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Original Research

Fourier transform infrared spectrometer analysis and antimicrobial screening of ethanolic extract of Operculina terpathum from cholistan desert

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Abstract

Aim of present study was to assess pharmacological (antioxidant, antibacterial & antifungal) potential of Operculina terpathum seeds. Ethanolic extract was prepared and its phytochemical evaluation show the different chemical compounds such as carbohydrates, phenols, tannin, flavonoids, cardiac glycosides, steroids, alkaloids and proteins. FTIR spectra showed the presence of organic acids, hydroxyl and phenolic compounds, amino groups, aliphatic compounds, functional groups such as amide, ketone, aldehyde, aromatics and halogen compounds. Antioxidant activity of the Operculina terpathum alcoholic extract was performed by DPPH method and it showed 97.13%whereas IC50±SEM (µg/ml) was 1.425±0.16. Antibacterial activity was performed against different bacterial strains and results were comparable with that of standard. Maximum antibacterial activity was exhibited by Bacillus subtillis (28.33±2 mm) and Bacillus pumilus (25.33±2 mm) respectively. Antifungal activity was also performed and it showed maximum activity against Aspergillus flavous and Candida albicans6±1, 5±1mm respectively. These results showed that Operculina terpathum has good antibacterial and antifungal activity against different microbes and it could be used as an alternative to antibiotics, as the antibiotics resistance is very common now a days

Keywords: Anti-bacterial activity; Anti-fungal activity; Agar disc diffusion method

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INTRODUCTION

Herbs have a long history of being used as antimicrobial agents in the past and also have a wide curative capacity. Plant contains

many active compounds that have antimicrobial properties and used to treat different diseases. In the study, 70 herbal plants were reported to have antimicrobial activity. Microorganisms recorded in the sample included Gram +ve, Gram-ve bacteria and fungi.[1] Plants have been used for the cure of human illnesses for thousands of years. Until the 18th century, the careers of physician and botanist were closely related. Each plant species produces chemicals that can adversely affect certain animals or micro-organisms and strongly supports the interpretation that secondary metabolites play a vital role in disease control. Antifungal medications are medicinal agents used against a range of pathological conditions caused by fungi, most commonly involving candidiasis, cryptococcal meningitis, ringworm, athlete's foot, fungal infection of the nails, aspergillosis, etc.[2] Operculina Turpethum is a perpetual sweet-smelling creeper with a straightforward stem, three-sided or rectangular stems with numerous leaves as well as has oval appearance. It belongs to Asian locales; Pakistan, Nepal, Sri Lanka, India, Bangladesh, Myanmar, China and Taiwan. Mainly it is used as expectorant, brain tonic and laxative. Pharmacological studies have revealed that Operculina Turpethum exhibit anti-inflammatory, antidiabetic, anti-microbial, antispasmodic, antidiarrheal, antiulcer, bronchodilator and anticancer properties. The additional use of useful plants has an enormous pragmatic effect in the producer countries, taking into account easy availability.[3] These assessments have yielded impressive results from useful antimicrobial plants and are planned to be applied to validate antimicrobial potential against various microbes.[4] This change in drug resistance is expected to cause 10 million causes worldwide by 2050.[5] More than 84% of Pakistanis use alternative medicines to cure different diseases, and more than 200 local plants are used as an optional adjacent treatment for various infectious contaminations.[6] Since the nation has different regions and social values, herbal remedies frequently fluctuate in such a way that approximately 600-700 plants are used to treat disease.[7]

Present study was designed to investigate chemical constituents of Operculina turpethum by FTIR (Fourier Transform Infrared Spectrometer) and screening of antioxidant, antibacterial and antifungal activities for technical evidence for its use as a folk medicine in the treatment of different bacterial and fungal diseases.

MATERIAL AND METHODS

Collection and identification of plants

Operculina terpathum (Turbad Sufaid), plant was bought from neighborhood market and Cholistan desert near to Bahawalpur, South Punjab, Pakistan. The Operculina terpathum was authenticated and identified by Assistant Professor Dr. Ghulam Serwar, Department of Botany, The Islamia University of Bahawalpur and voucher numbers were obtained for Turbad Sufaid 36/Botany.

