Preliminary phytochemical analysis and antioxidant activities of different parts of *Crescentia cujete:* An important medicinal plant

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Abstract

Crescentia cujete L. plant belongs to the family Bignoniaceae growing on tropical forest was collected from Western Ghats of Karnataka and subjected for qualitative phytochemical analysis, total phenolic content and antioxidant activity. In the present study, different parts like stem, leaves and fruits of *C. cujete* were subjected for preliminary phytochemical analysis in different solvent extracts, which revealed the presence of reducing compounds, alkaloids, flavonoids, tannins, sterols, terpenoids, glycosides, carbohydrates, resins, phenol and proteins. The methanolic fruit extract of the sample showed the presence of most of the phytochemicals when compared to leaves and stem extracts of different solvents. The methanolic fruit extract of the showed total phenolic content of about 2.64 mg of GAE, which is followed by leaves and stem 2.27 and 2.03 mg GAE/g respectively. Antioxidant activities of methanol extract showed dose dependent activity, which increased with increase in the concentration of the extract. Methanol fruit extract showed highest free radical scavenging activity which was almost equal to the standard with the IC_{50} value of 4.925 where the IC_{50} value of ascorbic acid was found to be 3.389. The present investigation provides insights on the phytoconstituents and antioxidant activities of stem, leaves and

fruits extracts of *C. cujete*, so it can be further subjected for purification of compounds which may act as an alternative for the current synthetic compounds which are used as pharmaceuticals.

Key words: *C. cujete*; Antioxidant; phytochemicals; phenol

Introduction

Nature has been a repository of medicinal compounds for thousands of year because of their traditional medicinal practice from pre historic time especially in Asia (Mithraja et al., 2012). According to World Health Organization (WHO), 20,000 medicinal plants are used worldwide, and these plants were considered as tremendous source of versatile phytochemicals (Agarwal and Chauhan, 2015). Herbal medicine plays a vital role among rural communities of developing countries for the establishment of wellbeing in the absence of a competent primary health care system (Tabuti, et al., 2003).Several plants are prospective sources of wide range of phyto-constituents such as alkaloids, oils, tannins, steroids, terpenoids, glycosides, flavonoids and other phenolic compounds, which have a cohesive part of defence system against several diseases and stress conditions, so it can be employed in the pharmacological industry(Singh, 2005).

The major welfares of using plants derived medicines are that they are ridiculous in several secondary metabolites and comparatively they are safer than synthetic medicines to combat against various illnesses (Erkan et al., 2008). Phytochemicals derived from different parts of the plants like stem, barks, leaves, fruits, flowers, roots, seeds and the information of chemical constituents of plants is significant to offer value for synthesis of synthetic complex chemical substances (Parekh and Chanda, 2007). Henceforth, techniques like Gas chromatography Mass spectroscopy (GC-MS) are a potent tool for the detection of various volatile and semi-volatile compounds (Vinodh et al., 2013).

The *Crescentia* genus comprises 7 species such as *Crescentia cujete*, *Crescentia plectantha*, *Crescentia amazonica*, *Crescentia alata*, *Crescentia linearifolia*, *Crescentia portoricensis* and *Crescentia latifolia* and some of these species are considered to have potent medicinal values (Madhukar et al., 2013). *Crescentia cujete* is a small sized tree belongs to the family Bignoniaceae. It grows about 6– 8m tall with a wide crown and long branches covered with clusters of leaves and ball like fruit which are borne on the stem and these branches are arranged as simple elliptical leaves clustered at the node (Julia, 1968). Different parts of the *Crescentia* species possess several medicinal properties such as leaves are used to reduce the blood pressure; bark is used to treat wounds and also to cure tumours. Fruit decoction is used to treat diarrhoea, bronchitis, cough, asthma and urethritis (Arango-Ulloa et al., 2009). Hence the present study was carried out for analysing the presence of different phytoconstituents and their antioxidant activities in extracts of *C. cujete*.

