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Results : Among all the extracts Ethanolic and Methanolic extracts showed maximum antibacterial activity against all the bacterial strain used with a zone of inhibition ranges from 12-21mm and the least activity was observed in Aqueous cold extract with zone of inhibition ranges from 7-9mm. The test results were compared with standard antibiotics chloramphenicol and Ciprofloxacine.

Conclusions : The qualitative analysis of different extracts of Raphanus sativus seed reveals the presence of Alkaloids, Flavonoids, Glycosides, Phenols, Tannins, Saponin, Sterols and Protien which may be responsible for the observed antibacterial activity. The results suggest that ethnolic and methnolic extracts can be used in the treatment of infection caused by these bacterial strains used in this study.

Keywords : antibacterial activity, phytochemical analysis, raphanus sativus, zone of inhibition.

I. Introduction

According to World Health Organization (WHO), the increase of resistance to antibiotics by bacterial pathogens is a growing problem in both developed and developing countries (1). The problem of microbial resistance is growing and the outlook of the use of antimicrobial drugs in future is uncertain. Therefore action must be taken to reduce this problem, for example, to control the use of antibiotics, to develop research to better understanding of the genetic mechanism of resistance and to continue study to develop new drugs either synthetic or natural (2).

For along period of time, plants have been a valuable source of natural products for maintaining human health (3). Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Medicinal plants represent a rich source of antimicrobial agent (4). Different parts of plants, herbs and spices have been used for many years for the prevention of infection. The use of plants with known antimicrobial properties can be of great significance in treatment of infections (5).

A renewed interest in plant based antimicrobials has arisen during the last twenty years, but still plant based antimicrobials are poorly explored. Screening of plants extracts for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective compounds (6). The antimicrobial compounds from plants may inhibit bacteria through different mechanism than the conventional antibiotics, and could therefore be of clinical value in the treatment of microbial infection (7).

Radish, ***Raphanus sativus Linn.*** (Brassicaceae family) is an annual herb, consumed as vegetable. Commonly known as Mooli. It is coarse, rough or glabrous. Leaves are lyrate, pinnate or pinnatifid. Flowers are large yellow, white or pale lilac, veined with purple, in long ebracteate racemes. Seeds are pendulous, globose; cotyledons conduplicate. Cultivated all over sub-continent up to 16,000 ft in temperate and warm countries (8). It is well reputed in Unani System of Medicine, useful for urinary complaints and piles. Almost all parts of the plant including leaves seeds and roots are utilized in medicine. The fresh juices obtained from leaves are diuretic, laxative. Roots are used for urinary complaints and syphilitic disease; they are a reputed medicine for piles and gastrodynic pains. The seeds are expectorant, diuretic, laxative, carminative, antitussive and stomach tonic (8, 9, 10, and 11). The present study aims at assessing the antibacterial property of R. *sativus* seed extract, to substantiate the use of radish in Unani System of medicine in infectious diseases.

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II. Materials and Methods

a) Plant Materials

The sample of seeds of *Raphanus sativus* [Tukhm-e-Mooli] were collected from local market of Hyderabad, Andhra Pradesh, and was properly identified authenticated on the basis of literary description available in the Unani classic as well as modern literature by Botanist Dr. V.C. Gupta, Deputy director, Central Research Institute Of Unani Medicine, Hyderabad (C.R.I.U.M.) and Dr. Hakeem. Mohd Yadullah Ex. C.M.O. Govt. Nizamia General Hospital Hyderabad and renowned Unani practitioner. Voucher sample was prepared and preserved in the Herbarium of C.R.I.U.M., Hyderabad for further reference.

b) Preparation of plant Extract

Different extracts of *Raphanus sativus* seeds were prepared for analysis in the present study (a) Ethanol (b) Methanol (c) Ethyl Acetate (d) Chloroform (e) benzene (f) Aqueous Hot (g) Aqueous Cold. Ten (10) grams powdered drug soaked in 100 ml of different solvents for 24 hrs & filtered through whattman's filter paper No.1. The filtrate was concentrated by evaporation of solvent on hot plate and water bath at room temperature. All extracts were stored at 4^0 C until further use.