Glass ware, equipments and chemicals

4-gram negative bacterial strains (Micrococcus luteus, E. coli, Pseudomonas aeruginosa and Bordetella bronchiseptica) and also tested against 4-gram positive bacterial strains (Staphylococcus epidermidis, Bacillus subtillis, and Bacillus pumilus). 4-fungal strains (Aspergillus flavous, Aspergillus terreus, Aspergillus fumigates and Candida albicans) Petri dishes, Flasks, Test tube, stand, Gloves, L-shaped rod, Micropipette, Facial masks, Nutrient Broth, Nutrient Agar, Sabouraud's Dextrose Agar, Methyl alcohol, Ciprofloxacin, Terbinafine, Microscope, Autoclave, Laminar Flow, FTC 90 Refrigerated Incubator.

Preparation of extract

The plant content (Seeds) sunk into the hydro-ethanol solution with a combination of 30/70 for the fifteen-days duration proceeded by the filtration initially with the muslin fabric and subsequently with filter paper. At that point, the acquired filtrate instilled for solvent evaporation into the revolving evaporator to obtain the unrefined concentrate of considerable plant numbers. Within the unrefined system the acquired plant separates was then placed away in the completely closed-off container for further use. Various plant sections have been used for medicinal purposes and extracts prepared in particular solvents have been used for various disorders of treatment.[8]

Phytochemical screening

Various chemical tests were performed using hydro ethanolic extracts of Operculina Terpathum to detect the presence of phytoconstituents like terpenes, flavonoids, saponins, steroids, cardiac glycosides, proteins, carbohydrates, alkaloids, tannins and phenolic compounds

Fourier transformed infrared (FTIR) spectroscopic analysis

The plant extract was checked utilizing Fourier Transform Infrared Spectrometer in the scope of 4000–400 cm−1. The resultant spectral information contrasted with reference graph to recognize functional groups presence in the extracts.

Antioxidant assays by DPPH

Samples reacted with a stable DPPH radical in an ethanol solution. The reaction mixture consisted of the addition of 0.5 ml of sample, 3 ml of absolute ethanol and 0.3 ml of DPPH radical solution 0.5 ml of ethanol. As DPPH reacts with an antioxidant compound that can give hydrogen, it is reduced. Changes in color (from deep violet to light yellow) were read Absorbance (Abs)] at 517 nm after 100 min of UV VIS spectrophotometer response (DU 800; Beckman Coulter, Fullerton, CA, USA). The ethanol mixture (3.3 ml) and the sample (0.5 ml) act as a blank mixture. The control solution was prepared by combining ethanol (3.5 ml) with DPPH radical solution (0.3 ml). The scavenging activity percentage (AA%) was determined according to Mensor et al.[9] The entire methanol plant separates at a dose of 100 ug / ml examined by DPPH, ferric decreasing power test, radical reduction of nitric oxide free, photometric testing and reduction of the OH radical effect.[10]

$$\text{Inhibition (\%)} = 100 - \frac{(\text{Abs. of test solution})}{\text{Abs. of control}} \times 100$$

Bacterial and fungal strains (Test organisms)

Bacterial strains that are Escherichia coli, Bacillus subtulus, Bacillus pumilus and Micrococcus luteus were given by the First Fungal Culture Bank of Pakistan (FCBP), Institute of Agricultural Sciences (IAGS) Lahore, University of Punjab, in the form of stock culture agar. Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis and Bordetella bronchiseptica were isolated from microbiologic Inc. Bacterial strains used for the antibacterial assay are shown in table 1.

Table 1. Bacterial strains used for the antibacterial assay are given below

Sl No.	Name	Type	Voucher Number
1.	Bacillus subtulus	Gram +ve	45
2.	Bacillus pumilus	Gram +ve	074
3.	Staphylococcus aureus	Gram +ve	ATcc 6539
4.	Staphylococcus epidermidis	Gram +ve	ATcc 9027
5.	Micrococcus luteus	Gram -ve	072
6.	E. coli	Gram -ve	088
7.	Pseudomonas aeruginosa	Gram -ve	147
8.	Bordetella bronchiseptica	Gram-ve	100

Fungal strains were delivered in form of stock culture agar by the First Fungal Culture Bank of Pakistan (FCBP), Institute of Agricultural Sciences (IAGS) Lahore, The University of Punjab. Fungal strains used for the antifungal assay are shown in table 2.

