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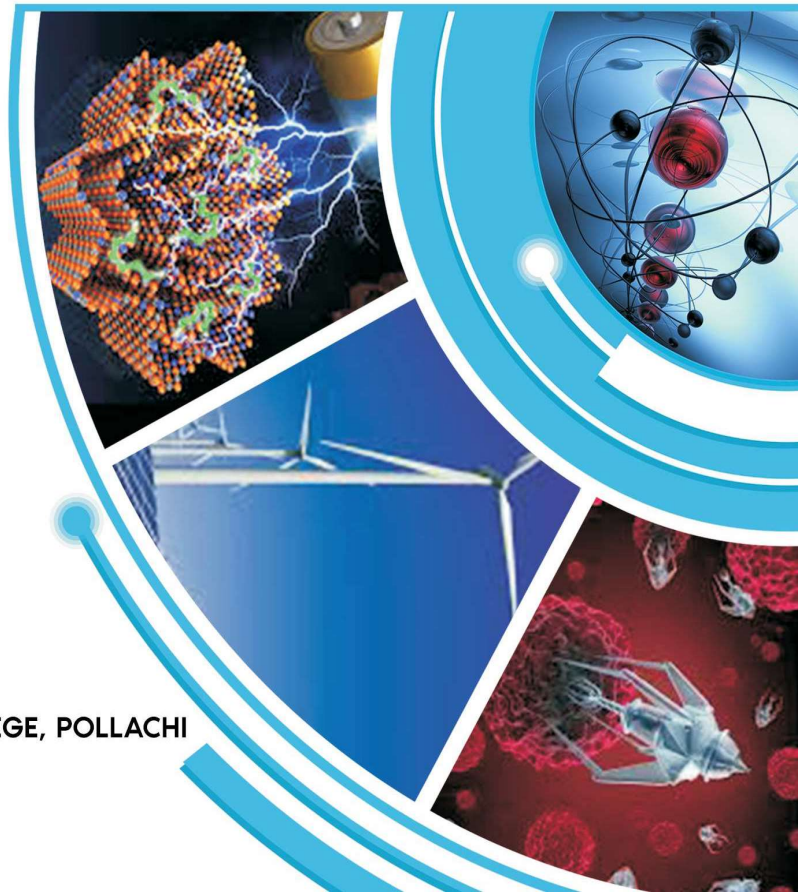

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CHEMICAL COMPOSITION, *INVITRO* ANTIOXXIDANT AND ANTIBACTERIAL ACTIVITY OF *PIPER BETEL*'S LEAVES ESSENTIAL OIL

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ABSTRACT

The present study examines the nature of phytoconstituents and *in vitro* antioxidant, antibacterial activity of essential oil of *P.betel* leaves. GC-MS analysis of *P.betel* leaves essential oil revealed the presence of thirty three compounds. In the present study, the major chemical constituents are safrole, (31.210%), 4- Allyl-1, 2 Diacetoxy benzene (12.140%), Eugenol (12.113%) and the minor compounds are β-Phellandrene (3.698%), Methyl Eugenol (3.566%), 4-Terpineyl acetate (1.199%) respectively. The *in vitro* antioxidant activity was carried out by DPPH assay. The essential oil of *P.betel* leaves showed maximum 87% scavenging activity at 5 µg/ml. The antibacterial activity tested against *S.aureus, S.aeurginosa* and *K.pneumoniae.* The essential oil showed moderate zone of inhibition of 10mm and 12mm against *S.aureus and K.pneumoniae* respectively.

Keywords*: Piper betel, GC-MS and in vitro antioxidant activity, antibacterial activity.*

INTRODUCTION

The Genus P. betel distributed worldwide tropical and subtropical region[1]. P.betel belongs to the family piperaceae known as pan comprises about 10 Genera, 2000 Species. The Genus piperaceae is largely distributed in tropical and subtropical regions of the world. Over 700 species of piper betel has been distributed in both of the hemisphere of world. Of these 30, species have been record in India, 18 in Srilanka and 3 are endemic[2]. It is used for therapeutics with the increase of resistant pathogen to commonly use anti biotics and the emergence of new infections diseases. It is mostly cultivated in most parts of South India, Myanmar, Thailand, Srilanka, and Bengal. 28 varieties are found in Kerala[3]. The biological activities like anti- microbial activity[4], anti-oxidantactivity[5], anti-bacterial activity[6], anti-fungal activity[7]. So our present aim is to investigate chemical composition, antioxidant and antibacterial activity for essential oil from leaves of *P.betel*.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *P.betel* were collected from commercial places of pollachi. The plant material was identified and authenticated by Department of Botany, NGM College, Pollachi, and Coimbatore.

