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Research article

Phytochemical analysis and estimation of Lycopene and β-carotene content in n-hexane extracts of different spices

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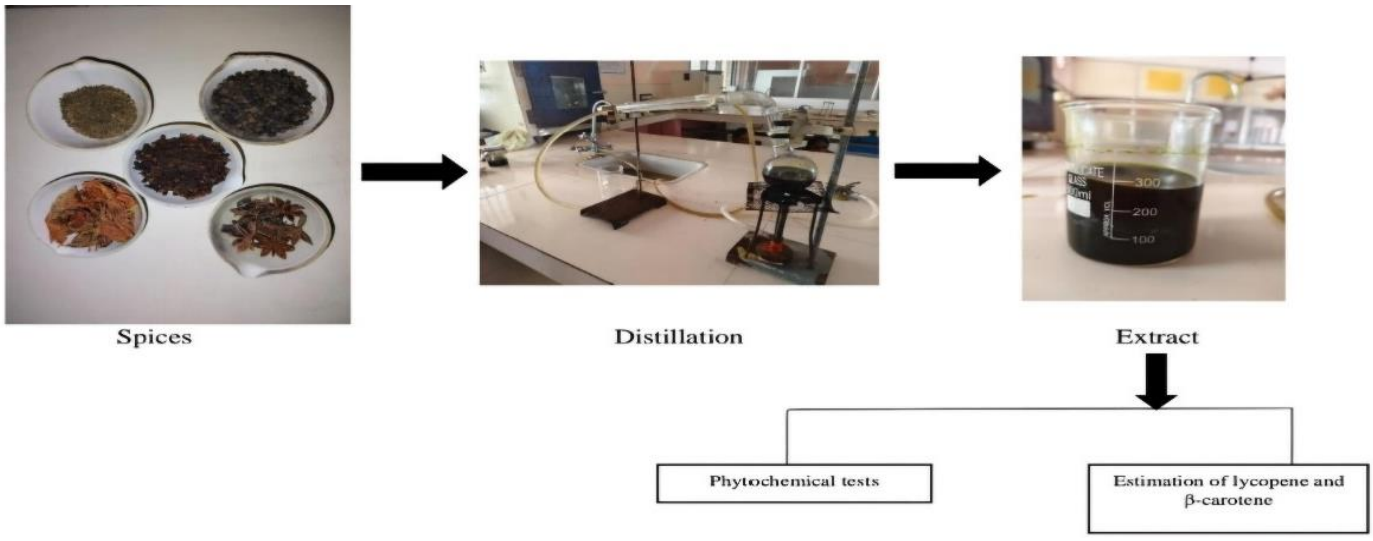
ABSTRACT

Spices are an important commercial crop in India. These Spices constitute an essential group of agricultural commodities. A spice is a seed, fruit, root, bark, and also other plant substance with an aromatic or strong taste used to enhance the flavor of foods. Spices like leaves, flowers, or stems of plants are used for flavoring and garnish. Spices are sometimes used in medicine, religious rituals, cosmetics, or perfume production there are over 80 pieces grown in different parts of the world, and around 50 pieces are grown in India. In India, abundant quantities of spices are pepper, ginger, turmeric, chili, cardamom, fenugreek, dill, cumin ajowan, cinnamon, clove, nutmeg and mace. Spices and herbs are plant-derived substances that add flavor to any dish. This study was carried out on the five spices, star anise family Schisandraceae, Mace family Myristicaceae, Black pepper family Piperaceae, Eugenia caryophyllata, family Myrtaceae, Caraway seeds, family Apiaceae, and to determine the phytochemical constituents like alkaloids, carbohydrates, glycosides tannins, flavonoids, saponins phenols, and terpenoids, reducing sugars, amino acids, and also to study the βcarotene and lycopene contents in all the five species.



Keywords: Star anise, Clove, Black pepper, Shah Jeera, Mace, Lycopene, Beta Carotene.

