BIOCHEMICAL AND PHYTOCHEMICAL EVALUATION OF BETEL LEAF VARIETIES

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Abstract

Piper betel belongs to Piperaceae family. It is a vine with heart shaped leaves. In India, the leaves are eaten as 'paan', after meals. It is given to mothers during and after pregnancy. Betel leaf is used for treatments like bad breath, constipation, ringworm, rheumatism. The betel leaves are appetisers, digestives and their chewing has shown to reduce the pathogenic microorganisms in the sub gingival microflora. Yet a complete and comprehensive report of its biochemistry and nutrition is not available. Also the type and specific activity of digestive enzymes in betel leaves have not been found out. To bridge these gaps, the current paper aims to evaluate biochemical and phytochemical aspects of three important and widely used varieties of betel leaves in India, namely Nagarvelli, Banarasi and Kolkata leaves.

Kolkata leaves have highest chlorophyll and carotene content. The leaves show seasonal variation in chlorophyll and carotene content. The leaf extracts contain 14% ash. They are a rich source of calcium, iron and manganese and good source of copper and zinc. The total protein content is around 23.58 to 26.544 mg/ml. The leaves show proteolytic, lipolytic and glycolytic activity. The concentrations of tannins and sugars are inversely proportional. Benarasi leaves have highest concentration of tannins and lowest of total sugars. They show the presence of essential amino acids Tyrosine, Tryptophan and Arginine. These results thus justify and emphasize the nutritional significance of betel leaf to be eaten as quid after meals and also during and after pregnancy.

Keywords: *Betel Leaf, Biochemical, Phytochemical, Nutritive value, Digestive enzymes.*

Introduction

Piper betel belongs to Piperaceae family. It is a vine and has heart shaped leaves. Traditionally the leaves are eaten as a quid in South East Asian countries. In India, besides this, the leaves are eaten as 'paan', after a sumptuous celebratory meal on special occasions. Also it is given to mothers during and after pregnancy. Betel leaf has been traditionally used for treatments like bad breath, constipation, ringworm, rheumatism and many more (1, 2). The betel leaves are easily available appetisers, digestives and their chewing has been shown to reduce the pathogenic microorganisms in the sub gingival microflora (3). Eugenol which is a major essential oil present in betel leaves has been quantified. It has a lot of applications in food and cosmetic industry.(4)

References have been found where the nutritive values of the betel leaf are mentioned. P.Guha (2006) has comprehensively reviewed about this neglected green gold as he describes the betel leaves. He has listed out the medicinal and nutritive value of betel leaf. In his paper he mentions that a complete biochemical analysis is not available from any single source. Consequently, the analytical values from two or more reports reveal a very wide or even contradictory trend. Hence, research work in this direction becomes inevitable.(5).Pharmacognostical and phyto-physiochemical profile of only the Pachaikodi variety of betel leaf has been studied by Periyanayagam et a in 2012.(6) In the book by Gopalan, betel leaf nutrition is given but not about its digestive enzymes.(7)

Thus the major gaps in the research of betel leaves is that complete and comprehensive report of its biochemistry and nutrition is not available. Also the type and corresponding specific activity of digestive enzymes in betel leaves have not been found out. Thus to bridge these gaps, the current paper aims to evaluate biochemical and phytochemical aspects of three important and widely used varieties of betel leaves in India, namely Nagarvelli, Banarasi and Kolkata leaves.

Materials and Methods

Samples

Three betel leaf varieties, namely, Nagarvelli, Banarasi and Kolkata were obtained from the local market.

