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ANTIMICROBIAL ACTIVITY OF CRUDE METHANOLIC EXTRACT AND VARIOUS FRACTIONS FROM PARROTIOPSIS JACQUEMONTIANA (DCNE) REHDER

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Abstract:

Plants contain such substances which can be used to treat different kind of disease conditions. These medically important substances are known as phytoconstituents or phytochemicals and they have the major responsibility of protecting the plant against infectious diseases and protect them from the attack of the pests. Crude Methanolic Extract (CME) and different polarity-based solvents fractions of P. jacquemontiana was evaluated for biological activities. Efficiency of each extract was checked. CME fraction gave maximum efficiency, followed by ethyl acetate, and n-hexane was least efficient. All the extracts were showed more or less effect on different bacterial strains and have different inhibition zone. CME, n-hexane and ethyl acetate (EA) extracts were showed effect on S. aureus and E.coli strains, and do not showed any effects against K. pneumoniae. Extracts were also checked on different fungal strain in which Ethyl acetate and CME of stem were showed maximum zone of inhibition against A. Flavus strain and A. nigar do not have effected by any extracts. Finding suggest that instead of fraction CME have greater effect on antimicrobial agents.

Key words: *P. jacquemontiana, CME, n-hexane and ethyl acetate, antifungal, antibacterial.*

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INTRODUCTION:

Plants have been used as therapeutics substances since ancient times. [1] These plants contain such substances which can be used to treat different kind of disease conditions. [2, 3] One of the major problems in these diseases are the infectious diseases and reports says that annually 57 million deaths occur worldwide through these infectious diseases. [4] Plants contain secondary metabolites which are of great importance. These metabolites have good or bad effect on plants and this phenomenon is known as allelopathy. [5]

Traditional medicines consisted mostly of the naturally occurring products and these natural products were the backbone of some major states like Egyptian, Chinese and ayurvedic medical systems Egyptian. [6] The reports show that almost 25% of the world medicine is obtained from the natural products. [7] According to WHO, a herbal plant is having therapeutic products in one or more of its organs. Such type of plant will have leaves, rhizomes, roots, flowers, barks, stems, grains, fruits and seeds which can be used to control disease condition. Such of plants have chemical substances which are very important from medical point of view. These medically important substances are known as phytoconstituents or phytochemicals and they have the major responsibility of protecting the plant against infectious diseases and protect them from the attack of the vermin, rats and rabbits etc. [8] [9] [10]

Most of the plants which are mostly used today in medicines have been used since ancient times. First experiment on antimicrobial properties of plants was conducted in 19th century. Use of the plant products and phytochemicals, can have remarkable output if their properties are already known. In the recent past many efforts have been done to explore such properties of the plants [11-13] Plants have been reported to have antimicrobial properties through many experiments and majority of them are being used as curative agents because of their antimicrobial properties. [14] Different substances in these medicinal plants such as alkaloids, phenolic compounds etc. have strong antimicrobial properties.

Parrotiopsis jacquemontianais, common name of which is beranj in Pashto. [15] It is usually found in the northern areas of Pakistan, in Nuristan area of Afghanistan and Kashmir. P.jacquemontiana mostly grows in mountain area at 1200-2800 m altitudes. It grows as small shrub or deciduous tree. Leaves of this plant are large; margins are crenate. Sometimes insects' galls are present on the leaves. P. jacquemontiana's Flowers appear before the leaves. Flowers are bisexual and are surrounded by large bracts. [16]

The current research was accompanied to evaluate antimicrobial effectiveness of the crude Methanolic (CME) extract and different polarity-based fractions of Parrotiopsis jacquemontianais.

MATERIALS AND METHODS:

Plant material:

The plant was collected in august 2016 from Usheri Darra of 'District Dir upper, Khyber Pakhtunkhwa'.The plant was collected from Usheri Darra, District Dir upper, Khyber Pakhtunkhwa on Aug 2017. Stem, root and leaf along with apex parts were collected from hilly areas. Parts of plant were then cut into fine pieces with the help of Sharp meterial and identification process was done by "Prof. Dr. Lal Badshah", Botany Department University of Peshawar.

Extraction:

Within 24 hours of the collection, plant pieces were carried to the Centre of Biotechnology and Microbiology, University Peshawar. The plant was placed on the ground in dark room and was dried in the absence of direct sunlight or other artificial type of light. The samples dried within two weeks at room temperature and then was grinded with the help of pistil and mortar in order to obtain the extract. The samples were placed in methanol for one to two weeks and were regularly stirred and pressed with the help of glass rod. After two weeks the methanol extract was filtered out with the aid of filtration apparatus. Filtrate were separated and obtained in separate bottle devoid of the methanol. End process of extraction goes on to Stirred the extract through muslin's cloth finally and filtered, using Whatman filter paper No.1, and then place on rotatory evaporator at low pressure at 40ºC. [17]

Fractionation:

Four different solvents were used based on polarity from low polar *n-hexane* (NH) to high polar water. Fractions were made by soaking crude extract in dist. Water (500ml) for 24h and then partitioned with NH and EA to obtain their respective fractions. The same procedure was applied for all fractions. The final fractions were saved in labeled bottles and placed at -80ºC to obtained semi-solid residues.