Isolation of Essential Oil

The fresh leaves of *Piper betel* (400g) were collected and washed with distilled water. The essential oil was isolated by using Clevenger's apparatus on 4hrs. The isolated fraction showed two distinct layers-an upper oily layer and a lower aqueous layer. Both the layers were separated and the moisture from the oily layer was removed by adding

anhydrous sodium sulphate. The collected essential oil was transferred into a dark glass bottle and kept at a temperature of 4 °C prior to GC-MS analysis.

GC-MS Analysis

GC-MS analysis of the phyto constituents of *P. betel* was carried out using thermo GC-trace ultra version: 5.0 coupled with thermo MS DSQ II instrument. Compounds were separated on DB-35, MS capillary standard non-polar column (30×0.25mm), film thickness 0.25µm. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 70°C and held for 2 minutes and the temperature of the oven was raised to 260°C for 10 minutes and raised 6°c per minute and final temperature was 300°C for 10 minutes. The sample of 100 ml was dissolved in 1 ml of acetone injected with split less mode. Mass spectra were recovered over 50-500 amu range with electron impact ionization energy 70ev, while injector and MS transfer line temperature were set at 230°Cand 280°C respectively.

Identification of phytoconstituents

The components were identified by comparison of their mass spectra with those of National Institute of Science and technology (NIST) mass spectral library version 2.0d, as well as on their comparison of their retention time either with those of authentic compounds or with their literature values.

Anti-bacterial activity

Preparation of discs for anti-bacterial activity

Whatmann No.1 filter paper was taken and punched in to small circular discs (6mm dia) and then wrapped in aluminium foil and sterilized using an autoclave.

Preparation of media for disc diffusion assay

Muller Hinton Agar (MHA) (38g) was dissolved in 100ml of sterile sea water and kept for sterilization autoclave for 45 minutes. The prepared MHA was poured in the Petri plates and then allowed to solidify under sterile condition. Pathogens were subjected to preliminary screening in order to test their anti- microbial activity against the extracts using disc-diffusion method. The sterilized filter paper discs were treated with the extracts under laminar air flow chamber. 200µl of indicator strains of human pathogens were spread plated over the Petri plate to form a lawn on the agar. The extract treated discs was placed in their respective place in the Petri place using sterile forceps under aspectic conditions. The plates were incubated for 24 -48hrs for bacteria and their fungi respectively. After 24 hrs of incubation the plates were observed for activity against the bacterial pathogens. The zone of growth inhibition was measured in millimeter for those extract which showed activity.

***In-vitro* antioxidant activity**

DPPH assay

The oxidative stress was considered as an important risk fact in the pathogenesis of maximum number of chronic diseases. The reactive oxygen species and free radicals leads to numerous degenerative diseases. The Free radical scavenging activity of different extracts was measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH) method. In brief, 0.1

mM solution of DPPH in ethanol was prepared. This solution (1 ml) was added to 3 ml of different extracts at different concentration (5, 10 and 15 µg/ml respectively). The mixture was shaken vigorously and allowed to stand at room temp for 30 min. Finally, the absorbance was measured at 517 nm by using spectrophotometer (UV-VIS Shimadzu). Reference standard compound being used was ascorbic acid and experiment was done in triplicate. The percent DPPH scavenging effect was calculated by using following equation:

DPPH scavenging effect (%) or Percent inhibition = $A_0 - A_1 / A_0 \times 100$.

Where A_0 was the Absorbance of control reaction and A1 was the Absorbance in presence of test or standard sample.

