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QUALITATIVE PHYSICOCHEMICAL, PHYTOCHEMICAL ANALYSIS AND QUANTITATIVE ESTIMATION OF TOTAL PHENOLS, FLAVONOIDS AND ALKALOIDS OF *CASSIA GRANDIS*

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ABSTRACT

The aim of present study was to investigate the physicochemical,phyto-constituents present within the hexane,ethylacetate and methanol extract of *Cassiagrandis*and to estimate the total phenolic,flavonoid and alkaloid contents. The amount of total phenols, were analyzed using a spectrophotometric technique, based on Folin-ciocalteau reagent. Gallic acid was used as standard compound and the total phenols were expressed as mg / g gallic acid equivalents (Standard curve equation: y= 0.0106x + 0.041, R2 = 0.996). Total flavonoid contents of the plant were determined by using quercetinreference standard method.The total alkaloid content was determined by using rutin as standard. Phytochemical analysis indicated the presence of cardiac glycosides, reducing sugars, flavonoids, phenolic compounds and alkaloids.The Physicochemical analysis includes Total Ash (0.072 to 0.666), Water insoluble Ash (0.053 to 0.551), Water soluble Ash (0.019 to 0.170), Acid insoluble Ash (0.033 to 1.946) and Loss on drying (0.471 to 0.924) as per standard methods.

INTRODUCTION: [1]

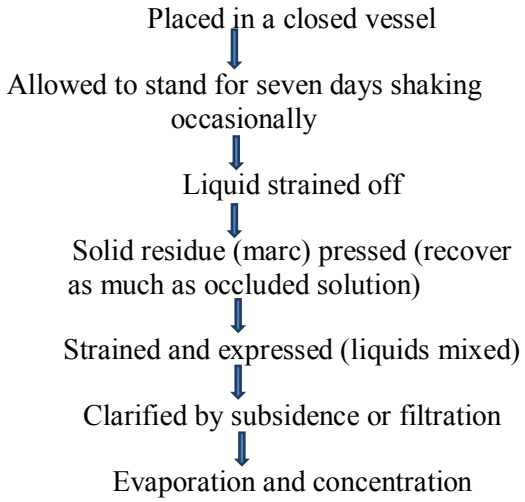
Cassia grandis, commonly known as coral shower tree, is a semi-deciduous tree, growing up to a height of about 18 m. The tree bears a high, irregular, spreading crown, made from dangling branches. *Cassia grandis* leaves are paripinnate, with 10-20 leaflets, measuring 3-6 cm in length, and obtuse or rounded at base and apex. Flower are in long, drooping, pink or purple colored axillary racemes, without bracts subtending the pedicels. Pods are compresssed-cylinderical, glabrous and transversely rugose. The pulp of the pods is edible, sweet in taste and foul smelling, and possesses laxative properties.[2] *Cassia grandis* seeds are elliptic, oblong-obovate, obovate or obovoid-ellipsoid, with slightly emerginate base. The seeds have biconvex cross section and are ventrally flattened, enclosed in a light brown, cartaceous, smooth and opaque seed coat. The treeis generally planted for its ornamental value, and the heavy, hard wood is also used in construction. Fruits ripen in summer. The sweet pulp and bad smelling of fruit is edible and used as laxative.

EXTRACTION PROCESS

The collected leaves and stem bark were dried under shade and powdered. The powdered materials were carried out successive maceration using different solvents such as hexane, ethylacetate and methanol.

Procedure:

Plant material (Crushed or cut small or moderately coarse powder)



The extract thus obtained were concentrated and dried completely, weighed and stored in a desiccator.

FLOURESCENCE ANALYSIS [4][5]

Fluorescence analysis of the drug was observed in UV light (245nm) using various extract of the drug. The drug powder was treated separately with different solutions.

Phytochemical Analysis

The prepared extract was tested for the type of chemical constituents present by known qualitative tests.*The following tests were carried out on the extracts to detect various phyto constituents present in them.

