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INVESTIGATION OF ANTIMICROBIAL ACTIVITY OF SOME SELECTED WEED SPECIES

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ABSTRACT

Medicinal plant species are more important because they are dependable sources for the curing of various ailments. The medicinal value of plant species increase due to the presence of chemical constituents which cause different pharmacological action on human body. The methanol leaf extract of Ziziphus jujuba, Withania coagulens, Tinospora cordifolia, Abutilon indicum and Acacia modesta showed an active antibacterial activity against Escherichia coli, Pseudomonas fluorescence, Bacillus subtilis, Staphylococcus aureus, Xanthomonas axonopodis and X. malvacearum. Alternatively the extract of plants also shows a significant antifungal activity against Aspergillus flavus, Drechslera turcica and Fusarium verticillioides as compared to bark and root extracts. The extract obtained from the leaf of Abutilon indicum and Acacia modesta showed a significant antibacterial activity against B. subtilis while the leaf extract which is obtained from the Z. jujuba showed an awesome result against X. axonopodis and X. malvacearum. The extract is also obtained from other parts than leaf i.e. including root and stem. The extract from the leaf and root of Abutilon indicum showed its antibacterial activity against all the test bacteria. The leaf and bark extracts of A. modesta showed a progressive result against fungal activity of A. flavus. Beside A. Modesta, the Tinospora cordifolia and Z. jujuba showed active activity against fungi while the methanolic extract of Abutilon indicum showed an important antifungal activity against F. verticillioides.

Key words: Antimicrobial activities, medicinal plants, weed species.

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INTRODUCTION

The utilization of the medicinal plants against many severe diseases in past is still widely used in ethnomedicines around the world. The capability of higher plant species is still largely unexplored as a source for drugs (Clark, 1996). An estimated data of plant species is about 245000-480000, out of these huge amounts of the plants only a very small percentage has been reported for phytochemicals and also their antimicrobial activity as well. The plant especially medicinal one is an awesome source of antimicrobial agents. Medicinal plants are used in many countries having great influence and also take a vital role in making power full drugs. A large quantity of medicinal plants parts is utilized for extract as a raw drug which has also importance and possesses variable medicinal properties. The important parts which are used include root, stem, flower, fruit etc. While, their collection also is made in very small quantity by the local community (Cunningham, 2001). Although a large number of plant species have been investigated for their chemical and antimicrobial activity, a very precise investigation was undertaken to notice the native flora for antifungal activity and also for antibacterial activity. The investigation give us analysis about plants chemicals which taken a vital role in the antimicrobial activity. The members of few families were notable and there specie are reported in the survey. *W. coagulens, Acacia modesta, Tinospora cordifolia, A. indicum* and *Z. jujuba*. The bark is very important, Decoction of bark is obtained which is used as gargle and pods in urino-genital disease. *A. indicum* belong to Malvaceae their root is very important and used in nervous disorder, coryza, and cardiac disease. While the stem of the wild species that is *Tinospora cordifolia* which belong to family Malvaceae is used as important ingredient. Root is a strong emetic i.e. induces nausea and vomiting. Its watery extract used in leprosy. *W. coagulens* which belongs to family Solanaceae is used in inflammatory condition and ulcers as well. It is also used in the cough, dropsy, hiccup etc. *Ziziphus jujuba* belongs to family Rhamnaceae which is also very important. The honey of *Ziziphus* is very famous and used for many diseases (Adams *et al*., 2007). Besides, the fruits are used as anodyne, tonic and also for chest infection. The seeds also play an important role in diarrhoea (Arndt *et al*., 2001).

MATERIALS AND METHODS

Developments and maintenance of test microorganisms for antimicrobial studies

The culture of bacteria is well established with essential nutrient for the growth of bacteria like *Escherichia coli* (*E. coli*), *Pseudomonas florescence*, *Xanthomonas axonopodis* and X.

