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Assessment of Antimicrobial Property of *Piper betel* Leaf against Some Human Pathogenic Bacteria

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ABSTRACT

The leaves of Piper betel (locally known as Paan) have long been in use in the Indian local system of medicine for its antioxidant and antimicrobial properties. In the present work, the antimicrobial activity of Piper betel leaves was evaluated against human pathogenic bacteria (both gram-positive and gram-negative). The leaves of Piper betel (locally known as Paan) have long been in use in the Indian local system of medicine for its antimicrobial property. In the present work, the antimicrobial activity of ethanol extract of Piper betel leaves was evaluated against human pathogenic bacteria (both gram-positive and gram-negative) such as Bacillus subtilis, Bacillus cereus, Bacillus frimicutes, Escherichia coli, Entrobacter, Klebsiella, Escherichia coli. The leaves of Piper betel were also showed the presence of alkaloids, saponins, glycoside, proteins, Phytosterols, flavonoids terpenoids, tannins fixed oil and fats in the extract. The antimicrobial activities were evaluated using agar disc diffusion method. The results indicated that the antimicrobial of Piper betel Linn inhibit the growth of microorganism's as dose dependently manner. The results also indicate that scientific studies carried out commonly use herbs having traditional claims of effectiveness might warrant fruitful results.

Key Words*: Phytochemical, Antimicrobial, Piper betel, hydro methanolic, Bacteria, E. Coli, seeds, antibiotics.*

INTRODUCTION

In many developing countries, medicinal plants are being used as alternative to treat wounds (Rowan, et, al., 2015). In Indonesia, boiled Piper betle L. leaf has been widely used as an alternative treatment, commonly for halitosis, vaginal and oral anti-candidiasis, and nonetheless conjunctivitis. Piper betel Linn (Piperaceae) leaves is widely used as a post meal mouth freshener and the crop is extensively grown in India, Sri Lanka, Malaysia, Thailand, Taiwan and other Southeast Asian countries. Due to strong pungent aromatic flavour betel leaves are used as masticatory by the Asian people. Its common names are betel (in English), paan (in Indian), phlu (in Thai) and sirih (in Bahasa Indonesian). Grown abundantly in many parts of India, betel is an evergreen dioecius herb that needs warm and moist growth conditions for its growth. Piper betel Linn (Piperaceae) leaves is widely used as a post meal mouth freshener and the crop is extensively grown in India, Sri Lanka, Malaysia, Thailand, Taiwan and other Southeast Asian countries. Due to strong pungent aromatic flavour betel leaves are used as masticatory by the Asian people. Its common names are betel (in English), paan (in Indian), phlu (in Thai) and sirih (in Bahasa Indonesian). Grown abundantly in many parts of India, betel is an evergreen dioecius herb that needs warm and moist growth conditions for its growth.

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Leaves of betel vine are used with various condiments such as areca nut (kattha), cloves, cardamom, arecanut, candied rose and fennel for chewing purposes (Verma et al., 2004). Indian system of medicine and health has adopted the use of betel leaves in various ways. In Indian folkloric medicine, betel leaf is popular as an antiseptic and is commonly applied on wounds and lesions for its healing effects. This particular property has paved way for further experimental studies, which have established paan extract to have antimicrobial and antileshmian properties (Sarker et al., 2008). Fresh juice of betel leaves is also used in many ayurvedic preparations. Betel leaves have long been studied for their diverse pharmalogical actions.

Traditional healers from different remote communities in India claim that their medicine obtained from these betel leaves is cheaper and more effective than modern medicine. Patients belonging to these communities have a reduced risk of acquiring infectious diseases from resistant pathogens than the people from urban areas who may be treated with regular antibiotics. A novel approach to the prevention of antibiotic resistance of pathogenic species is the use of new compounds that are not based on existing synthetic antimicrobial agents (Shah et al., 2005). It is imperative that evaluation of the potential use of folkloric medicine for the treatment of infectious diseases produced by common pathogens be performed on a scientific base. Many plants are thus becoming probable sources of important drugs and pharmaceutical industries, nowadays, have come to consider this traditional medicine as a source of bioactive agents which can be used in the preparation of synthetic medicine. Furthermore, they are possible source for new as well as potent antibiotics to which the pathogenic strains are not resistant. Reports of various researches show that betel extract and betel oil exhibit antimicrobial and antioxidant activities in model systems (Salleh et al., 2002; Lei et al., 2003; Bhattacharya et al., 2006). The objective of this study includes the evaluation of phytochemical and antimicrobial activity of the betel leaf and investigating the efficacy of the same as an antimicrobial agent on different types of pathogenic bacteria species.

