

Preliminary Phytochemical Screening of Selected Indian Spices

Shobha. N^{1*}

Abstract: Spices are commonly used in Indian kitchen as flavouring agent in food and used as food preservatives. Many of the spices are used in folk medicine. The objective of the present study is to analyse the phytochemical composition of few of the selected spices viz., clove, cinnamon and star anise. Phytochemical compositions of the spices were carried out with water and methanol extracts. The experimental result showed that the different extracts of these spices contain alkaloids, flavonoids, steroids, terpenes, tannins, saponins, glycosides, carbohydrates, free amino acid, crude protein and phenolic compounds.

Key words: phytochemical analysis, tannins, saponins, alkaloids, flavonoids

1. Introduction:

Since ancient times spices have served various roles such as coloring agents, flavouring agents, preservatives, food additives. A spice is a dried seed, fruit, root, bark or flower of a plant or a herb used in small quantities for flavor, color or as a preservative in small quantities for flavor, color or as a preservative.

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(Kunnumakkara et al 2009). Beyond their culinary uses spices are also used in traditional medicine by majority of the population worldwide. The medicinal property of spices is due to the rich sources of phytochemicals, bioactive compounds and this also contribute to their flavor, aroma, and health benefits. Phytochemicals are regarded as secondary metabolites because the plant that manufactures them may have little need for them. They are naturally synthesized in all parts of the plant body; any part of the plant body may contain active components (Tiwari *et al.*, 2011).

Cinnamomum L. is an evergreen tropical tree belongs to the family of Lauraceae. The bark and leaves of the cinnamon are used as spice, flavouring agent in foods and as a potent therapeutic agent in ethnomedicine (Seidemann J 2005, Unlu M et al 2010).

Cloves are the dried flower buds of *Syzygium aromaticum* (L.) Merr. et Perry — a tree of the myrtle family (Myrtaceae). They are sources of anti-microbial agents against oral bacteria that are commonly associated with dental caries and periodontal disease (Cai & Wu, 1996). Cloves are the dried flower buds of a tree *Syzygium aromaticum* (L.) belonging to the family Myrtaceae. It is used in traditional medicine against dental caries and associated diseases (Cai & Wu, 1996), used to treat asthma and other allergic disorders (Kim et al., 1998). Star anise is a spice from the tree *Illicium verum* (L.). The name "star anise" is due to the shape of the fruit which is star-shaped, with five to ten boat-shaped sections radiating from the center. Has tough skin and rusty colour (Encyclopedia of spices, 2003). It is used in traditional Chinese medicine to treat infant colic (Ize-Ludlow D et al 2004), and as flavoring agent in foods (De M et al 2001).

^{1*}Assistant Professor, Department of Biochemistry, Maharani's Science College for Women, Mysore

2. Materials and Methods:

Sample Collection

All the chemicals used in the experiment were of analytical grade. The spices Cinnamon, Cloves and Star anise were purchased from the local market of Myore. The spices were cleaned and washed using distilled water and dried at room temperature for two days. The dried samples were then ground into fine powder and collected in polythene bags for the study.

Preparation of the extracts

10gms each of the above powdered spices was weighed and dissolved with 100ml of distilled water and in 100ml of methanol in separate conical flasks. This mixture was warmed on hot plate at 55^oC for 2 hours with regular shaking for 2 days. After 2 days, it was filtered using Whatman No.1 filter paper. The filtrate of aqueous extract and methanol extract was evaporated in a hot air oven at 50 ^oC. One-gram of each dried extracts were resuspended in 10 ml of Dimethyl Sulphoxide (DMSO) in separate containers and stored in a refrigerator at 4 ^oC for further study.

3. Qualitative analysis of Phytochemicals

Analysis of phytochemicals ((Primary and Secondary metabolites) for carbohydrates, proteins, steroids, alkaloids, flavonoids, glycosides, tannins, phenols, saponins, terpenoids and coumarins in water and methanol extracts of all the four spices was performed using the standard methodology of Harborne (1998) and Kokate (1997), Chitravadivu et al (2009).

Test for carbohydrates (Molisch's test):

0.5ml of alcoholic α -naphthol solution was added to 1ml of each of the spice extracts were taken in test tube, mixed well followed by addition of few drops of conc. Sulphuric acid along the sides of the test tube. Appearance of violet ring at the junction indicates the presence of Carbohydrates.

Test for proteins (Biuret test):

1ml of extract was taken in a test tube followed by 4% NaOH and 1% CuSO₄. Appearance of Violet pink colour indicated the presence of proteins.

