Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(3): 774-778
Received: 15-03-2017
Accepted: 16-04-2017

Preethy TT
Cardamom Research Station, Pampadupara, Kerala Agricultural University, Kerala, India

Elsy CR
Professor, College of Horticulture, Kerala Agricultural University, Thrissur, Kerala, India

Dr. C Beena
Professor, College of Horticulture, Kerala Agricultural University, Thrissur, Kerala, India

Correspondence
Preethy TT
Cardamom Research Station, Pampadupara, Kerala Agricultural University, Kerala, India

Profiling of essential oil in *Tirur vettilai* (*Piper betle* L.), a group of unique betel vine land races from Malappuram district, Kerala

Preethy TT, Dr. Elsy CR and Dr. C Beena

Abstract
In Kerala, Tirur and nearby areas of Malappuram district are famous for betel vine cultivation covers an area of 183 ha (FIB, 2014) [1]. Betel vine cultivars from Malappuram district, popular as *Tirur vettila*, possess some special biochemical characters like unique flavor and aroma. The present investigation was carried out to study the chemical composition of betel vine land races in Tirur and nearby areas of Malappuram district and to characterize the land races based on biochemical components of essential oil. Essential oil studies were undertaken with *Muvattupuzha local* as check variety, a variety from Ernakulum district, Kerala. *Puthukodi, Chelan, Karinadan* and *Nadan* were the betel vine cultivars recorded from Malappuram District. *Puthukodi* and *Nadan* were the most common cultivars whereas *Chelan* and *Karinadan* were the cultivars conserved by few farmers. Essential oil content was maximum in *Muvattupuha Local,* a cultivar with high pungency and it was low in *Chelan*. GC studies revealed that, eugenol was the major component of essential oil in all land races with high content (20.80 percent) in *Chelan*. Possible compounds identified from the chromatograms of oil from different land races were hydroxychavicol, β caryophyllene and 5-(2-propenyl)-1, 3- benzodioxole.

Keywords: Essential oil, chromatograms, eugenol, *Chelan*, hydroxychavicol

Introduction
Betel vine (*Piper betle* L.) is a dioecious, evergreen creeper belonging to the family Piperaceae. It is an indigenous medicinal plant with glabrous, deep green, heart shaped leaves as economically important part.
Betel leaves have many medicinal properties and are used in Indian system of medicine to cure indigestion, stomach ache, diarrhoea, flatulence and to heal wounds, scales, burns, swelling *etc* The leaves are nutritive and contain anticarcinogens, showing future opportunities in anticancer drugs. In *Susruta Samhita*, it is mentioned as aromatic, sharp, hot, acrid and beneficial as laxative and appetizer (Pradhan *et al.*, 2013) [2]. The main biochemical component betel leaf is essential oil and it contributes flavor to the leaf. It is reported that essential oil contributed to the medicinal property of betel vine also (Pradhan *et al.*, 2013) [2]. Betel vine was reported to have immunosuppressive activity and antimicrobial property (Banerjee, 2012). Essential oil content is an important character for identification of types for commercial cultivation with the objective of oil extraction, as betel oil fetches high price (186 dollar per 100 ml) in international markets (New Directions Aromatics, nd).
In India, it is cultivated in an approximate area of 45,000 ha as cash crop. In Kerala, Tirur and nearby areas of Malappuram district are famous for betel vine cultivation with an area of 183 ha (FIB, 2014) [1]. Betel vine cultivars from Tirur area possess some special biochemical characters like unique flavor and aroma because of geographical features, traditional cultural practices, specific genotypes, special soil characters and peculiar climatic features of area of production. Studies on biochemical components of essential oil are undertaken to identify the best cultivars for commercial cultivation and to use in both medicine and cosmetic production.

Materials & methods
Essential Oil Content
Essential oil was extracted from fresh leaves by hydro distillation using Clevenger apparatus (Augustin, 1998) [5]. One fifty grams of fresh leaves were taken in the round bottom flask of Clevenger apparatus. Water was added into it up to 60 percent of its capacity. Glass beads were added to avoid bumping. Continuous cold water supply was provided. Fresh leaves were steam distilled in Clevenger apparatus for four hours to obtain essential oil. The oil being light in weight was collected over water in the delivery tube of the condenser. However oil was mixed with water and no separate layer of oil was seen.

