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Research Article

ANTIMICROBIAL EFFICACY OF *RAPHANUS SATIVUS* ROOT JUICE

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ABSTRACT

Raphanus sativus L. belongs to family *Brassicaceae* and is more commonly known as *Radish*. Radish has long been grown as a food crop and are of high medicinal value. *R. sativus* seeds and leaves contain 'raphanin' which has been found to possess antibacterial and antifungal potential. In the present study, *R. sativus* root juice was evaluated for its antimicrobial potential against five bacterial strains, viz. *Klebsiella pneumoniae*, *Staphylococcus aureus, Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Escherichia coli*. The results obtained from the present study, reveal that *R. sativus* root juice exhibits considerable antimicrobial activity against all the tested microorganisms at a Minimum Inhibitory Concentration (MIC) ranging from 0.078 to 0.625 mg/ml. A reference drug, ampicillin was used for comparing the results.

Keywords: *Raphanus sativus* root juice, Antimicrobial, Minimum Inhibitory Concentration and Ampicillin.

INTRODUCTION

The use of natural products with therapeutic properties is as ancient as human civilization. Plants have provided man with all his needs in terms of shelter, clothing, food, flavours, fragrances and most important of all medicines. Herbal medicine is the oldest form of healthcare product known to mankind and was used by all cultures throughout history[1]. It was an integral part of the development of modern civilization. About 80% of the world's population in the developing countries rely on plants and plant derived products for treatment of various diseases, primarily because of their cultural acceptability, better compatibility with the human body and lesser side effects.

It is thus that in recent years, there has been growing interest in alternative therapies and therapeutic use of natural products, especially those derived from plants[2-7]. Moreover, a large percentage of the world's population does not have access to conventional pharmacological treatment and have to depend heavily on folk medicine.

Infectious diseases are responsible for approximately one half of all deaths in tropical countries. However, it was only after the recent developments in the field of microbiology that has made possible the study of infectious diseases associated with microbes. The search for new molecules, nowadays, has taken a slightly different route where the science of ethnobotany and ethnopharmacognosy are being used as guide to lead the chemist towards different sources and classes of compounds. The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the spectre of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies[8]. Although a large number of synthetic antibiotics are available in the market, yet because of the increasing resistance to antibiotics of many bacteria, plant extracts and plant compounds are of new interest as antiseptics and antimicrobial agents[9-11]. Over 50% of all modern clinical drugs are having their origin in natural products[12]. The pharmacological properties of the medicinal plants have been attributed to the presence of active chemical constituents which are responsible for important physiological function in living organisms.

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as microorganisms, animals and plants. One such resource is folk medicine. Systematic screening of folk medicine may result in the discovery of novel effective compounds[13]. Scientific experiments on the antimicrobial properties of the plant compounds were first documented in the late 19th century[14]. Extracts of many plants are now known to produce certain bioactive molecules which react with other organisms in the environment, inhibiting bacterial or fungal growth (antimicrobial activity)[15-16]. The substances that can inhibit pathogens and have little toxicity to host cells are considered candidates for developing new antimicrobial drugs. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoides, coumarins, alkaloids and flavonoids, which have been found *in vitro* to have antimicrobial properties[17]. Condensed tannins have been determined to bind cell walls of ruminal bacteria, preventing growth and protease activity[18].

Contrary to synthetic drugs, antimicrobials of plant origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases[19]. Numerous research work has been done aiming to know the different antimicrobial and phytochemical constituents of medicinal plants and in using them for the treatment of microbial infections (both topical and systemic applications) as possible alternatives to chemically synthetic drugs to which many infectious microorganisms have become resistant. A number of plants have been evaluated for their antimicrobial properties[20-22].

The Indian traditional plant, *Raphanus sativus* was selected for the present study for systemic and scientific research of its antimicrobial potential. *R. sativus* seeds and leaves contain 'raphanin' which has already been reported for its antibacterial and antifungal properties[23-24]. The antibacterial principle 'raphanin' has been found to be strongly active on *Escherichia coli, Pseudomonas pyocyaneus, Salmonella typhi*, *Bacillus subtilis*[25], *Staphylococcus aureus, streptococci* and *Pneumococci*[26]. It is also active against many food borne pathogenic and food spoiling bacteria such as *Listeria, Micrococcus, Enterococcus, Lactobacillus* and *Pedicoccus* species[27]. Seeds are also rich in antibacterial proteins. Previous reports on the antibacterial activity of seeds and leaves encouraged us to carry out the scientific evaluation of antimicrobial activity of *R. sativus* root juice as well. Till date, *R. sativus* root juice has not been explored for its antimicrobial activity. The present study was undertaken to screen the antibacterial activity of *R. sativus* root juice against five bacterial strains, viz. *Klebsiella pneumoniae*, *Staphylococcus aureus, Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Escherichia coli.*

MATERIALS AND METHODS

Plant material

Fresh roots (about 10 kg) of *Raphanus sativus* (Family: Brassicaceae) were collected locally from Allahabad, U. P., India in the month of June, 2009 and were identified by Prof. Satya Narayan, Taxonomist, Department of Botany, University of Allahabad, Allahabad, India. A voucher specimen have been submitted to the University herbarium.

