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Research Article

Phytochemical screening and antibacterial assay of the crude extract and fractions of *Ferula oopoda*

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Abstract

The principal objective of the current study was to analyse phytochemical constituents and to determine the antimicrobial activity of the crude methanol extract and fractions of chloroform, ethyl acetate and hexane from the whole plant of *Ferula oopoda* against three bacterial strains *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. Phytochemical assay confirmed the presence of terpenoid, flavonoids, saponins, tannins, phenolic compounds, carbohydrates, steroids and glycosides. Agar disc diffusion method was used to determine the zone of inhibition of the tested sample for antimicrobial activity. The crude methanolic extract showed activity against *E. coli* ZOI, 30.00±1.060 mm, for ethyl acetate fractions 50.00±4.18 mm, for chloroform fraction 27.00±0.060 mm and for n-hexane fraction 24.00±0.353 mm. This observation shows that ethyl acetate fraction possesses great potential against *E. coli*. Inhibition zone for *Salmonella typhi* was 23.25±1.050 mm for ethyl acetate, 14.00±0.353 mm for crude methanol extract, 22.00±1.753 mm for chloroform fraction and 08.00±0.352 mm for n-hexane fraction. This observation shows that n-hexane fraction possesses low potential against *Salmonella typhi.* Anti-bacterial potential against *Staphylococcus aureus* strain was maximum in ethyl acetate fraction and showed ZOI, 34.00±1.767 mm, for chloroform fraction 21.24±2.636 mm, for crude methanol extract 19.00±1.060 mm and for n-hexane fraction 16.00±1.412 mm respectively.

Keywords: Antibacterial activity, *Ferula oopoda;* Phytochemical screening

Introduction

Infectious diseases are caused by pathogenic microorganisms; some organisms in extreme circumstances can be fatal to the host. According to WHO 80 % of the world's population uses plant extracts or their active ingredients as folk medicine in several traditional treatments **[1]**. Plants are considered as a valuable source of therapeutic agents. The research based on the Indo-Pakistan subcontinent it was recorded that plant species were used for medication in *Rigveda* between 4500-1600 B.C. There is an extensive knowledge and research based applicability already exist in this region. Some native plants are still widely used in rural areas of Pakistan especially in Balochistan province due to their antimicrobial effect without any logical

evidence [2]. People use *Ferula* species for different medicinal purposes such as to get rid of gastric and intestinal worms, lowering blood pressure and controlling diabetes. The methanol extract of *Ferula oopoda* possess anti-plasmodial activity [3]. The continuous use of plants in folk medicine makes it important to screen the medicinal plants for the discovery of new antimicrobial compounds. The growing concern about the resistant bacterial strains against the antibiotics is of focus point in international communities. Plant constituents having antimicrobial activity can hinder bacterial growth by different mechanisms as compare to that shown by currently used antibiotics. They may also have a significant clinical value in the treatment of resistant microbial strain [4]. Among the plants of Balochistan, the *Apiaceae* family has a great representation, and several species are used because they contain antimicrobial compounds, anti-inflammatory and antifungal agents [5].

The Species *Ferula oopoda* belong to the family *Apiaceae*, subfamily *Apioideae* and genus *Ferula*.This plant is found in Balochistan region of Pakistan and also found in neighbouring countries of Pakistan such as Iran, Afghanistan and India etc. In Pakistan this plant is mostly cultivated in Northern areas and in Balochistan such as Ziarat, Harboi, Chautair, Chasnak and Sasnamana etc. This medicinal plant possesses amazing significance for the treatment of toothache and gastric disorders. Several biological activities have been described for the species of this genus, such as antibacterial, antioxidant, antifungal, anti-plasmodial and anti-inflammatory activities. These activities are often attributed to the presence of phytochemicals which are bioactive compounds also known as secondary metabolites [6]. Phytochemicals include terpenoids, flavonoids, saponins, tannins, phenolic, carbohydrates, steroids, proteins, glycosides, their essential oils and some non-volatile compounds [6]. These are produced in almost all parts of the plant like leaves, bark, stem, flower, root, seeds and fruits etc [7].