Malvacerum. Similarly, fungal culture were also prepared for the fungi like *Drechslera turcica, Fusarium verticillioides* and *Aspergillus flavus*.Cultre were collected from department of Biotechnology, Agriculture University Peshawar. They were used for the antimicrobial test. The culture bacteria were placed on nutrient broth (NB).

Plant materials and their collection

Different plant material were collected and root, bark and fresh leaves of five different plants *Withania coagulens* (L) *Tinospora cordifolia* (wild) *Acacia modesta* (wild) *Abutilon indicum* and *Ziziphus jujuba* were used. Healthy and free from disease plants were collected from different sites of Khyber Pukhtunkhwa. After collection of plant leaves were collected and washed thoroughly 4-5 times with running water. Sterile distilled water was used for washing and cleaning the leaf barks and root material. The materials were air dried on sterile blotter under shade.

Extraction from the plant material

After washing and drying the extracts was obtained from the bark of *Acacia modesta*, *Tinospora cordifolia* and *A. indicum*. The plant materials were dried for 7 days and then powder with the help of blender. Thirty grams of powder was dried and filled in the thimble and extracted successively with methanol solvents in Soxhlet extractor for 48 hrs. The solvent extracts were concentrated under reduce pressure and kept at 3^0C in air tight bottle.

Making of inoculum

Bacillus subtilis and *Staphylococcus aureus*, these are gram positive bacteria while the *Escherichia coli*, *Pseudomonas fluorescence* and *Xanthomonas axonopodis*, X. *malvacerum* are the gram negative bacteria .These bacteria were obtained and pre culture in special nutrients broth in a rotary shaker at 34^0C ,centrifuged at 90,000 rpm for 3 min. A small sphere (pellet) was suspended in a purified water and the density of the cell was standardized spectro photometrical ($A_6$10nm) .The fungal inoculum (*A. Flavus* and *D. turcica*, *F. verticillioides*) was prepared from 6 to 12 days old culture grew by the help of potato dextrose agar medium. The other tools i.e. the petri dishes were washed with 7 to 13 ml of distilled water and the fungal spore were also have a noticeable density and adjusted with spectrophotometer (A_{583}nm) to get last concentration of approximately 10^4 spore/ml.

Antibacterial activity

The methanolic leaf extract of the different plants which were reported in the investigation are *Acacia modesta, Tinospora cordifolia, Abutilon indicum, Withania coagulens* and *Ziziphus jujuba*, the extract of other plants rather than leaf including bark extract of *Acacia modesta*, *Tinospora cordifolia* and *Zizyphus jujuba* beside the bark

extraction the root extraction of the *Abutilon indicum* and *Withania coagulens*. All these extracts were tested by a special method i.e. disc diffusion method. Extract of different concentration (90uml^{-1}) were synthesized by reconstituting with methanol, the microorganisms tested were seeded into respective 84ll (10^3cells/ml) with the 22 hours cultures of bacteria growth in nutrient broth. When the sodification occurred, the filter paper discs (3mm diameters) filled at certain quantity with extracts were placed on test organism-seeded plats, the bacteria such as *B. subtilis*, *E. coli*, *P. fluorescence*, *S. aureus, X. axonopodis* and X. *Malvacerum.* They were including for antibacterial test. A salt or ester of sulphuric acid (8ugml^{-1}) is used as positive control and other solvent of methanol (90ugml^{-1}) were used for negative control. The antibacterial analysis plats were incubated at 35^0C for 22hrs, the inhibition zones diameter were measured in mm.

Antifungal activity

The antifungal activity was also tested by disk diffusion method. The agar of potato dextrose plates were inoculated with every fungal culture (7 days old) by point inoculation. The filter paper disc (3 mm in diameter) impregnated with 90 ugml^{-1}. The extract concentration was placed on test organism seeded plates. The function of methanol was that to dissolve the extract and also enhance the evaporation before application on test organism seeded plates. Blank disc impregnated with methanol solvent were followed by drying off was used as negative control and Nystatin (8ugdis^{-1}) were used for positive check. After determination the activity for 67 hrs of incubation at 26^0C the inhibition zones diameter were measured in mm.

