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Chemical composition and antimicrobial activity of vellaikodi variety of *Piper betle* Linn Leaf oil against dental pathogens.

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Abstract: The *Piper betle* (Tamil: vetrilai) is a glabrous climbing vine belonging to the family *Piperaceae* . Essential oil from vellaikodi variety of *Piper betle* L leaves (*Piperaceae*) was extracted by hydro-distillation method in a clevenger type apparatus. The essential oil thus obtained was analyzed by gas chromatography and gas chromatography – mass spectroscopy. Sixty five components were identified in the oil. The 5-(2-propenyl)-1,3-benzodioxole(25.67%) was determined as the first major constituent in the oil, The second was eugenol,(18.27%)and third 2-methoxy-4-(2-propenyl) acetate-phenol (8.00) were predominant components in this oil. The antimicrobial screening of the isolated essential oil was performed against dental pathogens such as *Staphylococcus aureus*, *Streptococcus mutans, Lactobacillus acidophilus, Candida albicans* and *Saccharomyces cerevisiae*. The pronounced antimicrobial potential of *Piper betle* oil against tested pathogens concludes that it may serve as a source of antimicrobial agent for dental caries in commercial tooth paste. The physical parameters and HPTLC finger printing will be useful to identify and authenticate this variety of *piper betle* leaf oil in herbal industry.

Key words: Clevenger apparatus, dental pathogens , vellaikodi ,GC-MS spectroscopy, *Piper betle* .

Introduction

The *Piper betle* (Tamil: vetrilai) is a glabrous climbing vine belonging to the family *Piperaceae* . It is abundantly distributed in many Asian countries. In India it is found in Bihar, Bengal, Orissa, Tamilnadu, Andhra pradesh and Karnataka . In Tamilnadu, three varieties of Piper betel leaves, Sirugamani, Karpoori and vellaikodi are available mostly[1]. The chief constituent of the leaves is a volatile oil varying in chemical composition from different countries and known as betel oil. The active ingredient of *piper betle* oil which is obtained from the leaves are primary a class of allyl benzene compounds, chavibetol (betel-phenol; 3-hydroxy-4-methoxyallylbenzene), Chavicol (p- allyl-phenol; 4-allyl-phenol), Estragole (p-allyl-anisole; 4-methoxy-allylbenzene), Eugenol (allylguaiacol; 4-hydroxy-3-methoxyallylbenzene; 2-methoxy-4-allyl-phenol), methyl Eugenol (Eugenol methyl ether; 3,-dimethoxy-allylbenzene) and hydroxycatechol (2,4-dihydroxy-allylbenzene) [2]. Previous studies on the betel leaves, roots and

whole extract (mixture of volatile and non-volatile) of the green variety showed a very strong anti microbial activity. In the South East Asia region, *piper betle* L, is among the plants that have been associated with the control of caries and periodontal diseases and to the control of bad breath in traditional practice [3]. Mouthwashes and tablets containing pulverized betel nuts were used for the treatment of dental and periodontal diseases [4]. In the current investigation, the chemical composition, physico- chemical parameters and antimicrobial potential of leaf oil of *Piper betle* from vellaikodi variety has been evaluated against dental pathogens .

Materials and methods

Plant Materials

Piper betle leaves of vellaikodi variety was collected from Sugarcane Research Centre, Trichy in the month of June 2010 and authenticated by Dr. S. Panneerselvam, Professor, Department of Agronomy, Tamil Nadu Agricultural University, Sugarcane Research Station, Sirugamani. The voucher specimen (APCP/S-87/2010) of the same was kept in Department of Pharmacognosy, Adhiparasakthi College of Pharmacy, Melmaruvathur for future reference.

Test microorganisms

Antimicrobial activity was evaluated against *Staphylococcus aureus* (MTCC 740)*, Streptococcus mutans* (MTCC*497), *Lactobacillus acidophilus* (MTCC *447), *Candida albicans* (MTCC 227) and *Saccharomyces cerevisiae* (MTCC 170). They were collected from Microbial Resources Division, Kings Institute of Preventive Medicine, Guindy, Chennai. The agar medium and sabouraud dextrose medium was purchased from HI Laboratories Ltd., Mumbai, India.

Isolation of the essential oil

Fresh leaves were subjected to hydro distillation for 4 h using a Clevenger apparatus to get the essential oil [5]. The oil was dried over anhydrous $CaCl_2$ and store in sealed vials at low temperature before analysis. The percentage yield of essential oil was estimated and reported.