MATERIALS AND METHODS

Plant Materials: The Piper betel leaf were collected from local market of Raipur, Chhattisgarh and dried for few days in shade, which were then powdered and preserved in airtight bottles for further studies.

Extract Preparation: Piper betel leaf (20g) was extracted in 50% of ethanol and Millipore water solvent the supernatant was collected and concentrated in water bath at 40-50 C .The dried powder was kept in air tied box.

Microorganisms: The tested microorganisms included the Gram positive bacteria; *Bacillus subtilis, Bacillus cereus, Bacillus frimicutes* and Gram negative bacteria; *Escherichia coli, Entrobacter, Klebsiella, Escherichia coli*. These bacteria's strains were procured from National Chemical Laboratory (NCL), Pune, India. The bacteria were grown in the nutrient broth at 37º C and maintained on nutrient agar slants at 4º C.

Antibacterial Assay: Antibacterial activity of Piper betel leaf extract was determined by agar disk diffusion method (Nair, et al., 2005) at four concentrations i.e., 100, 75, 50 and 25 mg/ml. Muller Hinton agar was prepared according to the manufacturer's instructions and the plates were seeded with appropriate microorganisms (Gram positive bacteria; *Bacillus subtilis, Bacillus cereus, Bacillus frimicutes* and Gram negative bacteria; *Escherichia coli, Entrobacter, Klebsiella, Escherichia coli*). Discs of 6 mm diameter were prepared from

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Whatmann filter paper No. 24 and sterilized. The discs were then impregnated with the extracts and solvent DMSO. Antibiotics for Gram positive (NX – Norfloxacin, OF-Ofloxacin, E-Erythromycin, CFM- Cefixime) and Gram Negative (NX–Norfloxacin, OF-Ofloxacin, E-Erythromycin, CFM- Cefixime). Bacteria were used as standard. The plates were incubate at 37º C for 24 hrs and the zones of inhibition were measured with a measuring scale. Above experiment was carried out in triplicate for their confirmation.

RESULT

The initiation of microbial growth was considered as zero hour and further accordingly reading was taken. Our present study shows that antimicrobial activity of 50% ethanol extracts of Piper betel leaf against *Enterobacter* is best in 100% concentration after 12 hrs. Although 75% concentration is having mild effect of inhibition. In B. cereus and *Bacillus frimicute* is best in 100% concentration of extract and in 75% concentration are inhibit the zone of inhibition respectively. In E. coli 100% concentration show maximum activity and also show a good zone of inhibition. In Klebsiella 100% concentration show minimum zone of inhibition.

The above observation suggested that the different concentration (50%, 75%, 100%) were having good anti-bacterial activity against some gram positive (+) bacteria B. subtilis, B. cereus, B. frimicute and some gram-negative bacteria E. Coli, Klebsiella, Entrobacter. Thus, the extract is showing varying activity against all microorganisms. On comparing the zone of inhibition of extract to that standard antibiotic extract showed better activity than Ciprofloxacin (CIP), Doripenem (DOR), Ofloxacin (OF), Maxifloxacin (OM) in these conditions.