Antibacterial assay:

All distillates of whole plant were inspected against four bacterial strains. All the four extract were checked on Five different bacterial strains *(Escherichia coli,*

Staphylococcus aureus, pseudomonas aeruginosa, Klebsiella pneumoniae and *Bacillus subtillis)* using well diffusion agar technique. Eighteen (18) hours old culture of bacterial strains from nutrient broth were taken to create bacterial lawn. At the same time nutrient ager media was made and transferred to sterile petri plates. After hardening of nutrient agar media, bacterial strains were inoculated on the same media for further evaluation. With the help borer (6mm) holes were made inside petri plate and the placed in incubator for 24 hours at 37ºC. The information was assembled as zone of restriction of bacterial growth. The stock solution of the samples was made by dissolving 3.0 mg of extract in 1ml of DMSO (3mg/ml). Cefixime were used as positive control. [18]

Antifungal assay:

Crude Methanolic Extract and different polarity-based solvents fractions of entire plant was checked against ***(Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Mucor specie, Fusarium solani)*** using agar tube dilution method. The stock solution (24mg/ml) was prepared to dissoled 24mg of extract in sterile DMSO (<1%). Sabouraud Dextrose Agar (SDA) media was used in this bioassay. Plant extracts (66.66µl) were added to the test tubes having SDA media and fungal strains and placed it in slanting position for the calculation of result. Clotrimazole was used as a Optimistic control while DMSO as a Negative control. [19]

RESULTS AND DISCUSSION:

Antibacterial assay:

The plant extracts contain compounds and the mechanism through which these compounds are thought to control the bacterial infection is that it interrupts nucleic acid synthesis, membrane function and metabolism process. [20] Antibacterial resistance especially among gram-negative bacteria is an important issue that has created a number of problems in treatment of infectious diseases and necessitates the search. [21] The extracts used in this study showed different zones of inhibition against the bacterial population. All the extracts were affective contrary to different bacterial strains except K. pneumonia. Overall gram positive strains were more effected then gram negative strains. CME extract gave higher effect against E. coli (50%), P. aeruginosa (39%), S. aureus (45%) and B. subtillis (30%) with respect to *n*-hexane i.e. E.coli 34%, P. aeruginosa 45%, S. aureus 39% and B. subtillis 30% and Ethyl acetate E.coli 45%, P. aeruginosa 30%, S. aureus 40% and B. subtillis 30% extracts.

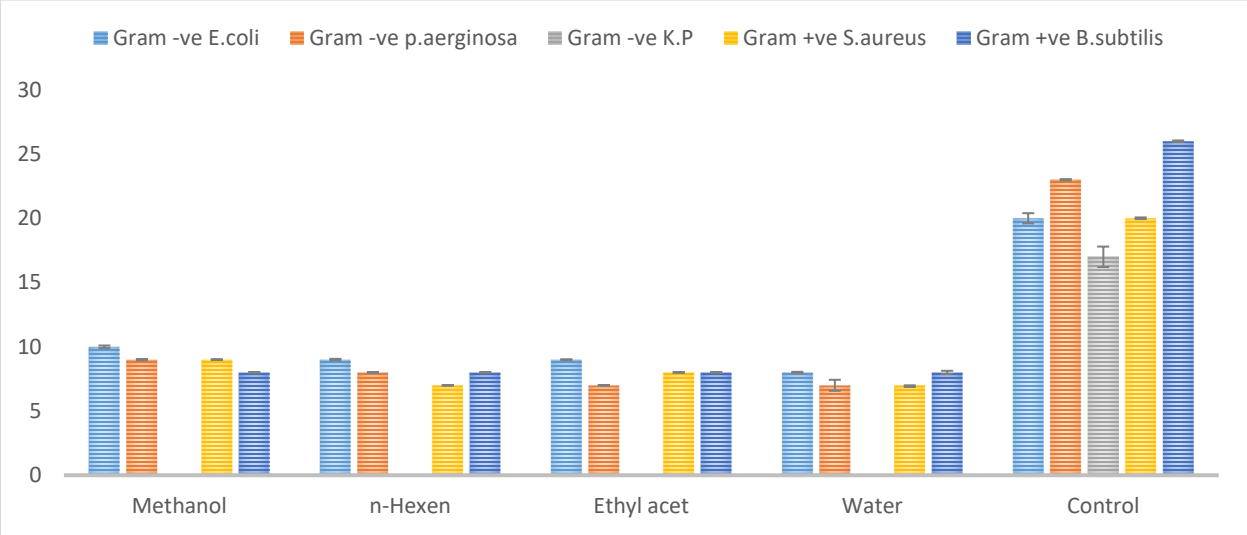


Fig 1.1 Antibacterial activity of Parrotiopsis jacquemontiana extracts.