Test for steroids (Salkowski test)

To 0.5ml of the extract in aa test tube, 2 ml concentrated sulphuric acid and 2 ml chloroform were added along the side of the test tube. The contents were mixed gently for few minutes. The development of red colour in the chloroform layer and greenish yellow fluorescence in the sulphuric acid layer indicates the presence of steroids.

Test for Alkaloids (Mayer's Test)

To 1ml of the extract, 2 drops of chloroform and 2 drops of Meyer's reagent is added. Appearance of white precipitate indicates the presence of alkaloids.

Test for Flavonoids (Alkaline Reagent Test)

4-5 drops of drops of 20% sodium hydroxide solution is added to 2ml of extracts. An intense yellow colour formed disappears on adding dilute hydrochloric acid. This indicates the presence of flavonoids.

Test for glycosides (Keller Kiliani Test)

To approximately 5ml of each extract, 2 ml of glacial acetic acid was added, followed by the addition of few drops of ferric chloride solution and 1ml of concentrated Sulphuric acid. Formation of brown ring at interface confirms the presence of glycosides.

Test for Tannins (Gelatin Test):

1ml of Gelatin solution (1%) containing sodium chloride was added to 1ml of the extracts. Formation of white precipitate indicates the presence of tannins.

Test for phenols (Ferric chloride test)

Few drops of 2% ferric chloride solution is added to 1ml of the extracts. Appearance of brownish green to blue-black colour indicates the presence of phenolic compounds.

Screening for Saponins (Foam Test)

2ml of extract was taken in a test tube and 6ml of distilled water was added to it. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Screening for Terpenoids (Salkowski Test)

5ml of extract was taken in a test tube and 2ml of chloroform was added to it followed by the addition of 3ml of concentrated sulphuric acid. Formation of reddish-brown layer at the junction of two liquids confirms the presence of terpenoids.

Test for Coumarins (Sodium hydroxide Test)

To 1ml of the extracts, 1ml of NaOH (10%) is added and mixed well. Appearance of yellow colour indicates the presence of coumarins.

4. Results and Discussion

Detection of phytochemicals is a method which determines the presence of specific classes of phytochemicals in the sample. The present study involved the analysis of phytochemicals in three different spices viz, Cinnamon, Clove and Star Anise in aqueous and methanol extracts. The details of the spices and the result is presented in Table 1, 2, 3 and 4.

Table 1: Botanical details of the selected spices

No	Botanical name	Common name	Family	Part used
1	<i>Cinnamomum verum</i>	Cinnamon	Lauraceae	Bark
2	<i>Syzygium aromaticum</i>	Clove	Myrtaceae	Flower bud
3	<i>Illicium verum hook</i>	Star anise	Illiciaceae	Fruit

Table 2: Phytochemical screening in Cinnamon

Phytochemicals analysed	Cinnamon	
	Aqueous Extract	Methanol extract
Carbohydrates	-	-
Proteins	-	-
Steroids	-	-
Alkaloids	-	-
Flavonoids	-	-
Glycosides	+	+
Tannins	-	-
Phenols	+	+
Saponins	+	+
Terpenoids	+	+
Coumarins	+	+

Note: (+) – present, (-) – absent

Table 3: Phytochemical screening in Clove

Phytochemicals analysed	Clove	
	Aqueous Extract	Methanol extract
Carbohydrates	+	+
Proteins	-	-
Steroids	+	+
Alkaloids	-	+
Flavonoids	+	+
Glycosides	+	+
Tannins	+	+
Phenols	+	+
Saponins	-	+
Terpenoids	+	+
Coumarins	-	-

Note: (+) – present, (-) – absent

Table 4: Phytochemical screening in Star Anise

Phytochemicals analysed	Star Anise	
	Aqueous Extract	Methanol extract
Carbohydrates	+	+
Proteins	+	+
Steroids	-	-
Alkaloids	+	+
Flavonoids	+	+
Glycosides	-	+
Tannins	+	+
Phenols	+	+
Saponins	-	+
Terpenoids	+	+
Coumarins	+	+

Note: (+) – present, (-) – absent

Results:

The result revealed that some of the phytochemicals analysed were present in the extracts of all the spices. A total of 11 phytochemicals were analysed for all the three spices taken. Glycosides, terpenoids and phenols were the common phytochemicals present in all the spices and in both the extracts studied. Cinnamon found to contain Glycosides, Phenols, Saponins, Terpenoids and Coumarins in both aqueous and methanol extracts whereas remaining phytochemicals showed negative result (Table 2). Similarly, Clove showed to contain Carbohydrates, Steroids, Flavonoids, Glycosides, Tannins, Phenols and Terpenoids in both aqueous and Methanol extracts but Alkaloids and Saponins were present only in methanol extracts and not in aqueous extracts. Proteins and Coumarins were totally absent in both the extracts (Table 3).

