

PHYTOCHEMICAL SCREENING OF HERBS USED IN THE PRESERVATION OF RAW MILK

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Abstract: A study was carried out to assess the phytochemical properties of betel and tulsi leaf extract used in preservation of milk. Fresh betel (*Piper betel* Linn) and tulsi (*Ocimum sanctum*) leaves were procured from the local market in Chennai. Phytochemical screening was carried out in which betel leaf extract showed the presence of flavonoids, tannins and phenolic compounds and tulsi leaf extract showed presence of glycosides, phytosterols, tannins and phenolic compounds. Phenols and polyphenols are water soluble compounds which can be easily mixed with milk. Phenolic compounds present in these leaf extracts possess of antimicrobial and antioxidant activity and can be used for increasing the shelf life of milk.

Keywords: Betel, tulsi, Phytochemical, antimicrobial, antioxidant.

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 Datta *et al.*(2011). *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. Tulsi (*Ocimum sanctum*) is a aromatic plant which has medicinal properties (Singh *et al.*2012). It contains several phyto-constituents such as eugenol, cubenol, borneol, vallinin (Kadian and Parle, 2012) due to which it possesses antibacterial, antiviral, antifungal, antioxidant properties (Cohen, 2014). In the present study phytochemical analysis of betel and tulsi leaf extract was carried out to assess the suitability in preservation of milk.

Materials and Methods

Fresh betel (*Piper betel* Linn) and Tulsi (*Ocimum sanctum*) leaves procured from the local market in Chennai. The leaves were shade dried and powdered as per the method of 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 extracts were 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 by 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.

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 colour 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 colour 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 colour 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 colour 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 coloured precipitate indicates the presence of alkaloids.

Detection of flavonoids

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

Results and Discussion

Table 1 shows the results of the phytochemical analysis of the herbs betel and tulsii. On phytochemical screening of herbs, it was found that tannins, phenolic compounds and flavonoids were present in betel leaves extract which correlates with the findings of Chaurasia *et al.* (2010). Phenols and polyphenols are water soluble compounds which can be easily mixed with milk. 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).

Likewise, tulsii leaves extract contained glycosides, tannins, phenolic compounds, phytosterols and alkaloids which coincides with the findings of (Joshi *et al.*, 2011; Shafqatullah *et al.*, 2013). Jeyaseelan *et al.* (2010) reported that water extracts of *Ocimum*

sanctum contain glycosides, saponins, flavonoids and ethanolic extracts contains tannins, alkaloids, glycoside and flavonoids. Many herbs and spices extracts contained high levels of phenols and exhibited antibacterial activity against food borne pathogens. Antibacterial substances can easily destroy the bacterial cell wall and cytoplasmic membrane resulting in a leakage of the cytoplasm. (Shan *et al.*, 2007).

Conclusion

In this present study it was concluded that betel leaf extract contains flavonoids, tannins and phenolic compounds and tulsi leaf extract showed presence of glycosides, phytosterols, tannins and phenolic compounds. These extracts possess antimicrobial and antioxidant activity and can be used in the preservation of milk.

References

- [1] Arani Datta, G. Shreya and M. Singh, 2011. Antimicrobial Property of Piper betel Leaf against Clinical Isolates of Bacteria; *J. of Pharm. Sci. and Res.* ,2(3): 104-109.
- [2] Chandra, V., T. Shalini, N.K. Verma, D.P. Singh, S.K. Chaudhary and R. Asha, 2012. Piper Betel: Phytochemistry, traditional use and pharmacological activity – a review *Int. J. of Pharm. Res. and Development.*, 4(4):216-223.
- [3] Chaurasia, S.C., T. Kulkarni and L.N. Shetty, 2010. Phytochemical studies and *invitro* cytotoxicity screening of *Piper Betle* leaf (PBL) extract, *Int. Res. J. of pharmacy*,1(1):384-391.
- [4] Cohen, M.M., 2014. Tulsi-*Ocimum sanctum*. A herb for all reason. *J. of Ayurveda and Integrate medicine.* 5(4):251-259.
- [5] Gad, A.S and M.H.A. Salam, 2010. The antioxidant properties of skim milk supplemented with rosemary and green tea extracts in response to pasteurization, homogenization and addition of salts. *Int. J. of Dairy Tech.*, **63**(3): 349-355
- [6] Hodzic Z, H. Pasalic, A. Memisevic, M. Srabovic and M. Poljakovic, 2009. The influence of total phenols content on antioxidant capacity in the whole grain extracts. *Eur. J. of Sci. Res.*, 28: 471-477.
- [7] Jeyaseelan, E.C., J.P. Jeyadevan, K. Uthayarasa and M.K. Pathmanathan, 2010. Antibacterial Activity and Phytochemical Analysis of Some Selected Medicinal Plants. *Int. Journal of Pharmaceutical and Biological Archives*, **1**(3):291 – 299.
- [8] Joshi, B.G., S. Prasad , B.B. Basnet, M. Raj Bhatt, D. Sharma, K. Subedi, J. Pandey and R. Malla, 2011. Phytochemical extraction and antimicrobial properties of different medicinal

plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). *J. of Microbiology and antimicrobials.*, (3(1):1-7.

[9] Kadian R, Parle M. 2012. Therapeutic potential and phytopharmacology of tulsi. *Int. J. of Pharmacy and life sciences*, 3(7):1858-1867,

[10] Preethi, R., V. Devanathan and M. Loganathan, 2010. Antimicrobial and Antioxidant efficacy of some medicinal plants against food borne pathogens. *Advances in biological Research*, 4(2):122-125.

[11] Shafqatullah, M., K. Asadullah, K. Rehman and F. Ali Khan, 2013. Comparative analysis of *Ocimum santum* stem and leaves for phytochemicals and inorganic constituents. *Middle East J. of Scientific Res.*, 13(2):236-240.

[12] Singh, E, Sharma, S. Diwivedi, J. Sharma S. 2012, Diversified potentials of *Ocimum sanctum* Linn (Tulsi). An exhaustive survey. *J. of National Products and Plant resources*, 2(1): 39-48.

[13] Smid, E.J and L.G.M. Gorris, 1999. Natural antimicrobials for food preservation. Inshah Rahman M (Ed). *Handbook of food preservation*. Marcel Dekkar, New York, pp 285-308.

[14] Shan, B., Y.Z. Cai, J.D. Brooks, and H. Corke, 2007. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. *Int. J. of Food Microbiology.*, 117(1):112-119.

[15] Trease, G.E and W.C. Evans, 2002. *Text book of Pharmacognosy*, 15th edition ELBS publications, New Delhi.

Table - 1
Phytochemical screening of the herbs[@]

Sl. No.	Constituents	Betel	Tulsi
1	Alkaloids	-ve	+ve
2	Carbohydrates	+ve	-ve
3	Fixed Oils	-ve	-ve
4	Flavonoids	+ve	-ve
5	Glycosides	-ve	+ve
6	Phytosterols	-ve	+ve
7	Protein	+ve	-ve
8	Saponins	-ve	-ve
9	Tannins and Phenolic compounds	+ve	+ve

@ Average of six trials