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PRELIMINARY PHYTOCHEMICAL ANALYSIS OF THREE SPECIES OF GENUS *CASSIA* SEEDS USING DIFFERENT ORGANIC SOLVENTS

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ABSTRACT:
In this study, preliminary phytochemical analysis of seeds belonging to three different species of Cassia viz. C. fistula Linn., C. occidentalis Linn. and C. tora Linn. were carried out. The seeds were obtained by manual separation of the fruits of the mother plants distributed in the Chandrapur district. The extracts of seeds were obtained using the Soxhlet successive extraction method with four different solvents such as methanol, acetone, petroleum ether and aqueous. The screening of phytochemical constituents was performed using a generally accepted laboratory technique for qualitative determinations. The constituents screened for were alkaloids, glycosides, tannins, flavonoids, phenols, quinones, lignins, resins, saponins, terpenoids and carbohydrates. During this investigation, different phytochemical constituents were detected in selected seeds. The seeds of these plants have a unique combination of phytochemicals.

Keywords :- phytochemical analysis, Cassia fistula, Cassia occidentalis, Cassia tora and seeds.

INTRODUCTION :

Throughout history, plants have proven to be the most effective means of treating diseases and they have been an important source of pharmaceuticals worldwide. They also provide a source of medicine since the earliest time. Plants in all facets of life have served as valuable starting material for drug development (Edeoga *et al.* 2005). The phytochemicals that plants produce are often referred to as secondary metabolites because the plants that manufacture them have little need for them. According to Solomon, *et al.* (2013), these phytochemicals are synthesized in all parts of the plant body i.e. any part of the plant body may contain bioactive compounds. Together with nutrients and fibers, these chemicals form an integrated defense system against diseases and stress (Thilagavathi *et al.*, 2015). The most important bioactive groups of plants are alkaloids, terpenoids, tannins, saponins, and phenolic compounds (Edeoga *et al.*, 2005). The seeds of *Cassia fistula* Linn., *Cassia occidentalis* Linn. and *Cassia tora* Linn. are rich in different biologically active compounds. The present investigation was undertaken to investigate and examine the phytochemical constituents of these seeds.

MATERIALS AND METHODS :

Plant Material:

For the present investigation seeds belonging to three species of genus *Cassia viz. C. fistula* Linn., *C. occidentalis* Linn. and *C. tora* Linn. were utilized.

METHODOLOGY:

To carry out a preliminary phytochemical investigation following methods were adopted.

i) Extensive exploration: Frequent visit to different areas of Chandrapur district Maharashtra, India was made.

ii) Collection of plant material: Seeds were collected from their mother plant along with fruits. Plant species under study were photographed along with certain flowering twigs for identification purposes. The flowers were also photographed for easier identification.

iii) Identification of collected plant species: Identification of collected plant species was

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carried out by referring to different floras, books and relevant journal articles.

iv) Processing of Plant Material: The dried seeds were grinded and sieved. The powder thus obtained was stored in zip-lock pouches.

v) Extraction: Powdered plant material was subjected to successive solvent extraction. For the extraction Soxhlet extractor (Harborne, 1973) with different solvents *viz.* methanol, acetone, petroleum ether and water were used. The obtained crude mixture was evaporated and stored in a closed container in the refrigerator. The condensed extracts were used for the preliminary screening of phytochemical constituents.

vi) Phytochemical screening:

A. Test for Alkaloids

(1) Dragandroff's test:

In a test tube, 2 ml seed extract was taken and acidified with 2 ml of 1 percent HCL. To this 1 ml of Dragendorff's reagent was added. The appearance of an orange-red precipitate indicates the presence of alkaloids.

(2) Mayer's test:

To 1 ml of seed extract, a few drops of Mayer's reagent was added. Cream precipitate confirms the presence of alkaloids.

(3) Wagner's test:

1 ml of seed extract was taken in a test tube and an equal volume of Wagner's reagent was added to it. The reddish precipitate appearance shows the presence of alkaloids.

(4) Hager's test:

In a test tube, 2 ml of seed extract solution was taken and a few drops of Hager's reagent was added. Bright yellow precipitate indicates the presence of alkaloids.