1. Test For Alkaloids [6][7]

About 50mg of solvent-free extract was stirred with little quantity of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows.

a) Mayer's test

To a few ml of filtrate, two drops of mayer's reagent was added along with the sides of test tube. If the test is positive, it gives white or creamy precipitate.

b) Wagner's test

To a few ml of the filtrate, few drops of Wagner's reagent were added along with the sides of the test tube. Formation of reddish brown precipitate confirms the test as positive.

c) Hager's test

To a few ml of filtrate 1 or 2 ml of Hager's reagent was added. A prominent yellow precipitate indicates positive test.

d) Dragendroff's test

To a few ml of filtrate, 1 or 2 ml of Dragendroff's reagent was added. A prominent reddish brown precipitate indicates positive test

2. Test for Carbohydrates [8]

About 100mg of the extract was dissolved in 5ml of distilled water and filtered. The filtrate was subjected to the following tests.

a) Molisch's test

To 2 ml of filtrate, two drops of alcoholic solution of α- napthol was added. The mixture was shaken well and 1 ml of concentrated sulphuric acid was added slowly along the sides of the test tube, the test tube was cooled in ice water and allowed to stand. A violet ring at the junction of two liquids indicates the presence of carbohydrates

b) Fehling's test

1 ml of filtrate was boiled on a water bath with 1 ml each of Fehling's solution A and B. Formation of red precipitate indicates the presence of sugar.

c) Barfoed's test

To 1 ml of the filtrate, 1 ml of Barfoed's reagent was added and heated on a boiling water bath for 2 minutes. Red precipitate indicates the presence of sugars.

d) Benedict's test

To 0.5 ml of filtrate 0.5 ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes. A characteristic brick red precipitate indicates the presence of sugar.

3. Test for Glycosides [9][10]

For the detection of glycosides, about 50 mg of extract was hydrolyzed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and filtrate was subjected to following tests.

a) Borntrager's test

To 2 ml of filtrate hydrolysate, 3 ml of chloroform was added and shaken, chloroform layer was separated and 10% ammonia solution was added to it. Formation of pink color indicates the presence of anthraquinone glycosides.

b) Legal's test

About 50 mg of the extract was dissolved in pyridine. Sodium nitroprusside solution was added and made alkaline using 10% sodium hydroxide solution. Presence of glycoside is indicated by a characteristic pink colour.

Taxonomical classification: [3]

Kingdom	Plantae
Sub kingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Fabales
Family	Fabaceae
Genus	*Cassia*
Species	*Cassia grandis*

Table no: 1. Extraction

Plant material	Solvents used	Weight of the extract
Dried powdered material (2kg)	Hexane	30gm
	Ethylacetate	75gm
	Methanol	150gm

Parameters	Results (% W/W)
Total ash	3.7
Water soluble	3.6
Acid insoluble	2

Table no 2: Extractive values

Extract/Solvent	Polarity	Extractive Values
Hexane	0	0.05
Petroleum ether	0.1	0.04
Dichloro methane	3.4	0.06
Chloroform	3.4-4.4	0.04
Ethyl acetate	4.3	0.12
Acetone	5.4	0.24
Methanol	6.6	0.24
Water	9	0.25

Table No: 3 Flourescence analysis of powder

Solution	Short wavelength (245 nm)	Long wavelength (365 nm)	Visible light
1N NaoH (aqu)	Yellowish green	Green colour	Green colour
1N NaoH (alcoholic)	Yellowish green	Green colour	Green colour
5% NaoH	Light green	Light green	Green colour
10% NaoH	Light green	Light green	Light brown
5%$Fecl_3$ (alc)	Dark green	Dark green	Dark green
5% $Fecl_3$ (aqu)	Light green	Dark green	Light green
Acetic acid	Yellowish green	Dark green	Dark green
Iodine solution	Dark green	Dark green	Yellowish green
Picric acid	Light green	Yellowish green	Green colour
Ammonia solution	Green colour	Light green	Yellowish green
Conc.Hcl	Yellowish green	Light green	Light green
Conc.H_2SO_4	Yellowish green	Light green	Light brown

