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Quantitative Analysis of Phytochemicals in the Bark Extracts of Medicinally Important Plant *Cassia fistula,* Linn.

Suman Kumar Ratnampally* and Venkateshwar Chinna

Department of Botany, Osmania University, Hyderabad, Telangana, India
**Corresponding author*

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A B S T R A C T

The present study was conducted to determine the yield of extract quantitatively for the crude bark extracts of *Cassia fistula,* family Fabaceae is a high value medicinal plant collected from Osmania University, Hyderabad, Telangana State. The basis for the present study is that the bark of this plant is used by Gondu tribes of Adilabad District, Telangana State, India to treat hyperthyroidism in human beings. The author has significantly documented the ethnobotanical relation of present plant under study. Since the advent of modern drug treatments, traditional medicine has greatly receded in occidental societies. Only a limited number of medicinal plants have received detailed scientific scrutiny thereby prompting the World Health Organization to recommend that this area be comprehensively investigated. The bark extracts were prepared by using Ethanol, Methanol, Acetone, Chloroform, Petroleum ether and also in water. The yields of extract were calculated as weight/weight in all the six solvents and are studied for eight phytochemical compounds. The present study revealed the presence of Alkaloids, Flavonoids, Total Phenols, Carbohydrates, Total Tannins, Saponins, Terpenoids, Total Glycosides in varying content and clearly shown that the more number of phytochemical compounds are maximum soluble in ethanol solvent.

Introduction

Phytochemicals are the chemicals produces by various parts of the plants. These bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins and glycosides. These compounds have various activities such as antimicrobial and antibacterial some have been reported to exhibit hemolytic and foaming activity reported (Feroz *et al.*, 1993). Qualitative phytochemical screening will help to understand a variety of chemical compounds produced by plants and quantification of those metabolites will help to extract, purify and identify the bioactive compounds for useful aspects to human beings. Plants have limitless ability to synthesize aromatic substances, mostly phenols or their oxygen-substituted derivatives (Geisssman, 1963). Most of the natural products are secondary metabolites and about 12,000 of such products have been isolated so far. These products serve as plant defence mechanisms against predation by microorganisms, insects and herbivores (Fransworth and Morris, 1976). Several bioactive constituents have been isolated and studied for pharmacological activities. During the last two decades, the pharmaceutical industry has made massive investment in

pharmacological and chemical researches all over the world in an effort to discover much more potent drugs, rather, a few new drugs (Santhi and Sengottuvel. 2016). The *Cassia fistula* plant extracts were shown as potent antibacterial, antifungal, anti-inflammatory and antioxidant (Gupta, 2010) properties and the findings were done using different solvent extracts and parts of the plant. The chemical analysis of different parts of *C. fistula* has been reported. It was found to contain flavonoids, phenolic compounds and pro anthocyanidins (Luximon *et al*., 2002). *C. fistula* extracts have been reported for various pharmacological activities including anti-inflammatory (Rajeswari *et al*., 2006), antioxidant (Irshad *et al*., 2012), antimicrobial (Irshad *et al*., 2013), wound healing properties (Kumar *et al*., 2006) and anticancer activity (Irshad *et al*., 2014).

The plant *Cassia fistula* is a tree 20-30 ft. high; trunk straight; bark smooth and pale-grey when young, rough and dark-brown when old; braches spreading, slender. Leaves 9-16 in. long; main rachis pubescent; stipule minute. Leaflets 4-8 pairs, ovate or ovate-oblong, acute, bright green and glabrous above, paler scent on the underside, base cuneate; main nerves numerous, close, conspicuous beneath; petiolules long, pubescent or glabrous. Flowers in lax racemes 12-20 in. long; pedicels long, slender, pubescent or glabrous. Calyx divided to the base, pubescent; segments oblong, obtuse. Corolla yellow; petals 5, sub-equal, obovate, shortly clawed, veined, stamens all antheriferous, Pods 1-2 ft. long, 1 in. in diam., pendulous, cylindrical, nearly straight, smooth, shinning, brown-black, indehiscent, with numerous (40-100) horizontal seeds immersed in a dark-colored sweetish pulp, and completely separated by transverse dissepiments. Seeds broadly ovate, slightly less in breadth, the plant flowers during March to May (Fig. 1).

