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Qualitative and Quantitative Comparison of the Phytoconstituents in the Leaf Extract of *Cassia fistula*

Archana Chaudhary, Vinay Pandit*

Department of Pharmaceutics, Laureate Institute ofPharmacy, Kathog, HP, India

Address for Corresponding:

E mail address: vinay2121@gmail.com

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Abstract:

Cassia fistula belongs to the family Caesalpinaceae commonly known as "Golden shower tree" has been used in different traditional system of medicines for various ailments since ancient times. *Cassia fistula*grows throughout in Bangladesh and in many other Asian countries such as India, China, Hong Kong, Philippines, Malaysia, Indonesia, and Thailand. Quantitative estimation of phytoconstituents present in ethanolic (EE), Aqueous (AE) and Hydroalcholic (HAE) leaf extract of *Cassia fistula.* Ethanolic leaf extract of *Cassia fistula* contain54.53 mg total tannin content, 8.31 mg total phenolic content, 204.6 mg of total Alkaloidal content, 296.8 mg total flavanoids content. Aqueous leaf extract of *Cassia fistula*contain10.61 mg total tannin content, 4.50 mg total phenolic content, 98.0 mg of total Alkaloidal content, 85.5 mg total flavonoid content. Hydroalcholic leaf extract of *Cassia fistula*contain25.03 mg total tannin content, 6.10 mg total phenolic content, 119.11 mg of total Alkaloidal content, 118.25 mg total flavanoids content. This study showed that the ethanolic leaf extract of *Cassia fistula* contain high percentage of tannins, Phenols, Flavanoids and alkaloids.

Keywords: *Cassia fistula L.*, Phytoconstituents, Alkaloid, Phenol, Flavanoids

Introduction

Cassia species are the well-known medicinal plants have different medicinal properties.The genus Cassia comprises of 600 species of herbs, shrubs and trees. [1] Traditionally, *Cassia fistula* is one of the most commonly used plants in Ayurveda, Siddha, Unani and Homoeopathy.[2]*Cassia fistula* is distributed and used as a traditional herbal medicine in India, China, Hong Kong, Philippines, Malaysia, Indonesia, and Thailand. [3,4] *Cassia fistula* is the national tree of Thailand and its flower is the national flower of Thailand. In India, it is the also state flower of Kerala.[5]

The plant is widely used in traditional Indian medicinal system reported to possess hepatoprotective, anti-inflammatory, antitussive, antifungal, antibacterial, antimicrobial and to improve wounds healing.[6] Traditionally, it has been also used in the treatment of diabetes, hematemesis, leucoderma, pruritis, intestinal disorder, antipyretics, antioxidant, antimutagenic, antitumor, analgesic and laxative.[7]*Cassia fistula*contain various phytoconstituents viz. tannins, flavonoids, glycosides, carbohydrates, linoleic, oleic, stearic, oxalic acids, tannins, oxyanthraquinones, anthraquinones derivatives. *Cassia fistula*also contain rhein glycosides, fistulic acids, sennosides A and B, anthraquinones, flavanoid-3-ol derivatives, ceryl alcohol, kaempferol, bianthraquinone glycosides, fistulin, essential oils, volatile components.[8,9]In this study, comparative quantitative phytochemical analysis was performed to identify the presence of various phytoconstituents in different leaf extract of *Cassia fistula* with standard procedures.

Material and Method

Collection and authentication of plant

Cassia fistula complete plant parts were collected within the kangra District (H.P.). The Herbarium of Plant was subjected to authentication from National Herbarium of cultivated Plants, New Delhi. The plant was identified by Dr. Anjula Pandey Principal Scientist at National Herbarium of cultivated Plants, New Delhi.

