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RESEARCH ARTICLE

BETEL VINE LEAVES – A GREEN TREASURE HOUSE OF USEFUL CHEMICALS

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ABSTRACT

Morphological characters are easily amenable to environmental characters due to their oligogenic character. So depiction of genotypes solitarily based on these characters is not reliable. Hence, biochemical characterization is essential for effective characterization of betel vine types.

INTRODUCTION

Morphological characters are easily amenable to environmental characters due to their oligogenic character. So depiction of genotypes solitarily based on these characters is not reliable. Hence, biochemical characterization is essential for effective characterization of betel vine types. The betel leaves were reported to possess anticancerous activity particularly against the tobacco carcinogens (Padma *et al*., 1989; Wu *et al.*, 2004; Chang *et al.*, 2002a) due to presence of ingredients like hydroxychavicol (Amonkar *et al.*, 1989) and chlorogenic acid without affecting the normal cells unlike the common anticancer drugs, applicable against wide ranges of environmental carcinogens in both prokaryotes and eukaryotes. Baliga *et al*. (2011) reported that the eugenol and hydroxychavicol in betel leaf were excellent antimutagens. Piperbetol, ethylpiperbetol, piperol A and piperol B, isolated from leaves, selectively inhibited platelet aggregation induced by platelet activating factor in a concentration dependent manner. It was concluded that betel leaf was a novel candidate for immunosuppressive activity. Betel leaf raised body temperature due to cholinergic responses. Leaves possessed broad spectrum of antimicrobial activity against various bacterial strains. Chewing of betel leaves not only accelerated the salivation, but also enhanced gastric juice secretion to aid digestion process. This might be the reason for chewing *pann* after food (Banerjee, 2012)

The chemo preventive potential of betel leaf was reported against liver fibrosis. Phytochemical investigation on leaf characters of betel vine revealed presence of biochemical molecules like eugenol, chavicol and amino acid. The betel leaf had moisture (85.4 per cent), protein (3.1 per cent), fat (0.8 per cent), carbohydrate (6.1 per cent), fibre (2.3 per cent), minerals (2.3 per cent), reducing sugars (0.38 to 1.46 per cent), all vitamins and iodine also (Pradhan *et al.*, 2013).

Betel leaf is considered as the best natural substance that contributed best oral hygiene to oral activity (Pradhan *et al.*, 2013). The medicinal importance of the herb as discussed above evidently proved that, betel leaf is one of the most promising commercial botanical with lot of therapeutic values. Biochemical compounds in betel leaf are separately reviewed below.

Essential Oil

Betel leaf is a very perishable commodity and therefore, is always subjected to wastage by quick spoilage due to dehydration, fungal infection, dechlorophyllation etc. This caused a post harvest loss ranging from 35 to 70 per cent during transport and storage. The chief constituent of the leaves is a volatile oil known as betel oil varying in chemical composition in betel vine varieties growing in different countries. The flavor of the leaf is due to the presence of essential oil (Banerjee, 2012).

Yield of Essential Oil from Betel Leaf

Guha (2006) found that the *Mitha*, *Sanchi* and *Bangala* varieties of betel vine had about 2.0, 1.70, and 0.80 per cent essential oil respectively. The average percentage yield of

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volatile oil of *P. betle* was 1.44 (Caburian and Osi, 2010). Sugumaran *et al.* (2011) obtained an essential yield of 0.31 per cent in volume by weight basis. Rani and Ramamurthy (2012) obtained 0.08 to 0.2 per cent of essential oil from betel leaves. The highest yield (15.6 per cent w/w based on betel powder) and high content of hydroxychavicol and eugenol (58 and 62.8 per cent w/w respectively) were obtained using ethyl acetate refluxed extraction (Singtongratana *et al.*, 2013). Pradhan *et al.* (2013) reported that the fresh new leaves had much more amount of essential oil.

Physical Features

Tyler *et al.* (1988) stated that pure volatile oils are colorless or with yellowish tinge when freshly prepared. Their taste varied like sweet, mild, pungent, hot acrid, caustic or burning. They had a characteristic aroma or odor. Most volatile oils are miscible in organic solvents but sufficiently soluble to form a saturated solution and impart its odour to the water. Caburian and Osi (2010) reported that the essential oil in betel vine was colorless to pale yellow when freshly extracted but acquired a darker yellow to orange color on exposure to light and heat. It had a strong aromatic odour (Sugumaran *et al.*, 2011), pungent taste and was greasy to touch. *P. betle* volatile oil had characteristics of most volatile oils. The *P. betle* volatile oil was miscible in all proportions in organic solvents like ethyl alcohol, chloroform, anhydrous ether and petroleum ether. It was immiscible in the water in the ratio of 0.1: 0.1 but was soluble in 50.0ml of water or no separation of phase was observed.