Table 2. Fungal strains used for the antifungal assay are given below

Serial No.	Name	Voucher No.
1.	Aspergillus terreus	002
2.	Aspergillus fumigatus	013
3.	Aspergillus flavous	005
4.	Candida albicans	007

Preparation of inoculums for antibacterial and antifungal activity

Bacterial Inoculums were made from cultures that were 24 hours old. The turbidity was altered to 0.5 McFarland turbidity level, which is comparable to 1-5x 108 CFU/ml cell densities, by taking a few colonies of the particular bacteria and shifting them to 5 ml of standard sterile saline solution (Sridhar, Duggirala, & Puchchakayala, 2014).

Fungal Inoculums are made from 3 days old crop plates by transferring a few colonies of a certain fungus to 5 ml of sterile saline solution and adjusting the turbidity to 0.5 McFarland turbidity levels. To reach an inoculum density of 1-5x103 CFU/ml, this 1:10 solution was diluted three times using a growth medium.[11]

Agar disc diffusion method used for antibacterial activity

Preparation of agar

28g of nutritional agar was dissolved in clean water and sterilized in an autoclave for 15 minutes at 121ºC and 15 pressure.

Preparation of sabouraud's dextrose agar

65g of Sabouraud's dextrose agar was combined with 1 litre of filtered water and autoclaved for 15 minutes at 121°C and 15 pressure.

Antibacterial assay

Petri dishes were sterilized in a heated air oven at 180 °C for 30 minutes before being installed in an aseptic laminar flow hood. At 35 °C, sterilized nutritional agar was cooled. In petri plates, 20 ml of sterilised nutrient agar was transferred and left to solidify at room temperature. On the 6mm filter paper disc, 30µl of 50mg, 25mg, and 12.5mg/ml 6.5mg/ml and 3.125mg/ml concentrate was put. After drying, the inoculated Petri plates were mounted on them. Petri dishes are placed in the refrigerator for diffusion after filtration of the filter paper bacterial culture, and then transported to the incubator for 24 hours of incubation at 37 °C. To compare antibacterial impacities, normal ciprofloxacin was used as a positive control of 30 µl per filter paper Disk and DMSO as a negative control. Following that, the inhibitory region is measured in millimetres (mm). Both experiments were carried out in triplicate, and mean data were calculated.[11]

Antifungal assay

Petri dishes were sterilized in a warm air furnace at 180°C for 30 minutes before being put in an aseptic setting in a laminar flow hood. The autoclave was used to sterilize Sabouraud's dextrose agar medium, which was then cooled to room temperature. At room temperature, 20 ml of Sabouraud's dextrose agar overflowed into Petri plates, causing it to solidify. The sterile paper disc was then produced. 60µl inoculums were placed in a Petri plate and disseminated using a sterile L-shaped rod. The disc was soaked in 30µl of 30µl of 50mg, 25mg, and 12.5mg/ml 6.5mg/ml and 3.125mg/ml extract, and the fungal culture Petri plates were placed in the diffusion refrigerator after that, it was placed to the incubator for 48 hours of incubation at 27 degrees Celsius. To compare antifungal effects, terbinafine (5 mg/ml) was utilized as a positive control while DMSO was used as a negative control. After that, the inhibitory region is measured in millimetres. Both processes were repeated three times, with the average values calculated.[11]

RESULTS

Phytochemical screening

Phytochemical examination of hydro-alcoholic extract of Operculina terpathum, revealed the existence of various chemical components. Operculina terpathum contains alkaloids, saponins, steroids, proteins, carbohydrates, flavonoids and glycosides (brown ring formation). Extracts of Operculina terpathum, tested for bioactive compounds by standard methods as shown in Table 3.

