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PHYTOCHEMICAL ANALYSIS OF COCCINIA GRANDIS (LINN.) VOIGHT FRUIT USING LC-MS

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Abstract
Coccinia grandis (Linn.) Voight commonly known as ivy gourd a member of family Cucurbitaceae, is one of the nutritionally and medicinally important plant. A number of medicinal properties such as anti- diabetic, anti-diarrheal, anti-inflammatory, hypoglycemic, anti-obesity, anti- ulcer, anti-oxidant, anti-angiogenic property have been ascribed to this fruit of high economic value. The phytochemicals are those chemicals that are not established as a food nutrients but acts as a healing agent for different diseases in human beings. Phytochemical analysis of fruit extract through LC-MS analysis reveals the presence of primary metabolites like carbohydrates, Proteins, fatty acids, amino acids and secondary metabolites like alkaloids, terpenoids, coumarins, plant sterols ,phenolics acids, flavonoids etc., The present study aimed to screen the phytochemicals present in the fruit using Liquid Chromatography Mass Spectrophotometry (LC-MS) and their activities predicted using Prediction of Activity spectra of Substance(PASS).

Keywords: LC-MS, Ivy gourd, Phytochemicals, Secondary metabolites ,PASS

Introduction

Vegetables are very rich sources of essential biochemicals and nutrients such as carbohydrates, carotene, protein, vitamins, calcium, iron, ascorbic acid and palpable concentration of trace minerals. These vegetables will continue to remain the basic source of energy for the developing countries (Akwaowo et al., 2000). Phytochemicals with antioxidant capacity naturally present in food are of great interest due to their beneficial effects on human health as they offer protection against oxidative deterioration.

Cucurbitaceae is economically very important being the major source of food and forage and its great diversity (ranked as fifth largest family in flowering plants) has also attracted much interest in ecological as well as systematic studies. Most of the members of Cucurbitaceae have tendrils, and this ancestral condition presents a clear morphological synapomorphy for the family; evolutionarily, these tendrils are modified shoots (Lassing, 1997)Cucurbitaceae species are valued for nutritional and medicinal purposes. Cucurbits are an excellent fruit in nature having composition of all the essential constituents required for good health of humans (Duke, 1999)

The *Coccinia grandis* (Linn.) Voight, commonly known as ivy gourd come under family cucurbitaceae, is a vegetable grown in subtropical and tropical areas of South East Asia, South Asia and Africa(Lim 1996). Every part of this plant is valuable in medicine and various preparations have been mentioned in indigenous system of medicine for various skin diseases, bronchitis and Unani systems of medicine for ring worm, psoriasis, small pox, scabies and other itchy skin eruptions and ulcers. It has antilithic, hypolipidimic, antimutagenic and hypoglycemic activities(Chopra and Bose,1925). Phytochemicals are defined as the substances found in edible fruits and vegetables that exhibit a potential for modulating human metabolism in a manner beneficial for the prevention of chronic and degenerative disease. Phytochemical screening is of

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paramount importance in identifying new source of compound having medicinal significance, to make the best and judicious use of available natural wealth (Tripoli et al., 2007).

The objective of the study is to identify the different compounds present in the fruit pulp of Coccinia grandis Linn Voight using LC-MS technique and also list out the various phytochemically important compounds.