RESULTS AND DISCUSSION

The present results revealed that five medicinal plant species with their extracts possessed good antimicrobial activity against *B. subtilis, E. coli, X. axonopodis , X. malvacerum, S. aureus, P. fluorescence* (Table 1) and also showed a fruitful results against fungi i.e. *F. verticillioides*, *D. turcica* and *A. flavus*. The test was conduct by a disk diffusion method so the methanol leaf extract of *Acacia modesta* showed a strong activity against *E. coli, S. aureus, X. axonopodis* and *X. malvacerum* around 12mm.The strong and highest activity against bacteria was 18mm in *B. subtilis* while least activity was recorded in *E. coli*. The extract of bark also showed a significant result against bacterial activity. The bark extract of *Acacia modesta* showed strong and high activity against *B. subtilis* and *S. aureus* (16mm) and lowest in *P. fluorescence*. The leaf extract of *A. indicum* has maximum and strong activity against *B. subtilis* and *S. aureus* (16mm) and showed a 15mm similar zone of inhibition, observed in *E. coli*, *P. fluorescence, X. malvacerum*. The root extracts of the *A. indicum* show a strong and

highest activity against *B. subtilis* and *S. aureus* while the weak action was noticed against *E. coli*. Beside these the leaf extract of *M. neglecta* also have exactly the same zone of inhibition activity against all the tested bacteria except X. *malvacerum* showed a strong activity against (16mm). *Tinospora cordifolia* bark extract shows a variable zone of inhibition from 8-12mm against all the tested bacteria. The root extract and the leaf extract of *Withania coagulens* recorded almost same antibacterial activity against the tested bacteria. The extract which is obtained from the leaf of *Zizyphus jujuba* having strong activity against *E. coli, X. axonopodis* and X. *malvacerum* (20mm) while the weak activity were observed against *S. aureus, P. fluorescence* and *B. subtilis* around 13mm zone of inhibition rather than root extract and the bark extract of such plant also showed good activity against *S. aureus* followed by *E. coli, X. axonopodis*, X. *malvacearum* and *P. fluorescence*. All the five plants, the leaf and bark extracts of *Acacia modesta, Abutilon indicum, Tinospora cordifolia, Withania coagulens* and *Ziziphus jujuba* showed strong antibacterial activity against the test pathogens. The leaf extract of all the five plants showed a strong activity against fungi in contrast to the root and bark extracts of the tested plants. The bark and leaf extracts of *Acacia modesta* showed fair activity against fungi i.e. *Aspergillus flavus* (14 mm) which are followed by *Ziziphus jujuba* leaf extract (13mm). The bark extract of *Tinospora cordifolia* and *Withania coagulens* showed a strong activity against *Drechslera turcica* in contrast to the *Tinospora cordifolia* and *Withania coagulens* leaf extracts which showed weak activity. *Fusarium verticillioides* recorded more exposed for all the five plants bark, root, leaf extracts. Rest of plant species showed a low activity against fungi.

In this study the important plants are reported which showed their strong antimicrobial activity. Different investigations are available on the antibacterial, anti-fungal, antiviral activities of plants. In the present report, the extract of different parts of the plant are introduced especially the bark, root extracts and the methanol leaf extracts of *Acacia modesta, A. indicum, Tinospora cordifolia* and *Withania coagulens.* Some investigation for *Withania coagulens* was recorded by Maurya (2010) and *Ziziphus jujuba* (Mahajan and Chopda, 2009). The plants in the current study showed a significant activity against microorganism including bacteria such as *B. subtilis, E. Coli, P. fluorescence, S. aureus, X. Axonopodis*, *X. malvacerum*, *A. flavus, D. Turcica* and *F. verticillioides.* The extracts of these plants have been used in making drugs effectively proved for their uses against microbial activity. For instance methanol extract of *Withania coagulens* showed strong activity against all the strain of *N. gonorrhoea*, while the variable solvent extract and the isolated chemicals of the leaves of

Acacia modesta showed strong antimicrobial specially pathogens of *Xanthomonas* species. Methanol extract was subsequently fractioned and monitored by bioassay leading to the separation of active fraction. Mahesh and Satish (2008) reported similar finding for *Acacia*.

