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Research Article

GCMS ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *PIPER BETLE*(LINN) LEAVES AGAINST *STREPTOCOCCUS MUTANS*

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ABSTRACT

*Piper betle (*Linn), a member of family Piperaceae is an edible plant. The leaves of *Piper betle* have been traditionally used in India for prevention of oral diseases. In the present study, the antibacterial activity against *Streptococcus mutans* for aqueous and ethanol extract of *Piper betle* leaves was studied by agar cup diffusion method. Minimum inhibitory concentration of extracts against *Streptococcus mutans* was detected by tube dilution method. GCMS[gas chromatography and mass spectroscopy] analysis of aqueous and ethanol extracts was carried out to detect the phytochemicals present in it. Both aqueous and ethanol extract inhibited the growth of *Streptococcus mutans*. Zone of inhibition of ethanol extract [20.6±1.12mm] was larger compared to aqueous extract [18.3±0.53].The MIC value for the aqueous and ethanol extract was 10mg/ml and 5mg/ml respectively. GCMS analysis revealed the presence of six compounds in each of the extract. The predominant compound in aqueous extract was 4 chromanol while phenol-2-methoxy-4(- 2propenyl) acetate was found in ethanol extract.

Keywords: *Piper betle*; Dental caries; *Streptococcus mutans*; GCMS analysis.

INTRODUCTION

Dental caries is a localized and transmissible disease that leads to the destruction of hard dental tissue. *Streptococcus mutans* is considered to be the main cause of the dental caries. It is an acidogenic and aciduric microorganism [1].Chemical approaches used for the management of caries include use of antimicrobial agents like fluorides, chlorohexidine in combination with tooth brushing daily. But there are some side effects in using these such as staining of tongue, disturbance of the taste and burning sensation. In addition, the most representative cariogenic bacteria are moderately resistant to antibiotics [2].These drawbacks justify the search for the new anticariogenic compounds that could be employed in caries prevention. There is long history of the use of plants to improve dental health and oral hygiene. In India most of the people use the traditional medicines which are derived from medicinal plants. The phytochemicals in medicinal plants are secondary metabolites of plants which act as antibacterial compounds [3].

*Piper betle (*Linn) belongs to family Piperaceae. It is commonly known as "Paan". It is extensively grown in Srilanka, India, Thailand, Taiwan and other Southeast Asian countries. The parts of *Piper betle* utilized are leaves, roots, stems, stalks and fruits. The plant has got large number of biomolecules which show diverse pharmacological activity. The leaves of *Piper betle* possess activities like antitumor, antimutagenic and antihelminthic [4].The present investigation was undertaken to determine antibacterial activity of *Piper betle* leaves against *Streptococcus mutans* and to find out the phytochemical profile of aqueous and ethanol extract of the leaves of *Piper betle* by GCMS analysis.

MATERIALS AND METHODS

Plant material: *Piper betle* (Linn) leaves were purchased from the local market in the month of January 2011.The leaves were identified in the Department of Botany, D.B.F.Dayanand College of Science and Arts, Solapur [Maharashtra].

Test Microorganism: Pure culture of *Streptococcus mutans* was obtained from MTCC, Chandigarh, India.

Preparation of plant extract: Fresh leaves were washed under the running tap water and were dried under shade at room temperature. Dried leaves were powdered in electronic grinder. Powdered leaves were then packed in Soxhlet apparatus and then the extraction was done [5].

- Aqueous extract: Fifteen grams of dry powder was subjected to Soxhlet extraction with 150 ml distilled water as solvent. Extraction was carried out for 6 hrs.
- Ethanol extract: Fifteen grams of dry powder was subjected to Soxhlet extraction with 150 ml of ethanol [Merck Company] as a solvent. Extraction was carried out for 6 hrs.

The solvent from extract was allowed to evaporate. Dried extracts were kept in freeze until further use.

Antibacterial activity of Plant Extract

Antibacterial activity of plant extract was determined by agar cup method. For this, fresh [overnight] isolated colony of *Streptococcus mutans* was suspended in sterile saline to get turbidity of 0.5 McFarland standards. 0.1 ml of this suspension was spread aseptically on sterile Muller Hinton agar medium [Hi media]. Then the wells [8 mm diameter] were bored by sterile cork borer. 0.2 ml. of each extract [100 mg /ml in 10% DMSO] was added to the wells separately. It was allowed to diffuse by keeping the plate in freeze for 20 minutes.10 % DMSO in one of the wells was used as negative control. As positive control gentamycin [10 mcg/ml][Hi Media] disc was used. After diffusion of extract, the plates were incubated at 37 ^{0}c for 24 hours. Zones of inhibition were then measured in mm. For each extract three replicates were maintained.