c) Preparation of Test Sample

A stock solution of the extracts was prepared at the concentration of 100mg/ml and store at 2^0C till further use.

d) Source and Maintenance of Organisms

A total 8 strains including gram positive (Staphylococcus aureus, ATCC25923) and gram negative (E.coli-ATCC25922, Pseudomonas aeruginosa-ATCC 27853, Shigella sonnei- ATCC 25931, Salmonella typhi-ATCC 25241, Proteus vulgaris- ATCC 6380, Klebsiella pneumonie-ATCC27736, Salmonella paratyphi -ATCC 9150) bacteria were selected to assess the susceptibility test against the drug extract. The strains were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. They were sub cultured on nutrient agar for every 15 days and maintained on nutrient agar slants at 4^0C. Fresh inoculums were taken for the test.

e) Culture Media

Muellar Hinton Agar (Himedia, India) was prepared according to the manufacturer's instructions, autoclaved at 15 lbs pressure and 121^0C for required time and dispensed into petridishes more than half. Set plates were incubated overnight at 37^0C to ensure sterility before use.

f) Preparation of inoculums

Select & label test cultures that are to be used for (plant extract) Sensitivity Assay. Prepare nutrient agar plates. 3-4 colonies should be selected from the agar plate culture. The top of the each colony is touched with loop & transferred in to into a test tube containing4-5 ml nutrient broth. The test tubes which containing broth cultured are incubated at 37°C until it achieves the turbidity.

g) Evaluation of Antibacterial Activity

The *in-vitro* antibacterial activity of the extracts was determined by agar well diffusion assay (Reeves, 1989). All strains were first grown in Mueller Hinton broth (MHB) under shaking condition for 4 h 37^0C and after the incubation period 0.1ml of the test inoculums was spread evenly with a sterile glass spreader on Mueller Hinton Agar (MHA) plates. The seeded plates were allowed to dry in the incubator at 37^0C. Wells were made using sterile 6 mm cork borer in the inoculated MHA plates. The wells were filled with 200µl of the extracts (re-suspended in respective solvents) and negative controls 1:1 (solvent: water). The concentration of stock extracts was 200 mg /ml. The inoculated plates were incubated at 37^0C for 24 h. The plates were observed for the presence of inhibition of bacterial growth that was indicated by a clear zone around the well. The size of zone of inhibition was measured and the bacterial activity was expressed in term of average diameter of the zone of inhibition in millimeters. The results were compared with the standard antibiotics, Chloramphenicol (25mcg) and Ciprofloxacin (25mcg). The photographs were taken in U.V-visible documentation system.

h) Statistical Analysis

Calculations of antibacterial activity were determined by Standard Deviation and Mean of replicates.

i) Screening for Secondary Metabolites

Secondary metabolites are identified in the extracts of R. *sativus* by using standard methods.1 mg of each extract was dissolved in 100 ml of the respective solvent and filtered through Whattman filter paper No.1. Thus, the filtrates obtained were used as test solutions for the screening. The details for the qualitative analysis (14, 15, 16) were described. Table**1**.

III. Results

The results are listed in Table**2**. Results obtained in the present study relieved that tested medicinal plant extracts posses potential antibacterial activity against all selected bacteria (agar well diffusion method). Among all the extracts Ethanolic and Methanolic extracts showed maximum antibacterial activity against all the bacterial strain used with a zone of inhibition ranges from 12-21 mm and the least activity was observed in Aqueous cold extract with zone of inhibition ranges from 7-9 mm. The test results were

compared with standard antibiotics Chloramphenicol (25μg) and Ciprofloxacin (25μg).