Materials and Methods

Selection of plant species

The plant material (bark) of *Cassia fistula* was collected from the Osmania University campus, Hyderabad, Telangana, India. The plant's bark washed thoroughly 2-3 times with running tap water and once sterile with distilled water. Then the plant parts were shade dried and coarsely powdered separately and stored in well closed bottles for further analysis in laboratory.

Extraction of the plant materials

The fresh plant bark was washed with running tap water and shade dried and crushed to coarsely powder. This coarse powder (25g) was then subjected to successive extraction in 250ml of ethanol, methanol, acetone, chloroform and also in water by using Soxhlet apparatus. The collected extracts were stored and then used for further analysis. The DMSO (Dimethyl sulfoxide) is act as dissolved solvents for these extracts.

Quantitative Phytochemical Analysis

Estimation of Alkaloids

Alkaloid determination by using Harborne (1973) method: One gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and it's covered and allowed to stand for 4 h. It was filtered and the extract was concentrated on a water bath to one quarter of the original volume. Concentrated NH_4OH was added by drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH and then filtered. The residue is the alkaloid, which was dried and weighed.

Estimation of Flavonoids

One grams of plant sample was repeatedly extracted with 100ml of 80% of various aqueous solvents at room temperature. The mixture was filtered through a Whatman No1 filter paper into a pre-weighed 250ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed (Krishnaiah *et al.,* 2009).

Estimation of total phenols

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. Five ml of the extract was pipette out into a 50 ml flask, then 10 ml of distilled water was added. Two ml of NH_4OH solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. The absorbance was read at 505nm.

Estimation of Carbohydrate

100 mg of sample was hydrolyzed in a boiling tube with 5 ml of 2.5 N HCl in a boiling water bath for a period of 3 hours. It was cooled at room temperature and solid sodium carbonate was added until effervescence ceases. The contents were centrifuged and the supernatant was made to 100 ml by using distilled water. From this 0.2 ml of sample was pipette out and made up the volume to one ml with distilled water. Then one ml of phenol reagent was added and followed by 5.0 ml of sulphuric acid. The tubes were kept at 25-30 C for 20 min. The absorbance was read at 490 nm (Krishnaveni *et al.,* 1984).

Total Tannins Content Determination

The tannins were determined by slightly modified Folin and Ciocalteu method. Briefly, 0.5 ml of sample extract is added with 3.75 ml of distilled water and added 0.25 ml of Folin Phenol reagent, 0.5 ml of 35% sodium carbonate solution. The absorbance was measured at 725 nm. Tannic acid dilutions (0 to 0.5mg/ml) were used as standard solutions. The results of tannins are expressed in terms of tannic acid in mg/ml of extract.

Determination of total saponins

The samples were ground and 20 g of each were put into a conical flask and 100 cm3 of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated (Obdoni and Ochuko, 2001)

Determination of total Terpenoids

5ml of aqueous extract of each plant sample is mixed with 2ml of $CHCl_3$ in a test tube 3ml of concentrated H_2SO_4 is carefully added to the mixture to form a layer. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

Determination of total Glycosides

1ml of concentrated H_2SO_4 is prepared in test tube 5 ml of aqueous extract from each plant

sample is mixed with 2ml of glacial CH_3CO_2H containing 1 drop of $FeCl_3$. The above mixture is carefully added to 1ml of concentrated H_2SO_4 so that the concentrated H_2SO_4 is underneath the mixture. If cardiac glycoside is present in the sample, a brown ring will appear indicate.