Table-1. Antibacterial activity of some medicinal plants methanol extracts (90µg/ml) and antibiotic (80µg/ml) against bacterial species tested by the disc diffusion assay

Bacterial species	Weed species									
	Acacia modesta		*Abutilon indicum*		*Tinospora cordifolia*		*Withania coagulens*		*Ziziphus mauritiana*	
	Bark	Leaves	Root	Leaves	Bark	Leaves	Root	Leaves	Bark	Leaves
B. subtilis	*16± 0.56*	*18± 0.40*	*15± 0.38*	*16± 0.67*	*13± 0.66*	*14± 0.42*	*14± 0.48*	*12± 0.47*	*12± 0.11*	*13± 0.15*
E. coli	*12± 0.26*	*12± 0.22*	*11± 0.26*	*12± 0.22*	*8±0 .76*	*13± 0.02*	*16± 0.11*	*15± 0.36*	*14± 0.13*	*17± 0.18*
P. fluorescence	*11± 0.47*	*11± 0.33*	*13± 1.11*	*17± 1.16*	*11± 0.63*	*13± 0.11*	*13± 0.66*	*14± 0.26*	*10± 0.90*	*13± 0.15*
S. aureus	*14± 0.77*	*12± 0.22*	*15± 0.38*	*18± 0.11*	*11± 0.63*	*14± 0.44*	*13± 0.13*	*14± 0.26*	*10± 0.13*	*12± 0.14*
X. axonopodis X. malvacearum	*12± 0.17*	*12± 0.22*	*13± 0.77*	*15± 0.38*	*13± 0.22*	*16± 0.11*	*12± 0.00*	*16± 0.55*	*12± 0.13*	*20± 0.18*

Negative values mean inhibition zone (mm) ± S.D of three replications.

Table-2. Antifungal activity of some medicinal plants methanol extracts (90 µg mlG1) and fungicide (80 µg mlG1) against fungal species tested by disc diffusion Zone of inhibition (mm)

Fungal species	Weed species									
	Acacia modesta		*Abutilon indicum*		*Tinospora cordifolia*		*Withania coagulens*		*Ziziphus mauritiana*	
	Bark	Leaves	Leaves	Bark	Leaves	Bark	Leaves	Bark	Leaves	Bark
Aspergillus flavus	*15± 0.34*	*11± 0.32*	*9±0 .78*	*13± 0.21*	*11± 0.63*	*15± 0.34*	*13± 0.20*	*14± 0.1*	*13± 0.20*	*14± 0.90*
Drechslera turcica	*16± 0.20*	*9±0 .33*	*11± 0.57*	*14± 0.53*	*5±0 .63*	*14± 0.20*	*12± 0.20*	*12± 0.26*	*10± 0.20*	*12± 0.10*
F. verticillioides	*15± 0.13*	*8±0 .01*	*12± 0.22*	*14± 0.23*	*4±0 .63*	*13± 0.20*	*10± 0.20*	*9±0 .17*	*09± 0.20*	*10± 0.22*

Negative values mean inhibition zone (mm) ± S.D of three replications

CONCLUSION

The consequences of the present report clearly showed the antibacterial and antifungal activities showed variable effects with plant extracts and plant species. The results of present investigation also give a general conscious awareness for making new drugs and medicine against the reported microorganisms. The extract from the leaf and root of *Abutilon indicum* has best antibacterial activity against all the test bacteria. The leaf and bark extract of *A. modesta* had a

progressive result against fungal activity of *A. flavus*. Besides *A. Modesta*, the *Tinospora cordifolia* and *Z. jujuba* performed actively against fungi while the methanolic extract of *Abutilon indicum* gave effective antifungal performance against *F. verticillioides*.

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