An Elsevier Indexed Journal ISSN-2230-7346

*Journal of Global Trends in Pharmaceutical Sciences*

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

**Kotipalli Harika*, Battu Ganga Rao and Devarakonda Ramadevi**

A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, 530-003, India

***Corresponding author E-mail:** *kotiharika@ymail.com*

**ARTICLE INFO**

**Key Words**

*Cassia grandis*, Physicochemical and Phytochemical analysis, Total Phenols, Flavonoids and alkaloids.

**ABSTRACT**

The aim of present study was to investigate the physicochemical, phyto-constituents present within the hexane, ethylacetate and methanol extract of *Cassia grandis* 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 quercetin reference 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:

*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 coloured axillary racemes, without bracts subtending the pedicels. Pods are compresssed-cylindrical, 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 tree is 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.

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

**Taxonomical classification: [3]**

**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, ethyl acetate and methanol.

**Procedure:**

Plant material (Crushed or cut small or moderately coarse powder) was placed in a closed vessel. Allowed to stand for seven days shaking occasionally and Liquid strained off. Solid residue (marc) pressed (recover as much as occluded solution) Strained and expressed (liquids mixed). Clarified by subsidence or filtration. Then Evaporation and concentration. The extract thus obtained were concentrated and dried completely, weighed and stored in a desiccators.

**Physico-chemical studies:**

**Ash values**: Used to determine quality and purity of crude drug. And to establish the identity of it. Also used to determine foreign inorganic matter present as impurity.
**Determination of total ash**:

Weigh about 2 g of powder into a porcelain dish. Heat with a burner using a flame about 2cm high and supporting the dish about 7cm above the flame. Heat till vapours almost cease to be evolved and tare the sample.

Cool in desiccators. Weigh the ash and calculate the percentage of total ash with reference to the air dried sample of crude drug.

**Determination of Acid insoluble ash:**

Using 25 ml of dil. hydrochloric acid, wash the ash from dish for total ash into 1000 ml of beaker. Place a mere gauze over a Bunsen burner. Boil for 5 min. Filter through ash less filer paper, wash the residue twice with hot water. Ignite the crucible in the flame and Cool in a desiccator. Weigh and calculate the percentage of acid insoluble ash with reference to the air dried sample of crude drug.

**Determination of water soluble ash:**

This is determined in a similar way to acid insoluble ash, using 25 ml of water in place of dilute hydrochloric acid. Boil for 5 min. Filter through ash less filter paper wash the residue twice with hot water. Cool in desiccator. Weigh and calculate the percentage of water soluble ash with reference to the air dried sample of crude drug.

**Loss on drying:**

**Procedure:**

Weight about 5 g of powder into a porcelain dish. Dry in oven at 100 & $105^0$c Cool in desiccator. Weigh and the loss in weight recorded as moisture content. Calculate the percentage of loss of drying recorded as moisture of sample.

**Swelling index:**

It gives an idea about the mucilage content of the crude drug. Hence it is useful in the evaluation of crude drugs containing mucilage.

**Procedure:**

Weigh about 1 g of powder into a 50 ml measuring cylinder. Add water up to 40 ml making. Shaking occasionally during 24 hrs. Keep a side for one hour. Measure the volume occupied by swollen powder.

**Foaming index:** It is evaluated by measuring the foaming ability in term of foaming index.

a) If the height of the foam in every tube is less than 1 cm it means foaming index is less than 100. If the height of foam is more than 1 cm every test tube; the foaming index is over 1000. In this case, repeat the experiment using a new series of dilutions o the decoction in order to get a result.
b) If the height of 1 cm in any tube, the volume of the plant material decoction in this tube(a) is used for determination of foaming index using formula follows :

$$\text{Foaming index} = 100/a$$

**Extractive values**:

- Useful in evaluation of crude drug.
- To determine give idea of nature of chemical constituents present in crude drug.
- Useful of estimation of constituents extracted with solvent used for extraction.
- Employed for material for which as yet no suitable chemical or biological assay exists.