Chemical Composition

Chemical components of oil and their quantities varied in different varieties. The qualitative and quantitative variation in the essential oil might be due to different factors like variety, soil, season and agronomic practices followed during growth season and plant part used for oil extraction (Garg and Jain, 1996).

The oil of *Bangala* variety was constituted by a mixture of about twenty one different compounds, of which eugenol was the chief ingredient constituting about 29.5 per cent of the oil. Terpenyl acetate was the chief constituent of some of the varieties (Guha, 2003). The chemical composition of common betel oil of Sri Lanka appeared to be closer to that of cultivar *Deshwari* in India. The chemical studies on *P. betle* of India revealed that composition of volatile oils in the leaves could be used as markers for identification of different cultivars (Arambewela *et al.*, 2005).
The GC – MS analysis of essential oil from different parts of common betel vine indicated that composition of stalk was different from that of the other parts. The stalk did not contain detectable amount of allylpyrocatechol diacetate (Arembewela *et al.*, 2005). It was observed that the content of major compounds like safrole and chavibetole acetate in the leaf was highest at the harvesting stage.

Hydroxychavicol was the major component of essential oil. It would vary based on different extraction procedure. Yield of hydroxychavicol from fresh leaves, extracted in boiling water, was reported to be 0.096 per cent in w/w determined by GC – MS (Tawastin *et al.*, 2006) and 5 per cent in w/w when determined by HPLC (Pandey and Bani, 2010). The compounds with highest retention time were 5-(2-propenyl) -1, 3-benzodioxole, eugenol isomers and 3-careen (Caburian and Osi, 2010). In GC - MS analysis conducted by Sugumaran *et al.* (2011), the total ion chromatogram retention time was about 34.10 minutes and most of the components of oil were isolated during the first 30 minutes of the analysis. The 5-1, 3-benzdioxole (25.67%) was identified as the major constituent in the betel oil. The hydroxychavicol content of dried leaf by ethanolic extraction was reported to be 0.9 per cent w/w (Bandopadhay *et al.*, 2011) when determined by HPLC.

According to Banerjee (2012), eugenol was present in betel leaves and flower. Isoeugenol and methyl eugenol were present in flower. A total of 65 components were identified by GC – MS, representing 100 per cent of the oil. Some of the major compounds identified in betel oil of Sri Lankan cultivars were β-phellandrene, 4-terpinol, eugenol, chavibitol acetate, safrole and allylpyrocatechol diacetate (DMI, 2013).

Singtongratana *et al.* (2013) obtained chromatograms of the standards of hydroxychavicol with concentration of 1000 mg per liter and eugenol with concenteration of 50 mg per liter with retention time of 6.6 and 8.6 minutes respectively. The chromatogram of hydroxychavicol and eugenol of extracted oil by liquid - liquid extraction had shown a retention time of 6.59 and 8.45 minutes respectively. Hydroxychavicol and eugenol were the major compounds belonging to the propenyl phenol group. Heat sensitive compounds like 5-(2-propenyl)-1 and 3-benzodiaxole (18.27 per cent w/w) from fresh betel leaves determined by GC - MS. Major essential oil components identified through the study by Pradhan *et al.* (2013) were safrole, allyl pyrocatechol monoacetate, terpinen-4-ol, eugenyl acetate and chavicol. Eugenol was identified as the antifungal principle in the oil and chavicol was four times potent as antiseptic agent, compared to carbolic acid.

Different potential uses had been reported for the biochemical molecules identified from the betel leaf. Isoeugenol has use in the manufacture of vanillin (Merck, 1996). Eugenol, a major constituent in betel oil, is used in perfumeries, flavorings and medicine as a local antiseptic and anesthetic. Eugenol could 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). As a flavoring agent, isoeugenol is added to nonalcoholic drinks, baked foods and chewing gums (NTP, 2010). Methyl eugenol is used in aroma therapy, massage oils and alternative medium (Government of Canada, 2010). It is also widely used as a fragrance ingredient in perfumes, toiletries and detergents. Methyl eugenol has been used as an insect attractant in combination with insecticides (NTP, 2000; HSDB, 2010). Methyl isoeugenol is natural food flavor and used for treating mood disorders (Fajemiroye *et al.*, 2011).