Table 4 presents the Phytochemical analysis of Star Anise. Both aqueous and Methanol extracts of Star Anise found to contain, carbohydrates, proteins, alkaloids, flavonoids, Tannins, phenols, terpenoids and coumarins whereas glycosides and saponins were present only in methanol extract and absent in aqueous extract. Steroids were absent in both the extracts of Star Anise.

Discussions:

Therapeutic uses of these spices is due to the various phytochemicals they contain (Okwu 2001). Different classes of phytochemicals are found to possess a wide range of physiological actions, which may help in protection against various diseases. Although these Phytochemicals present in herbs and spices are the essential nutrients in our diet, they proved to have anticancer (Kaefer and Milner, 2008; Lai and Roy, 2004), anti-inflammatory (Mueller et al., 2010; Steenkamp et al., 2006), antibacterial (Shan et al., 2007), antiviral (Aruoma et al., 1996), and antioxidant effects (Tapsell et al., 2006).

Talking about the secondary metabolites, in the present study, Steroids were found to be absent in cinnamon and Star Anise whereas it was present in both the extracts of Clove. Alkaloids were present in Star Anise and the methanolic extracts of Clove. Steroids bearing aromatic rings represent a fascinating subgroup of steroids that possess distinct chemical and biological characteristics [Kadis B.M.1957, Taub D.1973, Rutherford F.J 1972].

Alkaloids were absent in Cinnamon and aqueous extract of clove whereas it was present in methanol extract of clove and both the extracts of Star Anise. In spite of their therapeutic properties alkaloids have toxic effects on humans and animals (Trease and Evans, 2005, Beyer et al., 2009).

Flavonoids were absent in Cinnamon but present in both the extracts of Clove and Star Anise. Flavonoids have an excellent antioxidant activity (Nijveldt R.J., 2001). Dietary flavonoids show a favorable relationship between their consumption and reduction of cardiovascular diseases (Slavin J.L 2012)

Aqueous extract of Star Anise showed no Glycosides but Methanolic extract contained Glycosides but Cinnamon and Cloves showed the presence of Glycosides in both the extracts. Glycosides are a group of carbohydrate which have proved to possess antioxidant, anti-inflammatory, antihypertensive, and antidiabetic activities (Bernal et al., 2011).

Tannins were found to be absent in both the extracts of Cinnamon whereas both the extracts showed Tannins in Cloves and Star Anise. Tannins significantly influence the sensory attributes of beverages and foods, especially astringency, bitterness, aroma, and mouthfeel. Although they offer considerable health benefits, high tannin levels can intensify bitterness and astringency, potentially impacting consumption (Hufnagel J.C., Hofmann T. 2008). These tannins are easily hydrolyzed under acidic, basic, or enzymatic conditions, releasing phenolic acids and exhibiting strong antioxidant activity due to their hydroxyl-rich structure (Khanbabaee K., van Ree T 2001)

All the three spices under present study showed the presence of Phenols in both aqueous and Methanolic extracts. The biological potency of phenolic compounds includes possible pharmacological value (Ingold 1960). Phenolic compounds have long been recognized to possess antiallergenic, anti-inflammatory, antiviral and antiproliferative activities (Miliauskas, G et al 2004) Saponins are naturally occurring surface-active glycosides fungicidal, and other pharmacological properties.

Both the extracts of Cinnamon contain Saponins, whereas in Cloves and Star Anise, only the Methanolic extracts contained Saponins and it was absent in aqueous extract of Cloves and Star Anise. Saponins are naturally occurring surface-active glycosides, Saponins are bitter and reduce the palatability of livestock feeds. Due to their toxicity to various organisms, saponins can be utilized for their insecticidal, antibiotic, fungicidal, and other pharmacological properties. (Desai et al 2009).

Both the extracts of all the three spices showed the presence of Terpenes. Terpenoids are organic compounds that having tremendous applications in medical field and pharmaceuticals (Manfredi KP 2007).

Cinnamon and Star Anise have Coumarins in both the extracts whereas it was absent in Cloves. Coumarins are a family of benzopyrones having important place in medicinal chemistry research (Kennedy R, Thornes RD 1997)

5. Conclusion:

The spices under this study showed to have number of phytochemicals which are of potential physiological activity. However, it is important to conduct quantitative assay to prevent any toxic effects. Further research, including both in vitro and in vivo studies, to understand the mechanisms of action, therapeutic potential, and safety profile of these compounds is required.

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