RESULTS AND DISCUSSION

GC-MS Analysis

Hydro distillated essential oil was pale yellow color about 0.5 %(v/w) of yield. Its volatile composition was analyzed by GC-MS. Total of 33 components was identified and represents 99% of the detected oil composition. The oil was characterized by the abundance of safrole, (31.210%), 4- Allyl-1,2 Diacetoxy benzene (12.140%), Eugenol (12.113%),phenol-2 methoxy -4 (2- propenyl acetate (10.149%), γ-Muurolene(9.153%), Terpinen-4-ol (5.726%), β-Phellandrene (3.698%),Methyl Eugenol (3.566%),4-Terpineyl acetate (1.199%). To our knowledge there are few studies regarding the chemical composition of the essential oil from *P.betel* leaves since most studies are addressed to the investigation of the chemical composition and biological properties of the essential oil obtained from its leaves, Safrole (48.68%) was the major constituents and other components were 4-Allyl-1,2 diacetoxy benzene(9.7%), Eugenol (11.93%), γ-Muurolene (1.71%), β-Phellandrene (2.58%)[9]. Most of essential oil of *P.betel.*L contains Safrole, Terpinen-4-ol, β-Phellandrene, Eugenol, Caryophyllene, Hummulene, 4-Allyl-1,2-diacetoxy benzene (table 1).

Table 1: Chemical composition of essential oil of *P.betel* leaves leaves

SL.No	Retention Time	Compound Name	Molecular Formula	%of Compound
1.	3.424	β-Phellandrene	$C_{10}H_{16}$	3.698%
2.	4.460	4-Terpinenyl acetate	$C_{12}H_{20}O_2$	0.819%
3.	5.181	γ –Terpinene	$C_{10}H_{16}$	0.419%
4.	13.150	1,6 Octadien -3 –ol,3,7-dimethyl	$C_{10}H_{18}O$	0.676%
5.	13.707	Caryophyllene	$C_{15}H_{24}$	0.612%
6.	14.445	Terpinen -4-ol	$C_{10}H_{18}O$	5.726%
7.	15.446	Humulene	$C_{15}H_{24}$	0.625%
8.	15.710	γ-Muurolene	$C_{15}H_{24}$	9.153%
9.	16.959	Napthalene -1,2,3,5,6,8a hexahydro -4,7-dimethyl 1 (1-methylethyl)-(1s- cis)	$C_{15}H_{24}$	0.578%
10.	17.887	Methyl salicylate	$C_8H_8O_3$	0.111%
11.	16.400	Azulene,1,2,3,3a,4,5,6,7,Octahydro-1,4-dimethyl-7-(1-methylethenyl)-(1R- [1α,3aβ,4α,7β)]	$C_{15}H_{24}$	0.729%
12.	19.744	Safrole	$C_{10}H_{10}O_2$	31.210%
13.	20.379	Cubedol	$C_{15}H_{26}O$	0.161%
14.	20.786	Nonadecane	$C_{19}H_{40}$	0.064%

15.	21.435	4-Epicubedol	$C_{15}H_{26}O$	0.178
16.	22.344	2(3-Isopropyl- 4 methyl - pent-3-en-1-ynyl)-2 methyl –cyclobutanone	$C_{14}H_{20}$	0.077%
17.	22.675	Methyl Eugenol	$C_{11}H_{14}O_2$	3.566%
18.	23.291	Cubenol	$C_{15}H_{26}O$	0.319%
19.	23.634	1H-cycloprop[e]azulen-4- ol,Decahydro-1,1,4,7-tetramethyl- (1aR-(1aα,4β,4aβ,7α,7αβ,7bα)	$C_{15}H_{26}O$	0.433%
20.	24.986	Eugenol	$C_{10}H_{12}O_2$	12.113%
21.	25.448	Tau-Muurolol	$C_{15}H_{26}O$	0.176%
22.	25.715	1-Naphthalenol,1,2,3,4,4a,7, 8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-[1R(1α,4β,4aβ,8aβ)]	$C_{15}H_{26}O$	0.793%
23.	26.211	α –Cadinol	$C_{15}H_{26}O$	0.336%
24.	26.570	1-Naphthalenol,decahydro- 1,4a-dimethyl-7-(1-methylethylidene)-[1R-1α,4aβ,8aα)	$C_{15}H_{26}O$	0.179%
25.	26.889	Phenol,2- methoxy,4(2propenyl)-acetate	$C_{12}H_{14}O_3$	10.149%
26.	30.907	4-Allyl-1,2-diacetoxybenzene	$C_{13}H_{14}O_4$	12.140
27.	31.395	Phenol,2,6dimethoxy-4-[2-propenyl]	$C_{11}H_{14}O_3$	0.053
28.	31.946	Heptacosane	$C_{27}H_{56}$	0.045
29.	33.545	7,9-Di-tert-butyl-1-oxasipro[4,5]deca- 6,9diene-2,8-dione	$C_{17}H_{24}O_3$	0.045
30.	93.820	Octacosane	$C_{28}H_{58}$	0.052
31.	34.086	Benzenamine,2-nitro-5-[1-piperazinyl]	$C_{10}H_{14}N_4O_2$	0.079
32.	34.323	Estra-1,3,5[10]-trien-17β-ol	$C_{18}H_{24}O$	0.037
33.	34.611	Nonacosane	$C_{29}H_{26}O$	0.052