Chlorophyll content was found using 1 gm fresh leaves cut with scissors and homogenised in mortar and pestle in 5ml water for 10 min. Final volume was made 10 ml. An aliquot of 0.5 ml was mixed with 4.5 ml 80% acetone. The supernatant was collected after centrifugation at 3400 rpm for 10 min, and read at 480 nm, 645 nm, 663 nm. Total chlorophyll content, chlorophyll a, b and carotene content was calculated with formulae as given in reference.(8)

Proximate analysis of powdered leaves was done. Ashing was done by keeping 1gm leaf powder in muffle furnace at 550°C for almost 3 days till constant weight was

obtained. To find water soluble ash, 1 gm ash was dissolved in 10 ml water and filtered through ashless filter paper and heated and then weighed to give water insoluble ash. The difference was calculated as water soluble ash. Similar procedure was followed using 10 ml 1 N HCl to find acid soluble ash content. Moisture content was found by keeping 1 gm powder at 105°C for 3 hrs in oven. The difference in weight is loss due to moisture. (9)

Phytochemical screening was done using water extracts of powders prepared by keeping 10 gm of powder overnight in 120ml water. (10)

The ash obtained was dissolved in 5 ml 20% HCl by warming and adding 2 drops of conc HCl, filtered through Whatman filter paper 41, and volume made to 50 ml with deionized water. Further it was tested for Zn, Ca, Fe, Cu, Cr, Co and Mn using Atomic Absorption Spectroscopy.(AAS, Shimadzu AA 7000)

To estimate the total protein content, 1 gm fresh leaf was crushed in 4 ml of 100mM Tris HCl pH 7.8 in cold conditions. It was cold centrifuged at 5000rpm for 20 min. In the supernatant 1 ml 10% Trichloroacetic acid was added and was kept at -10°C for 1hr. Thereafter it was centrifuged, pellet was washed with 80% acetone, re-centrifuged and pellet was dissolved in 5 ml 100mM Tris HCl pH 7.8. This extract was used to determine total protein content by Biuret method (11) and also to determine enzymatic activities. Proteolytic activity was checked in presence of Bovine Serum Albumin as substrate. Glycolytic activity was checked in presence of starch as substrate by DNSA method. Lipolytic activity was studied by groundnut oil as substrate and estimating free fatty acids released by titration against NaOH using phenolphthalein as indicator. 1% oleic acid was used as standard.

Total tannins were estimated by Folin Denis method (8). Total sugars were estimated by Anthrone method (8, 12)

Vitamin C was estimated by titration using DCPIP method.(13)

Free amino acids were qualitatively determined by color tests like Xanthoproteic test, Hopkin Cole test, Sakaguchi test, Sulphur test and Millon`s test.(13)

GC MS of leaves was carried out using hexane extract and methanol extract. 1 gm leaf powder was kept overnight in 5ml methanol and 5ml hexane separately, then filtered and used for GCMS. (Shimadzu QP 2010 Ultra)

Results and Discussion

Kolkata leaves have highest chlorophyll and carotene content amongst the three varieties. All the leaves show seasonal variation in chlorophyll and carotene content. (Table 1)

Table 1: Chlorophyll Content

Leaf sample	Season	Total Chlorophyll (%)	Chlorophyll a (%)	Chlorophyll b (%)	Carotene (%)
Nagarvelli	Summer	0.2457	0.13938	0.10388	16.6814
Kolkata		1.2565	0.74825	0.4953	37.679
Benarasi		0.0930	0.0537	0.03841	10.8302
Nagarvelli	Rainy	0.186428	0.118361	0.066038	13.5606
Kolkata		0.94239	0.55602	0.37674	28.531
Benarasi		0.244858	0.14503	0.097348	13.5634
Nagarvelli	Winter	0.355538	0.226637	0.125018	13.2428
Kolkata		0.492282	0.266258	0.221312	40.5102
Benarasi		0.29405	0.160478	0.13076	23.2326
Nagarvelli	Average	0.26255	0.16145	0.098312	14.49493
Kolkata		0.89705	0.52351	0.364451	35.5734
Benarasi		0.21064	0.11974	0.088840	15.8754

The pH of leaf extracts of all the varieties is found to be acidic in the range between 5.3 to 6.5. Ash content is between 12.5 to 14%, of which acid soluble ash is 70.90% to 77.37%. Moisture content is 10.1% to 15%.(Table 2)

Table 2: Proximate Analysis

Test	Nagarvelli leaf powder	Kolkata leaf powder	Benarasi leaf powder
Colour	**Olive green**	**Green**	**Brown**
pH of 1% water extract	5.3	6.4	6.5
Ash content (%)	13.7	14	12.5
Water soluble ash (%)	47.48	53.28	36.92
Acid soluble ash (%)	77.37	76.06	70.90
Moisture content (%)	10.1	11.3	15.0