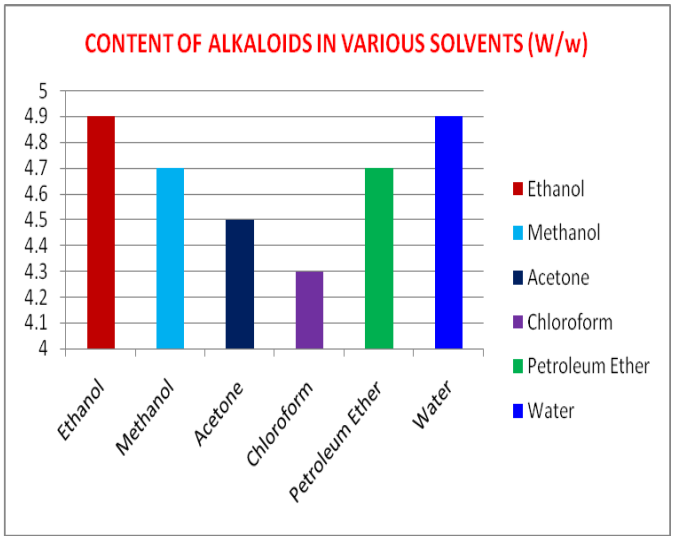
Results and Discussion

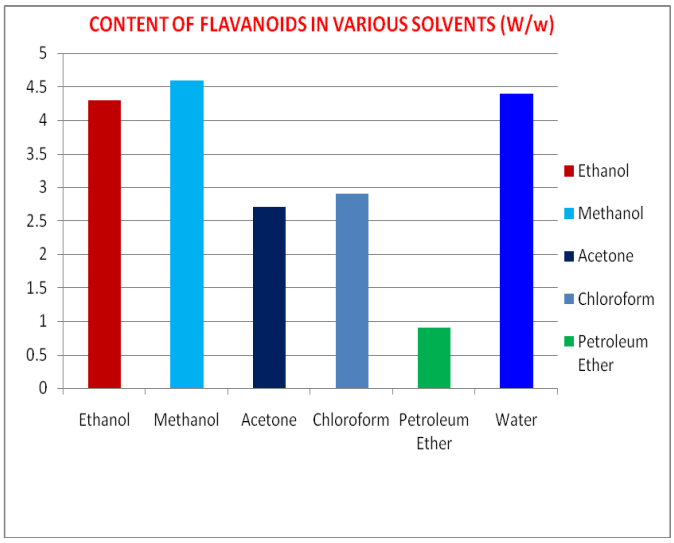
The present study was carried out on the bark extracts of *Cassia fistula* revealed the presence of active phytochemical constituents in various solvents and the results are mentioned in table 1 and also the same is represented through graph 1-8. The quantitative estimation of primary metabolites revealed that the various phytochemical constituents present in the plant extract is as the content of the Alkaloid is 4.9 W/w in ethanol and water extracts. While flavanoids and total phenols have shown their maximum content in water extracts as 4.4 W/w and 5.3 W/w respectively.

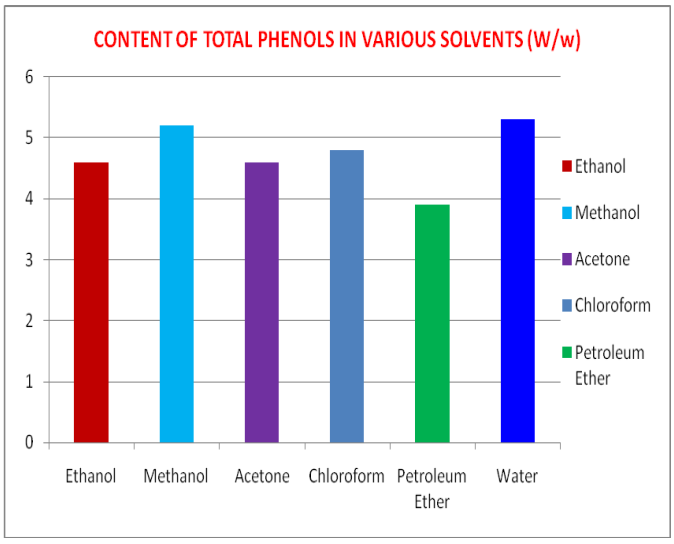
Table.1 Quantitative phytochemical analysis of Cassia fistula bark extracted with different polar, non-polar and aqueous solvents