Determination of minimum inhibitory concentration [MIC]

Tube dilution method was used to determine minimum inhibitory concentration of the extracts. A series of two fold dilutions of each extract ranging from 10 mg /ml to 0.3 mg/ml were done in Muller Hinton broth. 0.1 ml of suspension of *Streptococcus mutans* matched to 0.5 McFarland standard was seeded into each dilution. Two controls were used for each test batch. These included tube containing extract and growth medium without inoculum and organism control i.e. tube containing the growth medium and inoculum. The tubes were incubated at 37^{0}c for 24 hours and checked for turbidity. Minimum inhibitory concentration was determined as highest dilution of the extract that showed no visible growth.

GCMS analysis of extracts

GCMS analysis of ethanol and aqueous extracts of *Piper betle*(Linn) leaves was carried out in Indian Institute of Technology,

Mumbai.GCMS was performed by using Hewlett Packrd,GCD 1800 A model with electron ionization detector operated through a data system.1 µl of extract was used to inject in injection port of GC column.

Identification of compounds

The mass spectrum of unknown components was compared with spectrum of the known components stored in the NIST and Wiley library. Interpretation of mass spectrum of GCMS was done using data base of NIST library having more than 75,000 compounds. The name, molecular weight and structure of components were then ascertained. The relative percentage was calculated by comparing its average peak area to the total area.

Statistical analysis

For the determination of antibacterial activity, experiment was carried out in triplicates. Zone of inhibition was expressed as mean ± standard deviation.

RESULTS

Physical characteristics of extracts

Table1: **Physical characteristic of extracts**

S. No.	Solvent used	Physical characteristics		
		Color	Odor	Consistency
1	Ethanol	Dark green	pungent	solid sticky
2	Aqueous	Dark green	pungent	solid sticky

Physical characteristics of ethanol and aqueous extracts of *Piper betle* (Linn) leaves are depicted in table 1. Ethanolic extract was dark green in color with solid sticky consistency and pungent odour and aqueous extract was also dark green colored with solid sticky consistency and pungent odour.

Table2: Percentage yield of extracts

Sr. No.	Solvent used	Weight of dry powder [g]	Weight of dry extracts [g]	Percentage yield
1	Ethanol	15	1.45	9.0
2	Aqueous	15	2.03	13.5

The percentage yield [table 2] of ethanol extract was 9.0 and that of aqueous extract was 13.5

Antibacterial activity of extracts

Table3: Antibacterial activity of extracts against *Streptococcus mutans*

Sr.No.	Solvent used	Zone of inhibition [mm]± SD
1	Ethanol	20.6±1.12
2	Aqueous	18.3±0.53

Table 3 shows the results of antibacterial activity of ethanol and aqueous extracts of *Piper betle* (Linn) leaves. Both the extracts inhibited the growth of *Streptococcus mutans*. Ethanol extract showed more antibacterial activity as compared to aqueous extract. The inhibition zone of gentamycin was 32 mm.

Determination of minimum inhibitory concentration of extracts *Streptococcus mutans*

Table 4: Minimum inhibitory concentration of the extracts against *Streptococcus mutans*

Sr.No.	solvent used	MIC [mg/ml]
1	Ethanol	5
2	Aqueous	10

The minimum inhibitory concentration of the extracts against *Streptococcus mutans is* depicted in table 4. The MIC of ethanol extract was low [5.0 mg/ml] as compared to aqueous extract [10 mg/ml].The lower MIC is an indication of high effectiveness of extract.

GCMS analysis of extracts

GCMS Chromatogram of aqueous and ethanolic extract of leaves of *Piper betle* showed six peaks each indicating the presence of six compounds in each extract [fig 1 & 2].