INTRODUCTION

India is known as 'Home of Spices'. Spices can come from a seed, fruit, root, bark, or other plant substance[1-2] According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs [3]. In India pepper, ginger, turmeric, chilies, cardamom, celery, fenugreek, fennel, cumin, dill, coriander, cinnamon, ajowain, cassia, clove, nutmeg, and mace are abundant quantities cultivated. The spices are natural plant products, mainly used for flavoring, coloring, or preserving food [4-6]. These are also used in beverages, liquors, pharmaceuticals, cosmetics, and perfumery products. Many herbal constituents can enhance the bioavailability of drugs.

Illicium verum (star anise) is an evergreen medium-sized tree with star-shaped fruit and belongs to the family Schisandraceae. It is indigenous to northeast Vietnam and South China [7]. Anise is a delicate, reddish-brown color comprising six to eight follicles, the seeds are shiny and brittle brown, compressed and smooth, an annual herb that grows to about 18 inches high, with secondary feather-like leaflets of bright green color [8]. The fruit is mostly the medicinal part, collected in autumn and winter when it turns green to yellow [9]. Star anise oil is mostly used in cooking, perfumery, soaps, toothpaste, mouthwashes, and especially in response to the demand for eco-friendly and sustainable skincare solutions [10].

Mace is a delicate, green aromatic tree that belongs to the Myristicaceae family [11]. Nutmeg consists of two distinct spices, which are Nutmeg (seed) and Mace (aril), it is native to Indonesia (Moluccas Islands), and, the nutmeg tree grows abundantly and is now naturalized in the West Indies, Sri Lanka, India, and Philippines, Tropical America, and Pacific Islands[12,13]. The Mace (Aril) is dark orange in color, aromatic, pungent, irregular in shape, and has a rough surface. The center of the nutmeg contains a single, hard seed. Nutmeg plants can produce two different spices from a single plant. Though similar in taste, mace flavor is not as sweet as nutmeg and can offer a sharp bitter taste. Mace is taken in oral form for diarrhea, nausea, vomiting, stomach spasms, and intestinal gas. This may aid in digestion boost blood circulation and enhance appetite.

Black pepper scientifically known as Piper Nigrum, is one of the most widely used spices, and commonly used spices throughout the world it belongs to the family Piperaceae, Black pepper is native to Malabar, a tropical region on the Western Coast of Southern India [14, 15]. The plants of black pepper are either bushy or woody stems. The plant morphology is simple, with alternating leaves with an oval shape, and produces clusters, or spikes, of 50 to 150 flowers cultivated for its fruits (berries) that are used as spices. The fruit is about 5mm in diameter, dark red, and contains a single pepper seed [16, 17]. Black pepper is used as a medicinal agent, preservative, perfumery rich in vitamin B and active components are being used in different types of foods and as medicine [18, 19].

Eugenia caryophyllata is commonly known as Clove, from the family Myrtaceae. It is native to the Maluku Islands (or Moluccas often called the Spice Islands) of Indonesia [20]. The Clove tree is an evergreen tree that grows to a height ranging from 8-12m, having large square leaves and sanguine flowers in numerous groups of terminal clusters [21]. They have a deep brown color and are used as powerful fragrant odor, strongly sweet, and slightly astringent [22]. Clove is a food preservative, flavoring agent, and nutritional additive, coloring agent. It is commonly used for tooth care, tooth pain, and as a cough suppressant [23].

Caraway seeds also known as Shah Jeera (Sajeera or Kala Jeera) are a biennial plant species from the Apiaceae family [24, 25]. They are found mostly in Western Asia, Europe, and Northern Africa. The plant has finely split, fluffy white leaves with thread-like divisions, and grows on 20–30 cm stems. The main flower stalk is 40–60 cm tall, which are tiny white or pink blooms. The fruits have a strong, anise-like flavor and scent that originates from essential oils, primarily carvone, and limonene, and are generally utilized whole [26]. They are most commonly beverages, casseroles, and a variety of other dishes, such as sauerkraut. Shah jeera contains health-benefiting nutrients, minerals, vitamins, and antioxidants [27]. Caraway fruits are used as a remedy to cure indigestion, pneumonia, and carminative.

