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PHYTOCHEMICAL ESTIMATION OF ANTHRAQUINONES FROM CASSIA SPECIES

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ABSTRACT
Gastrointestinal problem mainly constipation is the major disorder in human beings in almost all regions. The present work aimed to study exclusively on various seeds of *Cassia* species for exploration and phytochemical estimation of anthraquinones and for its laxative activity. Three species of *Cassia* namely *C. fistula, C. angustifolia,, C. siamea* have been taken for the study in which three varieties of *Cassia fistula* has been taken viz. *C. fistula* seed marketed, *C. fistula* seed collected and *C. fistula* pod. The process was carried out in which initially the samples of different varieties were extracted by four methods namely maceration, percolation, decoction and Soxhlation. The crude extract obtained was subjected for qualitative and quantitative estimation of anthraquinones. The content of total anthraquinone glycoside in the crude extract prepared by each extraction method was determined by U.V. spectrophotometry. The extract prepared by maceration method (*Cassia siamea)* exhibit highest content of anthraquinone glycoside of followed by extract of percolation method, Soxhlation and decoction method. The investigation reviles that seed of *C. siamea* and *C. angustifolia* possess maximum amount of anthraquinone glycoside in majority of extraction processes.
Keywords: *C. siamea, C. fistula, C. angustifolia,* anthraquinone glycoside.

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INTRODUCTION

Anthraquinones are phenolic compounds based on a C6-C2-C6 ring structure whose stability is affected by extraction method and aging. There is a dynamic isomerism between anthranol and anthrone forms. Both of these are more drastic (more bioactive) compounds than the anthraquinone form. Many active plant anthraquinones are dimers (two molecules joined head to tail). They are often yellow in color and bitter to taste. They naturally exist in glycoside form, usually with glucose or rhamnose sugar moieties. Generally, the more powerful agents such as Frangula and Cascara bark are stored for a year prior to consumption: the aging process reduces the irritant activity of the herb as the anthraquinones condense to form dimers, or oxidize to the milder anthraquinone form. The ripe pods and leaves contain several anthraquinones both in aglycone and glycoside forms such as rhein, aloe-emodin, chrysophanic acid and sennosides[1]. The studies of physicological disposition of sennoside A & B, sennidin A & B, rhein and anthraquinone aglycone indicate the anthraquinone glycoside are less likely to enter the systemic circulation and thus able to exert their laxative effects at lower dose than aglycones[2,3]. Anthraquinones compounds are famous for their laxative property[4]. The leaf contains anthraquinones both aglycone and glycoside forms i.e. rhein, aloe-emodin, chrysophanol, glycosides of rhein, emodin, physcione and sennosides (A, B, C, D7-10) while rhein is a major component[5]. The aim of the investigation was to detect anthraquinones in some *Cassia* species exclusively on seeds which involves exploration and phytochemical estimation of the chemical marker anthraquinones through a series of process namely extraction of different species of *Cassia* by maceration, percolation, decoction and Soxhlation methods. Also quantitative estimation and isolation of chemical marker anthraquinone has been done with the help of U.V. spectral analysis.

MATERIAL AND METHODS

Collection of plant material

The ripe pods of *C. fistula Pod* (CFP) and *Cassia fistula seeds* (CFS), seeds of *Cassia Fistula* Marketed (CFM),

Cassia Simea (CS) and *Cassia Angustifolia* (CA) were collected from local market. The samples were authenticated identified in Department of Botany, Government Science College, Durg.

Extraction Methods

Determination of Appropriate Extraction Method for *Cassia species*

The extracting solvents used in Decoction process is Distilled water, while the extracting solvents for other three processes like Maceration, Percolation and Soxhelation was 70% Ethanol. The volume of solvent and time consumed in each extraction are given in table1

Decoction

Five drug samples viz. *C. fistula* pods (CFP) other species of cassia designated as *Cassia fistula* seed marketed (CFM), *cassia fistula* seed collected (CFC), *Cassia siamea* seed (CS), *cassia angustifolia* seed (CA) each weighing accurately 10 gram was taken, and boiled with 100 ml distilled water for one hour at 95-98°C various fraction has been obtained. The mixture was filtered through a muslin and the samples were re-extracted with to water for several times until the extraction was exhausted (tested by Borntrager's reaction). The decoction extracts were combined filtered again and the filtrate was evaporated to dryness on rota-vapour to yield a decoction crude extract.

Maceration

Five drugs samples viz. *C. fistula* pods (CFP) and other species of cassia designated as *Cassia fistula* seed marketed (CFM), *cassia fistula* seed collected (CFC) , *Cassia siamea* seed (CS), *cassia angustifolia seed* (CA) each weighing accurately 10 g was macerated with 100 ml of 70% ethanol. The extraction was repeated until exhausted. The maceration extracts were combined, filtered and evaporated to dryness rota- vapour, water bath and finally with lyophiliser to yield maceration crude extract.

Percolation

Five drug samples viz. *C. fistula* pods (CFP) & other species of cassia designated as *Cassia fistula* seed marketed (CFM) , *cassia fistula* seed collected (CFC) , *Cassia siamea* seed (CS), *cassia angustifolia* seed (CA) each weighing accurately 10 g was moistened with 70% ethanol (30 ml) for 15 minutes. The moistened material was put in a percolator and 70% ethanol was added. The percolation was adjusted at a rate of 1-3 ml/min until the extraction was exhausted. The extracts were combined, filtered and evaporated to dryness on a rota vapour water bath and finally with lyophiliser to yield percolation crude extracts.