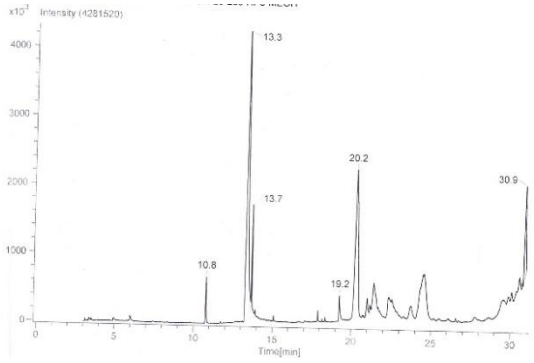

Figure1: GCMS chromatogram of aqueous extract of leaves of *Piper betle*

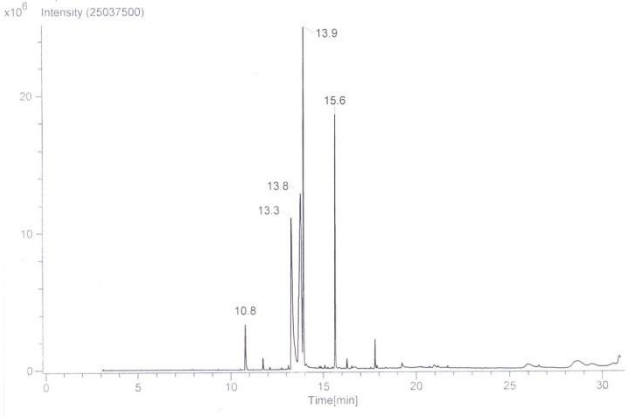

Figure 2: GCMS Chromatogram of ethanolic extract of leaves of *Piper betle*

Table5: Gas chromatographic and Mass spectral data for aqueous extracts of *Piper betle* (Linn) leaves.

S.No.	Retention time[min.]	Name of compound	Molecular formula	Molecular weight	% peak area
1	10.8	Eugenol	$C_{10}H_{12}O_6$	164	20.37
2	13.3	4 chromanol	$C_9H_{10}O_2$	150	27.81
3	20.2	Squalene	$C_{30}H_{50}$	410	21.78
4	30.9	Gamma tocopherol	$C_{28}H_{48}O_2$	420	12.62

Table 5 shows the phytochemical profile identified in aueous extract of *Piper betle* leaves.

Table 6: Gas chromatographic and Mass spectral data for ethanol extracts of *Piper betle* (Linn) leaves.

Sr.No.	Retention time[min.]	Name of compound	Molecular formula	Molecular weight	% peak area
1	10.8	Eugenol	$C_{10}H_{12}O_6$	164	20.37
2	13.3	4 chromanol	$C_9H_{10}O_2$	150	27.81
3	13.9	Phenol2-methoxy4-(2-propenyl)-acetate	$C_{12}H_{14}O_3$	206	61.15
4	17.8	2,5-dimethoxy-4-ethylamphetamine	$C_{13}H_{21}O_2$	223	4.5

The phytochemical profile identified in ethanol extracts is depicted in table 6.

GCMS analysis revealed the presence of 4 chromanol [27.81%] as major compound in aqueous extract while phenol 2 methoxy 4 – (-2 propenyl) acetate [61.15%] as major compound in ethanol extract.Euganol [20.37%] and 4 chromanol [27.81%] were present in both extracts. However, Squalene [21.78%] and Tocopherol [12.62%] were found in aqueous extract only. Ethanol extract showed phenol 2 methoxy 4 (2 propenyl) acetate and 2, 5-Dimethoxy-4-ethylamphetamine [4.5%] which were absent in aqueous extract of *Piper betle* leaves.

DISCUSSION

Herbal medicines are valuable and readily available resources for primary health care. They can be the best alternatives for the antibiotics against the pathogen. [6] studied antibacterial nature of *Piper betle* leaves. They have reported that methanol and ethanol extract exhibited antibacterial activity against various gram positive and gram negative bacterial pathogens.[6] The results of antibacterial activity performed in the present study are consistent with the present study and previous studies carried out by [7]. In the present study ethanol (20.6±1.1) extract showed larger zone of inhibition compared to aqueous (18.3±0.5) extract of *Piper betle* leaves against *Streptococcus mutans***.**Though the inhibition zone around gentamycin antibiotic is larger (32mm) than both of the extracts of *Piper betle,* considering the disadvantages of use of antibiotics against pathogens, justify the use of Piper as mouth fresheners in traditional folk medicine. [7]

GCMS is one of the best techniques to identify the constituents of volatile matter, Long chain & branched chain hydrocarbons, alcohols, acids, esters. The more precise information in qualitative analysis can be obtained by gas chromatography coupled with mass spectroscopy. [GCMS] [8]. The GCMS analysis of aqueous and ethanol extract revealed the presence of various secondary metabolites which constitute different pharmacological actions like antibacterial, antioxidant, anti-inflammatory and anti cancerous activities. Eugenol found in the present study in aqueous and ethanol extract has antibacterial and antifungal activities [9]. Aqueous and ethanol extract revealed the presence of 4 chromanol which has antioxidant activity. [10] Aqueous extract showed the presence of Squalene (isoprenoid) which is known to have antioxidant and anti cancerous activity [11]. Similarly gamma tocopherol, part of vitamin E family was also present in aqueous extract of *Piper betle* leaves. It has antioxidant, anti inflammatory & anti cancer activity [12].

This study is preliminary in nature. Further investigations on toxicology of herbal extracts are necessary to formulate and synthesize a new drug of medicinal value.

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