The procedure was again repeated twice for one sample. Once the distillation was over the mixture of oil and water collected in the delivery tube was transferred to a test tube containing small amount of sodium chloride to separate oil and water layers. Density of water was increased due to sodium chloride and oil became upper layer. The oil layer was separated carefully and dried over anhydrous sodium sulfate and kept in sterile appendorf tubes. The volume of essential oil was measured and expressed as oil percent (v/w) in the leaf tissue. The oil was stored in the refrigerator at 4 ^{0}C for further analysis using gas chromatography. Between the extractions of oil from two different samples, the condenser was washed with ether in order to remove any sticking oil.

Identification and Quantification of Components of Oil Using GC

Essential oil samples obtained were subjected to gas chromatography adopting following conditions; Gas chromatograph: DANI (Italy) make GC nodal Master with capillary column and FID detector; Column dimensions: length of 30mm, inner diameter of 0.25mm and film thickness of 0.25microns; Carrier gas: N_2 at flow rate of 1.2ml/min, H_2 at flow rate of 40ml/min, air at flow rate 280 ml/min; Injection volume: 1ml. The column was programmed as follows: held at 100 ^{0}C for 1 minute, heated to 150 ^{0}C @ 2.5 ^{0}C/minute and held at 0.5 minute, heated to 225 ^{0}C (@ 50 ^{0}C/minute), held at 0.5 minute. The injector temperature was 250 ^{0}C and detector temperature was 220 ^{0}C. The sample was injected in a split ratio of 1:99. Data on the chromatogram and peak were integrated on to the computer based programme, CLARITY (DANI INPUTS, Italy, 2005). Eugenol, isoeugenol, methyl eugenol and methyl isoeugenol were used as standards. Relative amounts of individual components were worked out using GC peaks of standards.

The other possible components of essential oil were identified by comparing the values of retention time and area of corresponding peaks with literature data. Compounds with a relative amount of more than two percent were identified as predominant chemical components of the specific oil sample.

Results & discussion

Essential Oil – Yield and biochemical components

Land races showed difference in yield of essential oil from leaves and it ranges from 0.45 to 0.57 percent. Comparatively higher yield (0.57 percent v/w) of essential oil was obtained from the check variety, *Muvattupuzha Local* and this could have contributed to the high pungency in this cultivar. The high content of essential oil makes *Muvattupuzha Local* cultivar more suited for oil extraction. *Karinadan, Puthukodi* and *Nadan* recorded 0.52, 0.50 and 0.47 percent essential oil respectively. *Chelan* recorded the minimum content of 0.45 percent (v/w) of essential oil and probably this might be the reason for low pungency in this cultivar. Sugumaran *et al*. (2011) [6] obtained an essential oil yield of 0.31 percent in volume by weight basis in the cultivar *Vellaikodi*. Rani and Ramamurthy (2012) [7] obtained 0.08 to 0.20 percent (v/w) of essential oil from betel leaf. Guha (2006) [3] found that *Mita*, *Sanchi* and *Bangla* varieties of betel vine had about 2, 1.70 and 0.80 percent essential oil respectively. The variations in the essential oil content in different studies might be due to varietal differences, cultural practices followed, plant part used, lab conditions *etc*. More studies are required to confirm the content of essential oil in different cultivars.

Biochemical components of essential oil extracted from different cultivars were analyzed through GC. Most of the biochemical components of essential oil were isolated during the first 30 minutes of the gas chromatography. In the present study, essential oil from most of the cultivars (Fig. 15, 16, 17, 18, 19) showed the presence of 55 – 56 components. Eugenol showed a retention time of 13.54 minutes (Fig. 1) whereas 16.05 minutes was recorded as the retention time of methyl eugenol. Chromatograms of isoeugenol and methyl isoeugenol had shown a retention time of 16.61 minutes and 18.78 minutes respectively.

Table 1: Essential oil and biochemical components present in different betel vine land races of Malappuram district during 2013 – 14

Sl. No.	Betel vine cultivars	Yield of essential oil (V/W %)	Eugenol (%)	Methyl eugenol (%)	Isoeugenol (%)	Methyl isoeugenol (%)
1	Puthukodi	0.50	15.30	-	0.80	0.30
2	Chelan	0.45	20.80	0.80	1.00	0.20
3	Karinadan	0.52	11.60	-	-	0.10
4	Nadan	0.47	16.30	0.80	0.80	0.10
5	Muvattupuzha Local	0.57	11.00	-	0.80	1.50