Preparation of Plant Material

Collected leaves were washed with Distilled water to remove dirt and shade dried. After drying leaves were crushed into coarse powder in a mechanical grinder and stored into a self sealing bag for further studies. [10]

Extraction of Plant Material

Dry leaf powder of *Cassia fistula* was extracted usingethanol, aqueous and hydroalcholic solution in Soxhlet apparatus. Place the thimble inside the extractor and pour the solvents sequentially in Round bottom flask. After about forty siphons of each solvent extraction step, the materials were concentrated by evaporation on water bath.The yield of each extract was calculated and stored in self-sealing bag for further use.[11]

Qualitative Phytochemical Studies

Ethanolic extract, aqueous extract and hydroalcholicleaf extract of *Cassia fistula*were investigated for the presence of carbohydrates, proteins, amino acids, steroids, glycosides, saponins, alkaloids, glycosides, tannins and flavonoids(Table 1).[12,13]

Quantification of Phytochemicals

Ethanolic, aqueous and hydroalcholicextractof*Casssia fistula*leaf wereanalysed for total phenolic content, total tannin content, total alkaloid content and total flavonoid content.[14, 15]

Total content of Alkaloids

Total Content of alkaloids was determined for ethanolic, aqueous and hydroalcholicextractof *Casssia fistula*leaf extract.Leaf extract (1 mg) was dissolved in dimethylsulphoxide and 1 ml of 2N HCl added and filtered. This solution was transferred to a separating funnel; add 5ml of bromocresol green solution and 5ml of phosphate buffer. The mixture was shaken with 1, 2, 3 and 4 ml of chloroform by vigorous shaking and collected in a 10 ml volumetric flask and diluted to the volume with the chloroform. Repeat the above procedure separately for each extract. A set of reference standard solutions of Atropine (10, 20, 30, 40 and 50 µg/ ml) were prepared in the same manner as described above. The absorbance for standard solutions and test

solutions were determined on the reagent blank at 470 nm with an UV/Visible spectrophotometer. The content of alkaloids was expressed as mg of AE/ 100g of plant extract.

Total Flavonoids content

Colorimetric assay was used to determine the total content of flavonoids content in ethanolic, aqueous and hydroalcholicextractof *Casssia fistula*leaf using aluminium chloride. In 10 ml flask 1ml of Plant extract and 4 ml of distilled water was taken. Add 0.30 ml of 5% sodium nitrite and after 5minutes, 0.3 ml of 10 % Aluminium chloride was mixed in the flask. 5 minutes later, 2 ml of 1M NaOH was treated and diluted using 10 ml distilled water. Repeat the above procedure separately for each extract. A set of standard solutions of Quercitin (20, 40, 60, 80 and 100 µg/ml) were prepared as mentioned above. The absorbance was measured for test and standard solutions using reagent blank at 510 nm wavelength by UV-Visible spectrophotometer. The total content of flavonoid was denoted as mg of QE/ 100g of extract.

Total Tannin content

Folin-Ciocalteu method was used for the quantification the tannin total content in ethanolic, aqueous and hydroalcholicextractof *Casssia fistula*leaf. About 0.1ml of plant extract was added in 10 ml of volumetric flask containing the distilled water of 7.5ml and Folin-Ciocalteu phenol reagent of 0.5ml, 35% Na_2CO_3 solution of 1 ml and diluted to 10ml using distilled water. The reagent mixture was well shaken and kept at 30°C temperature for 30 min. Repeat the above procedure separately for each extract. A set of gallic acid solutions (20, 40, 60, 80 and 100 µg/ml) were prepared as mentioned earlier. Absorbance of standard and test solutions was analyzed with blank at 725 nm wavelength using UV-Visible spectrophotometer. The tannin total content of tannin was expressed as mg of GAE/100 g of extract.

Total Phenolic Content

The phenolic compounds concentration in extract was quantified by Spectrophotometry method. Folin-Ciocalteu method was employed for the quantification of total phenolic content. The reaction mixture contains 1 ml of plant extract and 9 ml of distilled water. 1 ml of Folin-Ciocalteu phenol reagent was treated with the mixture and well shaken. After 5minutes, 10 ml of 7 % Na_2CO_3 solution was treated with the mixture. The volume was 25 ml. Repeat the above procedure separately for each extract.A set of gallic acid standard solutions (20, 40, 40, 60, 80

and 100 µg/ml) were prepared as earlier. Incubated for 90 min at 30°C and absorbance was analyzed for test and standard solutions with reagent blank at 550 nm with using UV Visible spectrophotometer. The content of total phenolic compound was denoted as mg of GAE/ 100 g of extract.

