Research Article

Analysis of Bioactive Compounds Present in *Syzygium caryophyllatum* (L.) Alston Fruit

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ABSTRACT

Syzygium caryophyllatum (L.) Alston is red listed as a threatened species according to International Union of Conservation of Nature and Natural Resources. The various phytochemicals present in the fruit of this plant were analysed using Liquid Chromatography Mass Spectroscopy (LCMS) and their bio activities were predicted using Prediction of Activity Spectra for Substances (PASS). Plants produce a variety of secondary metabolites that are not needed for the growth of plants but are essential for other uses such as protection, pollination etc. The results showed the presence of numerous phytochemicals such as terpenoids, carotenoids, alkaloids, carboxylic acids, and phenolic compounds such as flavanoids, other compounds such as coumarins, saponins, glycosides and sterols. The activity spectra of compounds were predicted using the chemical formula reveals that the compounds present in pulp and seed are active against numerous diseases. Many pharmaceutical industries use computer aided programs for the designing of new drugs for diseases. The present study clearly shows the medicinal uses of the plant, signifies the consumption of raw fruit for preventing diseases and will be helpful for pharmaceutical industries for the designing of new drugs for diseases. The work also emphasises the need for conservation of the species.

Keywords: Secondary metabolites, phytochemicals, LCMS analysis, PASS, sickle cell anaemia, gaucher disease.

INTRODUCTION

Plants have been used as source of food and medicine by man from the beginning. Effective remedy of ailments with minimal side effects is provided by use of plants. The use of herbal medicines in India can be cited about 5000 years back. Recent studies made by the WHO state that 80% of the world populations rely on herbal medicines for their preliminary healthcare and in developing countries its use may be about 95%. Some countries like US show continuous increase in its use each year.[1] Any part of the plant leaves, stem and stem bark, flowers, fruits, seeds, root, root bark or the plant as a whole may be used as medicine. The medicinal properties of plants are due to the wide variety of secondary metabolite like the flavanoids, alkaloids, tannins, phenols, terpenoids etc, that are produced as an intermediate or by product of the reactions of primary metabolites. They are sometimes produced as response to adverse environmental condition in particular stages of development, in specialized cells. Some secondary metabolites may also be species specific therefore these phytochemicals are also very important for taxonomic research.[2] They may be used by plants for defense mechanism, attractants as pollinators, seed dispersal, allelopathic agents, UV protectants, and signal molecules for nodule formation.[3] The secondary metabolites are not needed for the growth of the plant but they have wide range of chemical structures and biological reactions. These bioactive compounds provide medicinal property to a plant.

Therefore the identification and characterization of these compounds in crude plant extracts are very essential, which can further lead to isolation and purification so that it may be of use in pharmaceutical industry.

Syzygium caryophyllatum (L.) Alston, is a member of Myrtaceae family with edible fruits. Van Rheed (1678 – 1703) has mentioned it as '*njara*' in the *Hortus Malabaricus* the first published work about the plants of Malabar.[4] It is a small tree found in places where there is water.[5] It is categorised as Endangered under the Red List category of the IUCN Red List of Threatened species.[6] The leaves, bark and root of *Syzygium caryophyllatum* shows the presence of alkaloids, flavanoids, phenols, tannins, saponins, glycosides, triterpenoids, fats and fixed oils, the leaves of this plant shows hypoglycemic activity on alloxan induced diabetic mice and potent antioxidant activity was shown by root extracts of this plant.[7-9]

The tree bears black globose fruits which are edible. The fruits are green when unripe and then it turns pale pink, pink and purplish black on maturity. The fruit bears a crown of persistent calyx. So far little study dealing with its fruits have been reported. Therefore the study aims to identify the different compounds present in the pulp and seed of fruit of *Syzygium caryophyllatum* (L.) Alston by conducting the LCMS and PASS analysis and also to study its medicinal and nutraceutical properties.

MATERIALS AND METHODS

Taxonomic description of the plant

Syzygium caryophyllatum (L.) Alston, is a tree up to 6 m high; bark thick, reddish-brown; branchlets terete. Leaves simple, opposite, exstipulate; petiole up to 4 mm long, stout, glabrous; lamina 3-8 x 1.3-3.5 cm, obovate or obovate-oblong, base attenuate or acute, apex obtuse,

obtusely acute or emarginate, margin entire, glabrous, coriaceous, brown on drying, pellucid-dotted; lateral nerves many, close, slender, prominent looped at the margin forming intramarginal nerve. Flowers bisexual, white, up to 5 mm across, in terminal corymbose cymes, inflorescence branches moderately thick, ascending. Calyx tube 2-2.5 mm long, turbinate, no thick disc. Petals calyptrate. Stamens numerous, bent inwards at the middle when in bud, 2.5-3.5 mm long. Ovary inferior, 2-celled, ovules many; style 1; stigma simple. Fruit a berry, up to 5 mm across globose, green then turns purplish black when ripe.

