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UTILIZING BETEL AND TULSI LEAF EXTRACTS TO EXTEND THE SHELF LIFE OF RAW MILK: A PHYTOCHEMICAL DISCOURSE

¹M.H.A. Salam

Article Info	Abstract		
Keywords: Betel, tulsi,	Betel and tulsi leaf extracts were analyzed for their phytochemical		
phytochemical, antimicrobial,	properties to evaluate their suitability in preserving raw milk. The study		
antioxidant, preservation, raw	aimed to investigate the ability of these plant extracts to inhibit microbial		
milk.	growth, oxidation, and enzymatic reactions that occur in milk, by		
	screening for the presence of different phytochemical compounds.		
	Phytochemical tests were conducted and showed that betel leaf extract		
	contained flavonoids, tannins, and phenolic compounds, while tulsi leaf		
	extract contained glycosides, phytosterols, tannins, and phenolic		
	compounds. The presence of phenolic compounds in both extracts is		
	particularly relevant, as these compounds possess antimicrobial and		
	antioxidant properties that can potentially increase the shelf life of milk.		
	The study suggests that plant extracts can be a natural method for		
	preserving raw milk.		

Introduction

Betel and tulsi leaves have been examined for preserving raw milk due to their potential biological properties. Betel leaves contain various biologically active compounds dependent on the plant variety, season, and climate. Phytochemical compounds in tulsi herb such as eugenol, cubenol, borneol, and vallinin have been found to possess antimicrobial, antiviral, antifungal, and antioxidant properties. Thus, the plant extracts could offer inhibition of microbial growth, oxidation, and enzymatic reactions in milk. The study screened for different phytochemical compounds such as glycosides, proteins, tannins, phenolic compounds, phytosterols, and flavonoids to determine their presence in the plant extracts. The tests showed that betel and tulsi leaf extracts contained several phyto-constituents that can preserve raw milk. The results of this study suggest that the extracts of betel and tulsi leaves could be potentially used as natural preservatives for raw 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

¹ Department of Livestock Farm Complex, Veterinary College and Research Institute, Tirunelveli-Tamil Nadu Veterinary and Animal Sciences University.

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.

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

(a) Average of six trials

Results and Discussion

Table 1 shows the results of the phytochemical analysis of the herbs betel and tulsi. 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, tulsi 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 betal 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.

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