Chemical evaluation of volatile oil

Essential oil sample obtained was subjected to gas chromatography adopting following operating conditions :(Gas chromatogram- PerkinElmer Clarus 50, equipped with Capillary Column Elite-5ms (5% Phenyl 95% dimethylpolysiloxane); Oven temperature Program: 50 ºC@ 5 ºC/min to 150 ºC (5min) @ 10 ºC/min to 250 ºC; Injector temperature: 250 ºC; Carrier gas: Helium at flow rate 1ml/min; inlet line temperature – 200 ^{o}C. The sample injected in a Split ratio : 1:40. Gas chromatography-mass spectrophotometer data were obtained on a PerkinElmer Clarus 50 Capillary Column Elite-5ms (5% Phenyl 95% dimethyl polysiloxane) under same temperature programmed similarly as in gas chromatography. The mass spectra were obtained on electron impact at 70 eV with mass range was from m/z 40 - 450 amu. NIST'05 library was used for individual spectral matching of constituents in the oil [6].

Screening of Antimicrobial activity

The antimicrobial activity of the isolated oil from vellaikodi variety of *piper betle* was studied by disc diffusion method [7]. The oil was used in the concentration of 25, 50 and 100 µl /disc using a solvent DMF. Ciprofloxacin and Ketaconazole were used as standard for antibacterial and antifungal activity respectively. A suspension of the organisms, *Staphylococcus aureus, Lactobacillus acidophilus,* and *Streptococcus mutans* was inoculated to sterile nutrient agar medium at 45^{o}C, *Candida albicans* and *Saccharomyces cerevisiae* was added to sterile sabouraud dextrose agar medium at 45 ^{o}C. The mixture was transferred to sterile petri dishes and allowed to solidify. Sterile disc of 5 mm in diameter (made from Whatmann filter paper previously sterilized in U.V. lamp) was dipped in solution of different concentration (25 µl, 50 µl and 100 µl) of oil, standard (5 µl) and a blank was placed on the surface of plates. The plates were allowed to stand for 1 h at room temperature as a period of pre incubation to minimize the effects of variation in time between the applications of the different solutions. Then the plate was incubated for 24 h at 37 ± 1 ^{o}C for antibacterial activity and 48 h at 37±1 ^{o}C for antifungal activity. Then the diameter of zone of inhibition around the disc was measured .

Table No. 1: Organoleptic and Physical analysis of leaf oil of *Piper betle* L (vellaikodi)

Organoleptic property/ Physical constant	Description/ Value
Color	Colorless to pale yellow
Odour	Strong aromatic odor
Taste	Pungent
Characteristic feel	Greasy
Solubility	Immiscible in water and freely soluble in organic solvent
Specific gravity	1.0010
Optical activity	+4.392
Refractive index	1.529
Percentage yield	0.3144 % v/w

Table No 2: Antimicrobial activity of leaf oil of *Piper betle* L. (vellaikodi)

Test microorganism	Zone of inhibition (mm)					
	Betel oil			Standard		Vehicle
	25µl	50µl	100µl	Ciprofloxacin	Ketaconazole	
S .aureus	20	27	32	37	---	---
S.mutans	18	26	31	35	---	---
L. acidophilus	17	24	30	33	---	---
C .albicans	15	19	24	---	30	---
S. cerevisiae	16	20	26	---	33	---

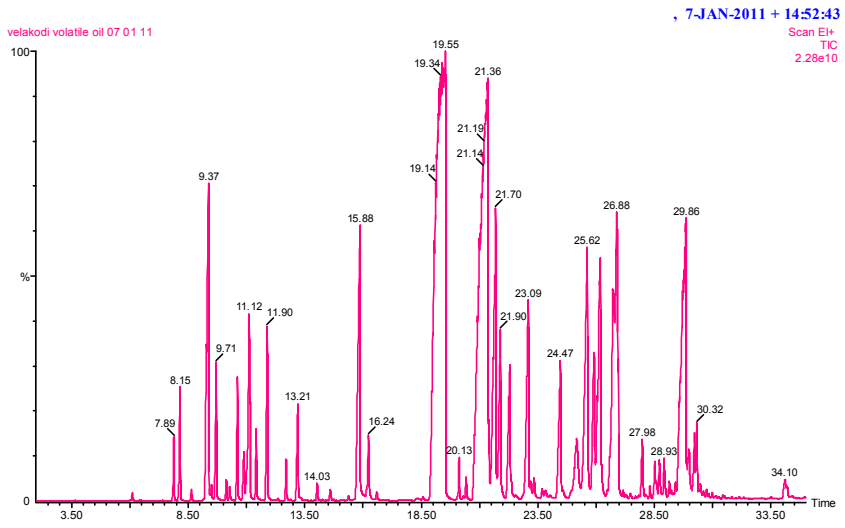

Figure 1: GC-MS Spectrum of betel oil (vellaikodi)