Collection and authentication

The ripe fruits of *Syzygium caryophyllatum* (L.) Alston were collected from various localities and these collected samples were authenticated from the Regional Herbarium Kerala (RHK) at S. B College, Changanacherry, Kerala.

About 100 fruits were collected from each locality and they were rapidly processed on the same day. The collected fruits were rapidly washed under running tap water to clean off dust and dirt particles. Wrinkled fruits were discarded. The rest of the fruits were dried at room temperature using an air blower. Fully dried fruits were packed in polypropylene bags sealed and stored at -20° C for future use.

Extraction

The pulp (fleshy part) along with its outer peel was separated from the seed manually. The residue of the pulp which was left on the seeds were washed off and the seeds were taken. Both (pulp with peel and seeds) were dried under hot – air oven and then ground into a fine powder in a coffee grinder and stored in airtight containers. 3 g of the pulp and the seed were separately extracted using petroleum ether (60 – 80◦C) and methanol sequentially using 30ml of the corresponding solvent. The extracts were then dried and dissolved in 10ml petroleum ether and ethanol (HPLC Grade, Merck). It was then filtered through 0.20mm membrane filter. The extract was used for this analysis.

LCMS analysis

10µl of the filtered sample was then injected to the manual injector using a micro syringe (1 - 20µl, Shimadzu).The mobile phase used was water: methanol (50: 50) in an isocratic mode. The column used was RP – C – 18 (phenomenex). The separated compounds were then ionized using APC method and using split mode (50: 50). The flow rate was maintained to 2ml/mn with a temperature 25± 2°C. The class VP integration software was used for the data analysis. The Library used for the analysis was Metwin – LS. The version of the library was version 1.0 – 52.09.

PASS

The activity spectra of the phytochemical compounds were predicted using the computer programme PASS (Prediction of Activity Spectra for Substance).[10-12]

RESULTS AND DISCUSSION

The primary and secondary metabolites are the wide range of organic compounds synthesized by plants. Apart from carbohydrates, lipids, proteins and nucleic acids primary metabolites include phytosterols, amino acids and organic acids. These have vital role in plant metabolism such as photosynthesis, respiration, growth and development whereas secondary metabolites that is comparatively produced in higher concentration in some species of plants is not used in plant metabolism rather have other functions like protection from microbes, attractants for pollination, allelopathic agents, UV protectants, signal molecules etc. Secondary metabolites are also used as waxes, gums, dyes, flavoring agents, drugs, perfumes etc.

Primary metabolites

The primary metabolites present in the pulp and seed of *Syzygium caryophyllatum* (L.) Alston are as follows:

Sugars

Fructose was detected in the pulp. The presence of sucrose and deoxyribose was detected from seeds.

Amino acids

Amino acids present in pulp were asparagine and cystine and serine was obtained from seed. Amino acid derivative like Diaminobutyric acid was obtained from pulp. Amino acid related compound (Betalins) like Betalamic acid was present in pulp and Dopaxanthin was present in seed.

Fatty acids and Lipids

Table 1: Shows the presence of Fatty acids present in pulp and seed.

	Pulp	Seed
Hexanoic acid	√	-
Linoleic acid	√	-
Linolenic acid	√	-
Alpha linolenic acid	-	√
Alpha licanic acid	-	√
Palmitic acid	**√**	**√**
Stearic acid	-	√
Decanoic acid	-	√

Palmitic acid is the only fatty acid present in both pulp and seed. Hexanoic acid, linoleic acid and linolenic acid were present in the pulp and alpha linolenic acid, alpha licanic acid, decanoic acid and stearic acid were present in the seed.

Secondary metabolites

The secondary metabolites are those that instigate more curiosity among the researchers since they are protective in function and lower the risk of most diseases. The

secondary metabolites that was present in the pulp and seed of *Syzygium caryophyllatum* (L.) Alston are as follows:

Terpenoids

Terpenoids play a very important role in the growth, development, reproduction and defense of plants. Its presence gives plants fragrance.[13] Both pulp and seed reported its presence.