Chlorophyll Content

Chlorophyll is beneficial in maintaining healthy teeth, clearing the mouth and throat and helping in digestion by encouraging

salivation and neutralizing excess acid (Loranty *et al*., 2010). Shivashankara *et al.* (2011) conducted a biochemical study on three types of betel vine. Among these, *Swee*t type and *Bengaluru local* type had different intensities of green color. The variation in color intensity was mainly due to the differences in the chlorophyll content. Leaves of *Swee*t type had higher total chlorophyll content, compared to *Bengaluru local* and *Madras* type. The higher chlorophyll content in *Simurali Sanchi* had given dark green color to the leaves which was preferred by customers and fetch higher price in comparison to other cultivars (Pariari and Imam, 2012a).

Total Chlorophyll

Different cultivars of betel vine showed significant variation (0.93 to 2.49 mg/g) in chlorophyll content (Balasubrahmanyam *et al*., 1990; Guha, 2006; Pariari and Imam, 2012a). Guha (2006) reported 0.01 to 0.25 per cent chlorophyll in betel leaves. An investigation by Pariari and Imam (2012a) to find out the most suitable cultivar with higher leaf yield and better quality in the gangetic alluvial plains of West Bengal, India revealed that the total chlorophyll content was highest (2.45 mg) in *Simurali Sanchi* among the cultivars.

Chlorophyll a

The investigation conducted by Pariari and Imam (2012b) concluded that chlorophyll a content in leaves varied significantly in various cultivars and maximum chlorophyll a content (1.61 mg per g) was found in *Sanchi*. Chlorophyll a content in leaves varied between 1.69 to 1.74 mg per g according to doses of applied nitrogen.

Chlorophyll b

In a study by Pariari and Imam (2012b) among different cultivars, significant variation was observed for chlorophyll b content and maximum chlorophyll b content (1.00 mg/g) was recorded in *Simurali Sanchi*. Source of organic manures had a significant effect on chlorophyll b content in leaves. Maximum amount (0.57mg/g) of chlorophyll b was recorded with the application of neem cake as nutrient source and minimum (0.48 mg/g) was in poultry manure. It was seen that nutrient supply through inorganic source or chemical fertilizer decreased chlorophyll b content in leaf.

Total Protein Content

Chandini (1989) found that nitrogen application at higher levels enhanced the protein content of marketable leaves and *Chilanthikarpooram red* contained 3.39 per cent protien. Guha and Jain (1997) reported that betel vine leaves contained significant amount of all the essential aminoacids except lysine, histidine and arginine, which were found only in traces. According to Guha (2000), six leaves of betel vine were comparable to about 300 ml of milk in relation to nutrient content. Akther (2004) found that an aqueous diffuse of bleached leaves had lucien (18.3 mg per 100 ml), phenyl alanine (14.2 mg per100 ml), arginine (2.4 mg per 100 ml), threonine (12 mg per 100 ml), aspartic acid (23 mg per 100 ml), glutamic acid (29.7 mg per 100 ml), valine (3.8 mg per 100 ml), tyrosine (1.2 mg per100 ml) and gama amino butynic acid (20.2 mg per 100 ml). The amount of total protein in betel leaf was reported to be 3 to 3.5 percentage (Akther, 2004; Banerjee, 2012; Pradan *et al*., 2013).

Total Phenol Content

Nair *et al.* (1986) stated that cultivar *Ambadi* had a higher disease index (anthracnose) and a lower phenolic content (7.76 mg/g) than the more resistant variety, *Kareyele* with a higher phenol content of 11.38 mg/g. Generally plants with significant therapeutic properties were found to be rich in phenols and had high antioxidant properties. Sazwi *et al*. (2013) found that total phenolic content of the betel leaf was 1.8 times less than betel quid without calcium hydroxide but 1.7 times higher than betel quid with calcium hydroxide. Balasubramanym and Rawat (1990) suggested that the characteristic clove like aroma of *Bangla* and *Sanchi* leaves was due to presence of phenolic compounds including eugenol (63.56 and 33.22 per cent respectively) and the sweet fennel like taste of *Meetha* leaves was due to anthole (19.13 per cent). The radical scavenging capacity of betel leaf was primarily due to its phenolic constituents. *Bengaluru local* recorded highest phenolics followed by *Madras* type. Sweet betel vine recorded lowest phenol and flavanoid content in leaf.