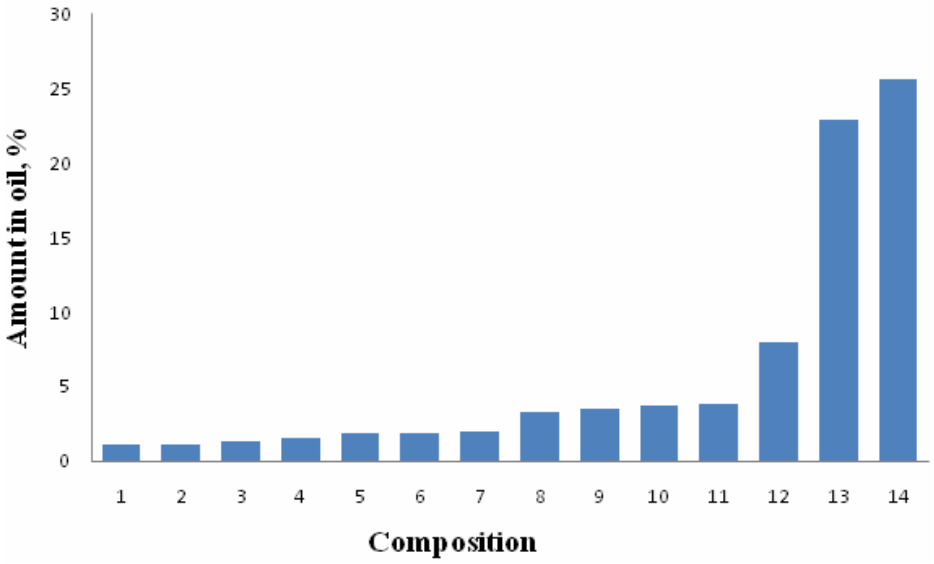

Figure 2 : Chemical composition of the leaf oil of *Piper betle* L (vellaikodi)

1.τ – Muurolene;2. τ – Terpinene;3.Eudesma-4(14), 11-diene;4.β – Elemene (-);5.1,2-dimethoxy-4-(2-propenyl)-benzene;6.β - Phellandrene;7.ά Caryophyllene;8.τ Gurjunene;9.Germacrene D; 10.Sabinene;

11.(-) – terpinene-4-ol;12.2 methoxy-4-(2-propenyl)-acetate- Phenol;13.Eugenol;14.5(2-Propenyl)- 1,3-benzodioxole

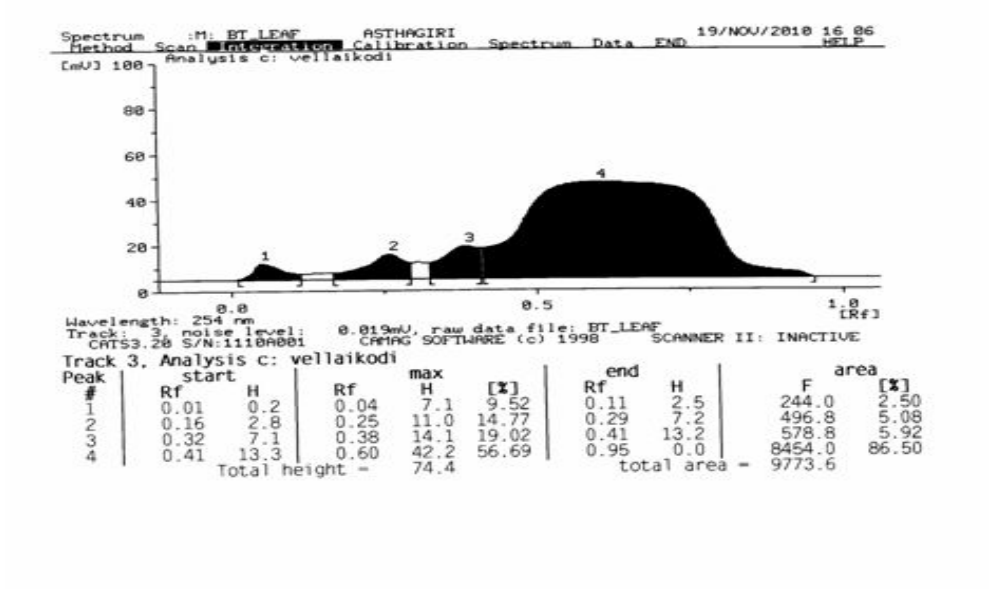

Figure 3: HPTLC Analysis of *Piper betle* volatile oil (Vellaikodi)

