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PHYTOCHEMICAL AND PROXIMATE ANALYSIS OF BETEL LEAF EXTRACT USED IN PRESERVATION OF MILK

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ABSTRACT

A study was carried out to assess the phytochemical constituents and proximate content of betel leaf extract used in preservation of milk. Fresh betel leaves (*Piper betel* Linn) procured from the local market in Chennai was identified and authenticated by Botanical survey of India. Phytochemical analysis was carried out that showed the presence of flavonoids, tannins and phenolic compounds. These compounds possess antimicrobial and antioxidant activity and can be used for increasing the shelf life of milk. Proximate analysis also showed the presence of Iron and Vitamin C and addition of extract in milk will improve the Iron and Vitamin C contents.

Keywords: *Betel Leaves, Tannins, Phenolic Compounds, Vitamin C*

INTRODUCTION

Piper betel Linn (Piperaceae) leaves is widely used as a post meal mouth freshener and the crop is extensively grown in India, Sri Lanka, and other Southeast Asian countries. Betel is an evergreen dioecious herb that needs warm and moist growth conditions for its growth (Arani *et al.*, 2011). Indian system of medicine and health has adopted the use of betel leaves in various ways. *Piper betel* contains a wide variety of biologically active compounds whose concentration depends on the variety of the plant, season and climate.

Currently, there is a growing interest to use natural antibacterial compounds like extracts of herbs and spices for the preservation of food (Smid and Gorris, 1999). The mode of action of natural preservatives is inhibition of microbial growth, oxidation and certain enzymatic reactions occurring in milk. In the present study phytochemical and proximate analysis of betel leaf extract was carried out to assess the suitability in preservation of milk.

MATERIALS AND METHODS

Fresh betel leaves (*Piper betel* Linn) procured from the local market in Chennai. It was identified and authenticated by Botanical survey of India, Southern Regional Centre, Coimbatore. The leaves were shade dried and powdered as per the method (Preethi *et al.*, 2010). Three grams of powder was dissolved in 20 ml of distilled water, boiled and cooled and then filtered through whatman No.1 filter paper. The extract was subjected to phytochemical analysis for the presence of carbohydrates, glycosides, fixed oils, protein, saponins, tannins, phenolic compounds, phytosterols, alkaloids and flavonoids as per the method described (Trease and Evans, 2002).

Carbohydrates

Two ml of extract was dissolved in 4 ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of carbohydrates and glycosides.

Molish Test

One ml of the filtrate was treated with 2 to 3 drops of Molish reagent and 2 ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids showed the presence of carbohydrates.

Glycosides

Two ml of extract was hydrolyzed with dilute hydrochloric acid for 10 minutes in a water bath at 37 °C and hydrolysate was subjected to the following test to detect the presence of glycosides.

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Borntrager's Test

Two ml of the hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal volume of dilute ammonia solution was added. Appearance of pink color on the ammonia layer indicates presence of glycosides.

Detection of Fixed Oils

0.5 ml of the extract was pressed between the filter paper. Appearance of oil stain on the paper indicates the presence of fixed oils.

Detection of Protein

Two ml of the extract was treated with Ninhydrin reagent and appearance of purple color indicates the presence of proteins.

Detection of Saponins

Four ml of the extract was diluted with 20 ml of distilled water and was agitated in a measuring cylinder for 15 minutes. The formation of 1 cm layer of foam shows the presence of saponins.

Detection of Tannins and Phenolic Compounds

Two ml of extract was taken and equal volume of water was added and tests for the presence of phenolic compounds and tannins were carried out with the following reagent.

5 per cent ferric chloride solution was added to 2 ml of the above solution. A violet color indicates the presence of phenolic compounds and tannins.

Detection of Phytosterols

Two ml of extract was dissolved in 5 ml of chloroform. Then, this chloroform solution was subjected to the following test to detect the presence of phytosterols.

Salkowski Test

To 1ml of above chloroform solution, few drops of concentrated sulphuric acid was added. Appearance of brown color indicates the presence of phytosterols.

Detection of Alkaloids

Two ml of extract was treated with few drops of dilute hydrochloric acid and filtered then the following tests were carried out.

