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# Preliminary phyto-chemical evaluation of the leaf extract of five *Cassia Species*

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## ABSTRACT

*Comparative studies of secondary metabolites using qualitative tests was performed on Cassia spectabilis, Cassia siamea, Cassia fistula, Cassia biflora and Cassia hirsuta. Qualitative analysis was done for various phytoconstituents like alkaloids, tannins, saponins, anthraquinones, anthocyanosides, phenolic flavonoids, flavonoids, carbohydrates, proteins, steroids, terpenoids, cardiac glycosides and phlobatannins.The leaf extracts were prepared using various solvents like ethanol, methanol and ethyl acetate to detect the presence of the active components. The phtyo chemical screening revealed the presence of all the above chemical constituents except anthraquinones, anthocyanosides and phenolic flavonoids.*

**Key words**: Cassia species, phyto-chemical constituents, leaf extracts, solvents.

## INTRODUCTION

Plant derived substances has obtained greater attention in the recent years to prevent and cure human diseases as they are considered to be more bio-friendly. It is generally estimated that over 6000 plants in India are in use in traditional, folk, and herbal medicine, representing about 75% medicinal needs of the third world countries [Rajashekaran PE, 2002].

Phyto-chemical investigations of crude plant extracts shows the presence of active principles in the plant parts like bark, leaves, flowers, roots, fruits, seeds etc. Phyto-chemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself but research works demonstrates that many phytochemicals can protect humans against diseases. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances.
In the present work, qualitative phytochemical analysis was carried out in five species of cassia.

Taxonomic classification:

| | | |
|---|---|---|
| Kingdom | : | Plantae |
| Sub Division | : | Spermatophyta |
| Division | : | Magnoliophyta |
| Class | : | Magnoliopsida |
| Sub Class | : | Rosidae |
| Order | : | Fabales |
| Family | : | Fabaceae |
| Genus | : | Cassia |

## EXPERIMENTAL SECTION

I. **a. Sample collection, Extraction and Processing**:
Dried plant material was used as a source for the extraction of secondary metabolites in plants.

Fresh plant parts were collected randomly from Bangalore region, India in the year 2011 [May and June]. The details of the plant/plant parts screened, their vernacular names and family are presented in table: I. All the five plant samples were authenticated by the senior scientists, Department of CES [Centre for Ecological Studies], IISc, Bangalore and plantation officer, Lalbagh Botanical Gardens, Bangalore.

Fresh plant material (leaf) of the Cassia species were separated, washed with tap water, rinsed with distilled water and air dried. The dried leaf of each plant was pulverized using a sterile electric blender, to a fine powder and stored in airtight dark bottles at room temperature. The aqueous extract of the plant samples were prepared by soaking 100gms of dry powdered samples in 200ml of different solvents:

a) Ethanol
b) Methanol
c) Ethyl acetate

for 12 hours. The extracts were filtered using Whatman filter paper No. 42(125mm) and the filtered extracts were stored in airtight dark bottles at room temperature for phytochemical analysis.

**b. Phytochemical screening**:
The different qualitative chemical tests were carried out on the aqueous extract using standard procedures to identify the constituents as described by Sofawara [1993], Trease and Evans (1989), Harborne [1973] and Edeoga [2005].

*Qualitative analysis on phytochemical constituents:*
**Alkaloids**: 1ml of the filtrate with 2ml of Drangendroff's reagent shows turbid orange colour.

**Tannins**: 1ml of filtrate with 2ml of Ferric chloride gives dark green colour.

**Saponins:** 1ml of filtrate with 2ml distilled water, shake vigorously allow it to stand for 10 minutes. Development of foam on the surface of the mixture, lasting for 10 minutes indicates the presence of saponins

**Anthraquinones**: 1ml of the filtrate with 10ml benzene, filter and now add 5ml of 10% (v/v) ammonia to the filtrate and shake well. Development of pinkish coloured solution indicated the presence of anthraquinones.