Preparation of plant material

The collected fresh roots were first washed well. They were then squeezed in an electric blender to obtain about 2 litres of fresh juice which was then filtered and concentrated in rotary evaporator at 35° ± 5°C under reduced pressure. The resulting material was then lyophilized to get a semisolid material (40 g, 11.3%, w/w). The semisolid material was then dissolved in distilled water for evaluation of antimicrobial activity.

Bacterial strains, stocks and growth *in vitro*

Five bacterial strains namely, *Klebsiella pneumoniae* (Gram-negative), *Staphylococcus aureus* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), *Enterococcus faecalis* (Gram-negative) and *Escherichia coli* (Gram-negative) were used to assess the antibacterial activity of *R. sativus* root juice. These bacterial strains were obtained from the Department of Biotechnology, All India Institute of Medical Sciences (AIIMS), New Delhi, India and the microbiologist of the department confirmed the identity based on microscopic examination, Gram's character and biochemical test profile. Bacterial stocks were maintained and stored as 1 ml aliquots at -80^{O}C in LB broth for all the five bacterial strains. Bacterial stocks were revived from -80^{O}C and grown in Luria Bertani (LB) broth for all the five bacterial strains. All cultures were grown overnight at 37^{O}C ± 0.5°C, pH 7.4 in a shaker incubator (190-220 rpm). Their sensitivity to the reference drug, Ampicillin (Sigma-Aldrich, New Delhi, India) was also checked.

Determination of antimicrobial activity based on MIC

Minimum Inhibitory Concentration (MIC) of the freshly prepared inocula of *Klebsiella pneumoniae, Staphylococcus aureus, pseudomonas aeruginosa, Enterococcus faecalis* and *Escherichia coli* was determined by the micro-dilution method using serially diluted (2-fold) plant extracts according to the NCCLS (National Committee for Clinical Laboratory Standards, 2000). A final concentration from 0.078 to 2.5 mg/ml was used for the plant sample. The effects were also compared with that of a standard antibiotic, ampicillin at the same concentration range. Finally, the test tubes closed with cotton plugs were incubated at 37^{O}C for 24 h in a shaker incubator. Control tubes without the tested sample was asssayed simultaneously. All samples were tested in triplicates.

Statistical analysis

Data were expressed as ±S.D. Two-way analysis of variance (ANOVA) was performed using Graph Pad Prism 4.00 for Windows (Graph Pad Software, San Diego, CA, USA).

RESULTS

Table 1 represents the antibacterial potential of *R. sativus* root juice. The results obtained from the present study, reveal that *R. sativus* root juice exhibits considerable antimicrobial activity against all the tested microorganisms at a MIC range from 0.078 mg/ml to 0.625 mg/ml. The standard antibiotic, ampicillin had MIC values ranging from 0.078 mg/ml to 0.312 mg/ml. The lowest MIC of 0.078 mg/ml was against the bacterial strains, *Escherichia coli* and *Klebsiella pneumoniae*. Whereas, *Enterococcus faecalis* showed inhibition at little higher MIC of 0.156 mg/ml. Moreover, *Staphylococcus aureus* and *P. aeroginosa* exhibited inhibition at even much higher MIC of 0.312 mg/ml and 0.625 mg/ml respectively.

Table 1: MIC values of *Raphanus sativus* root juice and standard drug, ampicillin against different bacterial isolates

Microorganism	MIC (mg/ml)	
	***Raphanus sativus* root juice**	**Standard drug, ampicilin**
Staphylococcus aureus (Gram-positive)	0.312 ± 0.2	0.156 ± 0.07
Escherichia coli (Gram-negative)	0.078 ± 0.03	0.078 ± 0.04
Pseudomonas aeruginosa (Gram-negative)	0.625 ± 0.4	0.156 ± 0.8
Klebsiella pneumoniae (Gram-negative)	0.078 ± 0.04	0.312 ± 0.3
Enterococcus faecalis (Gram-negative)	0.156 ± 0.09	0.156 ± 0.08

Values are mean ± S.D. of triplicate assays

Graph 1 represents the comparative MIC values of *R. sativus* root juice and ampicillin against the five tested bacterial strains. It is evident from the figure that *R. sativus* root juice possessed the greatest antibacterial activity against two bacterial strains, *E. coli* and *K. pneumoniae* (MIC 0.078 mg/ml). Though, ampicillin has highest activity against *E. coli* same as *R. sativus* root juice but it was least active against *K. pneumoniae*. These results clearly indicate that *R. sativus* root juice could be developed as an effective antimicrobial agent against *K. pneumoniae* which cannot be treated by ampicillin even.