Materials and methods

Collection of Plant material

Different parts of the plants like leaves, stem and fruit of *C. cujete* were collected from Western Ghats of Karnataka, India.

Preparation of the extracts

The fresh leaves stem and fruit of *C. cujete* was washed under running tap water to remove the surface debris followed by shade dried and powdered using mechanical blender. 50 gm of each sample of dried leaves, stem and fruit powder was filled in the thimble and effectively extracted with petroleum ether; chloroform; ethyl acetate and methanol using Soxhlet extractor. All the extracts collected were concentrated using rotary flash evaporator and stored at 4° C in air tight vials and used for further studies (Harborne, 1973).

Phytochemical Screening

The collected plant extracts were exposed to qualitative phytochemical screening for identification of different classes of active phytoconstituents like alkaloids, flavonoids, saponins, tannins, cardiac glycosides, glycosides, sterols, carbohydrates, proteins and amino acids following the method of Harborne (1973) and Trease and Evans (1987)

Determination of Total Phenolics

Total phenolic content was determined in methanolic extract by following the method of Singleton et al (1999). 20 µL of extract (5 mg/mL) was mixed with 0.75 ml of 20% sodium carbonate solution and 0.25 mL of Folin- Ciocalteau reagent. The reaction mixture was allowed to stand in light for 3 min and incubated for 2 h in dark. The absorbance was read at 765 nm using UV-Visible Spectrophotometer. Total phenolic content was determined by calibration curve obtained from measuring the absorbance of known concentrations of Gallic acid as standard (0- 100 g/mL). The concentrations were expressed as g of Gallic acid equivalents per mL.

DPPH assay:

DPPH free radical scavenging activity was performed as described by Sultanova et al., 2001. The DPPH solution was prepared by dissolving 4mg of standard DPPH in 100mL of methanol. Briefly 20µL, 40µL, 60µL, 80µL, 100µL of leaf, stem and fruit extracts were taken from the stock solution (1mg/mL) in different test tubes and made up to the 1mL using methanol. Similar concentrations of ascorbic acid (1mg/mL) was taken as standard. To each test tube 3mL of DPPH solution was added. DPPH and absolute methanol was used for reagent blank. The mixture was vortexed for 1 minute, kept for 30 minutes in dark and then the absorbance was measured at 517nm using spectrophotometer. The antioxidant activity of

each sample was expressed in terms of IC_{50}, which was calculated from the graph after plotting inhibition percentage against concentration.

Results

Phytochemical study

The present study of phytochemical screening of extracts of leaf, stem and fruit of *C. cujete* in different solvents like petroleum ether, chloroform, ethyl acetate and methanol was carried out using standard protocols. This qualitative analysis confirms the presence of various phytochemical constituents and the results are summarized in the table 1. Methanolic leaf, stem and fruit extracts showed the presence of various phytoconstituents whereas, petroleum ether and ethyl acetate extracts showed minimum results. The chloroform leaf extracts of *C. cujete* showed the presences of carbohydrates while the methanol leaf extracts showed the presence of sterols, triterpenes, saponins, alkaloids and glycosides. The chloroform stem extracts showed the presence of carbohydrates and ethyl acetate stem extracts showed the presence of resins. Carbohydrates, sterols, triterpenes, saponins and tannins were reported in the methanol stem extracts. The chloroform fruit extracts showed the presence of carbohydrates, where as the methanol fruit extracts showed the presence of sterols, triterpenes, saponins, tannins, flavonoids, resins, glycoside and carbohydrates.