Because of the scarcity of research studies about *Ferula oopoda*, the aim of this work was to comprehendon the first bioassay-guided isolation of the extract and phytochemical investigation of the compounds, to carry out the antimicrobial activity of plant extracts and its fractions against certain pathogenic bacterial strains.

Materials and methods

Plant collection and sample preparation

The plants were collected from different areas of Harboi (mountainous region of district Kalat, Balochistan, Pakistan). The species for this study was identified as *Ferula oopoda*. The whole plant was washed with the distilled water and dried naturally i.e. under shade. After completion of drying process, material was ground in a crusher and the powder was stored in sealed plastic bags for further analysis.

Extraction procedure

Solvent extraction method was used for extracting phytochemicals. Powdered plant sample was soaked in methanol, and then the mixture was subjected to rotary evaporator till the gummy extract was obtained after solvent evaporation. This extract was stored at room temperature for phytochemical and antibacterial analysis.

Fractionation of crude extract of methanol

Liquid-liquid extraction (LLE) commonly known as solvent extraction and partitioning, is a method where compounds are separated based on their relative solubility in two different immiscible liquids and separate into layers when shaken together. Solvents selected for the present study were ethyl acetate, chloroform and hexane. Selection of these solvent was based on polarity order. First, methanolic extract was dissolved in 200 ml of distilled water then poured in separating funnel followed by addition of 400 ml ethyl acetate solution. Layer between two different solvents appeared in separation funnel which was then sealed.

Funnel was shaken vigorously for 20-25 minutes and pressure was released at regular intervals. After 25 minutes two layers were separated, containing fraction in ethyl acetate and n-hexane solvent. The procedure was repeated twice and the obtained fractional extracts were placed in rotatory evaporator at 100 rmp speed to obtain gummy extract material of the fraction. These fractions were stored in refrigerator at -2^{0}C.

The methanolic extract was dissolved in 200 ml of distilled water then poured in separating funnel followed by addition of 400 ml chloroform solution and as mentioned above the same procedure was followed. The procedure was repeated twice. Obtained fractional extracts were placed in rotatory evaporator at 100 rmp speed to obtain gummy extract material of chloroform fraction. Exactly same procedure was followed to obtain n-hexane fraction. Obtained amount of fractional extracts for ethyl acetate was 0.8g, for chloroform was 0.65g and for n-hexane was 0.432g respectively. These obtained fractionations were used for phtochemical analysis, TLC, UV and FTIR spectroscopic analysis and antibacterial activity.

Phytochemical analysis

Terpenoid test (Salkowski test)

Crude methanol extract (5 ml) was added 2 ml of chloroform and 3ml of concentrated sulfuric acid on the test tube's side wall. Reddish brown colour shows the presence of Terpenoid [8].

Flavonoids test

Mixed methanol crude extract of plant with 3 ml ammonia solution followed by careful addition of sulfuric acid. Yellow colour appeared which indicated the presences of flavonoids in sample [9].

Saponins test (Foam test)

To the methanolic extract of the plant (0.5 ml) was added 20 ml of water. The mixture was shaken thoroughly for 15 min. The foam layer appeared which indicated the presence of Saponins [8].

Tannins test

In methanol crude extracts added 1-2 drop of ferric chloride. Resultant blue colour indicated the positive result [8].

Phenol test

In crude extract of plant 2-10 drops of ferric chloride solution was added. Greenish colour appeared which show presence of phenol [10].

Steroids test (Liebermann Burchard test)

In methanol crude extractwas added few drops of acetic anhydride, 2 ml chloroform and 2 ml of sulfuric acid. Presence of Red colour gave positive test [11].

Glycoside test (Keller-Kilian test)

Acetic acid (2 ml) and sulfuric acid (2 ml) were added in crude extract of plant. Reddish colour formation showed the presence of glycoside in sample [12].

Carbohydrates test (Benedict's reagent)

Benedict solution was prepared by mixing 17.3 grams of sodium carbonate, sodium citrate and copper sulphate. This reagent was used to confirm presence of carbohydrate. 2 ml of Benedict solution was added in plant extract. The mixture was kept on water bath for a few minutes. Presence of carbohydrate was confirmed by appearance of reddish brown colour [13].

Thin layer chromatography (TLC)

preparation of TLC plates

TLC plates coated with silica gel were used for the analysis. Each plate was of 30 cm length. A line was marked at 1.5 cm from bottom of TLC coated plate.