Carotenoid is a polygene backbone consisting of a series of conjugated C=C bonds and these are a group of pigments present everywhere in nature, and more than 600 different carotenoids have been identified and characterized. They are liable for the pigmentation of plants, animals, and microorganisms and also serve important roles in biological systems [28, 29, and 30]. The optimal intake of carotenoids reduces the risk of developing cancers, cardiovascular diseases, bone, and skin, and even provides cosmetic benefits. The primary bioactive property of carotenoids in organisms is their function as antioxidants, which helps in combating oxidative stress.

Lycopene is a non-pro-vitamin A carotenoid. It is a tetra terpene compound consisting of eight isoprene units and 11 double linear bonds and is an intermediate of carotenoid synthesis in plants [31-33]. Lycopene cannot be synthesized in the human body, so it should be consumed in daily diet. The absorbed lycopene is distributed to various tissues including, adrenals, skin, and prostate glands. The present study will increase the nutritive value of the food items, and antioxidants of the present study of these spices can lead to the development of therapeutic diseases like cancer diabetes oxidative stress.

The main aim of the present study was to detect the phytochemical constituents by phytochemical screening and to estimate the amount of lycopene and carotenoids present in Star anise, Mace, Black pepper, Clove, and Shah jeera [33].

MATERIALS AND METHODS

Solvent and Materials, n-Hexane was obtained from Ushodaya Scientific products Kakinada, high-speed centrifuge machine, Whatman filter paper grade No. 01 and 03. All the spices star anise, mace, black pepper, clove, and shah jeera were purchased from local vendors from the surrounding area of Yanam. They were dried for three days under shade and then they were powdered using mortar and pestle until a fine powder was obtained, then the powders were sieved using sieve no-44.

Extraction

50gm of each star anise, mace, black pepper, clove, and Shah jeera powders were taken in 100 ml of n-hexane each and they were macerated for 72 hours, After 72 hours filter the extract using Whatman filter paper then the filtrate using steam distillation. Then the obtained extract was subjected to phytochemical screening.

Phytochemical Screening

Test for alkaloids

Dragendroff's test: By adding 1 ml of Dragendroff's reagent to 2 ml of extract, anorange-red precipitate was formed.

Mayer's test: By adding 1 ml extracts to Mayer's reagent, a yellow or white precipitate formed.

Wagner's test: 2 drops of Wagner's reagent added to 2ml of extract mixed well. Areddish precipitate formed.

Test for carbohydrates

Molisch test: Add the sample to the test tube containing water and shake it to dissolve.Add 1 ml of Molisch reagent. Add concentrated Sulphuric acid along the walls of the test tube, and slowly purple ring formed.

Test for glycosides

Keller Kilian test: To the sample solution add 1 ml of glacial acetic acid, 1 drop of 5% ferric chloride solution, and 1 ml of Sulphuric acid. The Reddish brown color is formed.

Test for tannins

Gelatin test: To the Solution of gelatin add 1ml extract, and a white buff-colored precipitate formed.

Test for reducing sugars

Benedict's test: 1ml of the sample with 2ml of Benedict's reagent and heated in a bathof boiling water for 3-5 min. A brick-red colored precipitate of cuprous oxide is formed.

Fehling's test: mix equal parts of Fehling's A and B solutions, add to the sample, and heat. A reddish-brown precipitate indicates the presence of reducing sugars.

Test for steroids

Salkowski test: Add 5ml test solution, 2ml chloroform and 3ml of H_2SO_4. The Red color formed.

Test for amino acids

Ninhydrin test: A few drops of the 2% ninhydrin solution must be added to thissolution. Heat for 5 min in a water bath, it shows a deep blue color or violet.

Test for flavonoids

Alkaline reagent test: To the crude extract of 2ml, 1ml of NaOH was added, initially, a deep yellow color appeared but it gradually became colorless by adding 1 ml of dilute HCL.

Test for saponins

Foam test: 5ml of distilled water was mixed with aq. Crude plant extract in the test tube and mix vigorously to foam appearance.

Test for proteins

Millon's reagent: 1 ml of Millon's reagent was added to the extract and heated gently. A reddish-brown color forms.

Test for phenols**:** Sample dissolved in water, add $FeCl_3$ and NaOH. The Brown precipitate is formed.

