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RESEARCH ARTICLE

Qualitative estimation of bioactive compounds and evaluation of antimicrobial activity of leaf extracts of betel (Piper betle L.) varieties collected from the different locations of Odisha

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ABSTRACT

Since ancient days men have been using the biological resources for the food and medicines. The uses of plants are more common in traditional therapeutic practices. This is probably due to the presence of certain secondary metabolites or bioactive compounds in plants and /or their products. In the present days, synthetic medicines are more frequently used to cure microbial infections than the traditional medicines from natural sources. The inappropriate use of these medicines and antibiotics frequently results in antimicrobial resistance (AMR). Therefore, the need of the day is to screen new sources of bioactive compounds. *Piper betle* L. (betel) leaf is a common chewing materials in most part of Odisha. It is said to have medicinal values too. Keeping this in view, an attempt has been made to screen the possible bioactive compounds through qualitative screening and to asses the ant-microbial potential of selected varieties of betel (Vishnupuri, Desawari, Ghajipur, Desipaan and Jaleswar) leaf extracts (Ethanol, acetone, methanol and aqueous). In order to study the antimicrobial efficacy, the minimum inhibitory concentration (MIC) against five bacterial strains (*Streptococcus pyogenes, Streptococcus mutans*, *Shigella flexnerii*, *Salmonella typhi* and *Vibrio cholerae*) has been estimated and compared. The results revealed that leaf extracts of the experimental betel varieties contain saponin, tannin, flavonoids, phenolics and terpenoids. It was further observed that the methanol and acetone extracts of the leaves were more efficient and had more inhibitory effects against *S. pyogenes*, which was evident from the minimum inhibitory concentration (MIC) values. The results are encouraging and supportive of using this chewing leaves against bacterial infections. The study further suggests to extract and identify the specific components responsible and use of the same for formulation of new antimicrobial drugs.

INTRODUCTION

Since primitive time, plants are the major sources for the food and medicines. The uses of plants are quite common in treating microbial infections too. This might be due to the presence of some kind of secondary metabolites. Inappropriate uses of synthetic drugs are most often responsible for microbial resistance. Such drugs often become inactive and cannot fight against bacteria or pathogenic microorganisms. The clinical event is referred to as antimicrobial resistance (AMR). AMR is the resistance of microorganisms to antimicrobial agents which was once effective for the treatment of infections caused by them. It is an increasingly grievous threat to global health problem. Therefore, there is need to screen new antimicrobial agents from natural resources. Researchers have revealed that most of the plants posses antimicrobial potentials for which, many of the local/ common wild plants are used against microbial infections in traditional therapeutic practices. Betel (*Piper betle* L.) is one such plant which is used by majority of people as a chewing leaf. The leaf is said to have medicinal and antimicrobial effect too. It is the leaf of a vine belonging to the family Piperaceae. Betel leaf is mostly consumed in Asia and elsewhere in the world by some emigrants. The leaves are rich with antioxidants (Badmi et al., 2004; Parinitha et al. 2004). It is also used as potent antibacterial agent (Salleh et al. 2012; Akter et al., 2014). Keeping these in view an attempt has been made in the present study to qualitatively estimate the bioactive compounds present in the leaves of some common betel varieties available in Odisha and to evaluate the antimicrobial potential of the leaf extracts. The antimicrobial action has been determined and compared in terms of MIC against some common pathogenic bacteria. Such study not only supports the chewing of betel leaves against microbial infections but also suggests to identify the bioactive compounds responsible for these effects which might be used for formulation of antimicrobial drugs.

Materials and methods

2.1. Collection of plant parts and preparation of extracts

Leaves of selected plant varieties (Vishnupuri, Desawari, Ghajipur, Desipaan and Jaleswar) were collected from the different parts of Odisha. Soxhlet method was adopted to obtain the plant extracts (Tiwari *et al.*, 2011). The collected plant leaves were dried at room temperature under shade and powdered after drying using mechanical devices. The powdered material of the experimental betel varieties were kept in thimble and extraction was carried out using the Soxhlet apparatus. The residues were collected and left for air drying and dried crude extracts were stored in refrigerator for further experimental work.