Among the components studied, eugenol was the major component (11.02 – 20.80 percent) of essential oil in all the cultivars under study followed by methyl isoeugenol with a range of 0.10 – 1.50 percent. Highest percentage of eugenol (20.80) was seen in essential oil derived from *Chelan* cultivar. This is confirmation with the result of Guha (2003) [9], who reported eugenol as the chief ingredient of essential oil in betel vine with a content of 29.50 percent. Eugenol had antifungal and antioxidant properties (Pradhan *et al*., 2013) [2]. Baliga *et al*. (2011) [10] reported eugenol in betel vine as an excellent antimutagen. It could be used as a local anesthetic for tooth ache (Pradhan *et al*., 2013) [2]. In the present study, identification of eugenol as the major component of essential oil confirmed the medicinal properties of this crop. Eugenol with its antioxidant property makes betel vine as a probable candidate in the treatment of dreaded diseases. Moreover eugenol is used in perfumeries, flavorings and medicine as a local antiseptic and anesthetic. Eugenol can be combined with zinc oxide to form a material known as zinc oxide eugenol which has restorative and prosthodontic applications in dentistry (Jadhav *et al*., 2004) [11]. So *Chelan* with highest content of eugenol has more potential in manufacturing of drugs, perfumes and dentistry materials. Balasubramanyam and Rawat (1990) [12] also suggested that eugenol contributed to the clove like aroma of certain cultivars like *Bangla* and *Sanchi*. So it could be assumed that the high content of eugenol could impart mild aroma to this cultivar.

Methyl eugenol was found in *Chelan* and *Nadan* to the extent of 0.80 percent. Methyl eugenol is used in aroma therapy and as massage oil (Government of Canada, 2010) [13]. It is also widely used as a fragrant ingredient in perfumes, toiletries and detergents. Methyl eugenol is also used as an insect attractant in combination with insecticides (NTP, 2000; HSDB, 2010) [14, 17]. Hence *Nadan* and *Chelan* have more potential to use in perfume industry. Earlier the presence of high content of eugenol in *Chelan* had revealed its potential use in drugs, perfumes and dentistry materials.

