



Antioxidant capacity, radical scavenging ability, total phenols and flavonoids in three types of betelvine (*Piper betle* L.)

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Abstract

Betelvine (*Piper betle* L.) leaves from female (Bengaluru local), male (Madras type) and meeta paan (sweet type) were analysed for antioxidant capacity (FRAP) and radical scavenging ability (DPPH) using acidic methanol extracts. FRAP and DPPH values were higher in the leaves of Bengaluru local followed by Madras type and were lowest in sweet type of betelvine. However, chlorophyll was higher in female and sweet betelvine when compared to Madras type. Bengaluru local also recorded higher total phenols and flavonoids followed by Madras type and sweet betelvine recorded lowest phenols and flavonoids. Higher antioxidant and radical scavenging capacities of female and male types was mainly due to the higher phenols and flavonoids and not due to total chlorophyll content. The study indicated that the total phenol content of betelvine is comparable with that of tea powder.

Keywords: acidic methanol extracts, betelvine types, chlorophyll

Betelvine (*Piper betle* L.), commonly known as paan, is a perennial vine grown for its leaves used for chewing with arecanut and other supplements. Betel leaves are consumed after heavy meals as a digestive stimulant and mouth refreshner. Betelvine leaves from female (Bengaluru local), male (Madras type) vines and sweet type that are generally consumed vary in their pungency levels with female types being more pungent. A phenolic compound, hydroxy-chavicol having anti-carcinogenic property has been identified in leaves (Bhide *et al.* 1991).

The leaves also have very high antioxidant activity (Santhakumari *et al.* 2003) and were

reported to be more than that of tea in female types (Dasgupta & De 2004). Inhibition of low density lipoprotein (LDL) oxidation to the extent of 94% was reported for betelvine and was higher than cashew, japanese mint, chilli fruit, papaya shoot and rosella calyx (Salleh *et al.* 2002). Even though the leaves of male type of betelvine are reported to be having more antioxidants than tea, the consumption of Madras and sweet types are more because of less pungency when compared to the female (Bengaluru local) types. Therefore, in this study leaves from female (Bengaluru local), male (Madras type) and sweet type of betelvine were analysed for their antioxidant and radical

scavenging capacities and their relationship with the total phenol and chlorophyll content was also assessed.

The study was conducted at Indian Institute of Horticultural Research, Bengaluru. Leaves of Bengaluru local, Madras type and sweet type of betelvine were purchased from the local market. Ten good leaves were randomly selected and the midribs were excised. The excised leaves were washed with distilled water and cut into small pieces and a known quantity (3 g) of leaf material was incubated in acidic methanol (1:99 v/v) for 48 h and ground thoroughly in a glass pestle and mortar. The extract was filtered through two layers of muslin cloth and this was repeated twice using 100 ml of the acidic methanol. Five ml of the extract was centrifuged at 10000 rpm for 5 min and used for further analysis.

Ferric reducing antioxidant potential (FRAP) assay

The FRAP assay of the extract was done as per Shivashankara *et al.* (2004). The antioxidant capacity of the extract was estimated by its ability to reduce ferric iron to ferrous in a solution of 2,4,6-tripyridyl-2-triazine (TPTZ) prepared in sodium acetate buffer at pH 3.6. The reduction of iron in the TPTZ ferric chloride solution (FRAP reagent) by the sample resulted in the formation of blue product, the absorbance of which was read at 593 nm after 40 min of incubation. The activity was expressed as ascorbic acid equivalent antioxidant capacity (AEAC).

DPPH radical scavenging ability

DPPH radical scavenging ability of the extract was estimated as per Kang & Saltveit (2002). A 0.2 ml aliquot of methanol extract was mixed with 0.25 ml of ethanolic 0.5 mM DPPH solution and 0.5 ml of 100 mM acetate buffer (pH 5.5). The mixture was thoroughly mixed and incubated for 40 min under laboratory conditions and the absorbance was read at 517 nm. Radical scavenging ability was expressed as the concentration of the sample required for the 50% reduction of DPPH radical colour intensity.

Total phenol

Total phenol content of the methanol extract was estimated according to Folin-Ciocalteu method (Singleton & Rossi 1965) and expressed as gallic acid equivalents.

Total flavonoids

Total flavonoid in the methanol extract was determined as per Chun *et al.* (2003). Methanol extract (1 ml) 5% NaNO₂ followed by 0.3 ml of 10% AlCl₃. After 1 min, 2 ml of 1 M NaOH was added and diluted to 10 ml with double distilled water and mixed thoroughly. The absorbance of the pink mixture was read at 510 nm and expressed as catechin equivalents.