Test for terpenoids

5 ml of the extract is mixed with 2 ml of chloroform, and 3 ml of concentrated H_2SO_4 to form a layer with a reddish-brown coloration of the interface between the two liquids.

Estimation of βcarotenoid content

Beta carotene was estimated using the method described by Nagata and Yamashita. Hexane extracts (Star anise, Mace, Black pepper, Clove, Shah jeera) weighing 0.1 g were partitioned with 10 ml of acetone-hexane (4:6 by volume) at 37^0 c for 10 min. The resulting solutions were centrifuged and the absorbance of the supernatant layer was taken at the following wavelengths 663nm, 645nm, 505 nm, and 453nm.

B-carotene (mg/100 cm3) = 0.216A663 - 1.22A645 - 0.304A505-0.452A453.

Estimation of lycopene content

Lycopene was estimated using the method described by Nagata and Yamashita Hexane extracts of (Star anise, Mace, Black pepper, Clove, and Shah Jeera) weighing 0.1 g were partitioned with 10 ml of acetone-hexane (4:6 by volume) at 37^0 c for 10 min. The resulting solutions were centrifuged and the absorbance of the supernatant layer was taken at the following wavelengths 663nm, 645nm, 505 nm, and 453nm.

Lycopene (mg/100 cm3) = -0.0458A663 +0.204A645 +0.372A505-0.0806A453.

Where A663, A645, A505 and A453 are absorbance at 663, 645, 505, and 452.

RESULTS AND DISCUSSION

The results of Phytochemical screening suggest that the hexane extract of spices is rich in various bioactive constituents such

as alkaloids, carbohydrates, glycosides, tannins, flavonoids, phenols, saponins, steroids, phenols, and terpenoids. All the above spices contain flavonoids, saponins, phenols, and terpenoids but reducing sugars are absent. As Terpenes were present in all the extracts, further these extracts were studied for lycopene and carotene content. The highest percentage of β-carotene is observed in clove at 2.16% followed by mace at 1.31% and the star anise at 1.27%. The highest percentage of lycopene is observed in mace at 0.69% followed by clove at 0.53% and star anise at 0.44%.

Table 1: Phytochemical Screening of Different Spices

Phytochemical teat	Star anise	Mace	Black pepper	Clove	Shah jeera
Alkaloids	+	-	+	+	-
Carbohydrates	+	-	+	+	+
Glycosides	+	-	+	+	-
Tannins	+	+	-	+	+
Reducing sugar	-	-	-	-	-
Amino acids	-	-	+	-	-
Flavoneds	+	+	+	+	+
Saponins	+	+	+	+	+
Proteins	-	-	-	+	-
Steroids	+	-	-	+	-
Phenols	+	+	+	+	+
Terpenoids	+	+	+	+	+

Table 2: Estimation of β carotene content

Sample	A_{663}	A_{645}	A_{505}	A_{453}	0% of beta carotene content
Star anise	0.89	0.703	1.083	1.248	1.27
Mace	0.007	0.002	0.713	2.424	1.31
Black pepper	0.578	0.584	0.701	0.873	0.85
Clove	1.052	0.877	1.498	1.927	2.16
Shah jeera	0.714	0.676	0.838	0.99	0.89