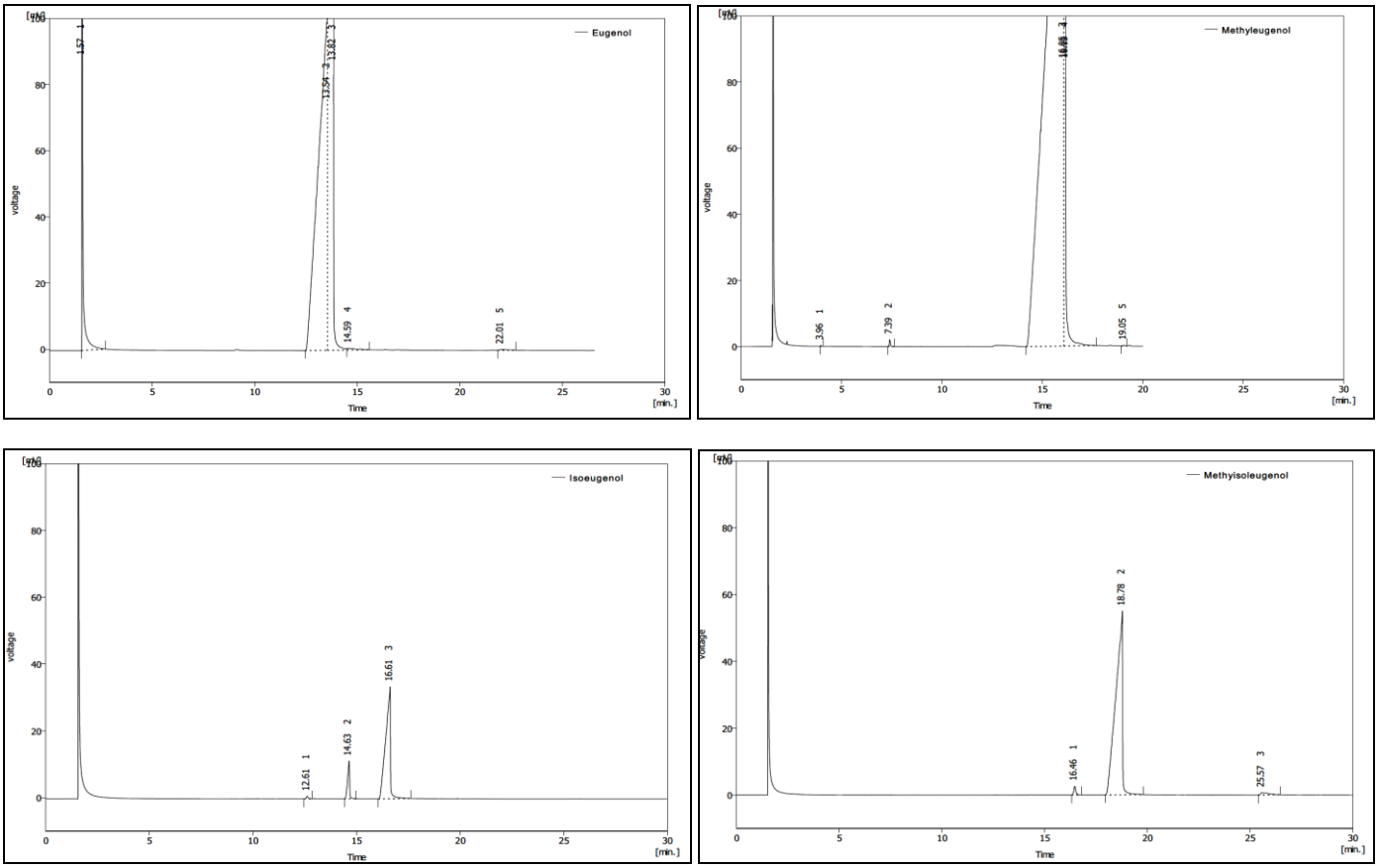

Fig 1: Chromatogram of eugenol, methyl eugenol, isoeugenol, methylisoeugenol.

Isoeugenol was also present in all cultivars except in *Karinadan* to the range of 0.80 – 1.00 percent. Isoeugenol had been used in the manufacture of vanillin (Merck, 1996) [16]. As a flavoring agent, isoeugenol is added to nonalcoholic drinks, baked foods and chewing gums (National Toxicology Programme, 2010) [17]. *Chelan* recorded higher isoeugenol content, indicating its potential value in the production of flavoring agents.

Like eugenol, Methyl isoeugenol in trace amounts, was identified from all the cultivars. Methyl isoeugenol is a natural food flavor and is used for treating mood disorders (Fajemiroye *et al*., 2011) [18]. *Muvattupuzha Local* had the highest content of methyl isoeugenol. Hence this cultivar has potential use in food flavours and medicines.

Number of peaks in essential oil, retention time with corresponding relative amount and its possible identity are presented in Table 2. A total of 55 possible components with eight compounds as predominant (>2 %) were identified in the essential oil of *Puthukodi*. Hydroxychavicol and β caryophyllene were the few predominant possible compounds identified in essential oil of *Puthukodi* and accounted for 48.10 percent of the total oil.

A total of 56 possible chemical components were identified in the oil from *Chelan*. In this cultivar, six predominant possible components, including hydroxychavicol accounted for 56.85 percent of essential oil. Compared to other cultivars, quantity of hydroxychavicol was less in this cultivar.

Analysis of the relative composition of betel oil of *Karinadan*, showed 39 possible compounds with seven predominant components. Hydroxychavicol, β caryophyllene and 5-(2-propenyl)-1, 3-benzodioxole were some of the possible predominant components with a relative amount of 44.50, 4.30 and 2.40 percent respectively.

Fifty six possible components were identified from the essential oil of *Nadan*. Hydroxychavicol with a relative amount of 44.60 was identified as the major predominant possible component in this cultivar.

GC analysis of essential oil of *Muvattupuzha Local* recorded a total of 55 possible components of which seven were predominant. Hydroxychavicol (41.10 percent), 5-(2-propenyl)-1, 3-benzodioxole (5.00 percent) and β caryophyllene (4.10 percent) were the major possible components identified from this cultivar.

Many studies have reported that hydroxychavicol is a major phenolic compound in the aqueous extract of betel leaves (Nalina and Rahim, 2007; Ali *et al*., 2010; Pin *et al*., 2010) [20, 21, 22]. Hydroxychavicol said to possess antibacterial (Ramji *et al*., 2002; Sharma *et al*., 2009) [23, 19], antioxidant and anticarcinogenic activities (Chang *et al*., 2002b) [24]. The betel leaves were reported to possess anticancerous activity particularly against the tobacco carcinogens (Padma *et al*., 1989; Chang *et al*., 2002b) [25, 24] due to the presence of hydroxychavicol (Amonkar *et al*., 1989) [25]. Baliga *et al*. (2011) [10] reported the use of hydroxychavicol as antimutagen. The possible presence of hydroxychavicol in all the cultivars indicated the medicinal properties of this crop. Betel leaves and betel juice if administered properly can contribute antibacterial and carcinogenic properties. The possible content of hydroxychavicol was more in *Puthukodi*.

