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QUANTITATION OF EUGENOL IN BETEL LEAF VARIETIES BY HPTLC

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ABSTRACT: Betel leaves have been eaten traditionally as a quid in many South East Asian countries. Its taste and aroma is because of the presence of essential oils, namely eugenol. Eugenol is an important phytochemical with analgesic, anti inflammatory and anaesthetic properties. Eugenol further finds applications in cosmetics and food industry. Betel leaves being perishable, become a major concern for agro waste. Yellow dry leaves and the petioles also go waste. In this paper, HPTLC fingerprint and eugenol content were studied in three varieties of fresh betel leaves as also in yellow leaves and in their petioles. Seasonal variation in eugenol content was also studied. The fingerprint of the leaves showed 10 bands with one of the marker peaks at 0.35 which corresponded to eugenol. It was found that Benarasi betel leaf has maximum eugenol content (average 1.1104%). Nagarvelli and Kolkata leaves have comparable eugenol content (0.659% and 0.637% respectively). In all the three leaf varieties, eugenol content was found to be the highest in winter season, the least in rainy season and intermediate in summer. Yellow leaves of Nagarvelli and Benarasi varieties showed presence of considerable amount of eugenol. The petiole of Nagarvelli leaf also showed presence of eugenol content.

INTRODUCTION: *Piper betle* Linn. is a dioecious perennial climbing lianas, cultivated throughout the hotter and damper parts of India [1]. Betel leaves have been eaten traditionally as a quid in many South East Asian countries. The leaf has a great potency to act as natural antioxidant. The antioxidant property is correlated with different biological activities like hepatoprotective, anti-diabetic and anti cancer properties, since free radicals are involved in all these diseases [2]. A complete review of biochemical characterization of betel vine types has been presented by Preethy T. T. *et al.*,[3] .

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The pharmacognostical and phytophysicochemical profile of betel leaves has been extensively studied by Periyanayagan K *et al.*,[4]. The pungency and spicy aroma of the leaf is because of the presence of essential oils. The most predominant of them all has been found to be eugenol. Eugenol is an important phytochemical with analgesic, anti inflammatory and anaesthetic properties. Eugenol can further find lot of applications in cosmetics and food industry.

Betel leaves are extremely perishable if not stored properly and there is lot of wastage by quick spoilage due to dehydration, fungal infection and dechlorophyllation. This causes post harvest loss ranging from 35 to 70% during transport and storage [5]. Sukanta K. Sen *et al.*, [6] have studied the antimicrobial activity of the betel leaf agro waste. In his paper, Guha [7] has brought to the fore that attempts have been made to minimize the wastage

of leaves by chemical treatments, manipulation of storage temperature, adopting better packaging materials and methods [8, 9]. Guha has further pointed out that such wastage may also be minimized by extracting essential oil from unsold, stocked up leaves. He found that Mitha, Bangla and Sanchi varieties of leaves contained 2.0%. 1.7% and 0.8% essential oil, of which chief ingredient was eugenol, which formed 29% of essential oil [10, 11]. Also when the leaves are used their petioles are removed and they generally go waste. The TS of petiole has been shown to have small oil cells with dark brown contents embedded in the phloem tissue [1]. But the oil content has not been quantified.

Thus it seems imperative to find the eugenol content from different varieties of betel leaves and also to find its maximum content through different seasons and also whether eugenol is present in dry yellow leaves and the petioles which otherwise go waste. Different extraction methods like Liquid-Liquid Extraction and Supercritical Fluid Extraction for eugenol and its separation by HPLC have been studied [12]. Also extractions by soxhlet, sonication and maceration methods have been compared, which showed higher extraction efficiencies by sonication followed by maceration and soxhlet in the order [13]. Although many such methods have been described, in this study eugenol was extracted by soxhlet method and quantitated by HPTLC method. Various mobile phases were tried namely; n hexane: ethyl acetate: glacial acetic acid (8.5:1.0:0.5 v/v/v) [14], toluene: ethyl acetate: formic acid (90:10:0.1 v/v/v) [15] and toluene: ethyl acetate (9.7:0.3 v/v) [1, 16]. Toluene: ethyl acetate (9.7: 0.3) was finally selected.

In this paper HPTLC fingerprint and eugenol content have been studied in three varieties of fresh betel leaves namely, Nagarvelli, Kolkalta and Benarasi, as also in yellowed leaves and in their petioles. Seasonal variation in eugenol content has also been studied. This study will thus help us to know which is the appropriate season of obtaining maximum yield of eugenol. Also we can find out if yellow dry leaves and the petioles can be of any use and prevent them from being wasted.

MATERIALS AND METHODS: 3 varieties of betel leaves were studied, namely; Nagarvelli, Kolkata and Benarasi.

Sample Preparation: Accurately weighed 2.0g of dried betel leaf powder was extracted with n-hexane (10.0 mL x 3) using soxhlet apparatus at 15% heat. The mixture was filtered through Whatman filter paper no. 41 and evaporated to dryness at 69°C. The extract obtained was reconstituted in n-hexane (8.0ml), filtered through Whatman filter paper no. 41 and the filtrate obtained was subjected to HPTLC analysis. Dried powders of petiole and yellow leaves were similarly treated and analysed.