2.2. Phytochemical screening

5 g of powdered leaf samples were soaked in 55 mL test tube (Borosil, India) containing 30 mL each of aqueous (distilled water), acetone, ethanol and methanol. All these were kept at room temperature for overnight. Then the solvent extracts were filtered through Whatman No. 1 filter paper (Himedia, India). The collected solvent extracts were used for the preliminary

qualitative phytochemical analysis following standard procedures reported by Harborne, 1973; Sofowara, 1993; Trease and Evans, 1989 and Misra et al. 2012.

2.3. Antimicrobial activity

Four different solvents as per their polarity index [acetone, methanol; (Merck, India), ethanol and aqueous] were used for antibacterial activity. The extracts of experimental plants were screened for antibacterial activity against five bacterial strains [*Streptococcus pyogenes* (MTCC-1926), *Streptococcus mutans* (MTCC-497), *Shigella flexnerii* (MTCC-1457), *Salmonella typhi* (MTCC-1252) and *Vibrio cholerae* (MTCC-3906)] collected from the IMTECH, Chandigarh, India (Figure 2). Nutrient broth (Hi-media, Mumbai, India) was used to maintain broth culture. An additional 1.5 gm of agar (Hi-media, Mumbai, India) per 100 ml made up the nutrient agar medium. The medium was autoclaved at pressure 15 psi at the temperature 121^0 C for 20 min to ensure sterilization.

Antibacterial activity was assessed by Minimum Inhibitory Concentration (MIC) using two fold serial dilution methods (CLSI, 2002; Moharana et al. 2014). Selected colonies of aforesaid microbes were picked off from a fresh isolation plate and inoculated in corresponding tubes containing 5 ml of Nutrient broth (Hi-media, Mumbai). The broth was incubated for 6±1 hours at 35±2 ^{0}C until there was visible growth. Mc Farland 0.5 standard was used to adjust the turbidity to get 10^5 colony forming per unit (cfu)/ml. MIC was calculated by two fold serial broth dilution method for leaf extracts/solvents with standard Kanamycin (Figure 4) for bacterial strains. After the incubation, the tubes of lowest concentration showing no visible growth after 8 hours till 12 hours were considered to be inhibitory to bacterial strains tested that represents MIC values. Inoculums control showed visible growth due to no antimicrobial agents and the negative control DMSO showed no growth due to absence of microbes. Triplicates were maintained and the experiment was repeated thrice. For each replicates the average readings were taken for all the experiments designed.

Results and discussion

Plants are nature's "Chemical factories" providing the richest source of organic chemicals on earth (Prabha et al., 2014). The results of qualitative phytochemical screening of experimental plants (leaf extracts) showed the presence of five different phytochemical like saponin, tannin, flavonoids, phenolic compounds and terpenoid (Figure 1). It was observed that all extracts showed presence of all bioactive compounds except terpenoids. It was found to be absent in aqueous extract of Jaleswar, ethanol and aqueous extracts of Vishnupuri variety (Table 1). Kumari and Rao (2015) reported the presence of tannin, flavanoides, alkaloids, terpenoides, saponin and reducing sugars in the ethanol extracts of betel leaf available in Telangana state.

Table 1: Qualitative phytochemical analysis of leaf extracts of betel varieties collected from different localities of Odisha

Betel verities	Solvents	Saponin	Tannin	Flavonoids	Phenolic Compounds	Terpenoids
Desipan	Acetone	+	+	+	+	+
	Methanol	+	+	+	+	+
	Ethanol	+	+	+	+	+
	Aqueous	+	+	+	+	+
Jaleswar	Acetone	+	+	+	+	+
	Methanol	+	+	+	+	+
	Ethanol	+	+	+	+	+
	Aqueous	+	+	+	+	-
Vishnupuri,	Acetone	+	+	+	+	+
	Methanol	+	+	+	+	+
	Ethanol	+	+	+	+	-
	Aqueous	+	+	+	+	-
Desawari	Acetone	+	+	+	+	+
	Methanol	+	+	+	+	+
	Ethanol	+	+	+	+	+
	Aqueous	+	+	+	+	+
Ghajipur	Acetone	+	+	+	+	+
	Methanol	+	+	+	+	+
	Ethanol	+	+	+	+	+
	Aqueous	+	+	+	+	+