Results and discussion

In the present investigation, essential oil of *Piper betel* leaves was obtained by hydro-distillation method using a clevenger type apparatus and analyzed by GC and GC/MS. Physical constants serve as a means of assessing the purity and quality of the volatile oil as well as for identification. So, the specific gravity, optical activity and refractive index were determined for the isolated oil of vellaikodi variety of *piper betle* leaf and reported in Table 1. The total ion chromatogram (TIC) retention time is about 34.10 min (Figure1). The most composition of the oil isolated around the first 30 min of the analysis procedure and condition. A total of 65component were identified by GC-MS, representing 100% of the oil. The 5-(2-propenyl)- 1,3-benzodioxole(25.67%) was determined

as the first major constituent in the oil, The second was eugenol,(18.27%)and third 2-methoxy-4-(2-propenyl) acetate-phenol (8.00) were predominant components in this oil. Figure 2 showed the percentage variation of major components (> 1%) in the oil. The composition with the lowest retention time was (Z)-3-hexen -1-ol, whereas 3-[3,4-bis (acetoxy) phenyl]- 2- propenoic acid had highest retention time. Cis-7-decen-1-al , Isoledene , Widdrol , varidiflorene were present only in vellaikodi variety of *piper betel* leaf oil . The chemical composition of leaf oil is comparable to the previous reports with some variations in the constituents[8]. This chemical variation in the constituents may be due to different chemo-types for the same species or result from environmental, developmental or other factors. The data summarized from the HPTLC studies would help in the authentication of the this variety of piper betle volatile oil (Figure 3).

The antimicrobial efficacy of leaf oil was determined using agar disc diffusion method and summarized in Table 2.The essential oil was found to be more or less active against almost all tested pathogenic strains with varied spectrum of inhibition zone. However, gram positive bacterial strains were found to be more susceptible. It may be due to that cell wall of gram positive bacteria is less complex and lack natural sieve effect against large molecules due to small pores in their cell envelope. The antimicrobial effect of Karpoori variety of *piper betle* leaf oil against the tested microorganisms present in the following order:

C. albicans < *S. cerevisiae* < *L. acidophilus*< *S.mutans* <*S.aureus*

As per the data obtained from antimicrobial activity, vellaikodi variety of *piper betle* leaf oil showed significant activity, is due to presence of high 5-(2-propenyl)- 1,3-benzodioxole content. So, essential oil obtained from this variety can be included in the commercial tooth paste to get maximum activity against dental pathogens. The zone of inhibition value of positive standards were found to be more than the essential oil tested against all the microorganisms because we performed the assay on highly purified reference standard and not in complex materials such as that of essential oil analyzed.

The present investigation justify the traditional claim of this plant as an antimicrobial agent in dental diseases.

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References

1. Nandkarni's K. M., Indian Materia Medica Vol, I. 3rd Ed, Popular Prakasan Pvt Ltd Mumbai , 2007960-964.
2. Ghosh K., and Bhattacharya T. K., Chemical Constituents of *piper betle* Linn. (*Piperaceae*) Roots, Molecules ., 2005, 10(7), 798-802.
3. Kirtikar R. K., and Basu D. B., Indian Medicinal Plant, Vol.III. 2nd Ed, International Book Distributors , Uthranchal, 2005, 2131-2133.
4. Jenie B. S. L., Antimicrobial activity of *Piper betle* Linn extract towards food borne pathogens and food spoilage microorganisms, FT Annual Meeting, New Orleans, Louisiana. 2001.
5. Ashutosh Kar., Pharmacognosy and Pharmacobiotechnology. 2nd Ed, New Age International Publishers, New Delhi, 2007,240-247.
6. Adeltrudes B., Caburian ., Marina O ., and Osi., Characterization and Evaluation of Antimicrobial activity of the Essential oil from the Leaves of *Piper betle* L. International Scientific Research Journal, 2010, 2(1), 1-11.
7. Quinto E. A., and Sntos G. M., Microbiology: A Guidebook to Plant Screening: Phytochemical and Biological, 2nd Ed, UTS Publishing House, Manila , 2005,67-87.
8. Lakshmi nirambewela ., Kumaratuga K. G. A ., and Kalyani Dias. Studies on *piper betle* of Srilanka. Journal of Science Foundation, Srilanka , 2005, 33(2), 130-133.

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