**Anthocyanides**: 1ml of filtrate with 5ml of dilute HCl shows the presence of pale pink colour.

**Phenolic falvonoids**: 1ml of filtrate with 2ml of 10% lead acetate gives brown precipitate.

**Flavonoids:** 1ml of filtrate with 2ml of dilute NaOH shows development of golden yellow colour.

**Carbohydrates:**
a.Take1m of the filtrate with 5ml Benedict's reagent and boil for 5 minutes. Bluish green colour indicates the presence of carbohydrates.
b. To 1ml of filtrate add few drops of Molisch's reagent and few drops of conc. $H_2SO_4$, gives purple colour.
c. To 1ml of filtrate add few drops of Fehling's 'A' which gives green colouration.
d.To 1ml of filtrate add few drops of Fehlings'B' which gives Brown colouration.

**Proteins:** 1ml of filtrate with 5 to 6 drops of Millon's reagent develops white precipitate which turns red on heating.

**Steroids**: To 1ml of the filtrate add 10ml chloroform and 10ml of $H_2SO_4$ slowly by the sides of the test tube. Upper layer turns red and sulphuric acid layer showed yellow with green fluorescence.

**Terpenoids**: Take 1ml of the filtrate with 2ml $CHCl_3$ and carefully add few drops of conc $H_2SO_4$. An interface with a reddish brown colouration is formed.

**Cardiac glycosides**: To 1ml of the filtrate add 1ml of $FeCl_3$ reagent (mixture of 1 vol of 5% $FeCl_3$ solution + 99 vol of glacial acetic acid) and a few drops of conc $H_2SO_4$. Greenish blue colour appears within few minutes.

**Phlobatannins:** To 1ml of the filtrate add few drops of 1% aqueous HCl. A Red precipitate is formed.

## RESULTS AND DISCUSSIONS

**Alkaloids** are a diverse group of secondary metabolites found to have antimicrobial activity by inhibiting DNA topoisomerase [Bonjean K, De Pauw-Gillet M-C, et al.,1998] .Screening of phytoconstituents from all the five species of *Cassia* leaf extract shows the following.(Table:2,3,4,5 & 6).

Alkaloids are present in higher amounts in ethanol,methanol and ethyl-acetate extracts of *Cassia spectabilis,Cassia siamea* and *Cassia hirsuta.*In *Cassia fistula* and *Cassia biflora* only ethyl-acetate extracts shows higher amount whereas in ethanol and methanol extracts it is present only in moderate amounts.

**Tannins** reduce the risk of coronary heart diseases [Janaky Ranjithkumar et al., 2010]. It is present in high quantities in ethanol, methanol extracts of *Cassia siamea* and *Cassia spectabilis*

and only in methanol extract of *Cassia hirsuta*.It is moderately present in all three extracts of *Cassia fistula,Cassia biflora,* only in ethyl-acetate extract of *Cassia siamea* and *Cassia spectabilis*. Where as in *Cassia hirsuta* it shows moderate amounts in ethanol extract and absent in ethyl-acetate extract.

**Saponins,** present in plants, have been suggested as possible anti-carcinogens. The proposed mechanisms of anticarcinogenic properties of saponins include direct cytotoxicity, immune modulatory effects, bile-acid binding and normalization of carcinogen-induced cell proliferation.However, the anticarcinogenic effects of saponins from commonly consumed plant foods have not been studied [Rao A V, et al 1995].Soybeans are the most important sources of dietary saponins ans the main protein supplier in many vegetarian diets.

Our results show that saponin is present in higher amounts in ethanol, methanol extracts and absent in ethyl-acetate extract of *Cassia spectabilis. Cassia siamea, Cassia biflora, Cassia hirsuta* show complete absence of saponins in all the three extracts. In *Cassia fistula* it shows moderate amounts only in methanol extracts and absent in ethanol and ethyl acetate extracts.

**Anthraquinones** are absent in all three extracts of *Cassia spectabilis, Cassia siamea, Cassia biflora Cassia hirsuta* and *Cassia fistula*.

**Anthocyanosides** are also absent in all three extracts of all the five species of *Cassia* except that it was found in moderate amounts in methanol extract of *Cassia siamea.*

Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [Singh et al 2007].Natural antioxidant mainly come from plants in the form of phenolic compounds such as flavonoids, phenolic acids, tocopherols etc [Ali et al., 2008]. A number of studies have focused on the biological activites of phenolic compounds, which are potential antioxidants and free radical scavengers [Rice-Evans et al. 1995; Cespedes et al., 2008; Reddy et al., 2008; Chanda and Dave, 2009].