Table 3. Compounds present in Operculina terpathum		
No.	**Tests**	**Present**
1	Carbohydrates	+ve
2	Phenols	-ve
3	Flavonoid	+ve
4	Saponins	+ve
5	Cardiac glycosides	+ve
6	Steroid	+ve
7	Terpenoids	-ve
8	Alkaloids	+ve
9	Protein	+ve
10	Tannin	+ve

Fourier Transform infrared spectroscopy (FTIR) analysis

The peaks at 3893.01 and 3860.20 cm^{-1} showed the presence of organic acids (-COOH). The peaks 3522.6 and 3421.74 indicate hydroxyl and phenolic compounds. The peak at 3138 cm^{-1} and 1632.63 cm^{-1} corresponds the presence of aliphatic compounds (C–H bend) and ester respectively. The peaks 1343.42, 1148.16 and 1074.91 indicated the presence of functional groups such as amide, ketone, aldehyde, aromatics (C–C & C-O) stretch (in–ring) and aliphatic amines (C–N stretch). The peaks from 988.74 cm^{-1} to 608.56 cm^{-1} revealed the presence of halogen compounds (C-Cl, C-F, C-Br). Spectra of operculina terpathum is shown in figure 1.

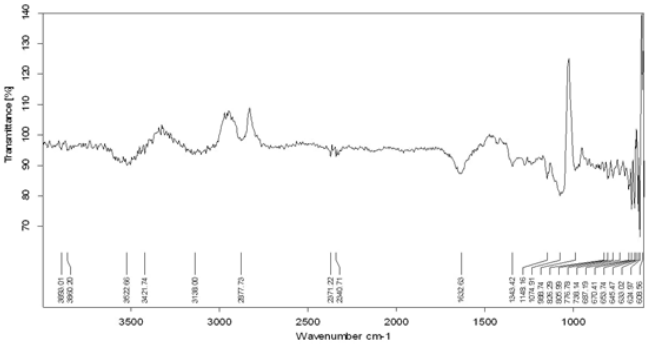

Figure 1. FTIR Spectra of Operculina terpathum.

Anti-oxidant assay

The ethanolic extract of Operculina terpathum, exhibited antioxidant potential with DPPH % inhibition are 91.703%, 88.059%, 77.379%, 59.172% respectively indicating significant results against DPPH. Results have shown that ethanolic extract has a significant free radical reduction capability by changing the color of DPPH from purple to yellow.[9] Antioxidant effect of Ethanolic extract of the Operculia terpathum, was might be due to the presence of total phenolic and flavonoids compounds in extracts and results are shown in table 4.

Table 4. antioxidant activity of Operculia terpathum Extracts at 0.5 mg/well		
Sr. No.	**% Inhibition**	**IC_{50}**±SEM (µg/mL)
1	97.13%	1.425±0.16

Anti-bacterial activity

Ethanolic extract of Operculia terpathum showed good susceptibility to almost all pathogens. Maximum antibacterial activity was exhibited by Bacillus subtillis (28.33±2 mm) and Bacillus pumilus (25.33±2 mm). Zone of inhibition was 14±2 mm by Pseudomonas aeruginosa and it was 13.33±2 mm by Bordetella bronchiseptica. Staphylococcus aureus and Staphylococcus epidermidis had inhibitory effect in the range of 15.33±2 mm and 16.66±3 mm respectively. 17.66±2 mm and 15±2 mm was zone of inhibition by Micrococcus luteus and E. coli respectively. Operculia terpathum showed very good antibacterial action against all bacterial strains, results are shown in table 5

Anti-fungal activity

Ethanolic extract of Operculia terpathum showed antifungal activity against Aspergillus flavous (6±1 mm) and Candida albicans (5±1 mm). Mild antifungal activity was reported by Aspergillus fumigates (4±1 mm) and (3±1 mm) Aspergillus terreus. Results are shown in Table 6.

DISCUSSION

The ethanolic extract of seeds showed very good antioxidant, antibacterial activity whereas some antifungal activity. Maximum antibacterial activity was exhibited by Bacillus subtillis (28.33±2 mm) and Bacillus pumilus (25.33±2 mm). Zone of inhibition was 14±2 mm by Pseudomonas aeruginosa and it was 13.33±2 mm by Bordetella bronchiseptica. Staphylococcus aureus and Staphylococcus epidermidis had inhibitory effect in the range of 15.33±2 mm and 16.66±3 mm respectively. 17.66±2 mm and 15±2 mm was zone of inhibition by Micrococcus luteus and E. coli respectively. This indicated that concentration of the extracts matters for its activity