The plant extracts were also screened for qualitative analysis to know the relative distribution of the secondary metabolites which may be responsible for the potent antibacterial activity. Flavonoids are extracted into Ethanol, Aqueous hot and Aqueous cold. Alkaloids are extracted into Ethanol, Methanol, Chloroform, Aqueous hot and Aqueous cold. Glycosides are present in all solvents. Carbohydrates are extracted only in Methanol, Aqueous hot and Aqueous cold. Phenols are extracted into Ethanol, Chloroform and Aqueous hot. Saponins are extracted into Methanol, Chloroform, Aqueous hot and Aqueous cold. Sterols are found in Ethanol, Methanol, Chloroform and Benzene. Tannins are extracted into Ethanol, Chloroform and Aqueous hot. While Protiens are present only into Benzene. Table**3**.

IV. Discussion

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antibacterial activity assay (17). Crude plant extracts are generally a mixture of active and non-active compounds. A number of medicinal plants described in Unani System of Medicine still need to be testify according to the modern parameters to ensure their activity and efficacy. Many reports are available on the antibacterial, antifungal and anti-inflammatory properties of plants (18, 19, 20, 21). Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings.

In India, mortality rate due to infections is largely due to *S. aureus, Ps. aeruginosa, K. pneumonia, E. coli, P.vulgaris, S.sonnie, S.typhi, S. paratyphi.* (22). The treatment and management of infections caused by these strains has become very difficult, therefore, the challenge to discover newer and potent drugs is ever increasing. Therefore, studies were undertaken to test the extracts of *R. sativus* against these pathogens. The highest activity was observed in Ethanol and Methanol extracts followed by Ethyl acetate, chloroform, Benzene, aqueous hot and aqueous cold.

The highest antibacterial effect of Methanol and Ethanol extract against these organism may be due to the ability of the Ethanol and Methanol to extract some of the active properties of these plants like Flavonoids, phenolic compounds, Saponins and other secondary metabolites which are reported to antibacterial (5). Flavonoids are found to be effective antimicrobial substances against a wide range of microorganisms, probably due to their ability to complex with extra cellular and soluble proteins and to complex with bacterial cell wall; more lipophilic Flavonoids may also disrupt microbial membrane (23). Phenol and polyphenols present in the plants are known to be toxic to micro-organism (24). Antibacterial activity of tannins may be related to their ability to inactivate microbial adhesins, enzymes and cell envelope transport proteins, they also complex with polysaccharides (25) .The broad spectrum antibacterial activity exhibited by R. *sativus* may be attributed to the various active constituents presents in it which either due to their individual or combined action. Thus, the study ascertains the value of R. *sativus* used in Unani System of Medicine. This could be of considerable interest to the development of new drugs.

V. Acknowledgments

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Table : 1

S.No.	Secondary metabolites	Experiment	Observation	Inference
1.	**Alkaloids**			
	Dragendroff 's Test	Few mg of alc. Or aq. Ext. of drug in 5 ml of dist. Water and add 2M HCL, then add few drops of Dragendroff's reagent	An orange or orange -red precipitate is formed	Present
2.	**Flavanoids**			
	(a) Shinoda test	To 0.5 ml of alc. ext. of the drug add 5 -10 drops of dil. HCL followed by addition of small piece of Magnesium. Boil the solution for	A pink or reddish pink or brown colour is produced.	Present
	(b) NaOH test	few minutes. 1 ml of 1N NaOH solution w as added to the 1ml of test solution.	Formation of yellow colour	Present

3.	**Glycosides**			
	(a) Conc.H_2SO_4 test	1ml of conc.H_2SO_4 was added to 1ml of test solution and is allowed to stand for 2 minutes.	Formation of reddish colour	Present
	(b) Aq NaOH test	To alc. Ext. of the drug add 1ml of water and adds aq.NaOH solution.	Formation of yellow colour	Present
4.	**Carbohydrates**			
	(a) Benedict's test	To o.5 ml of aq. Ext. of the drug add 5 ml of Benedict's solution and boil for 5 min.	Formation of colour ppt.	Present
	(b)Molisch's test	To 2 ml of aq. Ext. of the drug add 2 drops of freshly prepared 20% alc. α-naphthol and mix, pour 2 ml of conc. H_2SO_4 through the wall of the test tube.	A red-violet ring is formed at the junction of the two liquids, which disappears on addition of excess of alkali	Present
5.	**Phenol**			
	(a) Ferric chloride test	To alc. Or aq. ext. of the drugs add 2 ml of dist. Water and add few drops of 10% aq. $FeCl_3$ solution.	A blue or green colour is produced.	Present
	(b) Aq. Lead acetate test	To alc. Or aq. ext. of the drugs add 5 ml of dist. water and add few drops of 1% aq. lead acetate solution.	A yellow ppt. is formed.	Present
6.	**Saponins**			
	Foam test	To 5 ml of aq. ext. of the drug add drops of Sodium bicarbonate solution shake the mixture vigorously and leave for 3 min.	Honey comb like froth is formed.	Present
7.	**Sterols/Steroids**			
	Salkowski test	Add 1 ml conc. Sulphuric acid to 2 ml of chloroform ext. of the drug care fully through the wall of the test tube.	A red colour is produced in the chloroform layer or at the juncti on of the two liquids.	Present