**Phenolic flavonoids** are absent in all the three extracts of all the five species of *Cassia* but found in moderate amounts only in methanol extracts of *Cassia siamea*.

**Flavonoids** are also known as vitamin P or plant modifiers, present in high quantities in methanol and ethyl acetate extracts of *Cassia siamea* and ethanol extract of *Cassia hirsuta*.It is found in moderate amounts in ethanol extract of *Cassia siamea*, all extracts of *Cassia biflora* and ethyl acetate extract of *Cassia hirsuta*. It is completely absent in all extracts of *Cassia fistula* and only methanol extract of *Cassia hirsuta.*

**Carbohydrate** Benedict's test shows that high amounts of carbohydrates are present in all the five species of *Cassia.*

Molisch's tests show that high quantities of carbohydrates are present in all three extracts of *Cassia spectabilis, Cassia siamea, Cassia fistula, Cassia biflora* and only ethanol, methanol extract of *Cassia hirsuta* where as in ethyl acetate extract it is moderately present.

In Fehling's test, large amounts are seen to be present methanol extract of *Cassia siamea,* ethanol and methanol extract of *Cassia spectabilis, Cassia biflora, Cassia hirsuta* and in all three extracts of *Cassia fistula*.It is moderately present in ethanol extract, ethyl acetate extract of

*Cassia siamea* and only ethyl acetate extract of *Cassia spectabilis*, *Cassia biflora* and *Cassia hirsuta.*

**Proteins** are present in maximum quantities in all three extracts of *Cassia siamea, Cassia fistula, Cassia biflora* and ethanol extract of *Cassia spectabilis* and methanol extract of *Cassia hirsuta*. It is found in moderate amounts in methanol, ethyl acetate extracts of *Cassia spectabilis* and ethanol, ethyl acetate extracts of *Cassia hirsuta*.

**Steroids** in modern clinical studies have supported their role as anti-inflammatory and analgesic agents [Singh AP,2006]. It is found in large amounts in all three extracts of *Cassia siamea, Cassia fistula* and in *Cassia hirsuta* only in ethanol and methanol extracts. It is moderately found in all three extracts of *Cassia spectabilis, Cassia biflora* and ethyl acetate extract of *Cassia hirsuta.*

**Terpenoids** are found in large amounts in all three extracts of *Cassia siamea*,in *Cassia hirusta* only ethanol and methanol extracts. It is moderately found in all three extracts of *Cassia spectabilis, Cassia biflora*, only methanol extract of *Cassia fistula* and ethanol extract of *Cassia hirsuta*. It is totally absent in ethanol and ethyl acetate extracts of *Cassia fistula*.

**Cardiac glycosides** are found in maximum amounts in all three extracts of *Cassia siamea, Cassia spectabilis, Cassia fistula, Cassia biflora* and only in ethanol extract of *Cassia hirsuta*. It is moderately present in ethyl acetate extract of *Cassia hirsuta*.

**Phlobatannins** have diuretic property [Awoyinka OA, et al ., 2007]. These are in high amounts in methanol extract of *Cassia siamea* and ethanol extract of *Cassia biflora*. It is moderately present in ethanol, ethyl acetate extract of *Cassia siamea*, all three extracts of *Cassia spectabilis*, *Cassia hiirsuta*, and methanol , ethyl acetate extracts of *Cassia fistula* and *Cassia biflora*. It is not seen in the ethanol extract of *Cassia fistula*.