Possible content of β caryophyllene in essential oil ranged from 2.80 - 4.30 percent among the cultivars. This is an FDA approved food additive and contributes to the spiciness of black pepper.

The other possible component identified in the present study is 5-(2-propenyl)-1, 3- benzodioxole, commonly known as safrole. More in depth studies are needed to reveal the biochemical ingredients and medicinal properties of betel vine.

Table 2: Distribution of predominant chemical compounds (>2%) in the essential oil of different betel vine cultivars of Malappuram district during 2013 – 14

Betel vine cultivars	No. of peaks obtained	Retention time (minutes)	Relative amount (v/w %)	Possible identity
Puthukodi	55	11.05	45.30	Hydroxychavicol
		13.3	15.30	
		7.16	7.80	
		24.39	2.90	
		19.7	2.80	β caryophyllene
		4.47	2.70	
		3.34	2.50	
		4.06	2.10	
Chelan	56	11.49	39.50	Hydroxychavicol
		13.95	20.80	
		7.37	9.85	
		3.35	2.70	
		4.51	2.60	
		4.08	2.20	
Karinadan	39	11.60	44.50	Hydroxychavicol
		13.71	11.60	
		1.53	9.30	
		7.07	8.70	
		20.17	4.30	β caryophyllene
		3.85	2.80	
		24.55	2.40	5-(2-propenyl)-1,3-benzodioxole
		19.48	2.20	
Nadan	56	10.78	44.60	Hydroxychvicol
		13.07	16.30	
		4.45	3.20	
		4.04	2.40	
Muvattupuzha Local	55	11.34	41.10	Hydroxychavicol
		1.58	11.70	
		13.48	11.00	
		7.19	5.60	
		24.67	5.00	5-(2-propenyl)-1,3-benzodioxole
		19.99	4.10	β caryophyllene
		3.34	2.40	

Conclusion

Land races showed difference in yield of essential oil from leaves and it ranges from 0.45 to 0.57 percent. Comparatively higher yield (0.57 percent v/w) of essential oil was obtained from the check variety, *Muvattupuzha Local* Among the components studied, eugenol was the major component (11.02 – 20.80 percent) of essential oil in all the cultivars under study followed by methyl isoeugenol with a range of 0.10 – 1.50 percent. Possible compounds identified from the chromatograms in comparison to known retention time were hydroxychavicol, β caryophyllene and 5-(2-propenyl)-1, 3-benzodioxole. The data showed the possible presence of hydroxychavicol in all the cultivars. It's possible presence in cultivars was to the tune of 39.50 (*Chelan*) to 44.60 percent in *Nadan*.

Acknowledgement

We thank Mr. Muhammed Moopan who co - operated in this venture by allowing me to carry my research work in his field and the timely help rendered by him for carrying out the field experiments. The award of KAU fellowship is thankfully acknowledged.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. FIB. Farm Guide. Farm Information Bureau. Government of Kerala. 2014, 256.
2. Pradhan D, Suri KA, Pradhan DK, Biswasroy P. Golden Heart of the Nature - *Piper betle* L. J. Pharmacognosy and Phytochemistry. 2013; 1(6):147-152.
3. Banerjee B. Extraction, isolation and identification of the active component of essential oil of betel leaf. ME (Chemical engineering) thesis. Jadavpur University, Kolkata. 2012, 110.
4. New directions Aromatics. [online]. Available: http://www.newdirectionsaromatics.com/. [20 Jul 2014].
5. Augustin A. Influence of plant competition, FYM and harvest schedule on flowering and metabolic production in Indian sarsaparilla. AICRP report on medicinal and aromatic plants, Kerala Agricultural University, Thrissur, 1998, 108.
6. Sugumaran M, Gandhi M, Sankaranarayanan K, Yokesh M, Poornima M, Rajasekhar SR. Chemical composition and antimicrobial activity of vellaikodi variety of *Pier betle* L. leaf oil against dental pathogens. Int. J. Pharm. Tech. Res. 2011; 3:2135-2139.
7. Rani OU, Ramamurthi K. Betel leaf: nature's green Medicine. Facts for you. 2012, 3.
8. Guha P. Betel leaf: The neglected green gold of India. J. Hum. Ecol. 2006; 19:87-93.
9. Guha P. Extraction of essential oil from betel leaves grown in and around Midnapur district. In: annual report of All India Coordinated Research project on post harvest technology (ICAR). IIT. Kharagpur, India. 2003, 15-23.
10. Baliga MS, Bhat HP, Rao S, Palatty PL, Thilkchand KR, Rai MP. *Piper betle* L., the maligned Southeast Asian medicinal plant possesses cancer preventive effects: time to reconsider the wronged opinion. Asian Pac. J. Cancer.