Result and Discussion

Percent yield of *Cassia fistula* extract

The percent yield of *Cassia fistula* leaf with different Solvents were 50 %, 12%, 25% with ethanol, distilled water and hydroalcoholic, respectively. The percentage yield of the extract was found to be more in ethanol (50%) as shown in Table 2.

Phytochemical screening of *Cassia fistula* leaf extract

Phytochemical screening of *Cassia fistula* leaf extract with different solvents showed the presence of various secondary metabolites. Ethanolic extract and hydroalcholic extract showed the presence of Protein, Amino Acids, Steroids, Saponins, Alkaloids, Tannins, Phenols, and Flavanoids. Aqueous extract showed the presence of Carbohydrates, Protein, Amino Acids Saponins, Alkaloids, Tannins, Phenols, and Flavanoids (Table 3).

Quantitative screening of *Cassia fistula* leaf extract

The results of quantitative estimation of total phenolic content, total tannin Content, total alkaloid content and total flavonoid content (as shown in Table 4) along with the standard curves plotted (by using the standard equation of the curve: $y= m x + c$, R_2 value) have been depicted in Figure 1,2 ,3 and 4.Ethanolic leaf extract of *Cassia fistula* contain54.53 mg total tannin content, 8.31 mg total phenolic content, 204.6 mg of total Alkaloidal content, 296.8 mg total flavanoids content. Aqueous leaf extract of *Cassia fistula*contain10.61 mg total tannin content, 4.50 mg total phenolic content, 98.0 mg of total Alkaloidal content, 85.5 mg total flavonoid content. Hydroalcholic leaf extract of *Cassia fistula*contain25.03 mg total tannin content, 6.10 mg total phenolic content, 119.11 mg of total Alkaloidal content, 118.25 mg total flavanoids content. This study showed that the ethanolic leaf extract of *Cassia fistula* contain high percentage of tannins, phenols, flavanoids and alkaloids.

Conclusion

The results of preliminary screening reveals that in ethanolic, aqueous and hydroalcholicextractof *Casssia fistula*leaf showed the presence of various phytochemical constituents like protein, amino acids, steroids, saponins, alkaloids, tannins & phenols, flavanoids. The ethanolic extract of *Cassia fistula* leaf showed the presence of many chemical constituents with high percentage yield and high percentage of tannins, phenols, flavanoids and alkaloids. Thus, it can be concluded that *cassia fistula*. Leaves extract can be used as hepatoprotective, anti-inflammatory, antitussive, antifungal, antibacterial, antimicrobial and to improve wounds healing. It can also be used in the treatment of diabetes, hematemesis, leucoderma, pruritis, intestinal disorder, antipyretics, antioxidant, antimutagenic, antitumor, analgesic and laxative.