**Table:1 Review of the various medicinal uses of the study plants**

| Sl No. | Species | Uses | References |
|---|---|---|---|
| 1 | *Cassia spectabilis* (Tree) | Leaves-inhibit oedema, treatment of constipation, poisoning and protozoic infections of gut. | Abatan M.O (2010), Mwinu-kikuyu(2009) |
| 2 | *Cassia siamea* (Tree) | Leaves-liver problems, loss of appetite from gastro intestinal trouble, insomnia. Flowers-antihypertensive. | W.Thongsaard (2001) |
| 3 | *Cassia fistula* (Tree) | Leaves-scabies. Seeds and fruit pulp-mild laxative, amoebiasis, constipation, urinary disorders, fungal infection. Root-fever, common cold. | M.Dhanish et al (2011) |
| 4 | *Cassia biflora* (Shrub) | Human blood group typing using lectins.Seeds-lectins-agglutination=*C.biflora*(in PVP)=O(not A, B, AB). | Dr.Sathyananda |
| 5 | *Cassia hirsuta* (Shrub) | Leaves-herpes, snakebite, antihelmentic, fix bone fractures and sprains-humans and cattle. Seeds – powder is used to massage teeth and gums to protect from plaque and caries.Bark-chronic gastric. | A.Biswas(2008), A K Das(2006), A K Das (2005) |

**Table-2: Qualitative analysis of the various phyto-constituents on the ethanol, methanol and ethyl-acetate extracts of *Cassia spectabilis*.**

| Sl No | Phytochemical Test | Ethanol Extract | Methanol Extract | Ethyl acetate Extract |
|---|---|---|---|---|
| 1 | Test For Alkaloids | | | |
| | Dragendorff's Test | ++ | ++ | ++ |
| 2 | Test for Tannin | ++ | ++ | + |
| 3 | Test for Saponin | ++ | ++ | - |
| 4 | Test for Anthraquinone | - | - | - |
| 5 | Test for Anthocyanosides | - | - | - |
| 6 | Test for Phenolic flavonoids | - | - | - |
| 7 | Test for Flavonoids | + | + | + |
| 8 | Test for Carbohydrates | | | |
| | Benedict's Test | ++ | ++ | ++ |
| | Molisch's Test | ++ | ++ | ++ |
| | Fehling's Test | ++ | ++ | + |
| 9 | Test for Proteins | ++ | + | + |
| 10 | Test for Steroids | + | + | + |
| 11 | Test for Terpenoids | + | + | + |
| 12 | Test for Cardiac glycosides | ++ | ++ | ++ |
| 13 | Test for Phlobatannins | + | + | + |
| *Present ++* *Moderately present +* *Absent -* | | | | |

**Table-3: Qualitative analysis of the various phyto-constituents on the ethanol, methanol and ethyl-acetate extracts of *Cassia siamea.***

| Sl No | Phytochemical Test | Ethanol Extract | Methanol Extract | Ethyl acetate Extract |
|---|---|---|---|---|
| 1 | Test For Alkaloids | | | |
| | Dragendorff's Test | ++ | ++ | ++ |
| 2 | Test for Tannin | ++ | ++ | ++ |
| 3 | Test for Saponin | - | ++ | + |
| 4 | Test for Anthraquinone | - | - | - |
| 5 | Test for Anthocyanosides | - | - | - |
| 6 | Test for Phenolic flavonoids | - | + | - |
| 7 | Test for Flavonoids | + | ++ | ++ |

| | | | | |
|---|---|---|---|---|
| 8 | Test for Carbohydrates | | | |
| | Benedict's Test | ++ | ++ | ++ |
| | Molisch's Test | ++ | ++ | ++ |
| | Fehling's Test | + | ++ | + |
| 9 | Test for Proteins | ++ | + | + |
| 10 | Test for Steroids | ++ | ++ | ++ |
| 11 | Test for Terpenoids | ++ | ++ | ++ |
| 12 | Test for Cardiac glycosides | ++ | ++ | ++ |
| 13 | Test for Phlobatannins | + | ++ | + |
| *Present ++* *Moderately present +* *Absent -* | | | | |