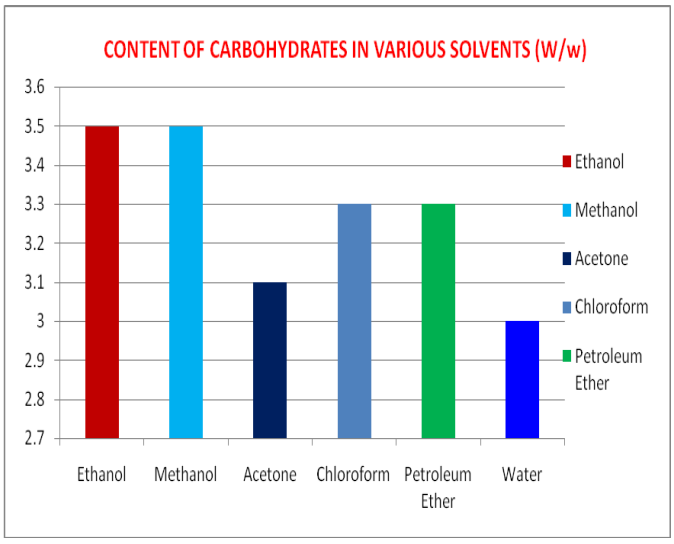
S.No.	Phytochemical Content in bark (W/w)	Name of the Solvent					
		Ethanol	Methanol	Acetone	Chloroform	Petroleum Ether	Water
1	Alkaloids	4.9	4.7	4.5	4.3	4.7	4.9
2	Flavonoids	4.3	4.6	2.7	2.9	0.9	4.4
3	Total Phenols	4.6	5.2	4.6	4.8	3.9	5.3
4	Carbohydrate	3.5	3.5	3.1	3.3	3.3	3.0
5	Total Tannins	4.1	4.7	4.8	4.5	4.5	4.7
6	Saponins	4.6	4.6	4.2	3.3	3.6	3.6
7	Terpenoids	5.1	4.8	4.7	4.1	2.8	2.7
8	total Glycosides	4.3	4.6	3.8	0.5	0.8	2.5

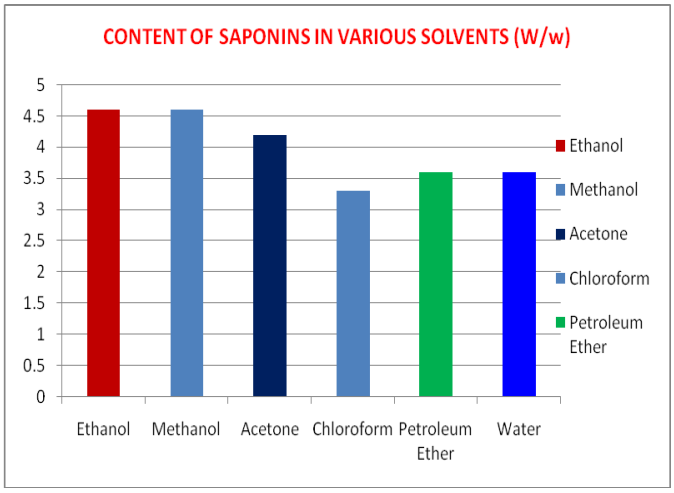
Fig.1 *Cassia fistula*-habitat

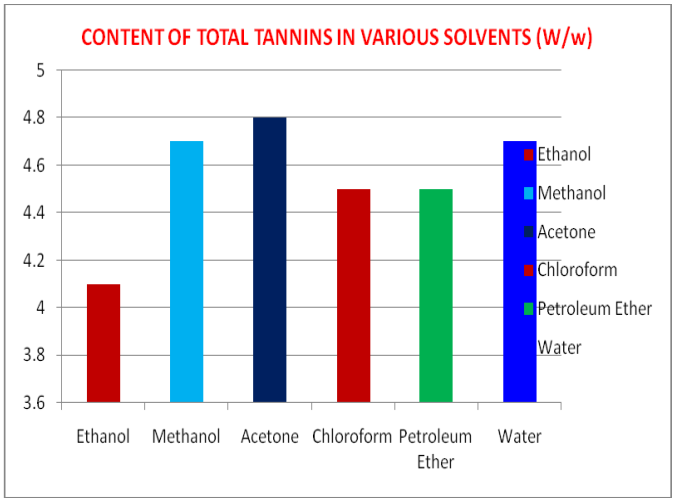

CONTENT OF ALKALOIDS IN VARIOUS SOLVENTS (W/w)
5
4.9
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4
Ethanol
Methanol
Acetone
Chloroform
Petroleum Ether
Water
Ethanol
Methanol
Acetone
Chloroform
Petroleum Ether
Water