**Procedure**

- Weigh about 1 g of powder in add different solvents like (Hexane, Dichloromethane, chloroform, ethyl acetate, acetone, methanol, water, petroleum ether) into a boiling tubes.
- Cork the flask and set aside for 24 hrs.
- Shaking frequently occasionally during 6 hrs and allowed to stand for 24 hrs.
- Filter into a 50 ml of measuring cylinder. Transfer into filtrate in thin porcelain dish.
- The fitrate evaporated to dryness in a tarred flat bottom dish.
- Weighed and calculate the % w/w of extractive values of different solvents

**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 phytoconstituents 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 colour 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.

**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.

**b) Salkowski test:** Few drops of concentrated sulphuric acid was added the chloroform extract, shaken on standing, red colour in the lower layer indicates the presence of steroids and golden yellow colour 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 colour 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 colour is produced after few minutes

## 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 – ciocalteau colorimetry 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 units 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 $4^0$C. 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, and 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.

**Table No 1: Extraction of dried powdered material**

| Plant material | Solvents used | Weight of the extract |
|---|---|---|
| Dried powdered material (2kg) | Hexane<br>Ethyl acetate<br>Methanol | 30gm<br>75gm<br>150gm |

**Table No: 2**

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

**Results:**

| **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 (--).

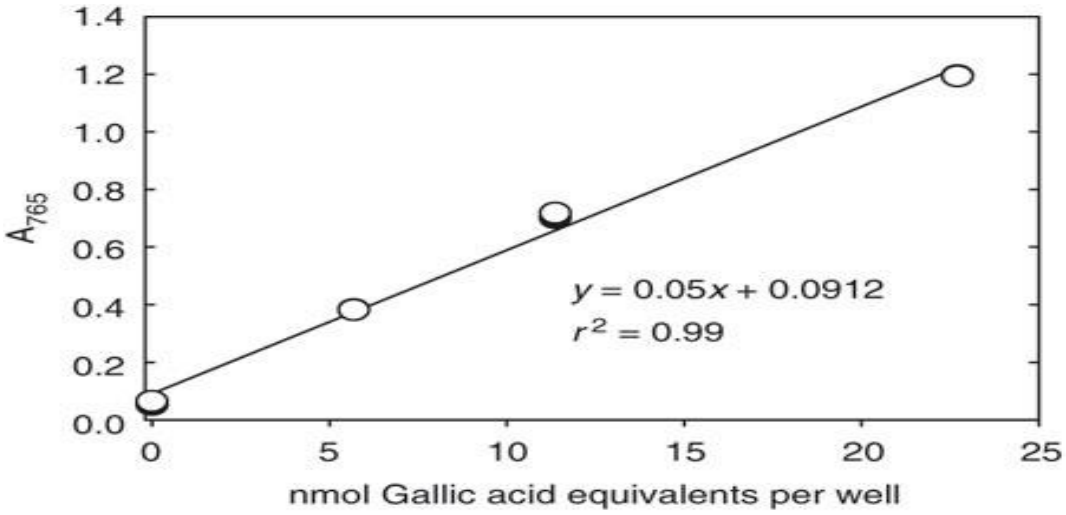



**Fig.no.1. Calibration curve of gallic acid**

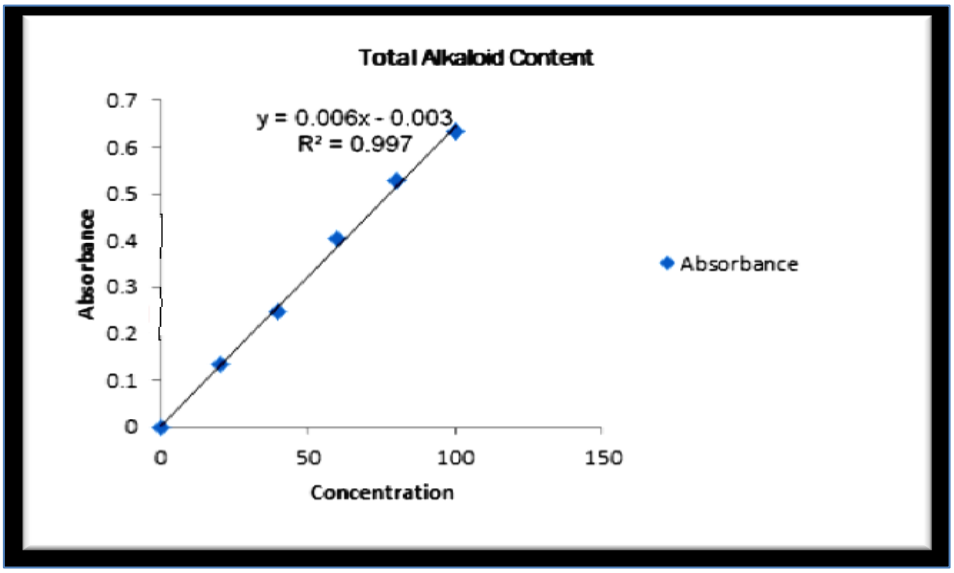



**Fig.no.2. Calibration of Rutin**

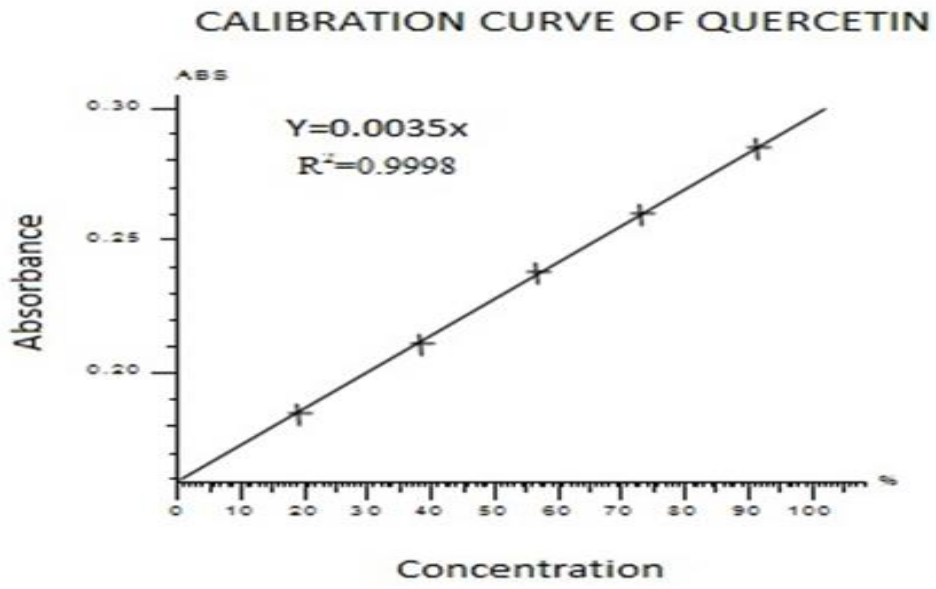
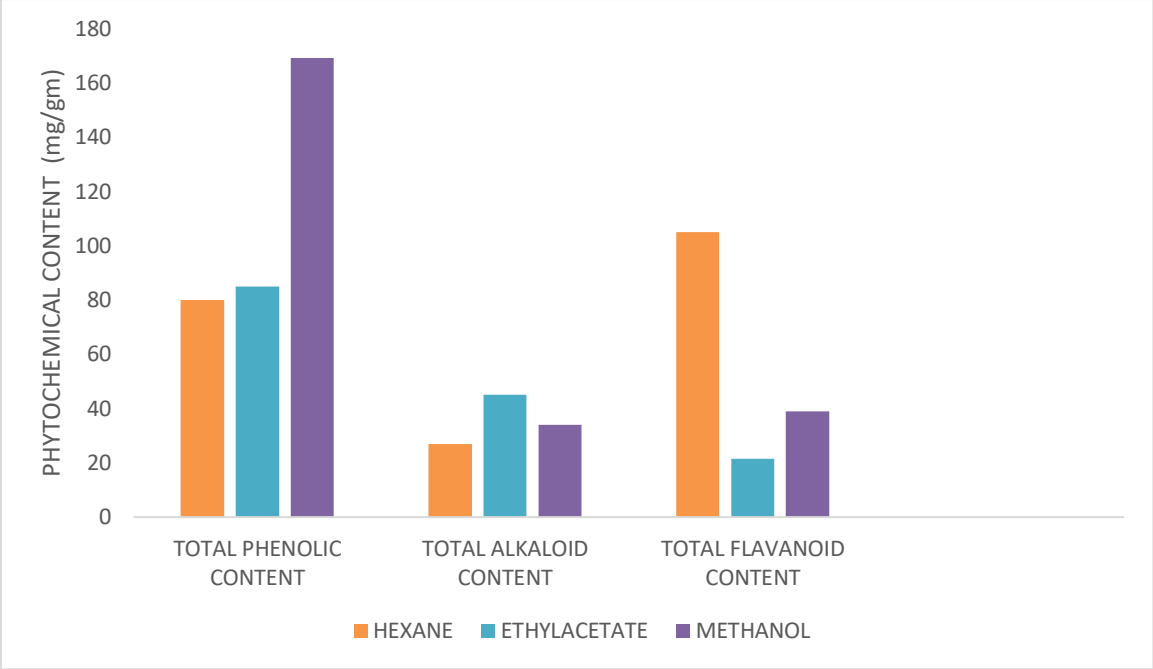