Mayer's Test

To 1ml of the filtrate, few drops of Mayer's reagent was added. Cream colored precipitate indicates the presence of alkaloids.

Detection of Flavonoids

Two ml of extract was dissolved in sodium hydroxide solution. Appearance of yellow color indicates the presence of flavanoids.

Proximate Analysis of the Betel Leaf Extract

Fat

Total lipid was analyzed gravimetrically after extraction with chloroform: methanol (2:1v/v) using Folch method as modified (Bligh and Dyer, 1959).

Moisture

Moisture content was estimated as per the method of the Association of Official Analytical Chemists (AOAC, 1990).

Carbohydrate

Total carbohydrate was estimated by calculation (Muller and Tobin, 1980) by using the following formula.

Total carbohydrates = 100 - (Protein + Fat + Moisture + Ash) in per cent

Protein

The protein content as estimated by Lowry's method (Sadashivam and Manickam, 1992).

Energy Value

The energy values (kcal/100 g) were estimated by multiplying the value of carbohydrate, protein and crude fat by the factors of 4, 4 and 9 respectively and the sum expressed in kilocalories (Onyeike and Ikru, 1998).

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Ash

Ash was estimated as per the method of the Association of Official Analytical Chemists (AOAC, 1990).

Analysis of Mineral and Vitamin

The ash was used for analysis of mineral and vitamins.

Iron was estimated by Spectrophotometric method (Hitachi 2010, Double beam) using 1, 10-phenanthroline (Vogel, 1988). Vitamin C (Ascorbic acid) was estimated by titrimetric method using 2, 6-dichlorophenol dye (AOAC, 1990).

RESULTS AND DISCUSSION

Phytochemical analysis of the betel leaf showed presence of carbohydrate, protein, flavonoid, tannins and phenolic compounds (Table 1). *Piper betel* extract showed the presence of carbohydrates, alkaloids, gums, oils steroids, glycosides, tannins, phenols, flavonoids, vitamins, organic acids and inorganic constituents (Chaurasia *et al.*, 2010). Aqueous extract of *Piper betel* leaf was found to possess high concentrations of flavonoids, tannins and phenols (Chakraborty and Shah, 2011). Phenols and polyphenols are water soluble compounds which can be easily mixed with milk. Piper betel leaf extract contained a large number of bioactive molecules like polyphenol, alkaloids, steroids, saponin and tannin and these phenolic compounds present in the betel leaf extracts possess broad spectrum of antimicrobial activity (Chandra *et al.*, 2012). Phenolics are the major contributor of antioxidant activity in plant extracts due to their higher value in total content (Hodzic *et al.*, 2009). The use of plant extracts as a source of phenols is preferred as a natural method of preservation (Gad and Salam, 2010).

Table 1: Phytochemical Analysis of Betel Leaf Extract

Sl. No.	Constituents	Betel Leaf
1.	Alkaloids	Negative
2	Carbohydrates	Positive
3	Fixed Oils	Negative
4	Flavonoids	Positive
5	Glycosides	Negative
6	Phytosterols	Negative
7	Protein	Positive
8	Saponins	Negative
9	Tannins and Phenolic compounds	Positive

Proximate analysis of the extract (Table 2) showed Iron and Vitamin C of 1.30±0.006 mg/100gm and 68.8±0.460 mg /100gm respectively. Majumdar *et al.*, (2002) reported that betel leaves contain volatile oils such as betel phenol and chavicol, tannin, sugar, vitamin-C, starch and diastase. Nutritive value of *Piper betel* leaves to be 9.89 per cent moisture, 14.75 per cent ash, 2.20 per cent fat, 14.89 per cent crude fiber, 38.50 per cent carbohydrates, 19.80 per cent protein, 7.64mg / 100 g vitamin C and 253 calories of energy (Babu *et al.*, 2011).