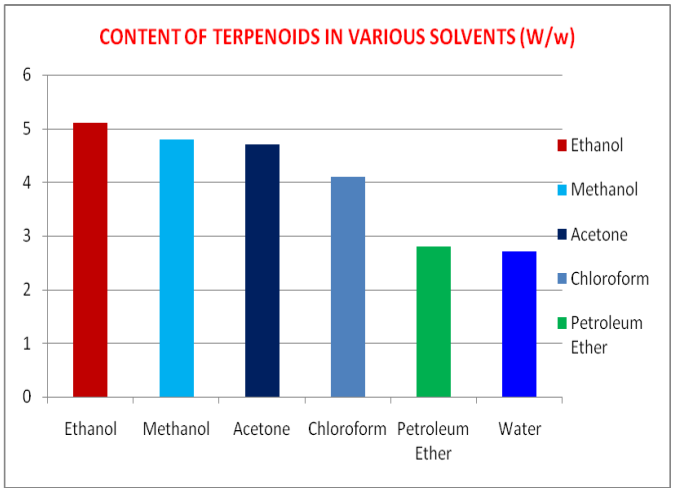

CONTENT OF FLAVANOIDS IN VARIOUS SOLVENTS (W/w)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Ethanol
Methanol
Acetone
Chloroform
Petroleum Ether
Water
Ethanol
Methanol
Acetone
Chloroform
Petroleum Ether
Water

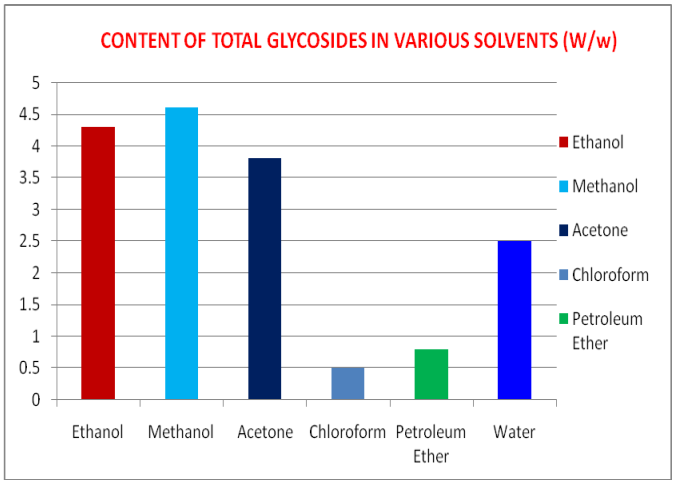

CONTENT OF TOTAL PHENOLS IN VARIOUS SOLVENTS (W/w)
6
5
4
3
2
1
0
Ethanol
Methanol
Acetone
Chloroform
Petroleum Ether
Water
Ethanol
Methanol
Acetone
Chloroform
Petroleum Ether
Water


CONTENT OF CARBOHYDRATES IN VARIOUS SOLVENTS (W/w)
3.6
3.5
3.4
3.3
3.2
3.1
3
2.9
2.8
2.7
Ethanol
Methanol
Acetone
Chloroform
Petroleum Ether
Water
Ethanol
Methanol
Acetone
Chloroform
Petroleum Ether
Water


CONTENT OF SAPONINS IN VARIOUS SOLVENTS (W/w)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Ethanol
Methanol
Acetone
Chloroform
Petroleum Ether
Water
Ethanol
Methanol
Acetone
Chloroform
Petroleum Ether
Water


CONTENT OF TOTAL TANNINS IN VARIOUS SOLVENTS (W/w)
5
4.8
4.6
4.4
4.2
4
3.8
3.6
Ethanol
Methanol
Acetone
Chloroform
Petroleum Ether
Water
Ethanol
Methanol
Acetone
Chloroform
Petroleum Ether
Water





On the other hand, the content of carbohydrates are maximum in ethanol and methanol extracts as 3.5 W/w. a key observation is that total tannins are maximum in acetone extract as 4.8 W/w. Furthermore, saponins and total glycosides content is maximum in ethanol and methanol extracts as 4.6 W/w and 4.6 W/w respectively. Finally, terpenoids are extracted maximum in ethanol extract as 5.1 W/w.

In conclusion, *Cassia fistula* is an important source of naturally occurring bioactive compounds. The work so far achieved on *Cassia fistula* also sets the basis of future studies on the effects of its polyphenol containing extracts, which may have important practical implications for food quality and their potential utilization in multi component biological/food systems. It is becoming clear that traditional systems of medicine have become a topic of global importance. *Cassia fistula* could be one of them particularly because of its low toxicity and its widespread use for its multiple medicinal effects. The phytochemical compositions and biological activities need to be well understood and the data gathered so far for *Cassia fistula* and its extracts aim at achieving that goal.

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