Preparation of Standard Solution: Eugenol (Sigma Aldrich) of 1000ppm strength was prepared by dissolving accurately weighed 10.0mg standard in 10.0mL methanol in a standard volumetric flask. From this, a stock solution of 100ppm was prepared. For obtaining linearity range for eugenol from the stock of 100ppm, 10.0ppm, 75.0ppm, 150.0ppm, 200.0ppm, 250.0ppm and 300.0ppm were prepared.

Preparation of Derivatizing Reagent: Anisaldehyde sulphuric acid: 5.0mL sulphuric acid was added to an ice cooled mixture of 85.0mL methanol and 10.0mL of acetic acid. To this 0.5mL of anisaldehyde was added.

Optimized Chromatographic Conditions: Merck Silica gel 60 F254 HPTLC pre coated plates were used as stationary phase. Mobile phase used was toluene: ethyl acetate (9.7: 0.3, v/v). 10.0µL sample was applied on the plate.

RESULTS:

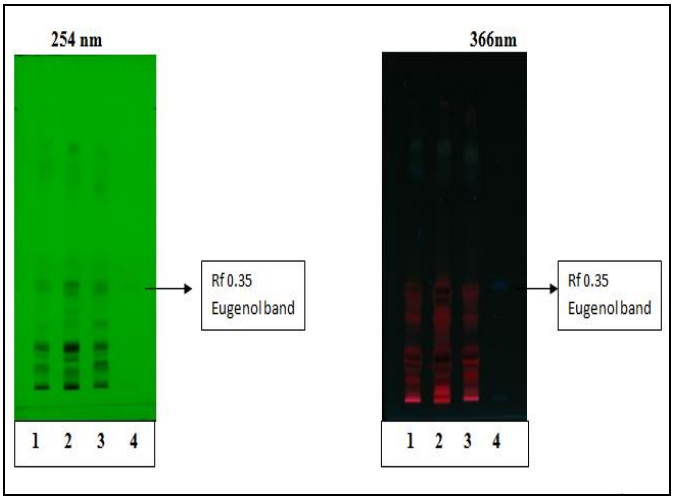

FIG. 1: DEVELOPED PLATES BEFORE DERIVATI - ZATION: 254nm AND 366nm

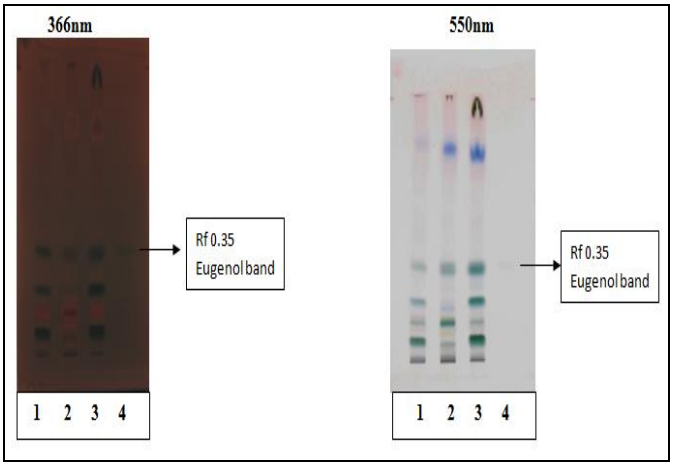

FIG. 2: DEVELOPED PLATES AFTER DERIVATIZATION: 366nm AND 550nm

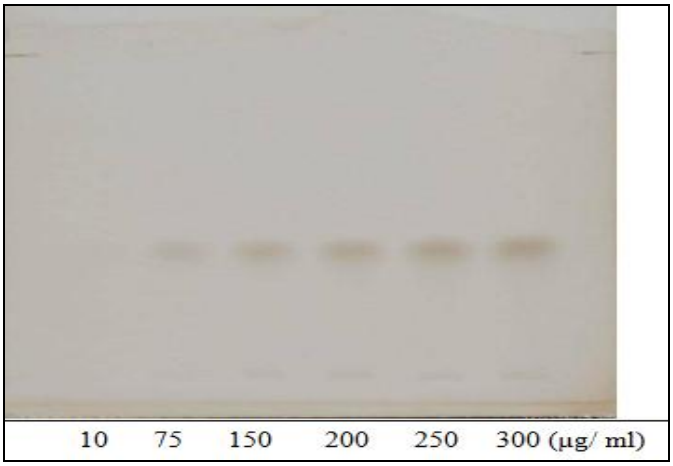

FIG. 3: PLATE AFTER DERIVATIZATION AT 550nm: FOR LINEARITY

Track 1: Nagarvelli leaf (N leaf)

Track 2: Kolkata leaf (K leaf)

Track 3: Benarasi leaf (B leaf)

Track 4: Eugenol

TABLE 1: LINEARITY RANGE FOR EUGENOL

Calibration standards ppm (µg/ ml)	Area under the curve (AUC)
10.0	1981.0
75.0	10215.4
150.0	16190.9
200.0	19715.7
250.0	22837.6
300.0	23970.6