All the leaf varieties contain tannins, essential oil, alkaloids, sugars, protein, free amino acids, glycosides, lipids and saponins. The leaf section showed presence of glandular hair. (Table 3)

Table 3: Phytochemical Analysis

Test	Nagarvelli leaf extract	Kolkata leaf extract	Benarasi leaf extract
Colour of extract	**Dark brown**	**Dark brown**	**Light brown to Yellow**
Tannins- FeCl3 test	+	+	++
Essential Oil	+++	+	++
Alkaloids- Lead acetate test	+	+	+++
Carbohydrates – Molisch test	+	+	+
Biuret test for proteins	+	+	+
Benedict`s test	+	+	+
Glycosides test	+	+	+
Ninhydrin Test	+	++	++
Sudan Red test for lipids	+++	+	++
Saponins	+	+	+
Section of leaf- Glandular hair	+	+	+

The leaves are rich in Calcium which is in the range of 9.750 mg/gm to 14.650 mg/gm. Iron content is fairly high from 74.49µg/gm to104.615µg/gm. They are also rich in Manganese content which is in the range of 46.07µg/gm to 56.49 µg/gm. There is presence of Copper and Zinc as well. (Table 4)

Table 4: Metal ion Content

Metal Ion µg/ml/gm	Nagarvelli leaf powder	Kolkata leaf powder	Benarasi leaf powder
Zn	16.55	23.50	19.50
Ca	12350	9750	14650
Fe	74.49	96.345	104.615
Cu	10.05	10.47	8.54
Cr	--	--	--
Co	--	--	--
Mn	53.445	46.070	56.490

The total protein content in the leaves is in the range of 23.58 to 26.544 mg/ml. The leaves show proteolytic, lipolytic as well as glycolytic activity. (Table 5)

Table 5: Total Protein Content and Enzymatic Activity

Per gm of leaf	Nagarvelli	Kolkata	Benarasi
Total protein mg/ml	24.321	26.544	23.580
Proteolytic activity- mg of protein digested/min/ml of Enzyme extract	0.1216	0.0608	0.1502
Specific activity- mg of protein digested/ min/mg protein	0.005	0.0023	0.0064
Lipolytic activity- mg of free fatty acids released/ min/ml of Enzyme extract	0.81	0.98	0.92
Specific activity- mg of free fatty acids released/ min/mg protein	0.033	0.037	0.039
Glycolytic activity- mg maltose produced /min/ml of Enzyme extract	0. 06	0.08	0.03
Specific activity- mg maltose produced /min/ mg protein	0.0025	0.0030	0.0012

The leaves have high content of tannins, with Benarasi leaf showing unusually highest level of 97.143 mg/gm of leaf. Total sugar content is also high in the range of 67.317 to 144.135 mg/gm of leaf. In this case Benarasi leaf shows lowest and Nagarvelli leaf the highest level of total sugars. (Table 6)

Table 6: Total tannins and sugars

	Nagarvelli	Kolkata	Benarasi
Total tannins mg/gm leaf	15.714	7.143	97.143
Total sugars mg/gm leaf	144.135	97.312	67.317

Vitamin C was found to be 19.01µg/ml/gm in Nagarvelli leaves, 18.46 µg/ml/gm Benarasi leaves and 12µg/ml/gmin Kolkata leaves.

Essential amino acid Tyrosine is present in all the leaves. Kolkata and Benarasi leaves show presence of essential amino acid Tryptophan as well. Sulphur containing amino acids are absent in all of the leaves. Arginine is present in Nagarvelli and Benarasi leaves. (Table 7)

Table 7: Free Amino acids

	Nagarvelli leaf powder	Kolkata leaf powder	Benarasi leaf powder
Xanthoproteic test for aromatic amino acids	++	+	+++
Hopkin Cole`s test for Tryptophan	-	+	+
Sakaguchi test for Arginine	++	-	+
Sulphur test for Cysteine and Methionine	-	-	-
Millon`s test for Tyrosine	++	+	+

GC-MS of both the extracts, methanol and hexane, showed some common components in the leaves like Caryophyllene, Eugenol, Phytol, Naphthol and Butanol.

