

Phytochemical profile of *Syzygium caryophyllatum* (L.) Alston

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Abstract

Plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Phytochemical techniques played a significant role in searching raw materials and resources for pharmaceutical industry. Phytochemicals are secondary metabolites in one or more parts of the medicinal plants. These have the ability to produce a definite physiological action on the human body. Medicinal plants are the local heritage with global importance and have curative properties due to the presence of various complex chemical substances of different composition which are found as secondary metabolites in one or more parts of the plants. Keeping this view in mind the present investigation was carried out in *Syzygium caryophyllatum* leaf, fruit, seed and bark collected from Edava, Thiruvananthapuram district, Kerala, India. Qualitative phytochemical analysis of these plants confirms the presence of various phytochemicals like alkaloids, flavonoids, tannins, saponins, terpenoids and quinone.

Keywords: *Syzygium caryophyllatum*, phytochemical constituents, crude extract, solvents

Introduction

Nature has been a source of medicinal agent for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Many of the isolations were based on the uses of these agents in traditional medicine. Prior to the nineteenth century, plant medicines were administered mostly in their crude forms as infusions, tinctures, decoctions and syrups or applied externally as ointments and herbal washes. However, during the late nineteenth and early twentieth century, scientists began to isolate, purify and identify the active ingredients (principles) from medicinal plant extracts. This endeavour led to the discovery of some of the most important drugs that are still widely used in modern medicine^[1, 2]. So it is assumed that a major part of traditional therapy involves the use of plant extracts or their active principles^[3]. These chemical constituents from plants may be therapeutically active or inactive also called inert chemicals, which are collectively called as phytochemicals or secondary metabolites^[4]. Some of these phytochemicals are alkaloids, phenolics glycosides, flavonoids, steroids, terpenoids, aminoacids and glycopeptides which are present in vegetables, fruits, flowers, leaves, stem, bark and roots that work with and act as a defence system more accurately to protect against diseases^[5].

Many species of Myrtaceae are used for several medicinal purposes. Among them, the most important species are *S. cumini*, *S. malaccense*, *S. aromaticum* and *S. travancoricum*. It may be due to the presence of active metabolites such as alkaloids, phenolics glycosides, flavonoids, steroids, terpenoids, aminoacids and glycopeptides. Despite the various bioactive phytochemical constituents and diverse medicinal properties attributed to the genus *Syzygium*, no detailed phytochemical studies have been carried out to shed light on these allied species of *S. caryophyllatum* which is also known as Malai naval in Tamil, Kattu njaval in Malayalam. In Ayurveda *Syzygium caryophyllatum* bark, leaves and fruits are used for the treatment of vata, pitta,

diarrhoea diabetes, leucorrhoea, fever, skin diseases and general debility. Therefore, the present study was designed to explore the preliminary phytochemical analysis of *Syzygium caryophyllatum* leaf, fruit, seed and bark for their pharmacological properties.

Materials and Methods

Plant material and sample preparations

S. caryophyllatum plant samples (Leaves, bark and fruits) were collected from Thiruvananthapuram district of Kerala, India during 2018 and voucher specimen (KUBH 10255) was prepared, identified and deposited at the Department of Botany, University of Kerala. The bark, leaves, fruit pulp and seeds were separately dried at below 40 °C. Powdered sample of *S. caryophyllatum* bark, leaves, fruit pulp and seeds was sequentially extracted with hexane, ethyl acetate, acetone, methanol and water in their increasing polarity by soxhletion. The extracts were filtered through Whatman No.1 filter paper and the solvents were evaporated under reduced pressure. Dried extracts obtained were stored in 4 °C until further analysis.

Preliminary phytochemical screening

Different qualitative chemical tests were done for establishing profile of given extract for its chemical composition. The following tests were performed on extracts to detect various phytoconstituents present in them^[6, 7, 8].

Detection of Alkaloids

Solvent free extract, 50 mg is stirred with few ml of dilute hydrochloric acid and filtered. The filtrate is tested carefully with various alkaloid reagents as follows.

Mayer's test

To a few ml of filtrate, one or two drops of Mayer's reagent was added through the side of the test tube. A white or creamy precipitate indicates the test as positive.

Dragendorff's test

To a few ml of filtrate, 1 or 2 ml of Dragendorff's reagent are added. A prominent yellow precipitate indicates the test as positive.

