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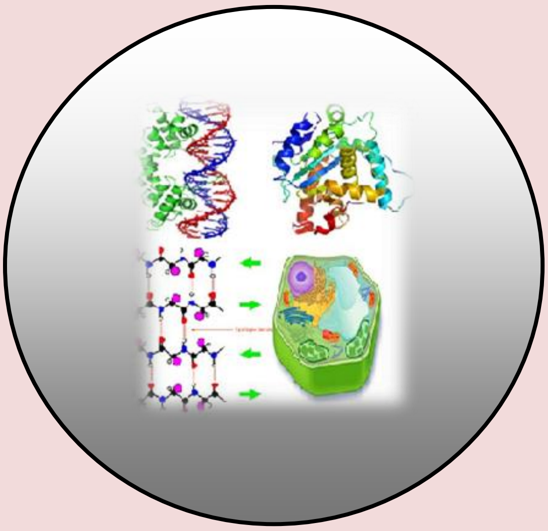

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Comparative Antibacterial Profile of two Medicinally Important Plants –*Citrus limonia* and *Phoenix dactylifera*

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ABSTRACT

The role of traditional medicines in the solution of health problems is invaluable on a global level. Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine. With the associated side effects of the modern medicine, traditional medicines are gaining importance and are now being studied to find the scientific basis of their therapeutic actions. Research work on medicinal plants has intensified and information on these plants has been shared with researchers at a global level. This research will go a long way in the scientific exploration of medicinal plants for the benefit of mankind and is likely to decrease the dependence on synthetic drugs. Antimicrobial agents can also be derived from herbs, and over 1000 plants exhibit antimicrobial effects. Traditionally, these herbs are said to provide safe and effective treatment against many diseases. Increasing bacterial resistance to antibiotics has led to the development of novel antimicrobial agents from plant extracts. Citrus limonia L. and Phoenix dactylifera L. are well known medicinal plants with immense medicinal value. These plants have not been scientifically explored so far for their antimicrobial property. In the present study, Citrus limonia leaf and Phoenix dactylifera seed extracts were evaluated for its antibacterial potential against K. pneumoniae, S. aureus, P. aeruginosa, E. faecalis and E. coli. Results reveal significant antibacterial activity at a Minimum Inhibitory Concentration ranging from 0.312 to 0.625 mg/ml in case of C. limonia and 0.156 to 0.625 mg/ml in case of P. dactylifera.

Keywords: Citrus limonia, Phoenix dactylifera, Antibacterial and Minimum Inhibitory Concentration.

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents (Mahesh and Satish, 2008). Plants generally produce many secondary metabolites which constitute an important source of microbiocides, pesticides and many pharmaceutical drugs. The effects of plant extracts against different bacteria have been studied by a very large number of researchers in different parts of the world (Ateb and Erdo Urul, 2003). However, only a few studies have focused on the development of medicinal plants as sources of antimicrobial compounds that could inhibit pathogenic microorganisms. Thus, the present study deals with the scientific and systematic exploration on antimicrobial efficacy of *C. limonia* leaves and *P. dactylifera* seeds as both these parts of these medicinal plants has not been evaluated so far.

Citrus limonia L. Osbeck (Family: Rutaceae) is more commonly known as 'lemon' or Mandarin lime' in English and 'nimbu' in Hindi. Despite many published reports dealing with bioactivity of compounds isolated from *C. limonia*, little was known about its antimicrobial activity prior to our investigation. Preliminary phytochemical screening of the aqueous extract of *C. limonia* leaves revealed the presence of phenolic compounds (Sofowara, 1993, Trease and Evans, 1989). Color test has revealed the presence of flavonoids which have been found to exhibit inhibitory effect against bacteria (Sato et al., 1996). Flavonoids constitute a large group of secondary plant metabolites that are ubiquitous among higher plants. These are polyphenolic compounds that generally occur as glycosylated derivatives. As dietary compounds, they are known as antioxidants that inhibit the oxidation of low-density lipoproteins and reduce thrombotic tendencies (Chang, 1990). *Phoenix dactylifera* L. (Family: Arecaceae) is known as 'date palm' in English and commonly known in India as 'khajur' in Hindi. The various parts of this plant are widely used in traditional medicine for the treatment of various disorders which include memory disturbances, fever, inflammation, paralysis, loss of consciousness, nervous disorders (Anonymous, 1985; Nadkarni, 1976). It is known as a healthy food since a long time, because of high tannin content and nutritive value. Tannins have been reported to be bacteriostatic or bactericidal against *Staphylococcus aureus* (Chung et al., 1993). Tannic acid, was found to be inhibitory to the growth of intestinal bacteria such as *Bacteroides fragilis*, *Clostridium perfringens*, *Escherichia coli* and *Enterobacter cloacae* (Miranda et al., 1996) because of its strong iron-binding capacity (Chung et al., 1998a). Many biological activities and antibacterial-promoting effects have been reported for plant tannins and flavonoids, and their investigation is now increasingly relevant (Haslam, 1989; Scalbert, 1991; Chung et al., 1998[b]). One of the benefits of *P. dactylifera* seeds is the capability to inhibit the growth of oral bacteria (Gunawan et al., 2009). Recently, because of increasing resistance to antibiotics of many bacteria, plant extracts and plant compounds are of new interest as antimicrobial agents (Augustin and Hoch, 2004; Blaschek et al., 2004; Norton, 2000). It was this aim which inspired us to determine the antimicrobial activity of extracts from medicinal plants like *Citrus limonia* (leaves) and *Phoenix dactylifera* (seeds) against pathogenic microorganisms.