Table1:Procedure for Phytochemical screening of *Cassia fistulaL.* leaf extract

S. NO.	Phytoconstituents	Test Procedure
1.	**Carbohydrates**	***Molisch'stest:***To2-3mlof extract solution,fewdropsofalpha-naphtholsolutioninalcoholwasadded andshakenwell.Concentratedsulphuricacidwasaddedfromsidesofthetesttubeandformationofvioletringwas observedatthejunctionoftwoliquids. ***Barfoed'stest:***EqualvolumeofBarfoed'sreagentandtestdispersionweremixedandheatedfor1-2mininboiling waterbath.Formationofredcolorprecipitatewasobserved.
2.	**Proteins**	***Biurettest****:*To3mltestSolution,4%sodiumhydroxideandfewdropsof 1% copper sulphate solution were added, and reaction mixture was observed for violet or pink color. ***Million'stest:***To3mltestSolution,5mlMillion'sreagentwasaddedandobservedforappearanceofwhiteprecipitate.On warmingprecipitateshouldturnsbrickredortheprecipitatedissolvesgivingredcoloredsolution.
3.	**Starch**	***Iodine test:*** To 3 ml of test Solution, few drops of dilute iodine solution was added and observed for the appearance of blue color. Blue color disappeared on boiling and reappeared on cooling.
4.	**Alkaloids**	Extract solution was evaporated and residue was collected. To the residue dilute hydrochloric acid was added and filtered. Filtrate was collected and following tests were performed: ***Wagner's test:*** To2-3 ml filtrate, few drops of Wagner's reagent was added and observed for the appearance of reddish brown color precipitate. ***Hager's test:*** To 2-3 ml filtrate, few drops of Hager's reagent was added and observed for the appearance of yellow color precipitate. ***Mayer's test****:* To 2-3 ml filtrate, few drops of Mayer's reagent was added and observed for the appearance of precipitate. ***Dragendroff's test:*** To 2-3 ml of filtrate, few drops of Dragendroff's reagents was added and observed for the appearance of orange- brown precipitate
5.	**Glycosides**	***Cardiacglycoside*** *Baljet'stest:*Adispersionofmucilagewasobservedforappearanceofyellowtoorangecolorwithsodiumpicrate. ***Anthraquinonesglycosides*** ***Borntrager'stest:***To3mldispersionofmucilage,equalvolumeofdilutehydrochloricacidwasadded,boiledandfiltered.Tocol

		dfiltrate,equalvolumeofchloroformwasaddedandshakenwell.Thenorganiclayerwasseparatedandammoniawasadde dtoit.Appearanceofpinkorredcolorinammonialayerconfirmsthepresenceofglyco-sides.
6.	**Flavanoids**	To each extract add NaOH and observed for yellow coloration.
7.	**Saponin**	***Foamtest:***Each extract wasshakenvigorouslywithdistilledwaterinatesttubeandobservedfortheappearanceoffoam.
8.	**Steroids**	***Salkowskireaction*:**To2mlofextractdispersion, chloroform(2ml)andconcentratedsulphuricacid(2ml)wereaddedandshakenwell.Reactionmixturewasobser vedfortheseparationofchloroformlayerandgreenishyellowfluorescenceinacidlayer
9.	**TanninsandPhen ols**	***$FeCl_3$(5%)solution*:**To2-3mlofalcoholicdispersionofmucilage,fewdrops5%ferricchloridesolutionwasadded, and reaction mixture was observed for the appearance of deep blue-black color.

Table 2:Percent yield of *Cassia fistula* leaf extract in different Solvents

S. No.	Solvent	Wt. of dried powder (g)	Wt. of dried Extract (g)	% yield
1	Ethanol	100	48	48
2	Distilled Water	100	12	12
3	Hydroalcholic	100	25	25

Table 3:Preliminary phytochemical Screening of extract of *Cassia fistula L.*

Test	Results		
	Ethanol	Distilled Water	Hydroalcholic
Carbohydrates	-	+	+
Protein	+	+	+
Amino Acids	+	+	+
Glycosides	-	-	-
Steroids	+	-	+

Saponins	+	+	+
Alkaloids	+	+	+
Tannins & Phenols	+	+	+
Flavanoids	+	+	+

Table 4: Quantitative estimation of phytoconstituents present in ethanolic (EE), aqueous (AE) and Hydroalcholic(HAE) Leaf extract of *Cassia fistula L*.

Total Alkaloidal Content (mg of AE/ 100 g)			Total Flavonoids Content (mg of QE/ 100 g)			Total Tannins Content (mg of GA/ 100 g)			Total Phenols Content (mg of GA/100 g)		
EE	**AE**	**HAE**	**EE**	**AE**	**HAE**	**EE**	**AE**	**HAE**	**EE**	**AE**	**HAE**
204.6	**98.0**	**119.1**	**296.87**	**85.5**	**118.25**	**54.53**	**10.61**	**25.03**	**8.31**	**4.50**	**6.10**