**Table-4: Qualitative analysis of the various phyto-constituents on the ethanol, methanol and ethyl-acetate extracts of *Cassia fistula.***

| Sl No | Phytochemical Test | Ethanol Extract | Methanol Extract | Ethyl acetate Extract |
|---|---|---|---|---|
| 1 | Test For Alkaloids | | | |
| | Dragendorff's Test | + | + | ++ |
| 2 | Test for Tannin | + | + | + |
| 3 | Test for Saponin | - | + | - |
| 4 | Test for Anthraquinone | - | - | - |
| 5 | Test for Anthocyanosides | - | - | - |
| 6 | Test for Phenolic flavonoids | - | - | - |
| 7 | Test for Flavonoids | - | - | - |
| 8 | Test for Carbohydrates | | | |
| | Benedict's Test | ++ | ++ | ++ |
| | Molisch's Test | ++ | ++ | + |
| | Fehling's Test | ++ | ++ | ++ |
| 9 | Test for Proteins | ++ | ++ | ++ |
| 10 | Test for Steroids | ++ | ++ | ++ |
| 11 | Test for Terpenoids | - | + | - |
| 12 | Test for Cardiac glycosides | ++ | ++ | ++ |
| 13 | Test for Phlobatannins | - | + | + |
| *Present ++* *Moderately present +* *Absent -* | | | | |

**Table-5: Qualitative analysis of the various phyto-constituents on the ethanol, methanol and ethyl-acetate extracts of *Cassia biflora.***

| Sl No | Phytochemical Test | Ethanol Extract | Methanol Extract | Ethyl acetate Extract |
|---|---|---|---|---|
| 1 | Test For Alkaloids | | | |
| | Dragendorff's Test | + | + | ++ |
| 2 | Test for Tannin | + | + | + |
| 3 | Test for Saponin | - | - | - |
| 4 | Test for Anthraquinone | - | - | - |
| 5 | Test for Anthocyanosides | - | - | - |
| 6 | Test for Phenolic flavonoids | - | - | - |
| 7 | Test for Flavonoids | + | + | + |
| 8 | Test for Carbohydrates | | | |
| | Benedict's Test | ++ | ++ | ++ |
| | Molisch's Test | ++ | ++ | ++ |
| | Fehling's Test | ++ | ++ | + |
| 9 | Test for Proteins | ++ | ++ | ++ |
| 10 | Test for Steroids | + | + | + |
| 11 | Test for Terpenoids | + | + | + |
| 12 | Test for Cardiac glycosides | ++ | ++ | ++ |
| 13 | Test for Phlobatannins | ++ | + | + |
| Present ++ Moderately present + Absent - | | | | |

**Table-6: Qualitative analysis of the various phyto-constituents on the ethanol, methanol and ethyl-acetate extracts of *Cassia hirsuta.***

| Sl No | Phytochemical Test | Ethanol Extract | Methanol Extract | Ethyl acetate Extract |
|---|---|---|---|---|
| 1 | Test For Alkaloids | | | |
| | Dragendorff's Test | ++ | ++ | ++ |
| 2 | Test for Tannin | + | ++ | - |
| 3 | Test for Saponin | - | - | - |
| 4 | Test for Anthraquinone | - | - | - |
| 5 | Test for Anthocyanosides | - | - | - |
| 6 | Test for Phenolic flavonoids | - | - | - |

| | | | | |
|---|---|---|---|---|
| 7 | Test for Flavonoids | ++ | - | + |
| 8 | Test for Carbohydrates | | | |
| | Benedict's Test | ++ | ++ | ++ |
| | Molisch's Test | ++ | ++ | + |
| | Fehling's Test | ++ | ++ | + |
| 9 | Test for Proteins | + | ++ | + |
| 10 | Test for Steroids | ++ | ++ | + |
| 11 | Test for Terpenoids | ++ | ++ | + |
| 12 | Test for Cardiac glycosides | ++ | ++ | ++ |
| 13 | Test for Phlobatannins | + | + | + |
| Present ++ Moderately present + Absent - | | | | |

## CONCLUSION

Secondary metabolite studies have shown the presence of large quantities of alkaloids, tannins, saponins, flavonoids, carbohydrates, proteins, steroids, terpenoids, cardiac glycosides and phlobatannins that are of great importance as a source of new useful drugs.

From these studies, it can be concluded that all five species of *Cassia* have many beneficial effects with respect to the presence of the above secondary metabolites which are likely to combat many diseases and also boost the immune system.

The phytochemical characterization of the extracts, the identification of responsible bioactive compounds and quality standards are necessary for future study.

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