TLC separation

Mobile phase was prepared by mixing ethyl acetate, chloroform and acetone with ratio 2:2:1. Chromatographic tank was covered and left for some time before analysis so that all tank gets saturated by vapours of mobile phase [14].Capillary tube was used for spotting of plants extracts on TLC plates. The ultra violet (UV) lamp was used to visualize all spots appeared after separation on TLC plates were placed under the UV lamp and circles were drawn to visible zones and followed

by R_f values calculations of different compounds.

UV-VIS spectroscopy

UV-visible spectrophotometric analysis was carried out at room temperature by using spectrophotometer (Perkin Elmer, USA Model: Lambda 950). The extract and fractions were examined under wavelength from 200-to-800nm and the wavelength selected for research was 300 nm. The crude extract of *Ferula oopoda* was diluted to 1:10 with distilled water. Distilled water was taken as blank and absorbance of the sample was measured and spectra were obtained. Same procedure was followed for chloroform ethyl acetate and n-hexane fractions [14]. All gummy samples were first dissolved in methanol solvent and then placed in sample cuvettes. Reference cuvette was filled with dilute methanol solvent. Each fraction was tested one by one. In each case absorption was recorded.

Fourier-transform infrared (FTIR) spectroscopy

FTIR spectrometer was used for the identification of the characteristic functional groups in the extract and in the fractions. This spectroscopic technique provides structural information of a molecule from the obtained absorption spectrum. Very small amount of the sample was used for analysis. The spectrum was obtained using Bruker, Germany Vertex 70 infrared spectrometer. The wavelength range used for sample analysis was 4000 to 625 cm^{-1} [15]. The spectrum was obtained and peak values were recorded

Antibacterial activity

For antimicrobial activity of an extract or pure compound a variety of laboratory methods can be employed. Broth/agar dilution or disk-diffusion methods are the most commonly used one [16].

Agar well diffusion method

The antibacterial activity was analysed for crude extracts and fractional extracts of selected medicinal plant. All samples were dissolved in dimethylsulphaoxide (DMSO) solution followed by filtration. The target bacterial species were refreshed in nutrient broth by inoculating them in nutrient broth and incubating at 37°C for 24h. The prepared bacterial culture were spread over the surface of sterile Mueller-Hinton agar plates using sterile cotton swabs. Well (6 mm) were bored in the media and 100 µl of each extract was poured aseptically. Plates were observed after 16-24 h incubation at 37 ^{0}C. The extracts activities in term of zone of inhibition (ZOI) were analysed visually and recorded in millimetre [17]. DMSO and Doxycycline (DO 30µg) were used as negative and positive control for this study.

Results and discussion

Ferula oopoda plant was screened for its phytochemical constituent and antimicrobial activity. Preliminary phytochemical analysis showed that the plant had considerable proportion of important phytochemicals that were detected by qualitative tests. From analysis it was cleared that *Ferula oopoda* is rich in flavonoids, steroid, glycosides, tannins and phenolic compounds (Table 1). From the literature survey it was found that flavonoids have a variety of biological properties such as antibacterial, anti-inflammatory, antiviral, anti-allergic, cytotoxic antitumor properties. It is used in the treatment of neurodegenerative diseases and has vasodilatory action. It is also reported that flavonoids involved in inhibition of platelet aggregation, lipid peroxidation, fragility and capillary permeability, lipoxygenase and cyclo-oxygenase enzyme activities etc. Flavonoids also inhibit wide range of enzymes like alkaline phosphatases, hydrolases, hyaluronidases, arylsulphatase, cAMP phosphodiesterase, lipase, α-glucosidase and kinases [17]. Moreover, saponins exhibit various biological activities like, it gives permeability to the cell membrane, lowers the serum cholesterol levels, it possesses abortifacient properties, it has immunomodulatory property, and it has

toxic effects on malignant tumor cells and enhances the synergistic toxicity of immunotoxins [18]. Saponins also show antidiabetic property [19]. Tannins are reported to have a cardio-protective, anti-inflammatory, anticarcinogenic and antimutagenic properties. Tannins are also involved in treatment of noninsulin dependent diabetes mellitus by enhancing the glucose uptake and inhibiting adipogenesis [20].