Prev. 2011; 12:2149-2156.
11. Jadhav BK, Khandelwal KR, Ketkar AR, Pisal SS. Formulation and evaluation of mucoadhesive tablets containing eugenol for the treatment of periodontal diseases. Drug. Dev. Ind. Pharm. 2004; 30(2):195-203.
12. Balasubrahmanyam VR, Rawat AKS. Studies on morphology and chemistry of Piper betle L. J. Plant. Crops. 1990; 18(2):78-87.
13. Government of Canada. Risk management scope for Benzene, 1, 2-dimethoxy-4-(2-propenyl)-Methyl Eugenol. Chemical Abstract Service Registry Number (CAS RN): 93-15-2. Environment Canada Health. 2010. Available at: http://www.ec.gc.ca/substances/ese/eng/challenge/batch9/batch9_93-15-2_rm_en.pdf.
14. NTP (National Toxicology Programme). Toxicology and Carcinogenesis Studies of Methyleugenol (CAS No. 93–15–2) in F344/N Rats and B6C3F1Mice. Natl. Toxicol. Program Tech. Rep. Ser. 2000; 491:1-412.
15. HSDB (Hazardous Substances Data Bank). Methyleugenol CASRN: 93–15–2. In: Hazardous Substances Data Bank. Bethesda, MD: U.S. National Library of Medicine, 2010. Available at: http://toxnet.nlm.nih.gov/cgi-bin/sis/search/. [13 Aug 2014].
16. Merck. The Merck Index, Twelfth edition. Merck & Co, Whitehouse. 1996, 35.
17. NTP (National Toxicology Programme). Toxicology and carcinogenesis studies of isoeugenol (CAS No. 97-54-1) in F344/N rats and B6C3F1 mice (gavage studies). Natl. Toxicol. Program Tech. Rep. Ser. 2010; 551:1-178.
18. Fajemiroye JO, Galdino MP, De Paula MAJ, Rocha FF, Akanmu MA, Vanderlinde AF *et al*. Anxiolytic and antidepressant like effects of natural food flavour (E)-methyl isoeugenol. Food Funct. 2011; 5:1819-1828.
19. Sharma S, Khan IA, Ali I, Ali F, Kumar M, Kumar A *et al*. Evaluation of the antimicrobial, antioxidant and anti-inflammatory activities of hydroxychavicol for its potential use as an oral care agent. Antimicrob Agents. Chemother. 2009; 53:216-222.
20. Nalina T, Rahim ZHA. The crude aqueous extract of Piper betle L. and its antibacterial effect towards Streptococcus mutans. Am. J. Biotechnol Biochem. 2007; 3:10-15.
21. Ali I, Khan FG, Suri KA, Gupta BD, Satti NK, Dutt P *et al*. *In vitro* antifungal activity of hydroychavicol isolated from Piper betle L. Ann. Clin. Microbiol. Antimicrob. 2010; 9:1-9.
22. Pin KY, Chuah AL, Rashih AA, Mazura MP, Fadurena J, Vimala S *et al*. Antioxidant and antiinflamatory activities of extracts of betel leaves (*Piper betle* L.) from solvents with different polarities. J. Trop. Forest Sci. 2010; 22(4):448-455.
23. Ramji N, Iyer R, Chandrasekaran S. Phenolic antibacterials from Piper betle in the prevention of halitosis. J Ethnopharmacol. 2002; 83:149-152.
24. Chang MC, Uang BJ, Tsai CY, Wu HL, Lin BR, Lee CS *et al*. Hydroxychavicol, a novel betel leaf component, inhibits platelet aggregation by suppression of cyclooxygenase, thromboxane production and calcium mobilization. Br. J. Pharmacol. 2002b; 152:73-82.
25. Padma PR, Lalitha VS, Amonkar AJ, Bhide SV. Anticarcinogenic effects of betel leaf extract against tobacco carcinogens. Cancer Let. 1989; 45(3):195-202.