(5) Tannic acid test:

The seed extract was treated with 10% tannic acid. A pale-yellow precipitate shows the presence of alkaloids.

(6) $FeCl_3$ test:

To 1 ml of seed extract solution, a few drops of neutral ferric chloride solution was added. Yellow precipitate confirms the presence of alkaloids.

B. Tests for Glycosides

(1) Raymond's test:

The seed extract was treated with dinitrobenzene in hot alkali. A violet or pink colour conforms to the presence of glycosides.

(2) Legal's test:

The seed extract was treated with pyridine and alkaline sodium nitroprusside solution, the cherry colour conforms to the presence of glycosides.

(3) Bromine water test:

The seed extract was treated with bromine water. The formation of a pale yellow colour indicates the presence of glycosides.

(4) Keller Kiliani's test:

1ml seed extract solution was taken into the test tube to which 1ml of glacial acetic acid was added and cooled. After cooling, 2-3 drops of ferric chloride were added. Afterward 2 ml of conc. H_2SO_4 was added to the test tube. The appearance of a reddish-brown ring at the junction of two layers shows the presence of Glycosides.

(5) Molisch's test

In a test tube, 1ml of seed extract solution was taken then 2-3 drops of Molisch's reagent was added and mixed well. After this conc. H_2SO_4 was added slowly along the walls of a test tube. A reddish-purple ring at the junction of two layers conforms to the presence of glycosides.

(6) Conc. H_2SO_4 test:

1ml of seed extract solution was taken in a test tube to which 1ml of conc. H_2SO_4 was added and allowed to stand for 2 min. The appearance of a red precipitate indicates the presence of glycosides.

C. Tests for Tannins

(1) Ferric chloride test:

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The seed extract solution was taken in a test tube to which a few drops of $FeCl_3$ solution was added. The formation of a blackish precipitate shows the presence of tannins.

(2) Gelatine test:

To 1ml of seed extract solution, a few drops of gelatine solution was added. The appearance of a white precipitate indicates the presence of tannins.

(3) Lead acetate test:

1 ml seed extract solution was taken in the test tube and basic lead acetate was added to it. The bulky red precipitate indicates the presence of tannins.

(4) Alkaline reagent test:

The seed extract solution was treated with NaOH solution. The formation of yellow to red precipitate confirms the presence of tannins.

(5) Mitchell's test:

The seed extract solution was taken in the test tube and treated with iron and sodium tartarate. The water-soluble iron-tannin complex was formed, which is insoluble in the ammonium acetate solution, indicating the presence of tannins.

D. Test for Flavonoids

(1) Shinoda test:

In a test tube, 1ml of seed extract solution was taken and a few fragments of Magnesium ribbon was added to it. After this conc. HCl was added dropwise along the walls of the test tube. The formation of Crimson red colour shows the presence of flavonoids.

(2) Zinc Hydrochloride reduction test

1ml of seed extract solution was taken in a test tube and a mixture of zinc dust and conc. HCl was added to it. The appearance of red colour after a few minutes confirms the presence of flavonoids.

(3) Lead acetate test

Basic lead acetate was added to 1ml of seed extract solution in a test tube. A bulky reddish-brown precipitate indicates the presence of flavonoids.

(4) Alkaline reagent test

The seed extract solution was taken in the test tube and treated with NaOH solution. The formation of yellow to red colour confirms the presence of flavonoids.

(5) Ferric chloride test

A few drops of FeCl3 solution was added to the seed extract solution in a test tube. The formation of a blackish precipitate indicates the presence of flavonoids.

E. Test for Phenols:

(1) Phenol test:

The 2ml of seed extract solution was taken in a test tube. Then 1ml of FeCl3 solution was added to it. The formation of intense colour indicates the presence of phenols.

(2) Ellagic acid test:

In a test tube, 2ml seed extract solution was taken and a few drops of 5% (w/v) glacial acetic acid and 5% (w/v) sodium nitrate solution were added to it. The appearance of Niger brown precipitate shows the presence of phenol.