**Fig.no.3. Calibration Curve of quercetin**

**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 |

**Fig 4: Total phenolic, alkaloid and flavonoid content (mg/gm) in the *Cassia grandis* extract.**




**REFERENCES:**


1. Jain, S.K., Medicinal Plants, National Book Trust, New Delhi., 1968, p.37.
2. World Agroforestry Centre. AgroforestryTreeDatabase, A tree species reference & selection guide [Online]. 2012; Available from: URL:http://worldagroforestrycentre. org/
3. Magalion, S.A., Sanderson, K.R. Absolute diversification rates in angiosperm clades. Evolution, 55 (9): 1762-1780. 2001.
4. Gupta MK, Sharma PK, Ansari SH, Lagarkha R. Pharmacognostical evaluation of Grewia asiatica fruits. Int J Plant Science 2006; 1 (2): 249-251.
5. Kokashi CJ, Kokashi RJ, Sharma M. Fluorescence of powdered vegetable drugs in ultra- violet radiation. J American Pharm Assoc1958; 47:715-717.
6. Egwaikhide P.A., Gimba C.E., Analysis of the Phytochemical Content and Anti-microbial Activity of Plectranthus glandulosis Whole Plant. 2007, 135-138.
7. Narasimhan Rangarajan, Mohan Ambilly, Phytochemical Screening of Sesamum Indicum seed Extract. Vol. 1, Issue 4, 2012, 1298-1308
8. Krishnadevi S, Theymoli B and Sadasivam S. Phenol Sulphuric acid method. Food Chem., 1984; 15: 229
9. Trease GE, Evans WC. Pharmacognosy. 11th edn; Brailliar Tiridel Can. Macmillian Publishers., 1989.
10. Harborne JB. Phytochemical Methods—A Guide to Modern Techniques of Plant Analysis. 1stedn: Chapman and Hall; London, UK., 1998.
11. Siddiqui AA, Ali M. Practical Pharmaceutical Chemistry. 1st edn; CBS Publishers and distributors, New Delhi, 1997; 126-131.
12. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. Internationale Pharmaceutica Sciencia, 2011; 1: 98-106
13. Wall ME, Krider MM, Krewson CF, Eddy CR, Willaman JJ, Correll DS, Gentry HS. Steroidal sapogenins – Survey of plants for steroidal sapogenins and othe constituents VII, Jour. Amer. Pharm. Ass., Sci Ed., 1954; 43: 1-7
14. Kokate CK. Practical Pharmacognosy. 4th ed. Reprint.

Delhi: Vallabh Prakashan; 1996; 10-321.
15. Gibbs RD. Chemotaxonomy of Flowering Plants. Vol.1, McGill Queen‘s University Press Montreal and London., 1974
16. Sadasivam S, Manickam A. Biochemical methods. New age International (P) Limited, Publishers, New Delhi, 1996
17. Siddiqui AA, Ali M. Practical Pharmaceutical Chemistry. 1st edn; CBS Publishers and distributors, New Delhi, 1997; 126-131.
18. Vabkova J, Neugebauerova J. Determination of total phenolic content, total flavonoid content and frap in culinary herbs in relation to harvest time. Acta Universitatis Agriculturae Et Silviculturae Mendelianae Brunensis 2012; LX(20):167-172.
19. Manjunath A, Mahadev BG, Shradda UN. Estimation of total alkaloid in Chitrakadivati by UV-Spectrophotometer. Anc Sci Life 2012; 31(4):198–201