Antibacterial activity

The anti-bacterial activities of the leaves of essential oil from *P.betel* were tested against 3 bacteria. *P.betel* leaves showed maximum zone of inhibition 26 mm at 12µg/ml against *K. pneumonia* and 12 mm at10µg/ml against *S.aureus*. Oil does not show any zone of inhibition against *S.aeurginosa.* The antibacterial activity is shown in the table 2 and figure 2.

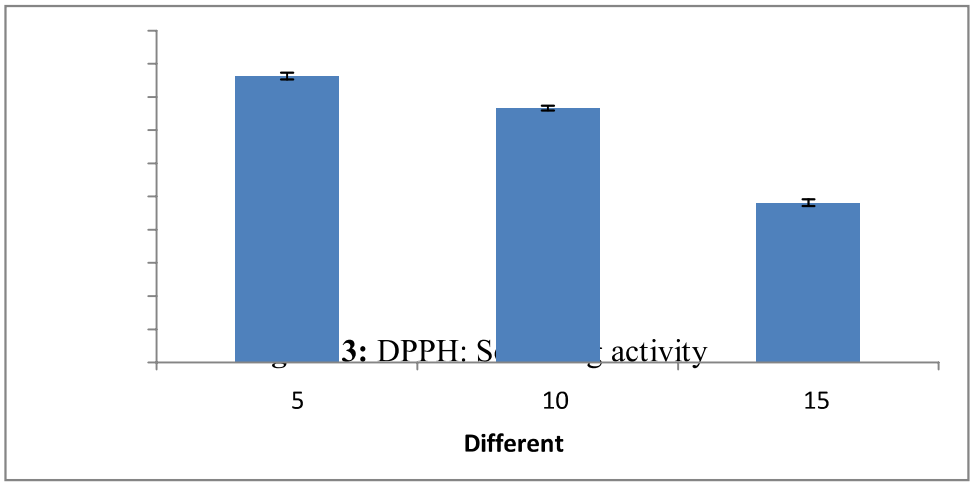
Table: 2 Anti-bacterial activities of *P.betel* leave against *S.aureus, S.aeurginosa, K.pneumoniae.*

Sl.No	**Test of Organism**	**Zone of Inhibition of Essential Oil of *P.betel* leaves**	**Zone of (Standard) Inhibition Tetramycin**
1.	*S.aureus*	10mm	12mm
2.	*S.aeurginosa*	-	-
3.	*K.pneumoniae*	12mm	26 mm

Figure: 2 Anti-bacterial activities against *P.betel* leave *S.aureus, S.aeurginosa* and *K. pneumonia*

Antioxidant activity

In the present study the effect of DPPH radical scavenging activity was evaluated for the essential oil and the result was given in the fig (3). The DPPH radical scavenging activity for the concentration of 5, 10 and 15 µg/ml was found to be at 87 %, 78 % and 48% respectively. Thus the essential oil of *P.betel* leaves can be used as an anti oxidant compound that scavenge the free radicals and Reactive oxygen species.



3: DPPH: S activity

CONCLUSION

The present study reveals that the essential oil of *P.betel* leaves has shows good antioxidant activity and moderate antibacterial activity. Further biological activities and their possible mechanism will explore in future.

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