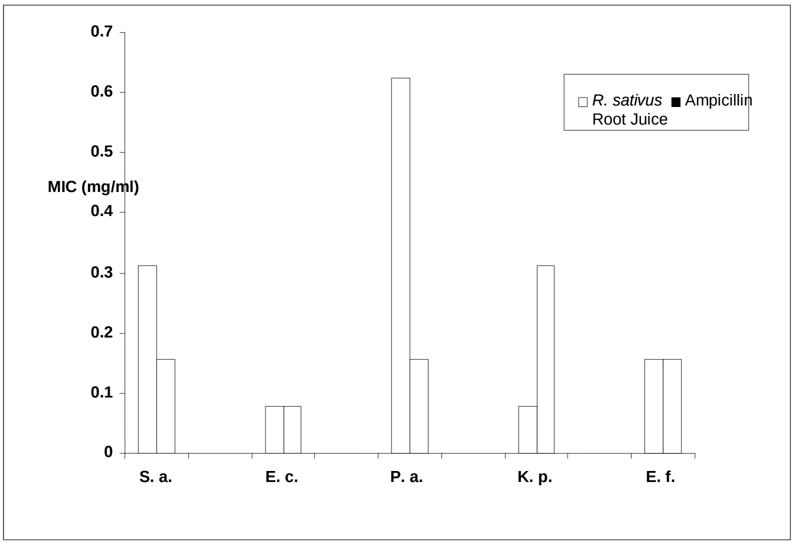

Fig. 1: MIC values of *R. sativus* root against different bacterial strains

P. a. (*Pseudomonas aeruginosa*), ***E. f.*** (*Enterococcus faecalis*), ***E. c.*** (*Escherichia coli*),

K. p. (*Klebsiella pneumoniae*), ***S. a.*** (*Staphylococcus aureus*).

DISCUSSION

The results indicate that *R. sativus* root juice possesses greatest antibacterial potential towards the Gram-negative bacteria viz. *E. coli* and *K. pneumoniae* as the most significant MIC value of 0.078mg/ml was observed in both the cases with *R. sativus* root juice. Next higher antibacterial activity of *R. sativus* was observed for *E. faecalis.* However, bacterial strains viz. *P. aeruginosa and S. aureus* were comparatively more resistant towards *R. sativus* juice. The antimicrobial activity of the plant was compared with reference drug, ampicilin and was found to be of the following order based on their MIC.

Ampicilin efficacy : *E. coli > E. faecalis = S. aureus = P. aeroginosa > K. pneumoniae*

R. sativus juice efficacy: *E. coli = K. pneumoniae* **>** *E. faecalis > S. aureus > P. aeroginosa*

The results were coherent with the earlier reports on antimicrobial activity against Gram-negative bacteria[17]. *E. coli* strain is reported to cause serious food poisoning in humans and is occasionally responsible for product recalls[28-29]. In our study, the growth of *E. coli* strain was remarkably inhibited by the root juice of *R. sativus* (MIC 0.078 mg/ml) and results could be compared even with the well known drug, ampicillin. The interesting as well as encouraging observation was that the *R. sativus* root juice was more effective against *K. pneumoniae* (MIC 0.078) than the reference drug, ampicillin (MIC 0.312).

Scientists from divergent fields are investigating plants with a new eye for their antimicrobial usefulness. A sense of urgency accompanies the search, as most of bacteria have developed resistance against the existing drugs. Laboratories of the world have found literally thousands of phytochemicals which have inhibitory effects on all types of microorganisms *in vitro*[30-31]. More of these compounds should be subjected to animal and human studies to determine their effectiveness including, in particular, toxicity studies as well as an examination of their effects on beneficial normal microbiota. It would be advantageous to standardize methods of extraction and *in vitro* testing so that the search could be more systematic and interpretation of results would be consistent. Also, alternative mechanisms of infection prevention and treatment should be included in initial activity screenings. Disruption of adhesion is one example of an anti-infection activity not commonly screened currently. Attention to these issues could pave way for a new era of chemotherapeutic treatment of infection by using plant-derived principles[17].

The results of the present study thus, seem to be promising and may enhance the use of highly potential *R. sativus* root juice in the treatment of various bacterial infections especially against *E. coli* and *K. pneumoniae* as, the existing drug ampicillin is also not that much effective against *K. pneumoniae* as the *R. sativus* root juice. Further studies on the chemical characteristics of the juice is in progress in order to identify the leads with antimicrobial activity.

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