F. Test for Quinones:

1 ml of seed extract solution was taken in a test tube and an alcoholic KOH solution was added to it. The appearance of the colour ranging from red to blue indicates the presence of quinones.

G. Test for Lignins:

To 1ml of the seed extract solution 2% (w/v) furfuraldehyde was added in attest tube. The formation of red colour shows the presence of lignins.

H. Test for Resins:

2-3ml of copper sulphate solution was added to 1ml of seed extract solution and the contents were mixed well for 2min. Then the solution was allowed to separate. The green-coloured precipitate formed indicating the presence of resin.

I. Test for Saponins:

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3ml of seed extract solution was taken in a test tube and shaken vigorously. The appearance of foam indicates the presence of saponins.

J. Test for Terpenoids:

(1) Salkowaski test:

2 ml seed extract solution was taken in a test tube; then 3 ml chloroform was added to it followed by the addition of conc. H_2SO_4. The formation of the reddish-brown ring at the junction of two liquids confirmed the presence of terpenoids in the extract.

(2) Libermann Burchard's test:

To 2 ml seed extract solution 3 ml chloroform was added followed by the addition of acetic anhydride. The solution was heated and cooled down then 2 ml of conc. H_2SO_4 was added to it. The appearance of the violet-coloured ring at the junction indicated the presence of terpenoids.

K. Test for carbohydrates:

(1) Fehling's test:

2 ml seed extract solution was taken in a test tube and 1ml of dilute HCl. The solution was neutralized with alkali and heated with Fehling's solution (A+B). The formation of red precipitate shows the presence of carbohydrates.

(2) Molisch's test:

In a test tube, 2 ml seed extract solution was taken and 2 drops of Molisch's reagent were added and shaken. Then conc. H_2SO_4 was added to it. The appearance of the violet ring at the junction of two liquids the presence of carbohydrates.

RESULTS AND DISCUSSION:

1) Preliminary phytochemical analysis of *Cassia fistula* Linn. seed extracts. (Table 1):

a) Methanol extract: The preliminary phytochemical analysis of seed methanolic extract revealed the presence of alkaloids, glycosides, tannins, flavonoids, phenols and terpenoids. The quinones, lignins, resins, saponins and carbohydrates were not detected in the analysis.

b) Acetone extract: In the acetone extract of seeds following compounds were detected alkaloids, glycosides, tannins, flavonoids, quinones, saponins and terpenoids. The phenols, lignins, resins and carbohydrates were not detected in the analysis.

c) Petroleum ether extract: The analysis of petroleum ether seed extract shows the presence of alkaloids, glycosides, tannins and flavonoids. The phenols, quinones, lignins, resins, saponins, terpenoids and carbohydrates were not detected.

d) Aqueous extract: The aqueous extract of seeds revealed the presence of the following compounds alkaloids, glycosides, tannins, flavonoids, lignins, saponins and carbohydrates. The phenols, quinones, resins and terpenoids were not detected in the analysis.

2) Phytochemical analysis of *Cassia occidentalis* Linn. seed extracts. (Table 2):

a) Methanol extract: The preliminary phytochemical analysis of seed methanolic extract revealed the presence of alkaloids, glycosides, tannins, flavonoids, resins, saponins, terpenoids and carbohydrates. The phenols, quinones and lignins were not detected in the analysis.

b) Acetone extract: In the acetone extract of seeds following compounds were detected alkaloids, glycosides, tannins, flavonoids, phenols, quinones, terpenoids and carbohydrates. The lignins, resins and saponins were not detected in the analysis.

c) Petroleum ether extract: The analysis of petroleum ether seed extract shows the presence of alkaloids, glycosides, tannins, flavonoids, phenols, quinones, resins, terpenoids and carbohydrates. The lignins and saponins were not detected.

d) Aqueous extract: The aqueous extract of seeds revealed the presence of the following compounds alkaloids, glycosides, tannins, flavonoids, phenols, resins, saponins, terpenoids

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and carbohydrates. The quinones and lignins were not detected in the analysis.