Table 1. Qualitative phytochemical contents of *Ferula oopoda* extract

Phytochemicals	Test	Observation	Results
Terpenoid	Salkowisk test	Reddish brown colour indicate	Positive (+)
Flavonoids	Ammonia solution +Sulfuric acid	Yellow colour indicates	Positive (+)
Starch	Iodine test	No confirmation colour indicate	Negative (-)
Saponins	1)Foam test and 2)Spots test	1) Foam will be formed. 2) spots form on filtered paper	Positive (+)
Tannins	Ferric chloride test	Blue colour indicates	Positive (+)
Steroids	Chloroform +Sulfuric acid	Red colour indicates	Positive (+)
Phenolic	Ferric chloride test	Greenish colour shown	Positive (+)
Carbohydrates	Benedict reagent test	bricks red colour indicates	Positive (+)
Glycosides	Acetate acid +Sulfuric acid	Brown colour indicate	Positive (+)

TLC profile

The retention factors (R_f) of crude methanol extract, ethyl acetate and n-hexane and chloroform fractions in solvents system are shown in (Table 2). The chromatogram revealed 3, 3, 2 and 2 spots for crude methanol, ethyl acetate, chloroform and n-hexane fractions respectively.TLC profiling of crude methanol extract, ethyl acetate and n-hexaneand chloroform fractions was very impressive which directs towards the presence of a variety of phytochemical. R_f values of different phytochemicals differ in different solvent system. These variable R_f values provide important information about the polarity and selection of solvent system for chromatographic separation of pure compounds in column chromatography. Mixture of solvents of different polarity in variableratio can be used for separating pure compound of plant extract. Therefore, R_f values of compounds in variable solvent system can be used for selecting appropriate solvent system for plant extracts [21].Secondary metabolites of plants can be identified accurately by TLC method. The obtained different R_f values in this presented study showeddifferent profile in a single extract.The results also gave an idea about the polarity which helps during the selection of suitable solvent system for further separation of compound from the plant extracts.

Table 2. Rf values of TLC solvent system for extract and fractions of *Ferula oopoda*

Interface	Mobile phase	Ratio	No of spot detected	R_f Values
Crude methanol extract	Ethyl acetate: chloroform: acetone	2:2:1	03	0.061 0.723 0.786
Ethyl acetate fraction	Ethyl acetate: chloroform: acetone	2:8.5:1	02	0.887 0.723 0.531
Chloroform fraction	Acetone: chloroform: ethyl acetate	2:2:1	03	0.89 0.43 0.901
n-Hexane fraction	Ethyl acetate: chloroform: acetone	2:2:1	02	0.87 0.071

UV-VIS Analysis

The UV-VIS analysis was carried out to study the phytochemicals present in methanolic extract and respective fractions of *Ferula oopoda*. Theanalysis was performed to confirm the compounds having σ and π-bonds, unshared electrons, aromatic rings and chromophores. Absorption bands of *Ferula oopoda* plant extract are showed in (Table 3). The UV profile showed the bands at 278, 279, 320, 327, 328, 287, 416, 600 and 663 respectively. Appearance of absorption bands in the wavelength range from 200 to 400 nm indicates the presence of unsaturation and heteroatoms like N, S and O [22]. The spectrums for *Ferula oopoda* extract and fractions show six peaks at positions 327nm, 278nm, 287nm, 320nm this sanctions the presence of organic chromophores within the *Ferula oopoda*.

Table 3. UV/VIS Interpretation data of extract and fractions of *Ferula oopoda*

Interface	Region of band (nm)	Interpretation
Crude methanol extract	278	Benzoyl band of flavonoids
	327	Cinnamoyl band of flavonoids
Ethyl acetate	328	Cinnamoyl band of flavonoids
	287	Benzoyl band of flavonoids
Chloroform	600, 663	Chlorophyll a and b carotenoids
	416	Chlorophyll a and b carotenoids
	320	Cinnamoyl bands of flavonoids
	279	Benzoyl band of flavonoids
n. hexane	235	Flavonoids band

FTIR analysis

The FTIR analysis was done for the identification of functional group of the constituent components on the basis of various peaks obtained in the IR region. The FTIR spectrum of the *Ferula oopoda* plant extract is shown in (Table 4). IR peak at 3200 cm^{-1}confirms the presence OH groups in the extract. Peak at 2913 cm^{-1} is because of symmetric stretching of saturated (sp3) C and IR peak at1601.00-1407.80cm^{-1} is due to C=C stretching associated with the aromatic skeletal mode of the extracts. A notable band at1234.86-1011.97 cm^{-1} can be due to C-O stretching [21].