(+ = presence, - = absence)

The estimation of MIC values of selected leaf extracts of betel varieties showed significant inhibitory effects. It was observed that Desipan showed lowest MIC values followed by Jaleswar, Vishnupuri, Desawari and Ghajipur (Table 2). The methanol extract of all experimental betel leaves showed lowest MIC values against all tested bacterial strains followed by acetone, ethanol and aqueous extracts (Table 2). It was also noted that the methanol and acetone extracts of Desipan leaf showed lowest MIC values (400 µg/ ml) against *S. pyogenes* (MTCC 1926) and *S. mutans* (MTCC 497) (Table 2). It was further noted that all leaf extracts taken, were more effective against Gram-positive bacteria then Gram-negative bacteria (Table 2). The results revealed variable antimicrobial activities with respect to the betel variety and type of pathogens studied. Earlier studies on betel varieties available in different

parts of the country have also revealed variable antimicrobial activities of plant extracts.

Table 2: Antimicrobial activity (Minimum Inhibition Concentration) of selected betel varieties

Plant extracts	Solvents	MTCC 3906	MTCC 1252	MTCC 1457	MTCC 1926	MTCC 497
Desipan	Acetone	500 µg/ ml	500 µg/ ml	500 µg/ ml	400 µg/ ml	400 µg/ ml
	Methanol	500 µg/ ml	500 µg/ ml	500 µg/ ml	400 µg/ ml	400 µg/ ml
	Ethanol	500 µg/ ml	500 µg/ ml	500 µg/ ml	500 µg/ ml	500 µg/ ml
	Aqueous	500 µg/ ml	500 µg/ ml	500 µg/ ml	500 µg/ ml	500 µg/ ml
Jaleswar	Acetone	500 µg/ ml	500 µg/ ml	500 µg/ ml	500 µg/ ml	500 µg/ ml
	Methanol	500 µg/ ml	500 µg/ ml	500 µg/ ml	500 µg/ ml	500 µg/ ml
	Ethanol	500 µg/ ml	500 µg/ ml	500 µg/ ml	500 µg/ ml	500 µg/ ml
	Aqueous	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml
Vishnupuri,	Acetone	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml
	Methanol	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml
	Ethanol	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml
	Aqueous	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml
Desawari	Acetone	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml
	Methanol	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml
	Ethanol	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml
	Aqueous	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml
Ghajipur	Acetone	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml	800 µg/ ml
	Methanol	Growth in all concentration	Growth in all concentration	Growth in all concentration	Growth in all concentration	800 µg/ ml
	Ethanol	Growth in all concentration	Growth in all concentration	Growth in all concentration	Growth in all concentration	Growth in all concentration
	Aqueous	Growth in all concentration	Growth in all concentration	Growth in all concentration	Growth in all concentration	Growth in all concentration
Inoculums control		Growth in all concentration	Growth in all concentration	Growth in all concentration	Growth in all concentration	Growth in all concentration
Broth control		No Growth	No Growth	No Growth	No Growth	No Growth
DMSO		Growth in all concentration	Growth in all concentration	Growth in all concentration	Growth in all concentration	Growth in all concentration

(MTCC: Microbial Type Culture Collection; MTCC 3906: *Vibrio cholerae*; MTCC 1252*: Salmonela typhi*; MTCC 1457: *Shigella flexneri*; MTCC 1926: *Streptococcus pyogenes*; MTCC 497: *Streptococcus mutans*; DMSO: Dimethyl Sulfo-oxide)