Table 2: Shows the presence of terpenoids

	Pulp	Seed
Alpha vetivone	-	√
Betulin	√	-
Isodonal	-	√
Lupulone	-	√
Phytol	√	-
Beta citronellal	**√**	**√**
Friedelin	**√**	**√**

Beta citronellal and Friedelin were found in both seed and pulp.

Carotenoids

Carotenoids give plants economical value in ornamental garden. They are also used as food addictives. They play a significant role in Vitamin A metabolism which is an uncompromising factor for growth and development in children.[14] The pulp of *Syzygium caryophyllatum* (L.) Alston contained crocetin.

Alkaloids

Very few alkaloids were present in pulp and seed. Alkaloids usually provide bitter taste but it also has other significant uses. They also exhibit numerous bioactivities.[13] Nicotine and nornicotine were present in pulp hydroquinidine and ambellin in seed.

Carboxylic acid

Table 3: Carboxylic acids present were as follows

Pulp	Mevalonic acid
	Ethyl paramethoxy cinnamate
Seed	Pipecolic acid
	Azelaic acid
	Lupic acid
	Succinic acid
	Melilotic acid

Phenolic compounds

Simple phenols

Simple phenol compounds like methyl phenol was present in pulp and hydroquinone in the seed.

Flavonoids

The flavonoids are the polyphenols with C_6– C_3 - C_6 structure. They show very good antioxidant activity, anticancer activity etc.[15]

Table 4: Shows presence of flavonoids

	Pulp	Seed
Liquiritegenin	√	
Quercetin	**√**	**√**
Kaempferol	√	
Isoquercetin		√
Dihydroxyflavan		√
Kaempferide		√
Quercemeritrin		√
Hydroxyflavan		√
Quercetin methyl ester		√

More number of flavanoids were found in the seed. Quercetin was present both in the pulp and seed.

Phenyl propanoids

They are organic compounds that are synthesized by plants from the amino acid phenylalanine. Caffeic acid glucoside was present in pulp and methoxy cinnamaldehyde in seed.

Phenolic acids

Phenolic acids are of two types as derivatives of benzoic acid and derivatives of cinnamic acid.

Table 5: Shows presence of phenolic acids

Pulp	Parahydroxy benzoic acid
	Hydroxymethyl benzoicacid
	Ferulic acid
	Caffeic acid
Seed	Gallic acid
	Ferulic acid

Caffeic acid is abundantly found in most fruits and vegetables and ferulic acid is common in cereals.[16] Ferulic acid was found to be present in both pulp and seed.

Coumarins

Coumarins mainly occur in higher plants its concentration being the highest in fruits followed by roots, stem and leaves. Coumarins are benzopyrones having anticoagulant, free radical scavenging, anticancer, anti-inflammatory and many such activities.[17]

Table 6: Coumarins present in the pulp and seed are tabulated below.

	Pulp	Seed
Xanthotoxin		√
Xanthatoxin	√	
Xanthatoxol	√	
Xanthotoxol		√
Scopoletin	√	
Wedelolactone		√

Coumarins such as Xanthotoxin, xanthotoxol and Wedelolactone were present in seed. Xanthatoxin, Xanthatoxol and scopoletin were present in pulp.

Saponins

Saponins are compounds that form foams and act as emulsifying agents. They have expectorant, anti-inflammatory activity and can inhibit cholesterol adsorption, besides they also have good hypertensive, cardiac depressant properties, and insecticidal, antifungal activities etc.[18,19] Acetyl oleonolic acid was found in pulp and seed.

Apart from these other phytochemicals like Jambolin a glycoside was present in pulp as well as seed. Ergosterol was present in seed. Ergosterol is considered as provitamin of calciferol.

PASS Activity of compounds

The biological activity spectrum is defined as a compounds intrinsic property depending on its structure and physiochemical activity. It depends on nature of compound such as structure and physicochemical property, the sex, age and type of the target species and also the mode and route of treatment. Various comparisons and algorithms are used to suggest its activity against specific target. Nowadays many of the pharmaceutical industries are using Computer Aided Drug Design for their new drug research and development, and PASS has proven to be 300% more than the estimation by skilled expert.[11]

