Antibacterial Activity of Raphanus Sativus Linn. Seed Extract

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Abstract

Raphanus sativus Linn. (Radish) is an annual herb of family Cruciferae or Brassicaceae and grown as an edible root. Objectives : The aim of the study is to test the potentiality of different solvent extracts (Ethanol, Methanol, Ethyl Acetate, Chloroform, Benzene, Aqueous hot and Aqueous cold) against various pathogenic bacterial strains E.coli (ATCC-25922), Klebsiella pneumonia (ATCC-27736), Proteus vulgaris (ATCC-6380), Pseudomonas aeruginosa (ATCC-27853), Staphalococcus aureus (ATCC-25923), Shigella sonnie (ATCC-25931), Salmonella typhi (ATCC-25241) and Salmonella paratyphi (ATCC-9150).Methods : The antibacterial activity was performed in vitro using Agar well diffusion assay and diameter of zone of inhibition was measured.

Index terms— antibacterial activity, phytochemical analysis, raphanus sativus, zone of inhibition.

1 I. Introduction

ccording 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 antiinfective 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 (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.

2 II. Materials and Methods

3 a) Plant Materials

The sample of seeds of Raphanus sativus

4 c) Preparation of Test Sample

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

5 d) Source and Maintenance of Organisms e) Culture Media

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

6 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.

7 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 0 C 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 0 C. 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 0 C 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.

8 h) Statistical Analysis

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

9 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. Table1.

10 III. Results

The results are listed in Table2. 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 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 0 C. Fresh inoculums were taken for the test. 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. **??**lavonoids

11 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 microorganism (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. Boil the solution for few minutes.

12 V. Acknowledgments

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,
Cultivated all over sub-continent up to 16,000 ft in temperate and warm countries

globose cotyledons

duplicate.

Figure 1:

Figure 2:

1 2 3

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3. 8.	Glycosides Tan- nins		
	(a) Conc.H 2 SO 4 Ferric chlo- ride test test	1ml of conc.H2SO4 was To 1-2 ml of aq. ext. of added to 1ml of test the drug add few drops of solution and is allowed 5% aq. ferric chloride to stand for 2 minutes. so- lution.	Form of A black reddi colou colou produ whicl
	(b) Aq NaOH test	To alc. Ext. of the drug add 1ml of water and adds aq.NaOH solution.	Form of disap on yellov colou addit a few n dil. Sulph acid
4.	Carbohydrates (a) Bene- dict' s test	To o.5 ml of aq. Ext. of the drug add 5 ml of Benedict's solution and boil for 5 min.	Form of ppt. solu follov by forma of a y A -viole brow
9.	(b)Molisch's test Pro- teins	To 2 ml of aq. Ext. of	ring forme

A pink or reddish pink or brown colour is Honey comb like froth is formed. 9±0.5 NA 12±0.5 12±0.5 9±0.5

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[Kabiruddin and Mufradat] , Makhzanul Kabiruddin , Mufradat . Ejaz Publishing house New Delhi. p. 558.

[Mason and Wasserman ()] , T L Mason , B Wasserman . *P Phytochem* 1987. 26 p. .

[Evans Wc In Trease et al. ()] , Evans Wc In Trease , Pharmacognosy Evans , Edn . *Bailliere Tindall. London* 1989. p. .

[Tsuchiya et al. ()] , H Tsuchiya , M Sato , T Miyazaki , S Fujiwara , S Tanigaki , M Ohyama , T Tanaka , M Iinuma . *J. Ethnopharmacol* 1996. 50 p. .

[Arya et al. ()] , Muktanjali Arya , K Prafull , Arya . *Indian J Pathol.Microbiol* 2005. 48 p. .

[Tona et al. ()] 'Antiamoebic and phytochemical screening of some Congolese medicinal plants'. L Tona , K Kambu , N Ngimbi , K Cimanga , A J Vlietinck . *J. Ethnopharmacol* 1998. 61 p. .