Table 4. FTIR spectrum of sample the *Ferula oopoda* extract

Wave numbers cm^{-1}	Type of bonds	Interpretation
3200 **cm^{-1}**	O-H	Alcohol, or phenolic groups
2913 **cm^{-1}**	C-H,	Alkyl groups like methyl, ethyl groups
1741 **cm^{-1}**	C=O	Carbonyl compound
1601,00-1407.80**cm^{-1}**	C=C	Aromatic compounds
1234,86-1011.97**cm^{-1}**	C=O	Alcohol, phenolic, ether, ester, glycosides linkages

Antibacterial assay

The antibacterial activity showed positive response in all fractions and in crude methanol extract (Table 5). The analysis for *E.coli* strains showed ZOI of 29 mm in crude methanol extract, 50 mm in ethyl acetate fractions, and 27 mm in chloroform fraction and 24 mm in n-hexane. These observations showed least potential of n-hexane fraction with significant potential *Salmonella* analysis with crude extract, ethyl acetate fraction, chloroform fraction and n-hexane fraction showed respectively ZOI 14 mm, 23 mm, 22 mm and 0.8 mm. significant result was showed by ethyl acetate fraction. *Staphylococcus aureus* showed maximum inhibition zones found in ethyl acetate fraction with value 34mm.

The chloroform fraction, crude methanolic extract and n-hexane showed ZOI as 21 mm, 19 mm and 16 mm respectively. These observations reveal significant potential of plant to act as antibacterial agent. Thus, plant active component may be useful in synthesis of effective drugs to treat bacterial infection.

Table 5. Inhibition zones of *Ferula oopoda* methanol extract and different fractions against pathogenic bacteria.

Bacterial strains	Mean zones of inhibition (mm)			
	Crude methanol extracts	Chloroform fraction	Ethyl acetate fraction	n-hexane Fraction
E. coli	30.00±1.060	27.00±0.060	50.00±4.182	24.00±0.353
S. typhi	14.00±0.353	22.00±1.753	23.25±1.050	08.00±0.352
S. aureus	19.00±1.060	21.24±2.636	34.00±1.767	16.00±1.412

Means ± SD

Conclusion

This study gave an insight and information for the determination of chemical composition of *Ferula oopoda* using different biochemical tests and results were confirmed by TLC, UV-VIS and FTIR techniques. In the present study flavonoids, steroid, glycosides, tannins and phenolic compounds were identified from methanolic plant extract and its different fractions. The presence of phytochemical constituents in *Ferula oopoda* gives credible evidence to its use by the human being. Novel drugs can be prepared by isolating these specific compounds. It could be concluded that *Ferula oopoda* contains various bioactive compounds which makes it an important pharmaceutical plant. However, further studies are needed to be undertaken to determinecomprehensively its toxicity profile, bioactivity, effect on the ecosystem and agricultural products.

Authors' contributions

Conceived and designed the experiments: S Iqbal, S Arifeen, S Zahoor & S Maher, Performed the experiments: S Iqbal, S Arifeen & A Akbar, Analyzed the data: S Iqbal, S Arifeen, S Zahoor, S Maher, A Sajjad & A Akbar, Contributed materials/ analysis/ tools: S Iqbal, S Arifeen, S Zahoor & S Maher, Wrote the paper: S Iqbal, S Arifeen, S Zahoor, S Maher & A Sajjad.