Table 2: Proximate Analysis of Aqueous Extract of Betel Leaf (Mean±SE)

Constituents	Level
Moisture (per cent)	98.5±0.456
Fat (per cent)	0.02±0.003
Protein (per cent)	0.60±0.006
Carbohydrate (per cent)	0.62±0.007
Ash (per cent)	0.26±0.006
Energy value (kcal/100gm)	5.06±0.009
Iron (mg/100gm)	1.30±0.006
Vitamin C (mg/100gm)	68.8±0.460

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Conclusion

In this present study it was concluded that betel leaf extract contains flavonoids, tannins and phenolic compounds which possess antimicrobial and antioxidant activity and can be used in the milk preservation. Since, milk is deficient in Iron and Vitamin C content addition of extract in milk will increase the Iron and Vitamin C level in milk.

REFERENCES

- AOAC (1990).** *Official Methods of Analysis*, 15th edition, (Association of Official Analytical Chemists, Washington, D.C., USA) 807-928.
- Arani D, Shreya G and Singh M (2011).** Antimicrobial Property of Piper betel Leaf against Clinical Isolates of Bacteria. *Journal of Pharmacological Sciences and Research* **2**(3) 104-109.
- Babu PR, Muniappan A, Rasool SK, Sheriff MA and Sekar T (2011).** In vitro antibacterial activity of *Piper betel* L. and Black betel CV. Kammar leaves against *Staphylococcus aureus* and *Streptococcus pneumoniae*. *Journal of Pharmacological Research* **4**(7) 2223-2225.
- Bligh EG and Dyer WJ (1959).** A rapid method of total lipid extraction and purification. *Canadian Journal Biochemical Physiology* **37**(8) 911-917.
- Chakraborty D and Shah B (2011).** Antimicrobial, anti-oxidative and anti-hemolytic activity of Piper betel leaf extracts. *International Journal of Pharmacy and Pharmaceutical Sciences* **3**(3) 193-199.
- Chandra V, Shalini T, Verma NK, Singh DP, Chaudhary SK and Asha R (2012).** Piper Bete: 1 Phytochemistry, traditional use and pharmacological activity – a review. *International Journal of Pharmacological Research and Development* **4**(4) 216-223.
- Chaurasia SC, Kulkarni T and Shetty LN (2010).** Phytochemical studies and *in vitro* cytotoxicity screening of *Piper Betle* leaf (PBL) extract. *International Research Journal of Pharmacy* **1**(1) 384-391.
- Gad AS and Salam MHA (2010).** The antioxidant properties of skim milk supplemented with rosemary and green tea extracts in response to pasteurization, homogenization and addition of salts. *International Journal of Dairy Technology* **63**(3) 349-355
- Hodzic Z, Pasalic H, Memisevic A, Srabovic M and Poljakovic M (2009).** The influence of total phenols content on antioxidant capacity in the whole grain extracts. *European Journal of Sciences and Research* **28** 471-477.
- Majumdar B, Chaudari SR and Roy A (2002).** A potent antiulcerogenic activity of ethanol extract of leaf *Piper betel* Linn. by antioxidative mechanism. *Indian Journal of Clinical Biological Chemistry* **17** 49-57.
- Muller HG and Tobin G (1980).** *Nutrition and Food Processing*, (Groom Helm Ltd, London, UK) 82-84.
- Onyeike EN and Ikru PR (1998).** Preliminary investigation of proximate composition of heat processed cashew nut seed flours. *Journal of Applied Science and Environment Management* **1**(1) 27-30.
- Preethi R, Devanathan V and Loganathan M (2010).** Antimicrobial and Antioxidant efficacy of some medicinal plants against food borne pathogens. *Advances in Biological Research* **4**(2) 122-125.
- Sadashivam S and Manickam A (1992).** *Biochemical Methods for Agricultural Sciences*, (Willy Eastern Limited, New Delhi, India) 173-175.
- Smid EJ and Gorris LGM (1999).** Natural antimicrobials for food preservation. In Shaust Rahman M (Edition). *Handbook of Food Preservation*, (Marcel Dekkar, New York, USA) 285-308.
- Trease GE and Evans WC (2002).** *Text Book of Pharmacognosy*, 15th edition, (ELBS Publications, New Delhi, India) 161-165.
- Vogel AI (1988).** *Vogel's Textbook of Quantitative Inorganic Analysis* 4th edition, (Longman's Publication, London, UK) 742-743.