Antioxidant activity

The results revealed that methanol extract of leaf showed excellent antioxidant activity when compared to methanol extracts of stem and fruit. There was complete discolouration of purple coloured solution to yellow colour in all the three extracts. As DPPH is relatively stable nitrogen centered free radical it can accept electron of hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agent as a result of which electron becomes paired off forming a corresponding hydrazine. The solution

therefore loses colour stoichiometrically depending on the number of electrons consumed. There is also a slight discoloration of the solution in lower concentrations compared to complete discolouration in higher concentrations. This indicates that the antioxidant activity increases with the increase in the concentration. Methanol extract of fruit showed highest free radical scavenging activity which was almost equal to the standard with the IC_{50} value of 4.925 where the IC_{50} value of Ascorbic acid was found to be 3.389 (Table 2).

Determination of Total Phenolic contents:

Phenolics are plant secondary metabolites and are very important by virtue of their antioxidant activity by chelating redox active active metal ions, inactivating lipid free radical chains and preventing hydroperoxide conversion into reactive oxy radicals. The present study was carried out for the determination of total phenolic contents in leaf, stem and fruits of *C. cujete* using methanol extracts. A total phenolics concentration equivalent of Gallic acid was estimated according to Folin-Ciocalteau method. Gallic acid being one of the most important polyphenol in natural products, was used to determine the phenolics of tested plant extracts. The phenolic content of plant extracts was calculated by Gallic acid equivalent. It is expressed as mg GAE/g (dry weight). The highest amount of phenolic content was found in fruit (2.64 mg GAE/g), followed by the leaves (2.27 mg GAE/g) and stem extracts (2.03 mg GAE/g) respectively. The results obtained have considerable value and the activity of this extract may be attributed to the phenolic contents (Fig. 1).

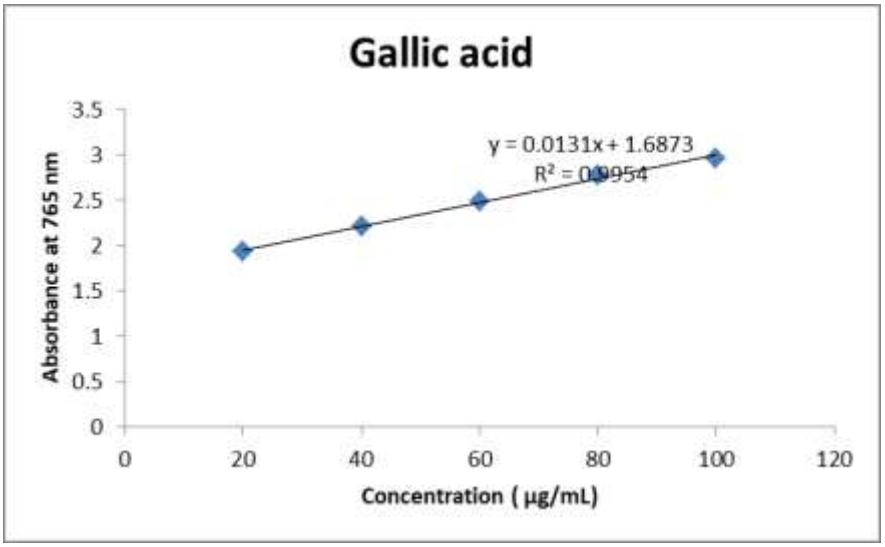

Fig 1: **Standard calibration curve for total phenolic contents**

Table 1: Phytochemical constituents of *C. cujete* fruit, leaf and stem extracts

TESTS	Types	FRUIT				LEAF				STEM			
		PE	CHL	EA	M	PE	CHL	EA	M	PE	CHL	EA	M
Test for sterols	1.Salkowski test	_	_	_	+	_	_	_	+	_	_	_	+
	2.Libermann Burchard's test	–	–	–	+	–	–	–	+	–	–	–	+
Test for Triterpenes	1.Salkowski test	_	_	_	_	_	_	_	_	_	_	_	_
	2.Libermann Burchard's test	–	–	–	+	–	–	–	+	–	–	–	+
Test for Saponins	1. Foam test	_	_	+	+	_	_	_	+	_	_	_	+
Test for Alkaloids	1. Mayer's test	_	_	+	_	_	_	_	+	_	_	_	+
	2. Dragendroff's test	_	_	_	_	_	_	_	_	_	_	_	_
	3. Wagner's test	_	_	_	_	_	_	_	+	_	_	_	_
Test for Tannins	1. Ferric chloride test	_	_	_	+	_	_	_	_	_	_	_	_
	2. Gelatin test	_	_	_	+	_	_	_	_	_	_	_	+
Test for Flavonoids	1. Shinoda test	_	_	_	+	_	_	_	_	_	_	_	_
	2. Ferric chloride test	_	_	_	_	_	_	_	_	_	_	_	_
	3. Lead acetate test	_	_	_	_	_	_	_	_	_	_	_	_