Table 7: Showing activity of compounds

Caffeic acid	Membrane integrity agonist, Mucomembraneous protector, Apoptosis agonist, Hypercholesterolemic, Choleretic, Sickle cell anaemia treatment, Cytoprotectant, Antihypoxic, Antiseborrheic, Eye irritation, Fibrinolytic, Pulmonary hypertension treatment
Ferulic acid	Membrane integrity agonist, Mucomembraneous protector, Apoptosis agonist, Hypercholesterolemic, Choleretic, Cytoprotectant, Pulmonary hypertension treatment, Myocardial ischemia treatment, Eye irritation, Fibrinolytic, Carminative, Vasodilator – cerebral, Sigma receptor agonist.
Gallic acid	Creatininase inhibitor, Sickle cell anaemia treatment, Fibrinolytic, Antiseptic, Mucomembraneous protector, Antiseborrheic, Antiinflammatory – intestine, Free radical scavenger, Coccolysin inhibitor, Pitrilysin inhibitor, Non mutagenic – Salmonella, Cardioprotectant.
Palmitic acid	Sickle cell anaemia treatment, Fibrinolytic, Mucomembraneous protector, Antiseborrheic, Skin disease treatment, Sclerosant, Carnosine synthesis inhibitor, Cholesterol synthesis inhibitor, Hypercholesterolemic, Lipid metabolism regulator, Antihypoxic, Antiviral (Arbovirus), Cytoprotectant, Alopecia treatment, Eye irritation (inactive), antithrombotic, platelet adhesion inhibitor, proline racemase inhibitor, acidifying agent gastric, Antimutagenic, erthropoietin, Leucolysin inhibitor, fibrinolytic, antitoxic, metabolic disease treatment, corticosteroid side – chain isomerase treatment.
Quercetin	Vascular (peripheral) disease treatment, membrane integrity agonist, capillary fragility treatment, membrane permeability inhibitor, antiseborrheic, antineurotoxic, cytoprotectant, mucomembraneous protector, chemoprotective.
Xanthotoxol	Radio protector, dependence treatment, vascular (peripheral) disease treatment, amyotrophic lateral sclerosis treatment, membrane integrity agonist, myocardial ischemia treatment.
Linoleic acid	Gaucher disease treatment, skin disease treatment, muco membraneous protector, lipid metabolism inhibitor, antiseborrheic, antithrombotic, Pulmonary hypertension treatment, antiviral (arbovirus), cytoprotectant, sickle cell anaemia treatment, eye irritation treatment, sclerosant, platelet adhesion inhibitor, skin irritation, hypercholesterolemic, cholesterol synthesis inhibitor.
Friedelin	Hypercholesterolemic, hepatic disorder treatment, cardiovascular analeptic.
Pipecolic acid	Proline racemase inhibitor, fibrinogen receptor antagonist, neuroprotector, convulsant, opoid dependency treatment, amyotrophic lateral sclerosis treatment, fibrinolytic, acute neurologic disorder treatment, gaucher disease treatment, dopamine release stimulant, urologic disorders treatment.

CONCLUSION

Syzygium caryophyllatum (L.) Alston is a small sized tree with edible fruits which is naturally distributed near ponds and water bodies. In the present study the phytochemical analysis of fruit and seed of *Syzygium caryophyllatum* using Liquid Chromatography Mass Spectroscopy (LCMS) shows the presence of various secondary metabolites. Terpenoids like beta citronellal and friedelin, flavanoids such as Quercetin and Phenolic acids such as ferulic acid were obtained from both pulp and seed. It is seen that more carboxylic acid and flavanoids compounds were obtained from seed. The coumarins such as Xanthotoxin, xanthotoxol and Wedelolactone were present in seed; xanthatoxin, xanthatoxol and scopoletin in the pulp. Other phytochemicals like acetyl oleonolic acid - saponin; jambolin – glycoside were obtained from seed and pulp. Very few alkaloids were obtained from the pulp and seed. The medicinal property of the plant may be due to the presence of these phytochemicals.

The Prediction of Activity Spectra for Substances of selected compounds points out that they are active against various diseases. Compounds such as Caffeic acid, Palmitic acid, Gallic acid and Linoleic acid showed >70% activity against sickle cell anaemia; along with these compounds Quercetin activity predicted as mucomembraneous protector. Pipecolic acid and Linoleic acid is predicted to be active for gaucher disease treatment. Pipecolic acid shows neuroprotector activity. The study serves as a stepping stone for designing novel drugs using this plant and can be recommended for use after preclinical trial using animals and clinical trials using humans. Even though the fruits are edible its consumption is less, it may be due to small size of fruit or lack of availability. *Syzygium caryophyllatum* is listed as endangered species by IUCN it may be due to the destruction of habitat of the species for urbanization and industrialization. The results obtained from our study shows that the plant has many significant compounds that are of pharmaceutical importance and therefore its conservation is very important.

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