[Tanaka et al. ()] 'Antibacterial activity of Indol alkaloids from Aspidosperma ramiflorum. Braz'. J C A Tanaka , C C Desilva , A J B De Oliveira , C V Nakamura , B P D Filho . *J. Med. boil. Res* 2006. 39 p. .

[Nascimento et al. ()] 'Antibacterial activity of plant extracts and phytochemicals on antibiotics-resistant bacteria'. G G F Nascimento , J Locatelli , P C Freitas , G L Silva . *Braz. J. Microbiol* 2000. 31 p. .

[Samy and Ignacimuthu ()] 'Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats in India'. R P Samy , S Ignacimuthu . *J. Ethnopharmacol* 2000. 69 p. .

[Parekh and Chanda ()] 'Antibacterial and phytochemical studies on twelve species of Indian medicinal plants'. J Parekh , S Chanda . *African Journal of Biology Res* 2007b. 10 p. .

[Reeves ()] 'Antibiotic assays'. D S Reeves . *Medical Bacteriology, A practical Approach*, P M Howkey, D A Levis (ed.) (Oxford) 1989. IRL Press. p. . (chapter)

[Aliero and Afolayan ()] 'Antimicrobial activity of Solanum tomentosum'. A A Aliero , A J Afolayan . *African Journal of Biotechnology* 2006. 5 p. .

[Press ()] 'Biodiversity: exciting prospects for drugs discovery and development. Meeting report of the Monroe Wall Symposium'. J B Press . *Chemtracts-Organic chemistry* 1996. 9 p. .

[Ya et al. ()] 'Carbohydrate polyphenol complexation'. C Ya , S H Gaffney , T H Lilley , E Haslam . *Chemistry and Significance of Condensed Tannins*, R W Hemingway, J J Karchesy (ed.) (New York) 1998. Plenum Press. p. .

[Chopra et al. ()] *Glossary of Indian Medicinal plants (Including the supplements) Council of Scientific and Industrial Research*, R N Chopra , S L Nayar , I C Chopra . 1986. New Delhi.

[Kritikar and Basu ()] *Indian medicinal Plants. Vol. I Ed. 2 nd . Edn., India: International Book Distributors*, R K Kritikar , B D Basu . 1987. 9/B, Rajpur Road, Dehradun. p. 178.

[Behera and Misra ()] 'Indigenous phytotherapy for genito-urinary diseases used by the Kandha tribe of Orissa, India'. S K Behera , M K Misra . *J. Ethnopharmacol* 2005. 102 p. .

[Sina and Book ()] *Institute of History of Medicine and Medical research*, A A Sina , Al-Qanun Fi´-Tib Book , II . 1987. New Delhi. p. 300.

[Srivastava et al. ()] *Medicinal Plants: An expanding role in development*, J Srivastava , J Lambert , N Vietmeyer . 1996.

[Forbes et al. ()] *Methods for testing antimicrobial effectiveness. Bailey and Scott's Diagnostic Microbiology MOS by Co*, B A Forbes , O F Sahm , A S Weissfeld , E A Trevomp . 1990. Missouri, PD: St Louis. p. .

[Anonymous ()] *Physiochemical standards of Unani formulations Part-3,. Central council for research in Unani Medicine CCRUM publication No.31 p*, Anonymous . 1993. p. .

[Harborne ()] *Phytochemical Methods, A guide to modern techniques of plant analysis, 3rd Edn*, J B Harborne . 1998. London: Chapman and Hall. p. .

[Cowan ()] 'Plant products as antimicrobial agent'. M M Cowan . *Clin. Microbiol. Rev* 1999. 12 p. .

[Eloff ()] 'Which extract should be used for the screening and isolation of antimicrobial components from plants'. J N Eloff . *Journal of Ethno pharmacology* 1998. 60 p. .

[WHO Global Strategy for containment of antimicrobial resistance www.who.int/emedocuments/antimicrobial_resistance/docs/E *WHO Global Strategy for containment of antimicrobial resistance www.who.int/emedocuments/antimicrobial_resistance/docs/E art*, 2001.