8.	**Tannins**			
	Ferric chloride test	To 1-2 ml of aq. ext. of the drug add few drops of 5% aq. ferric chloride solution.	A bluish black colour is produced which disappear on addition of a few ml of a dil. Sulphuric acid solu tion followed by the formation of a yellow-brown ppt.	Present
9.	**Proteins**			
	Millon's test	To aq. ext. of the drug add 1 ml of dist. water and add 5-6 drops of Millon's reagent.	A white ppt. is formed which turns red on heating.	Present

Table : 2 Antibacterial Activity.

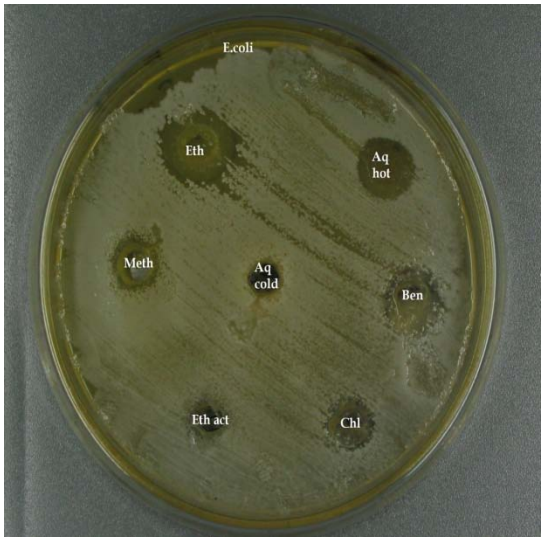
EXTRACTS	**Diameter of Zone of inhibition(mm)**							
	E.coli	***K.pneumoniea***	***P.valgaris***	***Ps.aeruginosa***	***S.aureus***	***S.sonnie***	***S.paratyphi***	***S.typhi***
ETHANOL	14.5±0.7	17±0.5	18±4.2	21.3±6.6	19±7.0	13.6±2.0	13.3±1.5	16.6±1.5
METHANOL	12.5±0.7	14.6±2.5	19.5±0.7	14.6±2.3	13.5±0.7	15.3±2.0	14.6±1.5	15.6±0.5
ETH. ACETATE	9±1.4	NA	22.5±4.9	18±0.5	18±0.5	NA	NA	19.6±0.7
CHLOROFORM	10±0.5	NA	19±0.5	18.3±3.5	10±0.5	NA	NA	14±2.0
BENZENE	12.5±0.7	NA	18±5.6	NA	9±0.5	NA	NA	16.3±1.5
AQ. HOT	12±0.5	11.6±0.5	9±0.5	13.3±2.0	12±0.5	12±0.5	NA	NA
AQ. COLD	NA	9.3±0.5	9±0.5	9.3±0.5	9±0.5	9.6±0.5	7±0.5	7±0.5
CHLORAMPHENICOL(25µG)	29±0.5	28±0.4	20±0.5	9±0.5	NA	16±0.3	14±0.4	21±0.5
CIPROFLOXACIN (25µG)	27±0.4	26±0.3	30±0.4	30±0.4	25±0.5	27±0.4	30±0.5	35±0.5