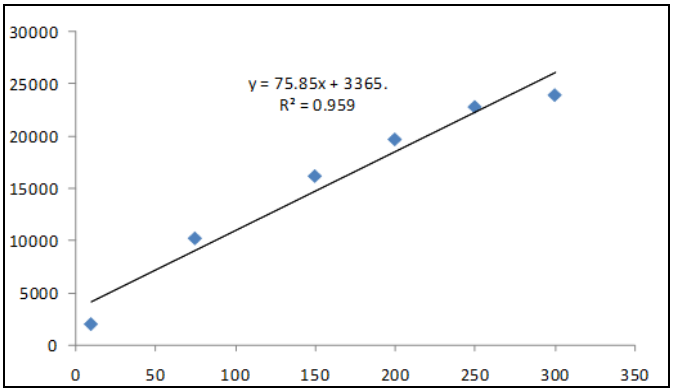

FIG. 4: LINEARITY CURVE AND THE EQUATION; X AXIS: EUGENOL STANDARD ppm, Y AXIS: AREA UNDER CURVE

Equation: y = 75.85x + 3365

TABLE 2: EUGENOL CONTENT IN SAMPLE (FRESH BETEL LEAVES) COLLECTED IN DIFFERENT SEASONS

Sample	Season	Area under curve	Content of eugenol (µg/ ml)	Content of eugenol (µg/ 8ml)	Weight of extract (g)	Content of eugenol (mg/ g of extract)	Average Content of eugenol (mg/ g of extract)	Content of eugenol (%)
Nagarvelli	Summer	757.0	34.38	275.04	0.0693	3.969	6.590	0.659
	Rainy	518.4	37.53	300.24	0.11	2.730		
	Winter	7876.5	59.47	475.76	0.0364	13.07		
Kolkata	Summer	5764.1	31.62	252.96	0.07	3.614	6.37	0.637
	Rainy	5218.5	24.43	195.44	0.0844	2.316		
	Winter	14865.5	151.61	1212.88	0.092	13.18		
Benarasi	Summer	10784.5	97.81	782.48	0.079	9.905	11.104	1.1104
	Rainy	12295.1	117.73	941.84	0.1267	7.434		
	Winter	17994.5	192.87	1542.96	0.0966	15.973		

TABLE 3: EUGENOL CONTENT IN YELLOWED BETEL LEAVES COLLECTED IN WINTER

Sample	Season	Area under curve	Content of eugenol (µg/ ml)	Content of eugenol (µg/ 8ml)	Weight of extract (g)	Content of eugenol (mg/ g of extract)	Content of eugenol (%)
Yellow leaves of Nagarvelli	Winter	13411.3	132.44	1059.52	0.0769	13.77	1.377
Yellow leaves of Benarasi	Winter	6902.9	46.64	373.12	0.0318	11.73	1.173

TABLE 4: EUGENOL CONTENT IN PETIOLE

Sample	Season	Area under curve	Content of eugenol (µg/ ml)	Content of eugenol (µg/ 8ml)	Weight of extract (mg)	Content of eugenol (mg/ g of extract)	Content of eugenol (%)
Petiole of Nagarvelli	Winter	2031.7	17.95	143.6	0.022	6.527	0.653

DISCUSSION: The fingerprint of the leaves showed 10 bands with their R_f at 0.01, 0.08, 0.15, 0.22, 0.35, 0.38, 0.43, 0.55, 0.70 and 0.85 as shown in **Fig. 1** and **2**. The band at R_f 0.35 corresponded to eugenol standard. **Fig. 3** shows the plate development for obtaining the linearity curve for eugenol. **Fig. 4** describes the linearity equation obtained based on the area under the curve values as shown in **Table 1**. The average content of eugenol in Nagarvelli leaves was 0.659%, in Kolkata leaves it was 0.637% and in Benarasi leaves it was 1.1104% (**Table 2**).

The best season to extract eugenol was found to be in winter in which the leaves showed its highest content. As shown in **Table 3**, yellow leaves also showed considerable quantity of eugenol, 1.377% and 1.173% in Nagarvelli and Benarasi leaves respectively. The petiole of Nagervelli leaf had 0.653% eugenol content (**Table 4**). Thus it is of economic and environmental importance to extract eugenol from yellow dried leaves and from petioles which otherwise go waste.

CONCLUSION: It can be concluded that Benarasi betel leaf has maximum eugenol content amongst the three varieties. Nagarvelli and Kolkata leaves have comparable eugenol content. Also in all the three leaf varieties, eugenol content was found to be the highest in winter season, intermediate in summer and the least in rainy season. Yellow leaves of Nagarvelli and Benarasi leaves show presence of considerable amount of eugenol. The petiole of Nagarvelli leaf also shows eugenol content. Once extracted, eugenol can be used for its wide applications in pharmaceutical, cosmetics and food industries.

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CONFLICTS OF INTEREST: There is no conflict of interest for the research findings

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