Test for Carbohydrates	1. Fehling's test	–	+	–	+	–	+	–	+	–	+	–	+
	2. Benedict's test	–	–	–	+	–	–	–	–	–	–	–	–
	3. Molisch test	–	–	–	+	–	–	–	–	–	–	–	–
	4. Schiff test	–	–	–	+	–	–	–	–	–	–	–	–
Test for Resins	1. Turbidity test	–	–	–	–	–	–	–	–	–	–	+	–
	2. Acetic anhydride test	–	–	–	+	–	–	–	–	–	–	–	–
Test for Phenols	1. Phenol test	–	–	–	+	–	–	–	+	–	–	–	+
	2. Ellagic acid test	–	–	–	–	–	–	–	–	–	–	–	–
Test for Glycosides	1. Keller-Kilani test	–	–	–	+	–	–	–	+	–	–	–	–
Test for Proteins	1. Biuret test	–	–	–	+	–	–	–	–	–	–	–	–
	2. Ninhydrin test	–	–	–	–	–	–	–	–	–	–	–	–
Test for Terpenoids	1. Terpenoids test	–	–	–	–	–	–	–	–	–	–	–	–

("+" indicates the presence of compounds and "-" indicates the absence of compounds)

Table 2: DPPH scavenging activity of Methanolic fruit extracts of *C. cujete*

Plant extracts	**Concentration (µg/mL)**					**IC_{50} value**
	20	**40**	**60**	**80**	**100**	
	DPPH % Scavenging					
Leaf	14.88	21.22	31.23	41.23	51.55	4.925
Stem	20.22	28.68	36.66	45.67	53.98	4.533
Fruit	15.60	24.54	34.58	56.87	61.25	3.924
Ascorbic acid	28.77	35.33	40.98	60.19	65.55	3.389

Discussion

Medicinal plants are one of the main source for new pharmaceutical and health care products as most of the plants contain phytochemicals which has protective properties against various diseases. Most phytochemicals, especially phenolics have been proved to benefit health of the human beings by scavenging free radicals or quenching reactive oxygen species. Phenolics in plants responsible for antioxidant activity, and their contents in the plants were

associated with antioxidant activity. Ascorbic acid also has antioxidant activity and is essential for the maintainence of normal function of the living cells. It has reported that the majority of drugs from natural resources and that approximately 60-80% of the world's population still believe in folk medicine (Prabhavathi et al., 2011).

Phytochemical analysis conducted on the plant extracts of *C. cujete* revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. The qualitative analysis carried out on leaf, stem and fruit extracts of the plant showed the presence of a good number of phytochemical constituents like triterpenes, carbohydrates, resins, tannins, flavonoids, saponins, glycosides, steroids, and alkaloids.

Similar type of study was conducted by Ejelonu et al., 2011, where he analysed the *C. cujete* fruit was chemical constituents, proximate and mineral composition as well as phytochemical properties and also sugar content, energy content, electrical conductivity and pH of the fruit were also determined. The results revealed comparatively low concentrations for the heavy metals; but high mean concentrations for manganese, iron, zinc and copper. The presence of phytochemicals like saponins, flavonoid, tannins and phenol as well as the presence of hydrogen cyanide were observed in the fruit sample.