Table : 3

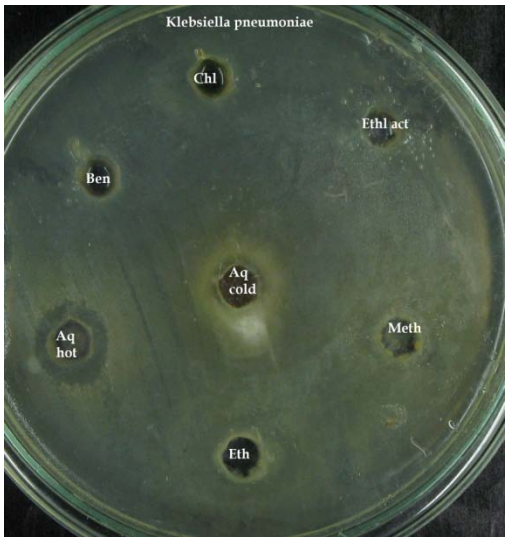
Secondary Metabolites	Name of Test	Results (+/-)						
		Et	Mt	Ea	Ch	Ben	Aq H	Aq C
Alkaloids	Dragendroff's	++	++	--	++	--	++	++
Flavonoids	Shinoda	++	--	--	--	--	++	++
	NaOH	++	--	--	--	--	++	++
Glycosides	Conc.H_2SO_4	++	++	++	++	++	++	++
	Aq NaOH	++	++	++	++	++	++	++
Carbohydrates	Benedict's	--	++	--	--	--	++	++
	Molisch's	--	++	--	--	--	++	++
Phenol	Ferric chloride	++	--	--	++	--	++	--
	Aq. lead acetate	++	--	--	++	--	++	--
Saponins	Foam	--	++	--	++	--	++	++
Sterols	Salkowski	++	++	--	++	++	--	--
Tannins	Ferric chloride	++	--	--	++	--	++	--
Proteins	Millon's	--	--	--	--	++	--	--

Et= Ethanol, **Mt**= Methanol, **Ea**= Ethyl acetate, **Ch**= Chloroform, **Ben**= Benzene, **Aq**=Aqueous, **H**=Hot, **C**=Cold