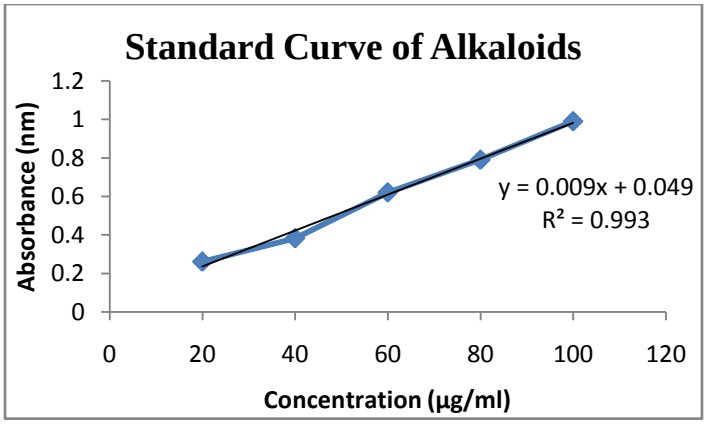

Figure 1: Standard curve of Alkaloids

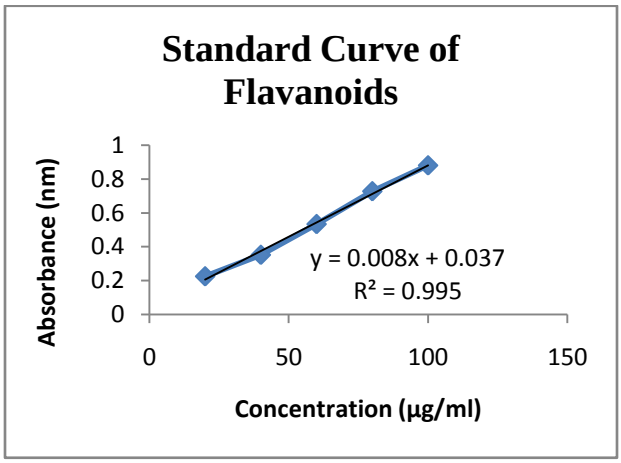

Figure 2:Standard curve ofFlavanoids

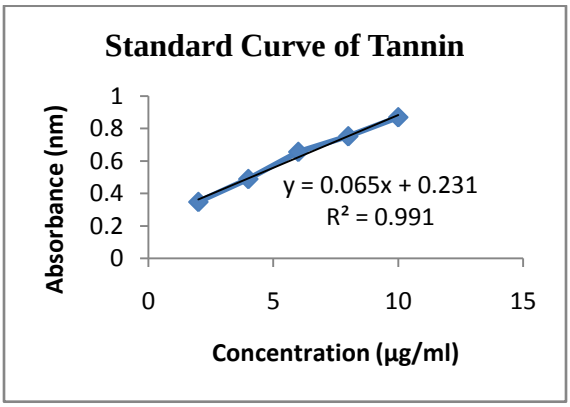

Figure 3: Standard curve of Tannin

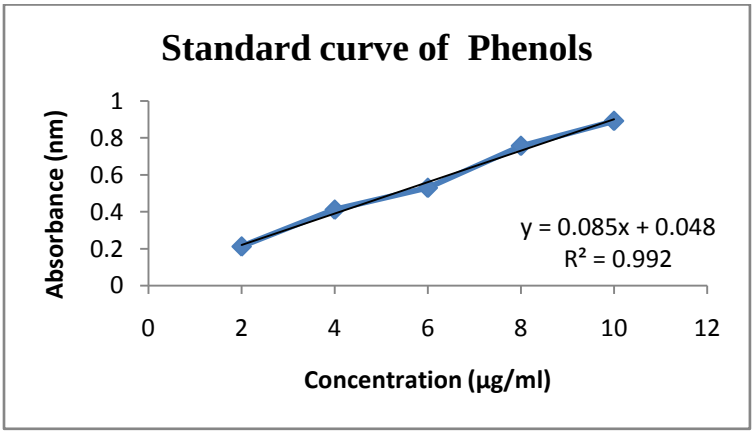

Figure 4: Standard curve of Phenols

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