CONCLUSION

Plants are rich sources of bioactive compounds. About 25% of all medicines available in the market has been derived directly or indirectly from plants (De Smet, 1997; WHO 2005). Herbal medicines are believed to be safe. However it is important to evaluate their biological safety before use in order to avoid fatal consequences (Kunle et al., 2012). Researches have further indicated that antibiotics are often becoming ineffective against common pathogens (Feng et al., 2010; Gracia et al., 2009) due to AMR. The present study aims to asses the antimicrobial potential of the betel leaves as well as to provide the scientific rationale for medicinal uses of betel varieties available in Odisha. The antimicrobial activity of the betel leaves could be attributed to the various phytochemical constituents present in the crude extract which has substantiated through qualitative estimation in the present study. The work carried out was a basic approach to find out the antimicrobial activity of the extracts of betel varieties of Odisha and the bioactive compounds available in these leaves. The results indicated the antimicrobial effect of leaf extracts against some common pathogenic bacteria. The study further suggest that betel leaf is a potential source of many bioactive compounds which might be utilized in formulation of new antimicrobial drugs. More of researches can be carried out on such phytoconstitutents from bioresources available to formulate novel pharmaceuticals.

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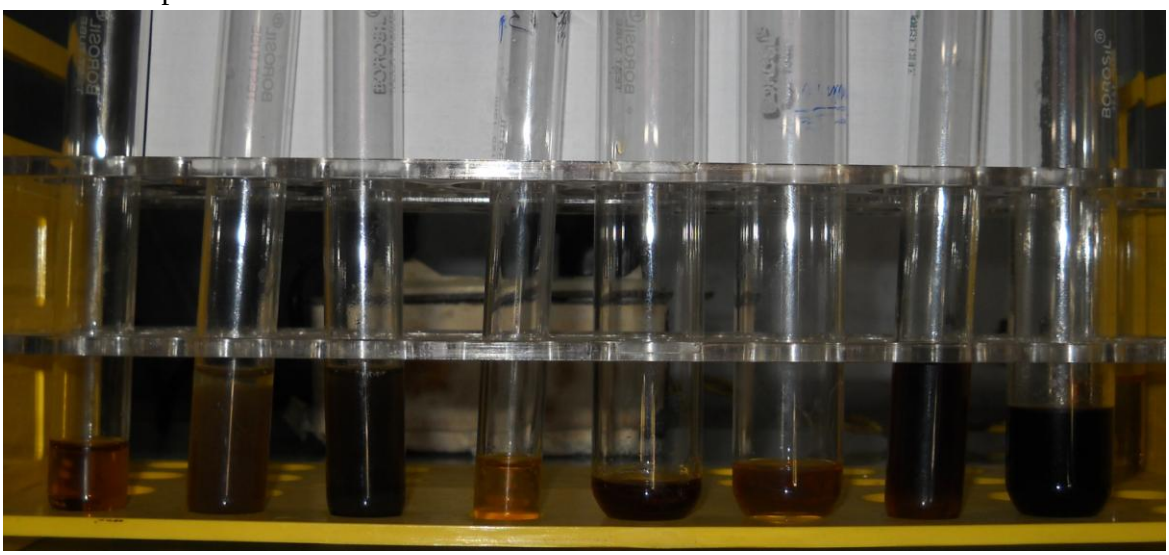