3) Phytochemical analysis of *Cassia tora* Linn. seed extracts. (Table 3):

a) Methanol extract: The preliminary phytochemical analysis of seed methanolic extract revealed the presence of alkaloids, glycosides, tannins, flavonoids, saponins, terpenoids and carbohydrates. The phenols, quinones, lignins and resins were not detected in the analysis.

b) Acetone extract: In the acetone extract of seeds following compounds were detected alkaloids, glycosides, tannins, flavonoids, quinones, saponins, terpenoids and carbohydrates. The phenols, lignins and resins were not detected in the analysis.

c) Petroleum ether extract: The analysis of petroleum ether seed extract shows the presence of alkaloids, glycosides, tannins, flavonoids, phenols, lignins, resins, terpenoids and carbohydrates. The quinones and saponins were not detected.

d) Aqueous extract: The aqueous extract of seeds revealed the presence of the following compounds alkaloids, glycosides, tannins, flavonoids, phenols, quinones, lignins, resins, saponins, terpenoids and carbohydrates.

In a review study conducted by Kabila *et al.* (2017), different *Cassia* species were analyzed for their phytochemical profiles. In this study, alkaloids, flavonoids, saponins and tannins have been identified in most *Cassia* species. They found that alkaloids are absent in ethanol and petroleum ether extracts of seed of *C. occidentalis*. According to our study, methanolic extract of *C. occidentalis* seeds also shows their absence in most of the conducted tests. In an investigation carried out by Ambikapathy and Panneer (2012) for *C. occidentalis* seeds alkaloids, flavonoids, saponins and tannins were detected. Roopashree *et al.* (2008) reported the absence of alkaloids and tannins while in aqueous extracts of *C. tora.* seeds flavonoid and saponins were not detected.

CONCLUSION:

The results of this study clarify that *C. fistula* Linn., *C. occidentalis* Linn., and *C. tora* Linn. have tremendous biological potential and can be utilised effectively for the treatment of a wide range of diseases. The findings of this investigation are quite encouraging, thus Ayurvedic and Chinese remedies use the seeds of these plants. However, there is a need for further standardization and isolation of its active constituent because there is tremendous scope for further research.

Conflict of Interest:

In the publication of this paper, the authors declare that they have no conflicts of interest.

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Table 1: Phytochemical analysis of *Cassia fistula* Linn. seed extracts.

Phytochemical test	Methanol extract	Acetone extract	Petroleum Ether extract	Aqueous extract
A. Test for Alkaloids				
(1) Dragandroff's test	+	+	+	+
(2) Mayer's test	+	+	+	-
(3) Wagner's test	+	-	+	+
(4) Hager's test	+	+	-	+
(5) Tannic acid test	+	+	+	+
(6) $FeCl_3$ test	+	+	+	-
B. Tests for Glycosides				
(1) Raymond's test	-	+	+	+
(2) Legal's test	-	+	-	+
(3) Bromine water test	-	-	-	+
(4) Keller Kiliani's test	+	+	+	-
(5) Molisch's test	-	+	+	+
(6) Conc. H_2SO_4 test	-	+	+	+
C. Tests for Tannins				
(1) Ferric chloride test	+	+	-	+
(2) Gelatine test	+	-	+	+
(3) Lead acetate test	-	-	+	-
(4) Alkaline reagent test	-	+	-	+
(5) Mitchell's test	+	+	+	+
D. Test for Flavonoids				
(1) Shinoda test	+	-	-	+
(2) Zinc Hydrochloride reduction test	-	-	+	+
(3) Lead acetate test	-	-	-	+
(4) Alkaline reagent test	+	+	-	-
(5) Ferric chloride test	-	-	-	+
E. Test for Phenols				
(1) Phenol test	+	-	-	-
(2) Ellagic acid test	+	-	-	-
F. Test for Quinones	-	+	-	-
G. Test for Lignins	-	-	-	+
H. Test for Resins	-	-	-	-
I. Test for Saponins	-	+	-	+
J. Test for Terpenoids				
(1) Salkowaski test	+	+	-	-
(2) Libermann Burchard's test	+	+	-	-
K. Test for carbohydrates				
(1) Fehling's test	-	-	-	+
(2) Molisch's test	-	-	-	+
+ = Detected and - = Not Detected				

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Table 2: Phytochemical analysis of *Cassia occidentalis* Linn. seed extracts.