The same study also indicated that the total phenol content of betel vine was comparable with that of tea powder (Shivshankara *et al*., 2011). The major phenolic compounds found in betel leaf were terpenoids which included hydroxychavicol, eugenole, chavibetole, 1, 8 – cineole, cadinene, camphene, caryophyllene, limonene, pinene, chavicol, allyl pyrocatechol, carvacrol and safrole. Hydroxychavicol was said to possess antibacterial, antioxidant, anticarcinogenic activities whereas eugenol had been used as a local anesthetic for toothache (Pradhan *et al*., 2013). The presence of phenols and terpene like bodies were the cause of pungent smell of the betel vine leaves. Phenol content was directly proportional to the quality of leaf; leaves with high phenol quantity would also have good quality with respect to shelf life, nutrient content and resistance against pest and disease. The total phenol content varied with gender. Female plants had three times higher phenol than male plants. The middle part of the main vein had largest quantity of tannin.

Antioxidant Capacity

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols.

Inhibition of low density lipoprotein oxidation to the extent of 94 per cent was reported for betel vine and this was higher than cashew, Japanese mint, chilli fruit, papaya shoot and rosella calyx (Salleh *et al.*, 2002). A study conducted by Santhakumari

et al. (2003) revealed that betel leaves possessed very high antioxidant capacity and it was more than that of tea in female types. The extract also showed strong hydroxyl radical and superoxide anion radical scavenging property (Dasgupta, 2004; Arambewela *et al.*, 2006; Rathee *et al.*, 2006; Pin *et al.*, 2010). It was also reported that the antioxidants could reduce mortality rate of cardiovascular disease (Devasagayam *et al.*, 2004; Agoramoorthy *et al.*, 2008) and protect against cancer and other chronic diseases (Anani *et al.*, 2005). Polyphenol compounds like catechol, allylpyrocatechol *etc*. in betel leaf extract inhibited the radiation induced lipid peroxidation process effectively. This could be attributed to its ability to scavenge free radicals (Anon, 2004). Betel leaf had great potency to act as natural antioxidant (Guha, 2006). Ascorbic acid content in fresh betel leaves varied from 0.005 to 0.01 per cent.

The antioxidant capacity, cytoprotective activity and total phenol content were positively correlated (Kondo *et al.*, 2007). The consumption of antioxidant rich foods would help to neutralize the free radicals in the body, thus preventing or delaying the oxidative damage of lipids, proteins and nucleic acids (Lim *et al.*, 2007). The extracts reduced most of the Fe^{3+} ions and possessed strong reducing ability (Maniguha *et al.*, 2009).

Differences in the antioxidant capacity and radical scavenging ability were found to be related to the pungency level. Pungent female type found to have more antioxidant capacity (Sivashankara *et al.*, 2011). It was also reported that total phenols, flavanoids, total antioxidant capacity and radical scavenging ability were highest in *Bengaluru* local type of betel vine and lowest in *sweet* type.

The study by Pariari and Imam (2012a) showed a significant difference among cultivars for ascorbic acid content with maximum (3.20 mg/100 g) in *Simurali Bhabna*. Pariari and Imam (2012b) reported that ascorbic acid content in betel leaves grown with different sources and combinations of nitrogen varied significantly with maximum ascorbic acid content of 2.75 mg/100 g of fresh leaves and minimum of 2.02 mg/100 g of fresh leaves. Banerjee (2012) stated that antioxidant activity included free radical scavenging capacity, inhibition of lipid peroxidation, metal ion chelating ability and reducing capacity. The high antioxidant activity was contributed by several biochemical molecules like phenols and terpenoids that included b-carotene, quinic acid, allylpyrocatachol, p –hydroxybenzoic acid, hydroxychavicol, ascorbic acid and eugenol. The antioxidant property was correlated with different biological activities like hepatoprotective, antidiabetic, antiarthritis, antistroke and anticancer properties (Pradhan *et al.*, 2013).

Sazwi *et al.* (2013) conducted a study on antioxidant and cytoprotective activities of *P. betle*, *Areca catechu*, *Uncaria gambir* and betel quid with and without calcium hydroxide. This study showed highest antioxidants (DPPH - IC_{50} = 6.4 ± 0.8 microgram/ml, FRAP - 5717.8 ± 537.6 micromol Fe (II)/ mg) for gambir. Betel quid without calcium hydroxide compared with betel quid with calicium hydroxide had higher antioxidants, total phenolic content and cytoprotective activities. Quinic acid was the major compound of gambir and betel quid.

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