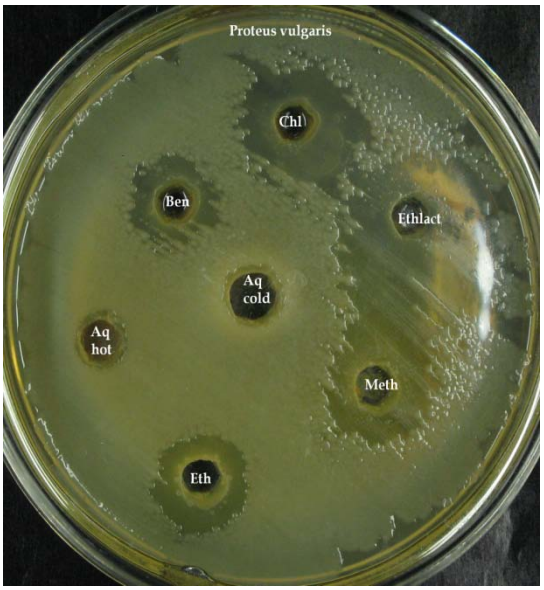
Figures



E.coli

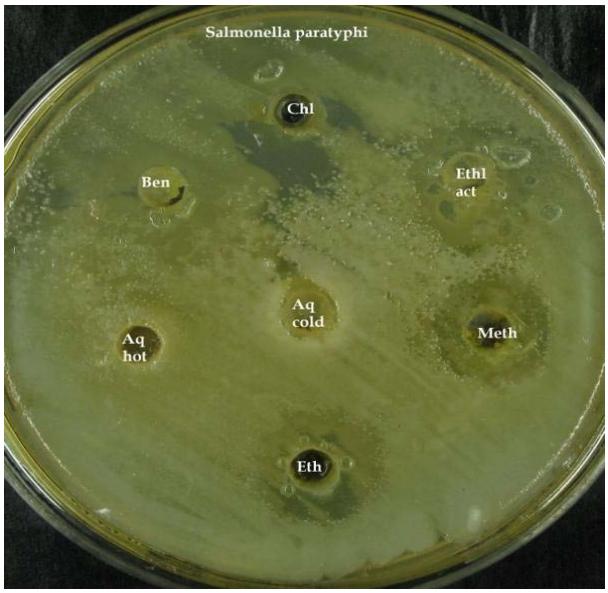


Klebsiella pneumonie

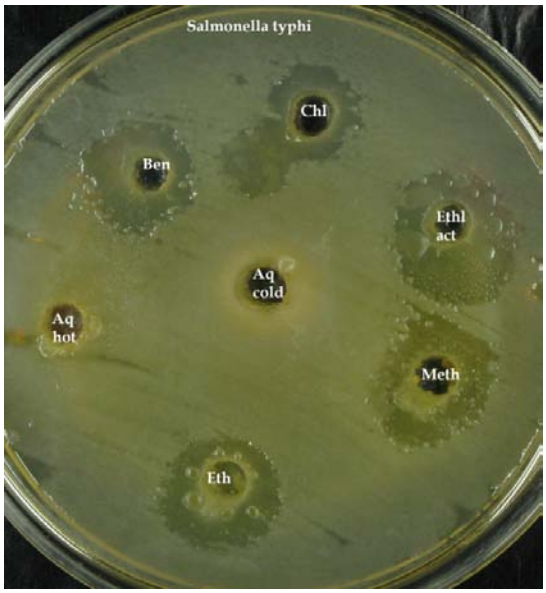


Proteus vulgaris

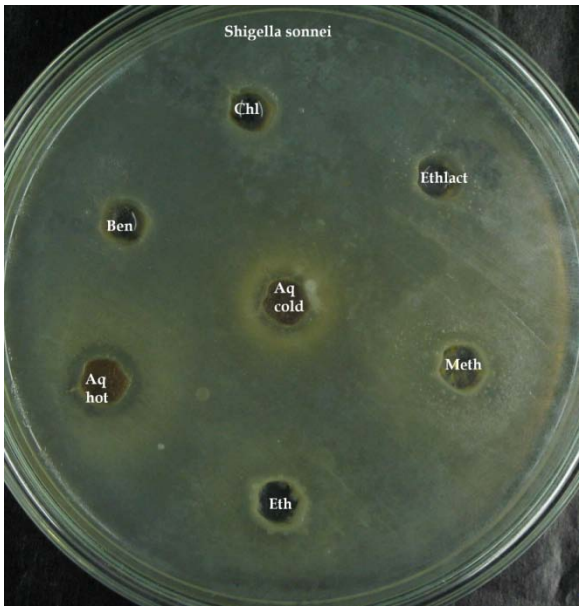
Pseudomonas aeruginosa



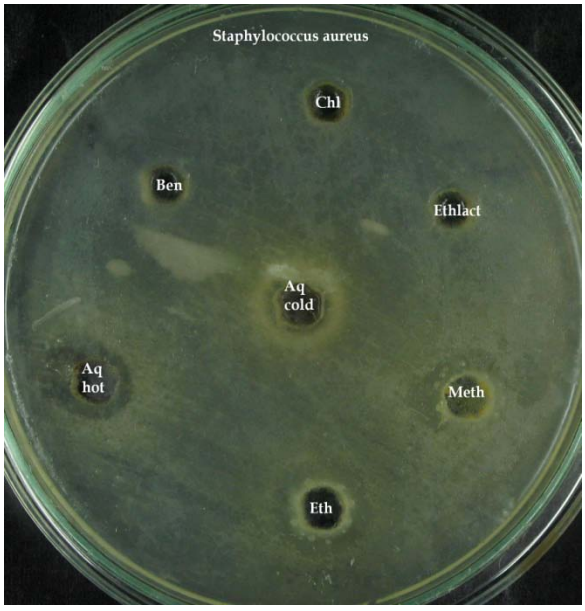
Salmonella paratyphi



Salmonella typhi



Shigella sonnei



Staphylococcus aureus

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