Materials and Methods

Plant selected for the study

Taxonomical description of *Coccinia grandis* (Linn.) *Voight*

Slender scandent or postrate herbs ; roots sometimes tuberous; tendrils simple. Leaves petiolate, deltoid or subrotund, angled or lobed, sometimes glandular beneath. Flowers large, white, dioecious; female solitary. Calyx campanulate, short; lobes 5. Corolla campanulate, shortly 5 - fid. Stamens 3; Filaments connate in a column , rarely free ; anthers connate, 1 1 – celled , 2 2- celled , cells conduplicate; in female flowers staminodes 3, oblong or subulate .Ovary ovoid, oblong or linear; ovules many, horizontal, from 3 placentas; style slender; stigma 3; pistillode in female flowers 0. Fruit an ovoid or oblong indehiscent berry. Seeds ovoid , compressed , margined ; the testa smooth. Rapidly growing, perennial climber, Stems are mostly glabrous, produced annually from a tuberous root stock with ovate or 5-angular Leaves alternate 5.9 X 4.9 cm, deeply cordate at the base, margins entire or sinuate and often with distinct reddish glandular teeth, glabrous, punctuate,petiole 1-5 cm long. Tendrils simple, axillary. Flowers white, arranged in leaf axil;unisexual, Calyx of five subulate, recurved lobes, 2-5mm long ; Corolla companulate 3 - 4.5 cm long ; Stamens 3, present as staminoids in female flower ; Ovary inferior; Fruits ellipsoid –oblongoid or cylindrical, 3.5 - 5 X 1.3 - 2.5 cm ; Seeds somewhat ovoid.rounded at the apex ,slightly papillose, much compressed.

Collection and Authentication

The fruits of *Coccinia grandis* were collected from different localities and are authenticated from the Regional Herbarium of S.B. College (RHK), Changanacherry, Kerala. The voucher specimens of the plant sample were deposited in the Herbarium of S.B. College Changanacherry.

Extraction

The fruits collected from the field and dirt were removed from the fruits prior to analysis. Then samples were manually washed with distilled water and dried at room temperature. The fruits were sliced in to two halves and dried under hot-air oven. Samples were ground in a Coffee grinder then stored in airtight container. 2g each of the fruit sample were separately extracted using Petroleum ether (60-80) and methanol sequentially using Soxhlet's apparatus for about 3 hours each using 30 ml of the corresponding solvent. The extracts were then dried and dissolved in 10 ml petroleum ether/methanol (HPLC Grade, Merck). It was then filtered through 0.20 mm membrane filter. The extract was used for this analysis.

LC-MS 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-C18(phenomenex). The separated compounds were then ionized using APC method using the split mode (50:50). The flow rate was maintained to 2 ml/mn with temperature 25±2° C. The class VP integration software were 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

Any biologically active compounds has a wide spectrum of effects. Computer Program PASS (Prediction of Activity Spectra for Substance) provides the activity spectra of compound (Filimonov *et al* 1995).

Result and Discussion

Phytochemicals are bioactive non-nutrient plant compounds that have protective or disease preventive property. Vegetables contain almost all the nutrients required for the functioning of the body. The diverse variety of organic compounds in vegetables represent the product of primary and secondary metabolism. The primary and secondary metabolites are the wide range of organic compounds synthesized by plants. Primary metabolites such as carbohydrates, amino acids, proteins, fats, chlorophylls *etc* are involved in the growth and development, respiration and photosynthesis, hormone and protein synthesis. Plant produce diverse compounds that have no direct participation in the role of growth and development are secondary metabolites. Secondary metabolites such as flavanoids, carotenoids, sterols, phenolic acids, alkaloids and glucosinolates determine the color of vegetables, protect plants against herbivores and microorganisms, attract pollinators and seed-dispersing animals and act as signal molecules under stress conditions. Some of the potential human benefits of Secondary metabolites include modulation of the immune system, anti-inflammation, anti-cancer, anti-viral, anti-bacterial, anti-toxic, hepatoprotective, antioxidant, anti-estrogenic, anti-atherosclerosis and cholesterol reduction (Crozier *et al* 2006).

Primary Metabolites

The primary metabolites present in the fruits of Coccinia grandis (Linn.) Voight are as follows.

Carbohydrates

Carbohydrates in vegetables occur as sugar monosaccharides, disaccharides, sugar alcohol, oligosaccharides and polysaccharides. Sugars like galactose, maltose, fructose *etc* are present in the fruit.

Amino acids

Aminoacids like proline, methonine, cystine, marasmic acid are obtained from the fruit. Amino acid derivative like Aminobutyric acid, methyl Amino L Alanine obtained from the fruit.