Phytochemical compounds	Hexane extract	Methanol extract	Ethylacetate extract
Cardiac glycosides	++	++	++
Saponin glycosides	+	+	+
Alkaloids	+	+	+
Amino acids	-	+	-
Starch	-	-	-
Reducing sugars	+	++	+
Phenols	++	++	++
Volatile oils	++	++	++
Tannin	++	++	++
Steroids	-	++	+
Flavanoid	+++	+++	+++

Table No: 4.Preliminary Phytochemical screening of the extract of *Cassia grandis*

Name of the test	Crude extract
Alkaloids	+
Carbohydrates	+
Amino acids	-
Phenols and tannins	++
Terpenoids	+
Saponins	+
Flavanoids	+++
Cardiac Glycosides	+++
Proteins	-
Fixed oils and fats	+
Steroids	++

*Weak (+), moderate (++), strong (+++), very strong (++++), absent (--).

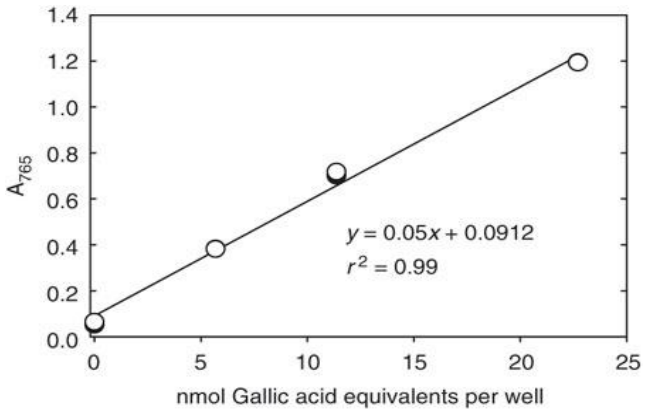


Fig no 3: Calibration curve of gallic acid

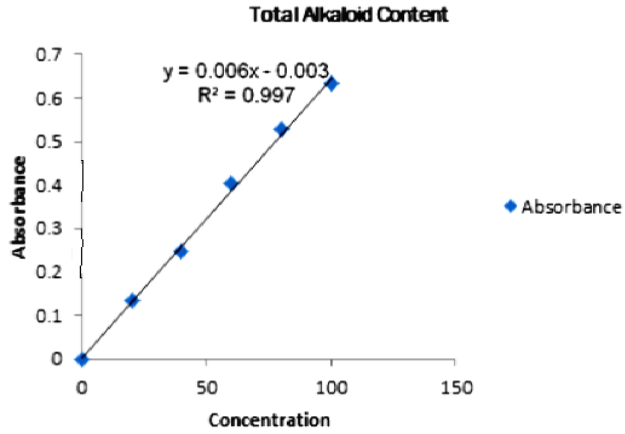


Fig. no 4: Calibration Curve of Rutin

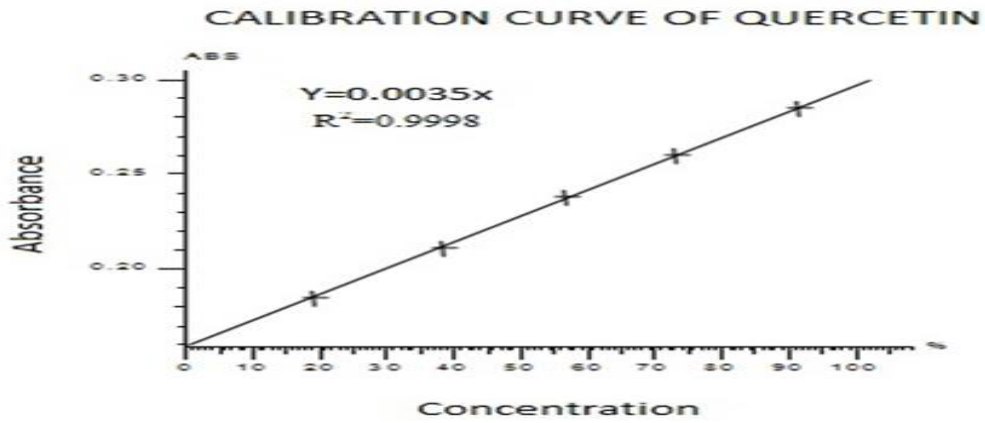


Fig. no.5. Calibration Curve of quercetin

Table No 5: Quantitative Estimation of Total Phenolic, Alkaloidal and Flavonoid Content

TEST TYPE	INFERENCES		
	Hexane Extract	**Ethyl Acetate Extract**	**Methanol Extract**
Total alkaloid content	27 ± 0.32 mg RU/g	45 ± 0.99 mg RU/g	33.9 ± 0.12 mg RU/g