Estimation of total chlorophyll

Leaf material (1 g) was incubated for 48 h in 25 ml of Dimethylsulphoxide (DMSO) reagent. The supernatant was collected and the volume was made up to a known quantity and the absorbance was read at 645 and 663 nms. Total chlorophyll, chlorophyll a and b were calculated as per the following equation.

$$\text{Chlorophyll-a (mg/g)} = \{12.7 \times (\text{OD663}) - 2.69 \times (\text{OD645})\} \times V / (1000 \times W)$$

$$\text{Chlorophyll-b (mg/g)} = \{22.9 \times (\text{OD645}) - 4.68 \times (\text{OD663})\} \times V / (1000 \times W)$$

Where, V = Volume of the extract (mL),

W = Weight of the sample (g)

Antioxidant capacity and radical scavenging abilities were estimated in methanol as well as DMSO extracts.

Among the three betelvine types, sweet type is known for its sweet taste and clove-like flavour. On the other hand Bengaluru local type is more pungent. Madras type is known for its mild pungency and good flavour. However, they have different intensities of green colour. The variation in colour intensity is mainly due to the differences in the chlorophyll content. Leaves from sweet type had higher total chlorophyll content when compared to Bengaluru local and Madras type (Fig. 1). Contribution of chlorophyll content for the variation in total antioxidant capacity was

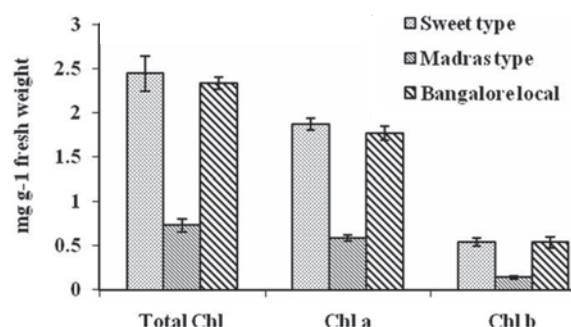


Fig 1. Chlorophyll concentration in betel vine leaf

assessed by extracting the chlorophyll using DMSO reagent and assessing the antioxidant capacity by DPPH method.

DPPH radical scavenging ability of the DMSO extract was very low in all the three types of leaves. However the activity was higher in male type followed by female and sweet type of betel vine (Fig. 2). Radical scavenging ability of the DMSO extract clearly indicated that the contribution of chlorophyll towards antioxidant capacity of these three major types of betel vine is very less.

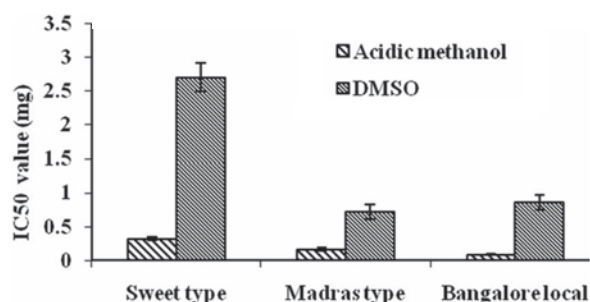


Fig 2. Quantity of betelvine leaves needed (IC50) (mg) to scavenge 50% of DPPH radical (0.2mM) (Values of DMSO should be multiplied by 10 to get the actual IC50 values (Lower the IC 50, higher is the efficacy).

Total antioxidant capacity of plant products is usually determined by total phenols and flavonoids. Total phenols, flavonoids, total antioxidant capacity (FRAP) and radical scavenging ability were higher in the Bangalore local type of betel vine and lowest in sweet type. Differences in antioxidant and radical scavenging capacities were found to be related to the total phenol content which is probably related to the pungency level.

Higher antioxidant capacity of Bangalore local type of betelvine was mainly due to higher phenol and flavonoid contents. Total chlorophyll content did not show any relationship with the total antioxidant capacity of leaves. Madras type showed higher antioxidant capacity than the sweet type even though the latter one had higher chlorophyll content. Green tea was reported to have 10% to 12% phenols (Gulati *et al.* 2003, Bramati *et al.* 2003) on dry weight basis. Total catechins were reported to be 6% to 12% of dry weight in Chinese rock and Olong teas (Lin *et al.* 2003). We have found that the total phenol content in betelvine leaf is 2%–4% on fresh weight basis. Pungent female type of betelvine (Bangalore local) was found to have more antioxidant capacity. The results indicated that total phenol content of betelvine is comparable to tea.

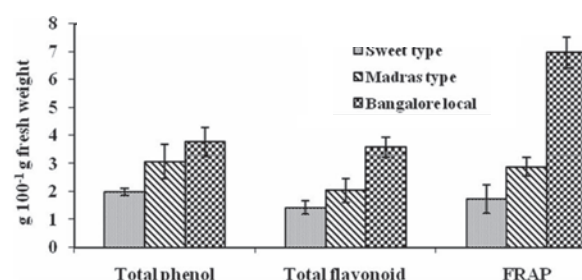


Fig 3. Total phenol, total flavonoids and ferric reducing antioxidant potential (FRAP) of betelvine leaf.

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