Essential fatty acids

The EFA mainly contribute towards the production of prostaglandins which regulate body functions such as heart rate, blood pressure, blood clotting, fertility, conception, and also play positive role in immune system by regulating inflammation and encouraging the body to fight against infections (Yehuda *et al* 2005). Fats play a vital role in maintaining health skin and hair, by insulating body organs against shock, maintaining body temperature and promoting health cell function.Arachidic acid, Linoleic acid, Cucurbic acid, Ricinoleic acid. are present in the fruits. Linoleic acid is one of the important Essential Fatty Acid. That play a key role in preventing many diseases and abnormal differentiation problems.

Organic acid

Organic acids give the vegetables tartness, and affect flavour by acting on the perception of sweetness (Fisher and Scott 1997), Organic acids influence the colour of vegetables since many plant pigments are natural pH indicators (Davies 1973) .Organic acids like Rosmarinic acid, Pipecolic acid, Succinic acid are present in the fruit.

Secondary metabolites present in the fruit

Coumarins

Coumarins are of great attention due to their therapeutic property. Coumarin comprise a very large class of compounds in plant kingdom. Xanthotoxol, Khellol glucosides are present in the fruit.

Phenolic compounds

Plant phenolics are the widest spread secondary metabolites in plant kingdom. Phenolics are defined as a class of polyphenols which are important secondary metabolites present in plants (Slade et al., 2005)and are also responsible for their antioxidant action and various beneficial effects in a multitude of diseases (Hotta *et al.*, 2002). Phenolic compounds are

considered beneficial for human health ,decreasing the risk of degenerative diseases by reduction of oxidative stress and inhibition of macromolecular oxidation(Larson, 1998). Flavonoids and phenolics acids are the most important groups of secondary metabolites and bioactive compounds in plants (Kim *et al.*, 2003).

Flavanoids

Flavanoids have been reported to exert a wide range of biological activities. These includes: anti-inflammatory, antibacterial, antiviral, antiallergic (Murray, 1998),cytotoxic antitumour, treatment of neurodegenerative diseases, vasodilatory action. Flavonoids are potent water soluble anti-oxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity (Okwu and Josaiah ,2006). Flavonoids like Hyperoside, Hydroxyflavan, Vitexin are present in the fruit. Vitexin is cardio protective. It exhibited potent hypotensive, anti-inflammatory, anti-metastatic potential and anti-spasmodic properties(Lu et al., 2013). Hyperoside is associated with several potent pharmacological activities which include anti-inflammatory, anti-thrombotic, ant diabetic, anti-viral, anti-fungal, hepato-protective, and antioxidant protective effects (Huang ,2008).

Phenolic Acid

Phenolic acids are secondary metabolites extensively spread throughout the plant kingdom. Phenolic compounds confer unique taste, flavour, and health promoting properties found in vegetables and fruits .Therefore, increasing the phenolic content in plants can enhance their quality**.** Phenolic Acids like Caffeic acid, Hydroxycinnamic Acid are present in the fruit.

Alkaloids

Alkaloids are significant for the protecting and survival of plant because they ensure their survival of plant against micro-organisms (antibacterial and antifungal activities), insects and herbivores (Feeding deterrens) and also against other plants by means of allelopathically active chemicals (Molyneux *et al.*, 1996). Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity. β Erythroldiene, Septentrionine, Vasicinol, Heliotrine etc are present in *Coccinia grandis* (Linn.)Voght. The presence of alkaloids in all the solvent fractions could be well correlated with the antimicrobial activities (Ramkumar, 2007). These phytochemicals possesses specific physical, chemical and biological activities that make them useful as drugs.

Sterols

Plant sterols may possess anti-cancer, ant atherosclerosis, anti-inflammation, and anti-oxidant activities(Awad and Fink, 2000). Ergosterol, Campesterol are present in *Coccinia grandis* fruit.