Phytochemical test	Methanol extract	Acetone extract	Petroleum Ether extract	Aqueous extract
A. Test for Alkaloids				
(1) Dragandroff's test	-	-	-	-
(2) Mayer's test	-	-	+	-
(3) Wagner's test	+	-	-	-
(4) Hager's test	-	-	-	+
(5) Tannic acid test	-	+	-	-
(6) $FeCl_3$ test	-	-	-	+
B. Tests for Glycosides				
(1) Raymond's test	+	+	-	+
(2) Legal's test	+	-	+	+
(3) Bromine water test	+	+	-	-
(4) Keller Kiliani's test	+	-	-	+
(5) Molisch's test	-	+	-	+
(6) Conc. H_2SO_4 test	+	-	-	+
C. Tests for Tannins				
(1) Ferric chloride test	+	+	-	-
(2) Gelatine test	+	-	+	+
(3) Lead acetate test	+	+	-	+
(4) Alkaline reagent test	-	-	-	+
(5) Mitchell's test	+	+	+	-
D. Test for Flavonoids				
(1) Shinoda test	+	+	-	+
(2) Zinc Hydrochloride reduction test	+	-	+	+
(3) Lead acetate test	+	+	-	-
(4) Alkaline reagent test	+	+	-	+
(5) Ferric chloride test	+	-	-	+
E. Test for Phenols				
(1) Phenol test	-	+	+	-
(2) Ellagic acid test	-	+	-	+
F. Test for Quinones	-	+	+	-
G. Test for Lignins	-	-	-	-
H. Test for Resins	+	-	+	+
I. Test for Saponins	+	-	-	+
J. Test for Terpenoids				
(1) Salkowaski test	+	+	+	+
(2) Libermann Burchard's test	+	+	-	-
K. Test for carbohydrates				
(1) Fehling's test	+	+	+	+
(2) Molisch's test	+	+	-	-
+ = Detected and - = Not Detected				

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Table 3: Phytochemical analysis of *Cassia tora* Linn. seed extracts.

Phytochemical test	Methanol extract	Acetone extract	Petroleum Ether extract	Aqueous extract
A. Test for Alkaloids				
(1) Dragandroff's test	+	+	-	-
(2) Mayer's test	+	+	+	-
(3) Wagner's test	+	+	-	-
(4) Hager's test	+	+	-	+
(5) Tannic acid test	-	-	-	-
(6) $FeCl_3$ test	+	+	-	-
B. Tests for Glycosides				
(1) Raymond's test	+	+	+	+
(2) Legal's test	+	-	+	+
(3) Bromine water test	+	+	+	+
(4) Keller Kiliani's test	-	-	+	-
(5) Molisch's test	+	+	-	+
(6) Conc. H_2SO_4 test	-	+	+	+
C. Tests for Tannins				
(1) Ferric chloride test	+	+	+	+
(2) Gelatine test	+	-	+	+
(3) Lead acetate test	+	+	+	+
(4) Alkaline reagent test	-	-	+	-
(5) Mitchell's test	+	+	+	+
D. Test for Flavonoids				
(1) Shinoda test	+	+	+	+
(2) Zinc Hydrochloride reduction test	+	+	+	+
(3) Lead acetate test	+	+	-	+
(4) Alkaline reagent test	+	-	+	-
(5) Ferric chloride test	+	+	+	+
E. Test for Phenols				
(1) Phenol test	-	-	+	+
(2) Ellagic acid test	-	-	-	+
F. Test for Quinones	-	+	-	+
G. Test for Lignins	-	-	+	+
H. Test for Resins	-	-	+	+
I. Test for Saponins	+	+	-	+
J. Test for Terpenoids				
(1) Salkowaski test	+	-	-	+
(2) Libermann Burchard's test	+	+	+	+
K. Test for carbohydrates				
(1) Fehling's test	+	+	-	+
(2) Molisch's test	+	+	+	+
+ = Detected and - = Not Detected				

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