Graph 1: Percentage of β carotene content of spices

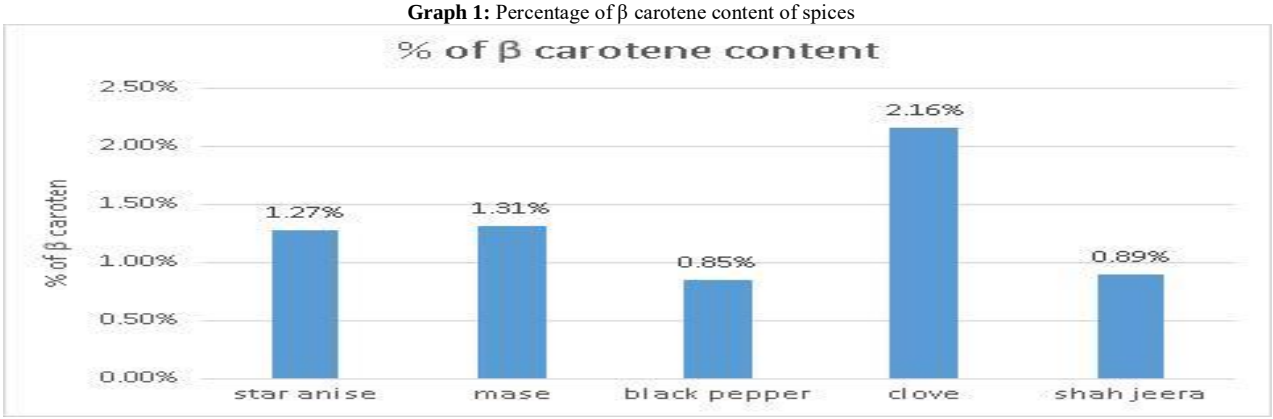


Table 3: Estimation of lycopene content

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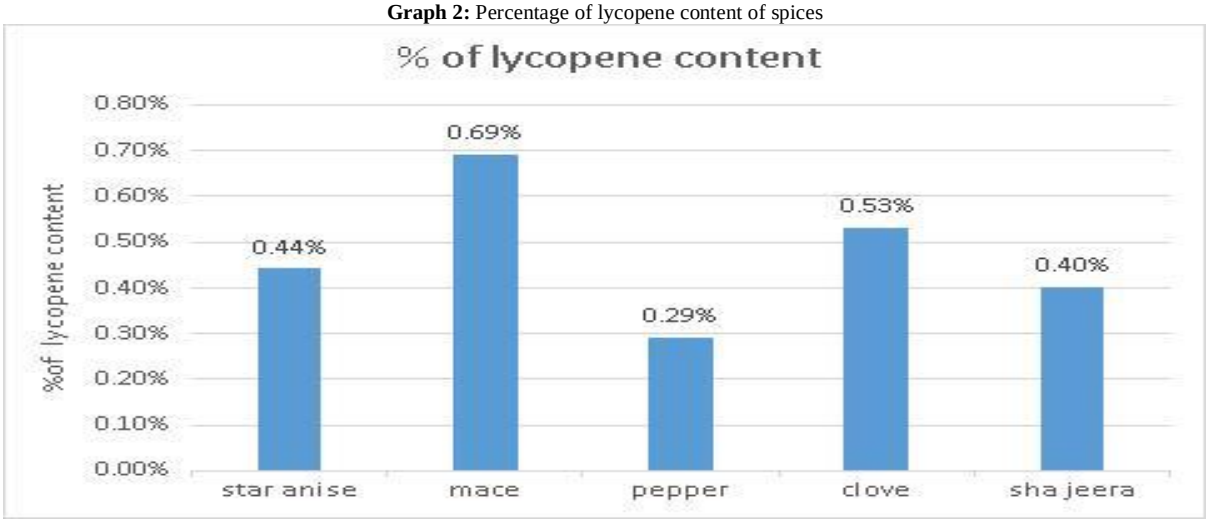
Clove	1.052	0.877	1.498	1.927	0.53
Shah jeera	0.714	0.676	0.838	0.99	0.4

Graph 2: Percentage of lycopene content of spices



CONCLUSION

In the present study, five different spices were extracted using hexane as a solvent, and the obtained extracts were analyzed for phytochemicals present in them where alkaloids, carbohydrates, glycosides, tannins, reducing sugars, amino acids, flavonoids, saponins, proteins, steroids, phenols, and terpenoids are present. Maximum phytochemical constituents appeared in Black pepper and Clove, and the minimum constituents were present in Shah jeera, Mace, and Star anise. As terpenes are present in all five spices, the extracts were studied for lycopene and β carotene content. The maximum percentage of β carotene is present in Clove and the least in Black pepper. The maximum amount of lycopene is observed in Mace and the least amount in Black pepper. So, from the above studies, we can conclude that using these spices will increase the nutritive value of the food items, and these spices can lead to the development of therapeutic agents for oxidative stress diseases and disorders.

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