Terpenoids

Terpenoids like Carnosol, betalin, delcorine ect are present in fruit. Carnosol is known as a promising anti-inflammatory, ant carcinogenic, antibacterial, and ant oxidative agent in both *vitro* and *in vivo* experimental models (Johnson, 2011).Carnosol was more effective at scavenging hydroxyl radicals and protecting DNA than vitamin C and vitamin E (Lo *et al.*, 2002). Carnosol had an inhibitory activity against lipid peroxidation and had a promoting effect on antioxidant enzymes in the liver of mice (Zeng *et al.*, 2001).

Pass activity

Caffeic acid

Membrane integrity, agonist, Mucomembrane protector, Apoptosis agonist, Hypercholed-terolemic, Cholerectic, Sickel cell anaemia treatment, Cytoprotectant, Antihypoxic, Antiseborrheic, Eye irritation, Fibrinolytic, Pulmonary Hypertension Treatment.

Linoleic acid

Skin disease treatment, mucomembraneous protector, lipid metabolism inhibitor, antiseborrheic, antithrombotic, Pulmonary

hypertension treatment, antiviral, Cytoprotectant, sickle cell anaemia treatment, eye irritation treatment, sclerosant, platelet adhesion inhibitor, Skin Irritation, Hypercholesterolemic, Cholesterol synthesis inhibitor.

Vitexin

Vascular disease treatment protector, Lipid metabolism regulator, reductant, Cardioprotectant, Anticarcinogenic, Antineoplastic, Hepatotoxic, Dermatologic.

Pipecolic acid

Convulsant, Neoroprotector, Fibrinolytic, Urologic disorders treatment, Dopamine release stimulant.

Table 1, Primary Metabolites Present in *Coccinia grandis* fruit

Sl. No	Amino acid	Organic acids	Lipids and Fatty acids
1	Methionine	Rosmarinic acid	Linoleic acid
2	Proline	Pipecolic acid	Cucurbic acid
3	Cystine	Succinic acid	Arachidic acid
4	Marasmic acid		Ricinoleic acid

Table 2. Secondary Metabolites present in *Coccinia grandis* fruit

Sl. No.	Flavonoids	Phenolic acids	Alkaloids	Coumarins	Terpenoids
1	Vitexin	Caffeic acid	Thebaine	Khellol glucoside	Decorine
2	Hyperoside	Hydroxycinnamic acid	Sptentrionine	Xanthotoxol	Carnasol
3	Hydroxyflavan		Vasicinol		Betalin
4			Heliotrine		
5			Beta Erythroldine		

Conclusion

Now a days people interested in the health benefits of food and have begun to look beyond the basic nutritional benefits of food to the disease prevention and health enhancing compounds contained in many foods. A number of medicinal properties such as ant diarrheal, ant obesity, ant diabetic, anti-inflammatory, antimicrobial, antioxidant, diuretic and nervous disorders have been ascribed to the *Coccinia grandis* fruits it may be due to the presence of functionally important compound such as flavanoids, terpenoids, alkaloids, steroids and glycosides.Over the years scientists have verified many of the traditional uses of *Coccinia grandis* that continue to be an important natural remedy for various diseases. Recently many researchers have taken a great interest in medicinal plants for their phenolic and flavanoid concentrations are related to total antioxidant potential .

Vegetables have important role in our life. Increase their consumption help to control various life alarming diseases. .Various types of diseases especially diabetes, atherosclerosis, cancer, aging, and inflammations are caused due to oxidative damage that reduced by the action of antioxidants. In this scenario the role of vegetable containing these anti-oxidants are very important. Majorly the phenolics provide the anti-oxidant capacity. Natural compounds

found in vegetables protect againt many life threatening conditions like diabetes, heart diseases, cancer etc. The application of computerized system PASS result shows several pharmacological activities such as fibrinolytic, anti-virus, anti-carcenogenic, anti-seborrheic, free radical scavenger, hypercholesterolemic, hypercholesterolemic, cardioprotectant, sickle cell anemia treatment, chemoprotective, lipid metabolism regulator etc. Based on the findings from this study we concluded that it is a potential source of high value components for pharmaceutical and neutraceutical industry.

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