Table 5. Antibacterial action of Operculia terpathum									
Zone of inhibition mean + standard deviation (mm)									
Extract Concentration (mg/mL)	**B. subtillis**	**B. pumilus**	**S. aureus**	**S. epidermidis**	**M. luteus**	**E. coli**	**P. aeruginosa**	**B. bronchiseptica**	**Ciprofloxacin**
50	28.33±2	25.33±2	15.33±2	16.66±3	17.66±2	15±2	14±2	13.33±2	46±3
25	14.66±2	19±3	7±2	8±2	6±3	5±2	10±2	5±2	22±2
12.5	8±3	9±4	3±0	4±1	2±0	1±0	2±1	1±1	10±2
6.25	3±1	5±1	1±0	2±0	1±0	0±0	0±0	0±0	5±1
3.125	0±0	1±0	0±0	0±0	0±0	0±0	0±0	0±0	3±1

Table 6. Antifungal action of Operculia terpathum

	Zone of inhibition mean + standard deviation (mm)				
Extract Concentration (mg/mL)	**Aspergillus flavous**	**Candida albicans**	**Aspergillus fumigates**	**Aspergillus terreus**	**Terbinafine**
50	6±1	5±1	4±1	3±1	9±2
25	3±1	2±1	1±00	00±00	6±2

results. Antibacterial activity offered by nonpolar compound(s) may also be a reason as it may fail to diffuse in agar media to exhibit antibacterial activity in disc diffusion assay (Anderson et al., 1988). Change in inculum size can change the results of test sample. Saponins are also reported from this plant that have different pharmacological activities, such as antimicrobial, analgesic, cytotoxic, anti-inflammatory, Anti allergic and anti-neoplastic. Other chemical constituents such as tannins, flavonoids, alkaloids other volatile compounds of this herb acts as defense mechanisms against different microorganisms. Flavonoids have antibacterial activity that may be due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. Alkaloids, phenolic, tannins, polyphenols, quinones, flavonoids and terpenoids are the main antimicrobial and antioxidant potential of separating plants. Many chemicals found in plants' secondary metabolism have strong antibacterial activity, making them ideal candidates for developing natural alternatives to synthetic preservatives in food.[12] Another study used varied concentrations of ethanol extract of Aloe vera leaves and roots on bacterial and fungal strains (15, 20, 25, 30ul). Bacillus cereus, Bacillus megaterium, Bacillus subtitis, Streptococcus pyogenes, Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, Acinetobacter baumannii, and other bacterial strains were employed in the study concluding that the use of plants in traditional medicine to treat a variety of ailments caused by these pathogenic strains is effective.[13] In an in vitro analysis, major active components of cinnamon stick such as (E)-cinnamaldehyde and proanthocyanidins exhibited important antibacterial potential against five foods containing bacteria, including Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, Salmonella anatum.[14]

In another study by 13 common flavonoids i.e. chrysin, flavone, apigenin, luteolin, vitexin, orientin, vitexin 2''-O-rhamnoside, isovitexin, isoorientin, kaempferol, quercetin, naringin and rutin were tested for their antibacterial activity and from the study it was revealed that these compounds inhibited both the gram +ve and gram -ve bacterial strain of which gram negative were strongly inhibited.[15]

In acute toxicity studies, Operculia terpathum did not cause any mortality up to the tested dose of as high as 5 g/kg, which is much higher than the routinely used dose.[16] Further studies are needed to evaluate its chronic toxicity.

CONCLUSION

It is concluded that hydro-alcoholic extracts of Operculina terpathum, have been found to be more effective Anti-oxidant properties. However further pharmacological investigations can be carried out to confirm their efficacy and mechanism of action Ethanolic extract of plants showed good susceptibility to almost all pathogens except very few when compared to standard ciprofloxacin as very good results seen against Bacillus pumilus, Bacillus subtillis, Staphylococcus aureus, Staphylococcus epidermidis Micrococcus luteus, Bordetella bronchiseptica, Pseudomonas aeruginosa and E.coli respectively against different concentrations of plant extract. Antifungal activity results showed that Aspergillus flavous, Aspergillus terreus having good sensitivity but Aspergillus fumigates and Candida albicans having less sensitivity as compared to standard medicine. Further investigations are needed to understand mechanism of action.

CONFLICTS OF INTEREST

Authors have declared that there are no conflicts of interest regarding this article.

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