zones of inhibition mm)

TABLE III. ANTIMICROBIAL ACTIVITY OF LEAVES OF *VISCUM ALBUM* L. LEAST SIGNIFICANT DIFFERENCE (LSD)

Concentration of extracts 100mg/ml, Ciprofloxacin 100µg/ml and Nystatin 1500u/ml								
S.No.	Microorganisms	Ethyl acetate	Chloroform	Ethanol	Methanol	Water	Ciprofloxacin	Nystatin
1	S. aureus	24.33 b	0.00 e	15.9 c	15.16 c	9.66 d	32.13 a	---
2	B. subtilis	19.66 b	0.0023 e	15.83 c	15.83 c	10.66 d	31.93 a	---
3	E. faecium	0.023 e	0.023 e	15.9 c	19.83 b	9.83 d	30.03 a	---
4	E.coli	24.96 b	19.76 c	16.66 d	16.93 d	9.16 e	31.83 a	---
5	B. bronchisiptica	24.83 b	0.023 f	16.9 c	16.13 d	10.00 e	31.5 a	---
6	P. aeruginosa	19.66 b	10.00 d	13.83 c	19.83 b	9.9 d	29.83 a	---
7	P. syringae	19.66 c	15.16 d	13.66 e	20.93 b	9.83 f	30.83 a	---
8	S. typhae	0.023 e	0.023 e	15.83 b	15.26 c	9.00 d	29.93 a	---
9	S. cerevisiae	---	---	---	---	---	---	23.00
10	A. flavus	---	---	---	---	---	---	15.00

TABLE IV. ANTIMICROBIAL ACTIVITY OF TWIGS OF *VISCUM ALBUM* L. LEAST SIGNIFICANT DIFFERENCE (LSD)

Concentration of extracts 100mg/ml and Ciprofloxacin 100µg/ml						
S.No.	Microorganisms	Ethyl acetate	Chloroform	Ethanol	Methanol	Ciprofloxacin
1	S. aureus	15.83 c	10.00 e	11.66 d	19.83 b	32.13 a
2	B. subtilis	15.06 c	9.66 e	11.83 d	19.9 b	31.93 a
3	E. faecium	20.06 b	9.66 e	12.00 d	15.03 c	30.03 a
4	E.coli	24.83 b	20.06 c	14.83 d	24.83 b	31.83 a
5	B. bronchisiptica	15.06 b	10.06 d	10.66 c	14.83 b	31.5 a
6	P. aeruginosa	19.66 b	15.1 d	12.76 e	17.03 c	29.83 a
7	P. syringae	22.66 b	9.66 e	11.66 d	15.83 c	30.83 a
8	S. typhae	15.06 b	9.66 c	9.06 d	10.06 c	29.93 a
9	S. cerevisiae	---	---	---	---	
10	A. flavus	---	---	---	---	

X. CONCLUSION

On the basis of present investigations it is concluded that there exists a great potential in the search of new and more potent antimicrobial substances from the natural sources. The potential for developing antimicrobials from plants appears rewarding as it will lead to the development of phytomedicines to act against microbes. Plant based antimicrobials have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. The tested drugs (organic solvents extracts of leaves and twigs of *Viscum album*) showed excellent antimicrobial activity against tested bacteria. So it can be concluded that leaves and twigs of the selected plant can be regarded as good natural antibiotics with considerable degree of antimicrobial activity.

As a consequence of this study, we will try to isolate pure compound, which is present in fractions showing large inhibitory activity to bacteria as well as any pharmacological or toxicological properties that such compound might have.

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