MATERIAL AND METHODS

Plant collection and identification

Fresh leaves (5 Kg) of *Citrus limonia* and fruits (7 Kg) of *Phoenix dactylifera* were collected locally from Allahabad, U. P., India and got identified by Prof. Satya Narayan, Taxonomist, Department of Botany, University of Allahabad, Allahabad, India. Voucher specimens have been submitted to the University herbarium. The leaves and fruits were first washed well. The *P. dactylifera* fruits were boiled at 70°C and pulp of fresh fruits was separated to take out the hard seeds. The *C. limonia* leaves and *P. dactylifera* seeds were then dried at room temperature and mechanically crushed separately. The crushed leaves and seeds were then extracted with distilled water (65°C) using Soxhlet upto 72 h. The two extracts were filtered and concentrated in rotary evaporator at 45-50°C under reduced pressure, to obtain semisolid materials, which were then lyophilized at -40°C to get powders. These powders were dissolved in distilled water and used for evaluation of their antimicrobial activity.

Bacterial strains, stocks and growth *in vitro*

Bacterial strains of *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Escherichia coli* were clinical isolates obtained from Department of Biotechnology, All India Institute of Medical Sciences (AIIMS), New Delhi, India. Bacterial stocks were maintained and stored at -80ºC in Luria Bertani (LB) broth for all the five bacterial strains. Bacterial stocks were revived from -80ºC and grown in LB broth and all cultures were grown overnight at 37ºC, pH 7.4 in a shaker incubator. Their sensitivity to the reference drug, Ampicillin was also checked.

Experimental design

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) of the freshly prepared inocula of the bacterial strains was determined by the micro-dilution method using serially diluted (2-fold) plant extract according to the NCCLS (National Committee for Clinical Laboratory Standards) (NCCLS, 2000). A final concentration from 0.078 to 2.5 mg/ml was used for the aqueous extracts. The effects were also compared with that of a standard antibiotic, ampicilin at the same concentration range.

RESULTS AND DISCUSSION

The antibacterial activities of the aqueous extract obtained from *C. limonia* leaves and *P. dactylifera* seeds under study by the micro-dilution method are shown in Tables 1 and 2, respectively. In this study, the results of the investigations show that the aqueous extract from the leaves of *C. limonia* possess antimicrobial activity against all the tested organisms at a concentration (MIC) ranging from 0.312 mg/ml to 0.625 mg/ml (Table 1). Whereas, aqueous extract of *P. dactylifera* seeds was shown to possess greater antimicrobial activity against all the tested organisms being effective at a lower concentration (MIC) ranging from 0.156 mg/ml to 0.625 mg/ml (Table 2). Results of both the extracts were compared with the standard antibiotic, ampicilin. The standard drug, ampicillin had MIC values even at a further lower concentration varying between 0.078 mg/ml and 0.312 mg/ml indicating thereby its greater activity than the extracts.

Table 1 reveals that the aqueous extract of *C. limonia* leaves screened, showed maximum inhibition at 0.312 mg/ml concentration against the bacterial strains, *Pseudomonas aeroginosa* and *Enterococcus faecalis*. The MIC values observed against *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were at double the concentration of 0.625 mg/ml. Thus, the most significant MIC value of 0.312 mg/ml observed against the bacterial strains, *P. aeroginosa* and *E. faecalis* was more effective being much lesser in concentration in comparison to the MIC value of 0.625 mg/ml against other three bacterial strains viz. *E. coli*, *K. pneumoniae* and *S. aureus*.

Table 2 shows that the aqueous extract of *P. dactylifera* seeds on the other hand, showed maximum inhibition at concentration of 0.156 mg/ml against the bacterial strain, *Escherichia coli*. The next maximum inhibition was observed against *Pseudomonas aeruginosa* at double the concentration of 0.312 mg/ml. The remaining three bacterial strains, viz. *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Enterococcus faecalis* showed maximum inhibition at even higher concentration of 0.625 mg/ml the most significant MIC value of 0.156 mg/ml in case of the aqueous extract was therefore, observed against the bacterial strain, *E. coli*. Bacterial strains viz. *P. aeroginosa*, *S. aureus*, *K. pneumoniae* and *E. faecalis* were comparatively more resistant to the extract used.