Table 1 showing the study of antibacterial activity of Piper beetle Linn extract using Disk Diffusion Method (Mean±SE)

SI	Bacterial Stain	Bacteria use	Zone of inhibition (In MM)			
			100%	**75%**	**50%**	**25%**
1.	**Gram Negative (-)**	***Bacillus subtilis***	10.12±0.11	14.33±1.85	10.33±0.32	8.00±0.8
		Bacillus cereus	18.66±0.65	12.00±0.10	11.66±0.40	9.00±0.15
		Bacillus frimicute	12.00±0.58	09.00±2.73	10.66±1.60	9.66±1.40
2.	**Gram positive (+)**	***E. coli***	10.33±0.87	08.33±0.46	09.0±0.00	10.33±0.32
		Klebsiella	9.66±0.87	10.00±1.23	11.66±0.65	12.87±0.87
		Enterobacter	12.66±2.32	09.33±0.87	10.66±0.19	8.33±0.12

Table 2: The study of anti-bacterial activities of standard antibiotics using disk diffusion method

SI	Bacterial Stain	Bacteria use	Zone of inhibition (In MM)			
			NX10	**OF5**	**E15**	**CFM5**
	Gram positive	***Bacillus Subtilis***	37.00	32.00	15.00	09.00

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1.	**(+)**	***Bacillus cereus***	27.00	28.00	24.00	21.00
		Bacillus frimicute	31.00	28.00	30.00	26.00
2.	**Gram Negative (-)**	***E. coli***	34.00	30.00	18.00	21.00
		Klebsiella	28.00	31.00	26.00	29.00
		Enterobacter	27.00	30.00	31.00	29.00

Higher and aromatics plants have been used traditionally in folk medicine as well as to extend the shelf life of foods, showing inhibition against bacteria, fungi and yeasts [1]. Biologically active compounds from natural sources have always been a great interest for scientists working on infectious diseases [2].

DISCUSSION

Higher plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Piper betle L., (betel vine, Pan) an indigenous medicinal plant, has a folk (Siddha and Ayurveda) reputation in the rural areas of southern India and is a member of family Piperaceae.

The higher activity of the methenolic extracts could be because of the fractions containing bio active phenolic and terpene compounds of different polarity (Bowers, et. al., 2000; Prasad, et. al., 1986). This result supported the previous findings as the leaves are the sources of essential oils (Hammer, et. al., 1999). Plants release them into the environment through volatilization (Naidu, 2010). This study is an experimental evaluation and offers a possibility to make use of P. belte in biopesticidal drug development to control food grain storage contaminants and soil borne pathogen (Nikhil, 2010). It has also been confirmed that there is no adverse effect on seed germination (Muhammad, et. al., 2005).

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In our experiment the different concentration (50%, 75%, 100%) were having good anti-bacterial activity against some gram positive (+) bacteria B. subtilis, B. cereus, B. frimicute and some gram-negative bacteria *E. Coli, Klebsiella, Entrobacter*. Thus, the extract is showing varying activity against all microorganisms. On comparing the zone of inhibition of extract to that standard antibiotic extract showed better activity than Ciprofloxacin (CIP), Doripenem (DOR), Ofloxacin (OF), Maxifloxacin (OM) in these conditions.

The relative efficiency of bactericidal activity by betel leaf ethanol extract to that of a broad-spectrum antibiotic such as Norfloxacin on the above-mentioned microorganisms suggest the possibility of a more cost effective and potentially harmless antibacterial agent. The results obtained support the fact that more work needs to be done on the purification, identification and quantification of the active components and the toxicity of active components, their side effects and pharmacokinetic properties with the view of their use for in vivo studies.

SUMMARY

The exploration of new medicinal properties of various plant species has induced the attention of the scientists towards the biologically active compounds since the last couple of

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decades. The reason behind this is that the bioactive compounds possess potent pharmacological activities and have low or no toxicity. Our reports also showed that the leaf of this plant different concentration (50%, 75%, 100%) were having good anti-bacterial activity against some gram positive (+) bacteria B. subtilis, B. cereus, B. frimicute and some gram-negative bacteria *E. Coli, Klebsiella, Entrobacter*. Thus, the extract is showing varying activity against all microorganisms. On comparing the zone of inhibition of extract to that standard antibiotic extract showed better activity than Ciprofloxacin (CIP), Doripenem (DOR), Ofloxacin (OF), Maxifloxacin (OM) in these conditions. In conclusion from the recorded data, it is demonstrated that the ethanol extract of leaves of P. betel has promising antibacterial effect. As the current study confirmed that leaves of P. betel showed several biological activities, so taking into consideration of all the findings it can be mentioned that P. betel leaves can contribute major role in drug research.

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