Detection of Anthocyanin

To the substance, 10% NaOH was added, blue color shows the presence.

To the substance, concentrated sulphuric acid was added. Yellowish orange color confirms the presence.

Detection of Coumarins

To the extract, added alcoholic KOH or NaOH. A yellow color appears which will disappear on adding Con. HCl.

Detection of Flavonoids

Three different methods were used for the determination of flavonoids in the plant samples.

Alkaline reagent test

Extracts were treated with few drops of NaOH solution. Formation of intense yellow color, which become colorless on addition of dilute acid.

To a portion of the aqueous filtrate of extract, 5 ml of dilute ammonia solution was added, followed by addition of concentrated sulphuric acid. Yellow coloration in extract indicates the presence of flavonoids.

A portion of the filtrate was heated with 10 ml of ethyl acetate over steam bath for 3 minutes. The mixture was filtered and 4 ml of filtrate was shaken with 1 ml of dilute ammonia solution. Yellow coloration indicates the presence of flavonoids.

Detection of Glycosides

Hydrolysed 50 mg of extract with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following test.

Bontrager's test ^[9]

To 2ml of filtered hydrolysate, 3ml of chloroform is added and shaken, chloroform layer is separated and 10% ammonia is added to it. Pink color indicates the presence of glycosides.

Detection of Phenolic compounds**Ferric chloride test**

The extract (50mg) is dissolved in 5ml of distilled water. To this few drops of neutral 5% ferric chloride solution were added. A dark green color indicates the presence of phenolic compounds.

Gelatin test

The extract (50mg) was dissolved in 5ml of distilled water and added 2ml of 1% solution of gelatin containing 10% sodium chloride to it. White precipitate indicates the presence of phenolic compounds.

Detection of Saponins ^[10]**Froth test**

Extracts were diluted with distilled water to 20ml this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam test

Shaken 0.5 gm. of extract with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

Detection of Steroids**Liebermann-Burchard's test**

Added 2ml of acetic anhydride to 0.5 g extract of each sample with 2 ml of sulphuric acid. The color change from violet to blue or green indicates the presence of steroids.

Detection of Tannins**Gelatin test**

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence.

To the extract, ferric chloride was added, formation of dark blue or greenish black showed the presence.

Detection of Terpenoids**Salkowski Test**

Mixed 5 ml of extract with 2 ml of chloroform and carefully added 3 ml of concentrated sulphuric acid to form a layer. A reddish brown coloration at interface was formed to show positive result for the presence of terpenoids.

Detection of Quinone

The extract is mixed with concentrated sulphuric acid; resulting red color indicates the presence of quinone.

Carbohydrate and Reducing sugar

The small quantities of the filtrate will be dissolved in 4ml of distilled water and filtered. The filtrate will be subjected to,

Fehling's test: The extract was treated with Fehling's reagent A & B. The appearance of reddish brown colour precipitate indicated the presence of reducing sugar.

Benedict's test: The extract was treated with Benedict's reagent. Appearance of reddish orange colour precipitate indicated the presence of reducing sugar.

Iridioids

The extract was added to 1 ml of reagent (10 ml acetic acid, 0.2% CuSO₄ and 0.5 ml conc.HCl). The mixture was heated over a small flame. Development of a light blue colour indicates the presence.

Results

The present study was carried out on the plant samples revealed the presence of medicinally important bioactive compounds. The plant *Syzygium caryophyllatum* (leaf, fruit, seed and bark) showed the presence of steroids, terpenoids, saponins, tannins, phenol, flavonoids, quinone from different solvent extract (Table 1- 4). Hexane extract, showed negative result for phytochemicals. Majority of the phytochemicals were present in methanolic extract of leaf, fruit, seed and bark. Phenols, flavonoids and terpenoids were present in both acetone and methanolic extract of plant parts.