References

1. WHO (2013). WHO traditional medicine strategy 2014–2023. World Health Organization.
2. Aziz-UL-Ikram NBZ, Shinwari ZK & Qaiser M (2015). Ethnomedicinal review of folklore medicinal plants belonging to family Apiaceae of Pakistan. *Pak. J Bot* 47(3): 1007-1014.
3. Akhgar MR, Moradalizadeh M, Faghihi-Zarandi A & Rajaei P (2011). Chemical Composition of the Essential Oils of *Ferula oopoda* (Boiss. &Buhse) Boiss. And Ferula badghysi (Korovin.) from Iran. *J of Essential Oil Bearing Plants* 14(3): 297-301.
4. Eloff JN (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants. *J of Ethnopharmacol* 60(1): 1-8.
5. Chanda S & Rakholiya K (2011). Combination therapy: Synergism between natural plant extracts and antibiotics against infectious diseases. Science against microbial pathogens: communicating current research and technological advance, A. Méndez-Vilas (Ed.) 520-529.
6. Da Silva Sa FA, de Paula JAM, dos Santos PA, de Almeida Ribeiro Oliveira L, de Almeida Ribeiro Oliveira G, Liao LM, & do Rosario Rodrigues Silva M (2017). Phytochemical Analysis and

Antimicrobial Activity of Myrciatomentosa (Aubl.) DC. Leaves. *Mol* 22(7): 1100.

7. Bansode TS & Salalkar BK (2015). Phytochemical analysis of some selected Indian medicinal plants. *Int J Pharm Bio Sci* 6(1): 550-556.
8. Savithramma N, Rao ML & Suhrulatha D (2011). Screening of medicinal plants for secondary metabolites. *Middle-East J of Sci Res* 8(3): 579-584.
9. Soni A & Sosa S (2013). Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. *J of Pharmaco and Phytochem* 2(4): 22-29.
10. Tamilselvi N, Krishnamoorthy P, Dhamotharan R, Arumugam P & Sagadevan E (2012). Analysis of total phenols, total tannins and screening of phytocomponents in Indigofera aspalathoides (Shivanar Vembu) Vahl EX DC. *J of Chem and Pharm Res* 4(6): 3259-3262.
11. Cook RP (1961). Reactions of steroids with acetic anhydride and Sulfuric acid (the Liebermann-Burchard test). *Analyst* 86(1023): 373-381.
12. Sheel R, Nisha K & Kumar J (2014). Preliminary phytochemical screening of methanolic extract of Clerodendron infortunatum. *IOSR J Appl Chem* 7(1): 10-13.
13. Teja M, Ramya B, Reddy SM, Sudha GJ, Janaki B, Rama S, & Kumar SAR (2016). Preliminary phytochemical evaluation of Caesalpinia sappan saraca indica. *Int J Res Pharm Chem* 6(1).
14. Benedict SR (1909). A reagent for the detection of reducing sugars. *J of Biol Chem* 5(5): 485-487.
15. Jayashree D (2013). Pytochemicals analysis and TLC fingerprinting of methanolic extracts of three medicinal plants. *Int Res J Pharm* 4(6): 123-1236.
16. Jain PK, Soni A, Jain P & Bhawsar J (2016). Phytochemical analysis of *Menthaspicata* plant extract using UV-VIS, FTIR and GC/MS technique. *J Chem Pharm Res* 8(2): 1-6.
17. Agarwal AD (2011). Pharmacological Activities of Flavonoids: A review. *Inter J of Pharm Sci and Nanotechnol* 4(2): 1394-1399.
18. Thakur M, Melzig MF, Fuchs H & Weng A (2011). Chemistry and pharmacology of saponins: special focus on cytotoxic properties. *Botanics: Targets and Therapy* 1: 19-29.
19. Zheng T, Shu G, Yang Z, Mo S, Zhao Y & Mei Z (2012). Antidiabetic effect of total saponins from Entadaphaseoloides (L.) Merr. in type 2 diabetic rats. *J of Ethnopharmacol* 139(3): 814-821.
20. Kumari M, & Jain S (2012) Tannins: An Antinutrient with Positive Effect to Manage Diabetes. *Res J of Recent Sci* 1(12): 1-8
21. Gujjeti RP, & Mamidala E (2013). Phytochemical analysis and TLC profile of Madhucaindica inner bark plant extract. *Inter J of Sci & Engin Res* 4(10): 1507-1510.
22. Jain PK, Soni A, Jain P & Bhawsar J (2016). *J of Chem and Pharm Res* 8(2): 1-6.