Figure 1: Phytochemical analysis of Desipan

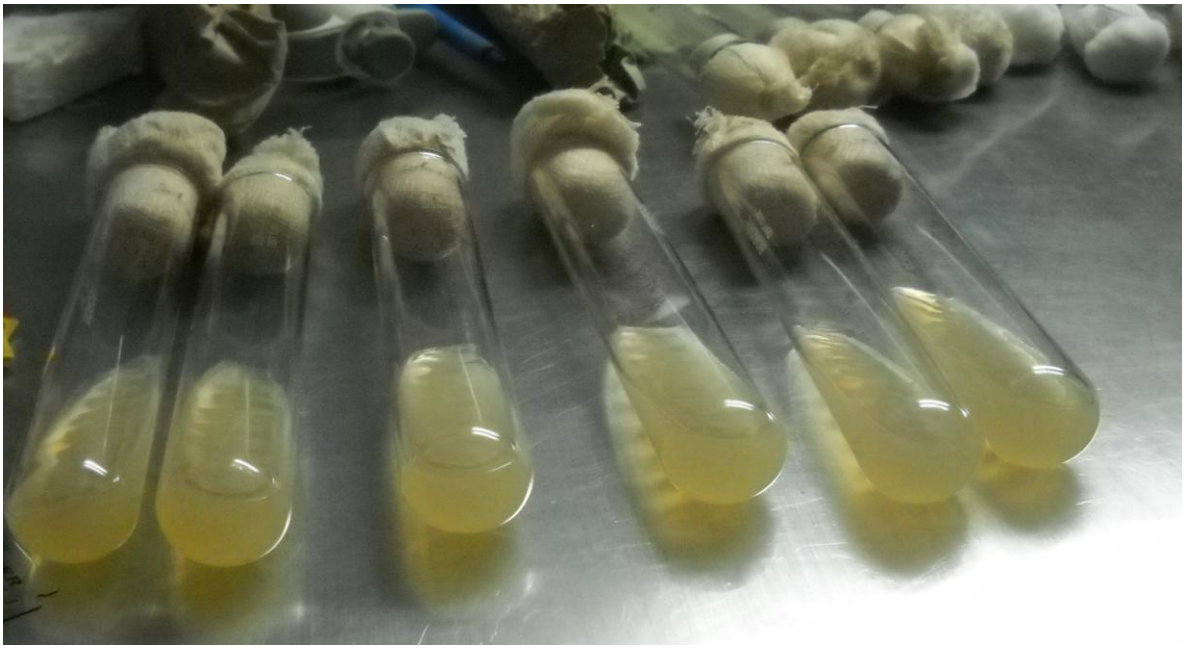

Figure 2: Bacterial strains used for the present antibacterial activity

Figure 3: MIC values of methanol extract of Desipan leaves against MTCC 1926 (*S. pyogenes*)

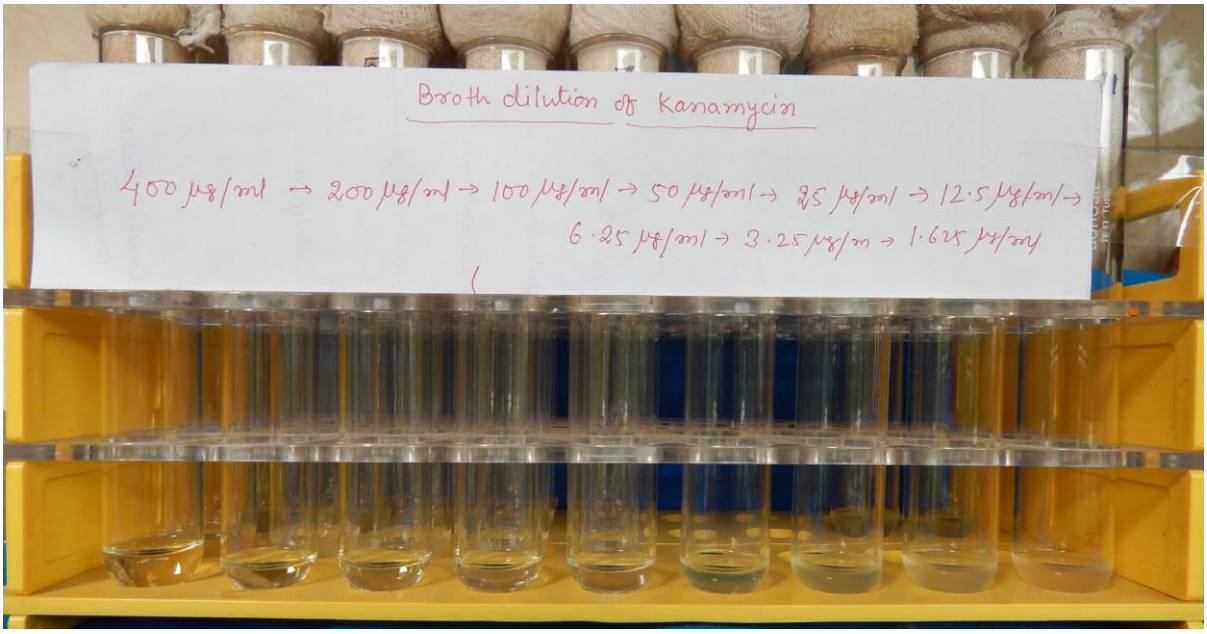

Figure 4: MIC values of Kanamycin against MTCC 1926 (*S. pyogenes*)

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