Discussion

Phytochemical analysis conducted on the plant extracts has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Analysis of the plant extracts revealed the presence of phytochemicals such as alkaloids, glycosides, terpenoids, steroids, saponins, flavonoids, phenols, quinones, coumarins and tannins. Majority of the phytochemicals were present in the methanolic extract. The various phytochemical compounds detected are known to have beneficial importance in medicinal sciences. For instance, Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity [11]. Several workers have reported the analgesic [12, 13] antispasmodic and antibacterial properties of alkaloids [14, 15]. Glycosides are known to lower the blood pressure according to many reports [16]. The terpenoids have been shown to decrease blood sugar level in animal studies [17]. The triterpinoids have analgesic properties [18]. Plant steroids are known to be important for their cardiostimulant activities and also possess insecticidal and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics [19]. Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions; hemolytic activity, cholesterol binding properties and bitterness [20, 21]. Flavonoids were found in the extracts and are potent water soluble antioxidants [22]. Flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities [23]. Tannins were reported to exhibit antiviral, antibacterial and anti-tumour activities. It was also reported that certain tannins were able to inhibit HIV replication selectively and was also used as diuretic [19]. The results obtained in this study indicates that phytochemical compounds are the bioactive constituents and this tropical plant *Syzygium caryophyllatum* is proving to be a valuable reservoir of bioactive compounds of potential health benefits. More research is needed to fully explain the actions of these phytochemical compounds in the human body.

Table 1: Preliminary phytochemical screening of *Syzygium caryophyllatum* leaf

Phytochemical Constituents	Hexane	Ethylacetate	Acetone	Methanol	Aqueous
Alkaloids	-	-	-	+	-
Coumarins	-	-	-	+	-
Flavonoids	-	-	+	+	-
Glycosides	-	-	-	+	+
Phenols	-	+	+	+	+
Terpenoids	-	+	+	+	+
Saponins	-	-	-	+	-
Tannins	-	-	-	+	+
Reducing sugars	-	-	-	-	-
Carbohydrates	-	-	-	+	-
Quinones;	-	-	-	+	+
Iridoids	-	-	+	+	-
Steroids	-	+	+	+	+
Anthocyanins	-	-	-	-	-

+ indicates presence; - indicates absence

Table 2: Preliminary phytochemical screening of *Syzygium caryophyllatum* fruit

Phytochemical Constituents	Hexane	Ethylacetate	Acetone	Methanol	Aqueous
Alkaloids	-	-	-	+	-
Coumarins	-	-	-	+	-
Flavonoids	-	-	+	+	-
Glycosides	-	+	+	+	+
Phenols	-	-	+	+	+
Terpenoids	-	+	+	+	+
Saponins	-	-	-	+	-
Tannins	-	-	-	+	+
Reducing sugars	-	-	-	+	-
Carbohydrates	-	-	-	+	-
Quinones;	-	+	+	+	+
Iridoids	-	-	-	-	-
Steroids	-	+	+	+	+
Anthocyanins	-	+	-	+	-

+ indicates presence; - indicates absence

Table 3: Preliminary phytochemical screening of *Syzygium caryophyllatum* seed

Phytochemical Constituents	Hexane	Ethylacetate	Acetone	Methanol	Aqueous
Alkaloids	-	-	-	+	+
Coumarins	-	-	-	+	-
Flavonoids	-	-	+	+	-
Glycosides	-	+	+	+	-
Phenols	-	+	+	+	+
Terpenoids	-	+	+	+	+
Saponins	-	-	-	+	-
Tannins	-	+	+	+	+
Reducing sugars	-	-	-	-	-
Carbohydrates	-	-	-	+	-
Quinones;	-	+	+	+	+
Iridoids	-	-	-	-	-
Steroids	-	+	+	+	+
Anthocyanins	-	+	-	+	-

+ indicates presence; - indicates absence

Table 4: Preliminary phytochemical screening of *Syzygium caryophyllatum* bark

Phytochemical Constituents	Hexane	Ethylacetate	Acetone	Methanol	Aqueous
Alkaloids	-	-	-	+	+
Coumarins	-	-	-	+	-
Flavonoids	-	-	+	+	-
Glycosides	-	+	+	+	+
Phenols	-	+	+	+	+
Terpenoids	-	+	+	+	+
Saponins	-	-	-	+	-
Tannins	-	+	+	+	+
Reducing sugars	-	-	-	-	-
Carbohydrates	-	-	-	-	-
Quinones;	-	+	+	+	+
Iridoids	-	-	-	-	-
Steroids	-	+	+	+	+
Anthocyanins	-	+	+	-	-

+ indicates presence; - indicates absence

Conclusion

The results revealed the presence of medicinally important phytochemical constituents in the methanolic extracts of *Syzygium caryophyllatum*. Additional work is encouraged to elucidate the possible mechanism of action of these extracts.

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