Total flavanoid content	105 ± 0.96 mg QE/g	21.5 ± 0.5 mg QE/g	39.03± 1.98mg QE/g
Total phenolic content	80 ± 0.46 mg GA/g	85 ± 0.45 mg GA/g	169.73±2.90mg GA/g

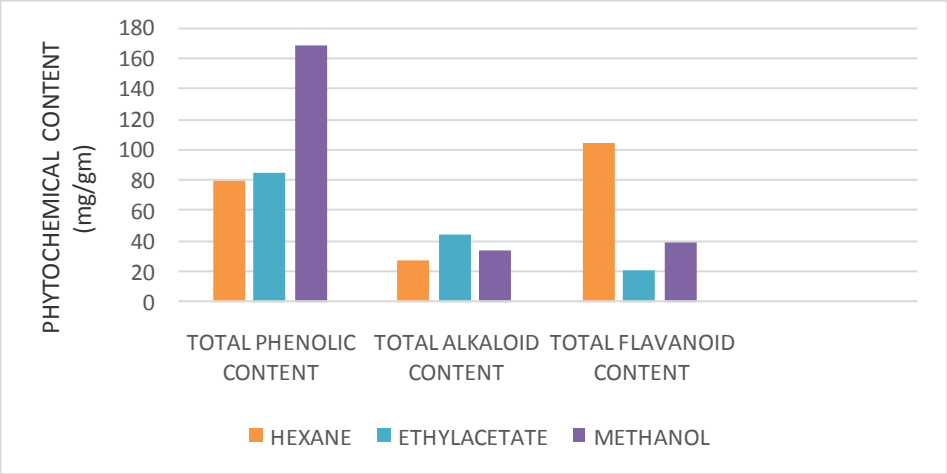


Figure 5: Total phenolic, alkaloid and flavonoid content (mg/gm) in the *Cassia grandis* extract.

4. Test for Saponins [11, 12]

a) Froth test

A small quantity of the extract was diluted with distilled water to 20 ml. The suspension was shaken in graduated cylinder for 15 minutes. A two centimeter layer of foam or froth which is stable for 10 minutes indicates the presence of saponins.

5. Test for Phytosterols and Triterpenoids [13]

a) Liebermann- burchard's test:

The extract was dissolved in acetic anhydride, heated to boiling cooled and then 1 ml of concentrated sulphuric acid was added along the side of the test tube. Red, pink or violet color at the junction of the liquids indicates the presence of steroidal triterpenoids and their glycosides.

c) **Salkowski test:**

Few drops of concentrated sulphuric acid was added the chloroform extract, shaken on standing, red color in the lower layer indicates the presence of steroids and golden yellow color indicates the presence of triterpenoids.