Thus, the significant antimicrobial activity observed against *E. coli* was of the following order in both the extracts:

Ampicilin > *C. limonia* extract > *P. dactylifera* extract

Whereas, it was of just the reverse order against *E. faecalis* in case of both the extracts as follows:

Ampicilin > *P. dactylifera* extract > *C. limonia* extract

Therefore, it may be summarized that the bacterial strains, *P. aeroginosa* and *E. faecalis* were inhibited most by the aqueous extract of *C. limonia*. In other words, *C. limonia* aqueous extract was found to show the most potent inhibition for *P. aeroginosa* and *E. faecalis* (MIC 0.312 mg/ml). Whereas, the aqueous extract of *P. dactylifera* possessed the highest antibacterial activity against the bacterial strain, *E. coli* (MIC 0.156 mg/ml). Thus, the most significant MIC value recorded by *P. dactylifera* was greater as compared to *C. limonia* against all the five tested bacterial strains. The MIC values for all the five bacterial strains were confirmed after subjecting the strains to even lower concentrations, which showed no further inhibition.

The results indicated that the aqueous extract of *C. limonia* leaves studied showed greatest antibacterial activities towards the Gram-negative bacteria, *P. aeroginosa* as well as the Gram-positive bacteria, *E. faecalis*. Similarly, the aqueous extract of *P. dactylifera* seeds showed highest antibacterial activity against the Gram-negative bacteria, *E. coli*. The results against *P. aeroginosa* and *E. coli* are consistent with previous reports on antimicrobial activity against Gram-negative bacteria (Cowan, 1999). The resistance of Gram-positive bacteria (*E. faecalis*) to plant extracts was unexpected as, in general, this class of bacteria is more resistant than Gram-negative bacteria. Such resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism (Adwan and Abu-Hasan, 1998). Infections caused by *P. aeruginosa*, especially those with multi-drug resistance, are among the most difficult to treat with conventional antibiotics (CDC, 1999). *E. coli* strain is reported to cause serious food poisoning in humans and is occasionally responsible for product recalls (CDC, 2007; Vogt and Dippold, 2005). In our study, the growth of *P. aeruginosa* and *E. coli* strains were remarkably inhibited by the aqueous extracts of *C. limonia* (MIC 0.312 mg/ml) and *P. dactylifera* (MIC 0.156 mg/ml), respectively.

It seems very likely, therefore, that the antibacterial compound if extracted from *C. limonia* and *P. dactylifera*, may inhibit bacteria by a different mechanism than that of currently used antibiotics and may thus, have therapeutic value as an antibacterial agent against multi-drug resistant bacterial strains. Infection with these organisms may result in life-threatening complications such as hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (Cowan, 1999). Our results demonstrate that the aqueous extract of *C. limonia* displayed highest antimicrobial activity against *P. aeroginosa* and *E. faecalis* (MIC 0.312 mg/ml). Whereas, the aqueous extract of *P. dactylifera* displayed highest antimicrobial activity against *E. coli* (MIC 0.156 mg/ml). These two plants may thus, be potential sources that could be useful in the treatment of infections caused by these microorganisms. Thus, it shows that the medicinal plants, *C. limonia* and *P. dactylifera*, used in traditional medicine are potentially effective antimicrobial agents and the information obtained from the above results will definitely contribute to a better understanding of the antimicrobial activity of both the plants. It is hoped that this study would lead to the development of more potent novel antimicrobial drugs of natural origin by elucidating the nature of compounds that could be used.

Table 1. MIC values of the aqueous extract of *C. limonia* leaves and standard drug, ampicilin against different bacterial strains.

Micro-organism	MIC (mg/ml)	
	***C. limonia* Aqueous Extract**	**Standard drug, Ampicilin**
Escherichia coli (Gram-negative)	0.625	0.078
Klebsiella pneumoniae (Gram-negative)	0.625	0.312
Pseudomonas aeruginosa (Gram-negative)	0.312	0.156
Staphylococcus aureus (Gram-positive)	0.625	0.156
Enterococcus faecalis (Gram-positive)	0.312	0.156

Table 2. MIC values of the aqueous extract of *P. dactylifera* seeds and standard drug, ampicilin against different bacterial strains.

Micro-organism	MIC (mg/ml)	
	***P. dactylifera* Aqueous Extract**	**Standard drug, Ampicilin**
Escherichia coli (Gram-negative)	0.156	0.078
Klebsiella pneumonia (Gram-negative)	0.625	0.312
Pseudomonas aeruginosa (Gram-negative)	0.312	0.156
Staphylococcus aureus (Gram-positive)	0.625	0.156
Enterococcus faecalis (Gram-positive)	0.625	0.156

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