Antifungal assay:

Fungus cause most diseases in plants like “Vascular wilt” caused by (F.oxyspoum), “rice sheath blight” caused by (Rhizoctonia solani), “fruit rot” caused by (Fusarium solani) (Saini & Sharma, 1978). Fungicides chemical are very effective against several diseases of fruitlets and vegetables, but have no use for long-term because of associated with strength and environmental problem related to threats and developmental tolrence. [22] With the determined of discovering new bioactive complexes from plant origin, the samples were tested against the selected pathogens. Figures show the effect of all extract against different fungal strains. A. nigar do not give zone of inhibition against any of the extract and further strains are maximum effected by methanolic and ethyl acetate extract. CME gave

effects against A. flavus 22%, A. Fumigatus 20%, Mucor sp. 43% and F. solani 25% and ethyl acetate against A. flavus 25%, A. Fumigatus 25%, Mucor sp. 20% and F. solani 23%. While *n*-hexan was 20% inhibition zone for A.Fumigatus and have no effect against other strains. It was observed that when the polarity of solvent was increased, the antifungal activity of the extract was also higher.

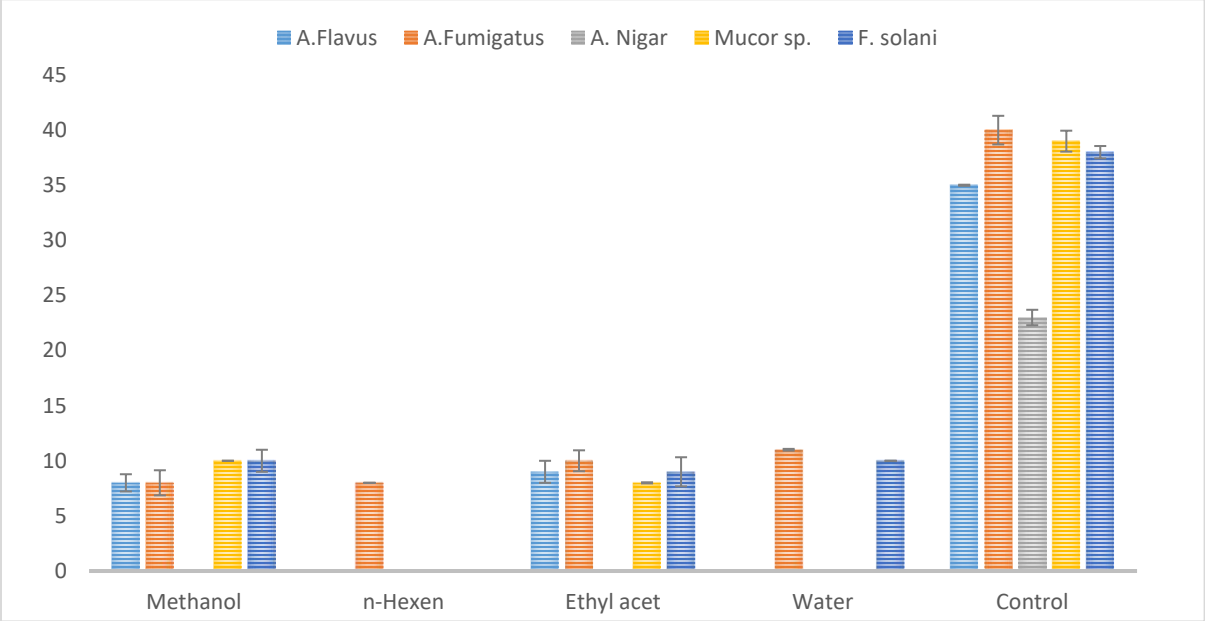


Fig 1.2 Antifungal activity of Parrotiopsis jacquemontiana extracts.

CONCLUSION:

In the current study, the deviation in the extract yields might be defined as the occurrence of different extractable components i.e. different chemical composition of the plant metabolites. In the present findings CME and ethyl acetate fraction showed maximum activity against various bacterial and fungal strains which could be served as a possible source of antimicrobial drugs after appropriate processing.

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