6. Test for Phenols and Tannins

a) Ferric chloride test[14,15]: About 50 mg of extract was dissolved in distilled water and to this few drops of neutral 5% ferric chloride solution was added. Formation of blue, green and violet color indicates the presence of phenolic compounds.

b) Gelatin test: A little quantity of extract was dissolved in distilled water and 2 ml of 1% solution of gelatin containing 10% sodium chloride was added to it. Development of white precipitate indicates the presence of phenolic compounds.

c) Lead acetate test [16, 17]

A small quantity of extract was dissolved in distilled water and to this; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of phenolic compounds.

7. Test for Flavonoids

a) Alkaline reagent test

An aqueous solution of extract was treated with 10% ammonium hydroxide solution- yellow fluorescence indicates the presence of flavonoids.

b) Shinoda test

A little quantity of extract was dissolved in alcohol and few fragments of magnesium turnings and conc. Hydrochloric acid (drop wise) were added. If any pink or crimson- red color develops, presence of flavonol glycoside is inferred.

c) Zinc- hydrochloric acid reduction test

The alcoholic solution is treated with pinch of zinc dust and few drops of conc. Hydrochloric acid- magenta color is produced after few minutes

RESULTS:

Quantitave Estimation of Total Phenolic, Alkaloidal and Flavonoid Content:

Quantification of Total Phenolic Content

Total phenolic content was determined by folin - ciocalteau reagent [18]. Folin – ciocalteaucolorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption at the wave length is proportional to the concentration of the phenols.

By using standard gallic acid calibration curve, measure the concentration of phenolic content in gallic acid total equivalents using unit's mg/gms (GAE).

Procedure:

Gallic acid was used as standard 0.5mg/ml (250 mg of gallic acid was dissolved in 1 ml of extract solvent and diluted to 500 ml with distilled water. This stock solution was stored at 4^0C. Working standards of 0.01 to 0.05 mg/ml was prepared by diluting the stock with distilled water. 100uL of extract was transferred into a test tube and 0.75 ml of FC reagent was added.0.7 ml of 6% (w/v) sodium carbonate was also added. Stand at room temperature for 90 minutes, then absorbance was read at 725nm using UV- visible spectrophotometer. Results were reported in table no.7.

QUANTIFICATION OF TOTAL ALKALOID CONTENT [19]

Procedure:

The plant extract 1 (mg/ml) was dissolved in 2N Hcl and then filtered. The pH of phosphate buffer was adjusted to neutral with 0.1 N sodium hydroxide. 1 ml of this solution was transferred to a separating funnel and then 5 ml of BCG solution along with 5 ml of phosphate buffer was added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extract was collected in 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. All the experiment was performed thrice, the results were averaged and reported in the form of mean or SEM.

Results were reported in table no.7.

QUANTIFICATION OF TOTAL FLAVONOID CONTENT [18].

Procedure:

The sample contained 1 ml of methanol solution of the extract in the concentration of 1 mg/ml of 2% aluminum tri chloride was dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determines at 415 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. Same procedure was repeated for quercetin (as standard) and the calibration curve was constructed. Results were reported in table no.5.

DISCUSSION

Phytochemical analysis for the extract (*Cassia grandis*) has been carried out by using standard procedures. In fluorescence the fluorescent light is always of greater wavelength than the exciting light. Light rich in short wavelengths is very active in producing fluorescence and for this reason ultraviolet light produces fluorescence in many substances which do not visibly fluoresce in daylight. The preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, phenols, tannins, terpenoids, flavonoids, cardiac glycosides, steroids, fixed oils and fats. All the three extracts contains moderate amount of cardiac glycosides, phenols,volatile oils and tannins and higher quantity of flavonoids are present. In quantitative estimation methanolic extract possess higher quantity of phenols. In ethyl acetate extract possess higher quantity of alkaloids and the hexane extract possess higher amount of flavonoids.

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