Phenol and tannins were found in the fruit sample of methanolic extracts. Phenol and phenolic compounds have been comprehensively used in manufacture of disinfections (Cater, 1979). Because of this properties *C. cujete* was used as disinfectant in emollient healing and in the treatment of burns (Micheal, 2004; Burkilli,1985). Tannins possess astringent properties that accelerate the wound healing processes, prevent further other secondary infections and have antimicrobial activity (Chukwuma et al., 2010). Tannins and their derived compounds are known to be responsible for preventing and treating urinary tract infections

and other bacterial infections (Michael, 2004). The presence of tannins in the fruit suggests that it may serve as a useful antibacterial agent.

The presence of alkaloids posses antimicrobial, antiviral, anticancer and many pharmacological activities (Parekh and Chanda, 2006), whereas tannins have antiviral, antibacterial, antiparasitic, antiulcer, anti- inflammatory and antioxidant activities. Flavonoids present in the *C. cujete* methanolic extracts can act as anti-oxidants and protect the cells from free radical scavenging damage (MacArthur, 1992). The presence of alkaloid was observed in the fruit, leaves and stem sample. Flavonoids have many therapeutic properties such as antirheumatism, antihypertensive, antimicrobial, anticancer anti- inflammatory, diuretic, antioxidant (Veerachari and Bopaiah, 2011) and give protection against free radicals, allergies, hepatotoxins, ulcers, platelet aggregation and viruses

Alkaloids have been used as basic medicinal agent for their analgesic, anti-spasmodic and bactericidal effects (Frantisek, 1998). Saponins were present in *C. cujete* leaf, stem and fruit methanolic extracts. Saponins are known to acts as natural antibiotics and also boost energy (Lipkin, 1995). Saponins posses anti inflammatory activity there by it are useful in reducing inflammation of the upper respiratory passage (Frantisek, 1998). Saponins in *C. cujete* might be serve as anti-inflammatory agent and as antibiotics in curing diseases and ailments.

Plants are excellent sources for antioxidant compounds, which can fight against reactive oxygen species (ROS) responsible for many health disorders such as anemia, ageing, cardiovascular disease, inflammation, cancer, degenerative disease, diabetes and ischemia (Cai et al,, 2004). The methanol extract of leaf showed excellent antioxidant activity when compared to methanol extracts of stem and fruit. There was complete discolouration of purple coloured solution to yellow colour in all the three extracts.Synthetic antioxidants such as BHT (Butylated Hydroxy Toluene), BHA (Butylated Hydroxy Anisole), TBHQ (Tertiary butylhydroquinone) and PG (Propyl Gallate) are used to reduce the risk of above mentioned

diseases (Hoffman and Garewal, 1995) but due to their toxicity they are responsible for carcinogenesis and liver damage (Grice, 1988). Hence many of the researchers screened plants for their antioxidant activity (Katalinic et al., 2006). *C. cujete* fruit might be suggest that the fruit can make useful to both human and animal nutrition as its possesses several medicinal values.

Conclusion

C. cujete possess immense medicinal properties which were selected to qualitatively to analyse the secondary metabolites. It revealed presence of several phytoconstituents like triterpenes, carbohydrates, resins, tannins, flavonoids, saponins, glycosides, steroids, and alkaloids. The results obtained from the current studies suggested that there is considerable antioxidant properties of various extracts of *C. cujete*. The methanol extracts of fruit showed excellent radical scavenging activity which was significantly comparable to free radical scavenging activity of gallic acid. The findings of this study suggested that the fruit of this plant could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the process of ageing and oxidative stress related degenerative diseases. The overall results explain the presence of several medicinally important constituents in *C. cujete*, providing health application at affordable cost. However, further investigation, isolation and characterization is essential to draw decisions about the properties of the secondary metabolites and their potency. The study provides justification for the therapeutic use of these plants as natural antioxidants in folklore medicine.

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