Conclusion

Kolkata leaves have highest chlorophyll and carotene content amongst the three varieties. All the leaves show seasonal variation in chlorophyll and carotene content. The leaf extracts are acidic and they contain around 14% ash ie inorganic ions of which 70% is acid soluble and 50% is water soluble. The leaves are a rich source of calcium, iron and manganese and good source of copper and zinc. Zinc is essential for conversion of beta carotene to vitamin A.(14)

The total protein content in the leaves is in the range of 23.58 to 26.544 mg/ml. The leaves show proteolytic, lipolytic as well as glycolytic activity.The concentrations of tannins and sugars are found to be reciprocal. Benarasi leaves have highest concentration of tannins and lowest of total sugars. Tannins are condensed polyphenolic compounds. They bind with Iron irreversibly and interfere with its absorption. But at the same time, Vitamin C which was found to be in the range of 12 to 19µg/ml/gm, is a strong reducing agent. It helps in absorption of dietary Iron by keeping it in the reduced ferrous form. Copper also helps in Iron absorption. (6). Vitamin C is essential for Tryptophan hydroxylation step in the synthesis of serotonin. (14)

Essential amino acid Tyrosine is present in all the leaves. Kolkata and Benarasi leaves show presence of essential amino acid Tryptophan as well. Sulphur containing amino acids are absent in all of the leaves. Arginine is present in Nagarvelli and Benarasi leaves.

These results thus justify the eating of 'paan' after meals, or during and after pregnancy. There is considerable requirement of Calcium and Iron during pregnancy and lactation. The habit of chewing betel leaf can increase Calcium and Iron uptake.

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References

1. Chopra.RN., Nayar. SL. and ChopraI.C. 1956. Glossary of Indian Medicinal Plants. CSIR, New Delhi: pg 194.
2. Quality Standards of Indian Medicinal Plants. 2008. Vol 7. Medicinal Plants Unit, ICMR, New Delhi: pg 198.
3. Vani K, Jose M, Rao S. 2011. Qualitative Evaluation of Subgingival Microflora after the chewing of Betel Leaf; Int J of Research in Ayurveda and Pharmacy. 2 (4): 1278-81.
4. Chitnis KS. 2017. Quantitation of Eugenol in Betel Leaf Varieties by HPTLC. International Journal of Pharmaceutical Sciences and Research. 8(11): 4858-4862.
5. Guha.P. 2006. Betel Leaf: The Neglected Green Gold of India; J Hum. Ecol. 19(2): 87-93.
6. Periyanayagam K., Jagadeesan M., Kavimani S., Vetriselvan T. 2012. Pharmacognostical and Phyto-physicochemical profile of the leaves of Piper belle L.var Pachaikodi (Piparaceae) - Valuable assessment of its quality; Asian Pacific Journal of Tropical Biomedicine. 2 (2): S506-10.
7. Gopalan C., Rama Shastri BV., Balasubramanian SC. 2011. Nutritive Value of Indian Foods. National Institute of Nutrition (ICMR), India.
8. Sadasivam S., Manickam A. 2015. Biochemical methods, 3rd Ed. New Age International Publishers.

9. Harborne JB. 1998. Phytochemical Methods- A Guide to Modern Techniques of Plant Analysis. Chapman and Hall London.
10. Dr Khandelwal KR., Dr Sethi V. 2013. Practical Pharmacognosy- Techniques and Experiments, 23rd Ed. Nirali Prakashan.
11. Plummer DT. 2013. An introduction to Practical Biochemistry, 3rd Ed.Mc Graw Hill Education (India) Private Limited.
12. Jayaraman J. 2011. Laboratory Manual in Biochemistry, 2nd Ed.New Age International Publishers.
13. Sawhney SK., Singh R. 2014. Introductory Practical Biochemistry, 10th Ed. Narosa Publishing House.
14. Srilakshmi B. 2016. Nutrition Science, 5th Ed. New Age International Publishers.