Soxhlet Extraction

Five drug samples viz. *C. fistula* pods (CFP) and other species of cassia designated as *Cassia fistula* seed marketed (CFM) , *cassia fistula* seed collected (CFC) , *Cassia siamea* seed (CS), *cassia angustifolia* seed (CA) each weighing accurately 10 g was extracted with 300 ml of 70% ethanol in a soxhlet apparatus. The extraction was continued until the extraction was exhausted. The extracts were then combined, filtered and evaporated to dryness on a rota- vapour, water bath and finally with lyophiliser to yield soxhlet crude extract.

Phytochemical Screening

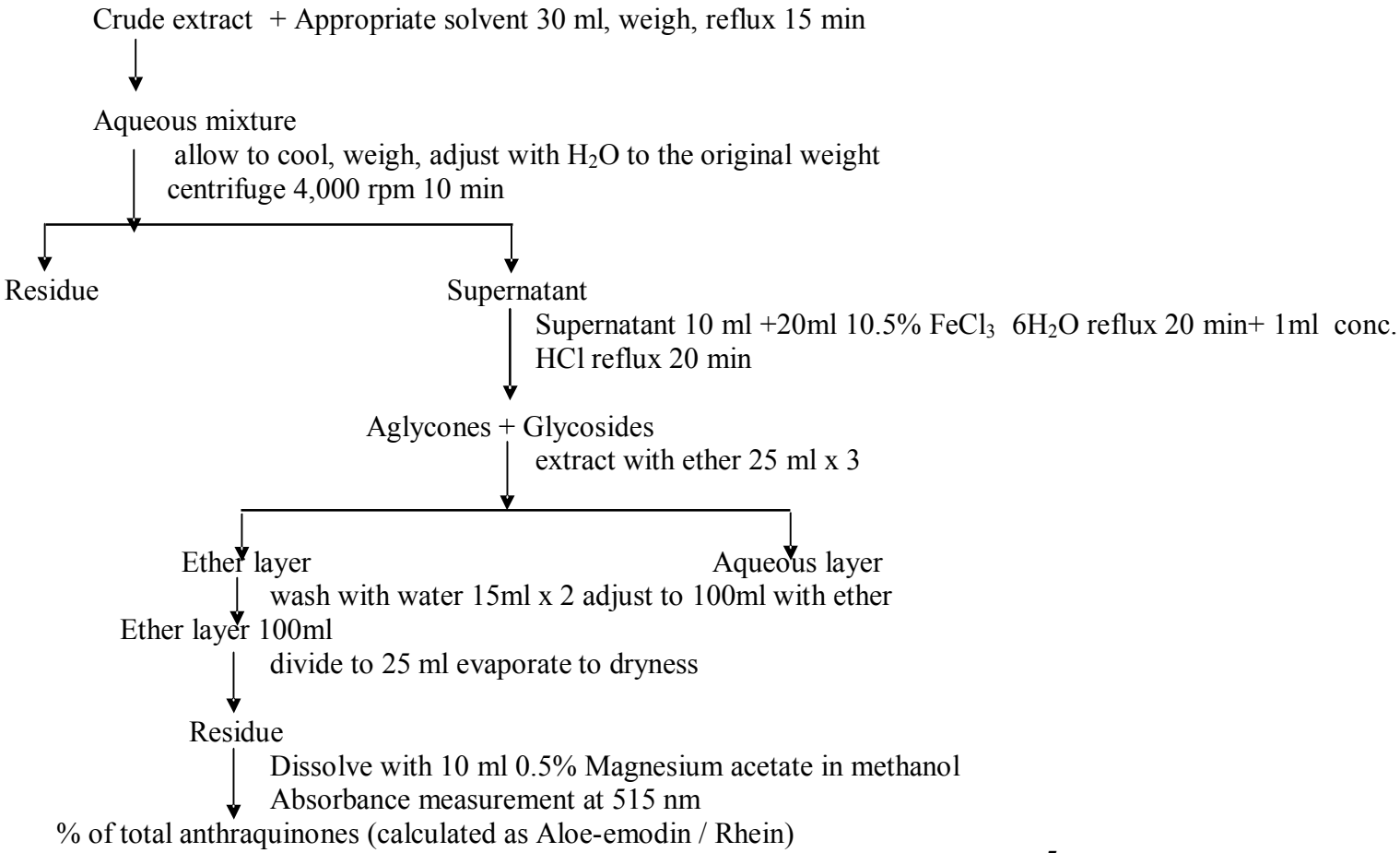
Identification of Anthraquinones

Borntrager's reaction was used to detect anthraquinone aglycones in the extract. Hydrochloric acid (2M) was added to the sample and the mixture was heated on a hot water bath for 15 minutes, then cooled and filtered. The filtrate was extracted with chloroform. The chloroform layer was separated and shaken with 10% potassium hydroxide solution. The upper aqueous layer becomes pink-red[6].

Quantitative Estimation

Determination of Total Anthraquinone Content in 70% Ethanolic Extracts of *Cassia species*

The 70% ethanolic extracts of ***Cassia species*** were separately analyzed for total anthraquinone content by UV spectrophotometer (Shimadzu 1800) at 515 nm. The analysis of each sample was done in triplicate and the content of total anthraquinone in each sample was reported as mean ± SD .



Scheme 1 Quantitative analysis of total anthraquinones [7]

RESULT AND DISCUSSION

Quantitative estimation of the anthraquinone reveals that seed of *Cassia siamea* and *Cassia angustifolia* possess maximum amount of anthraquinone mainly aloe-emodin in majority of extraction processes. U.V. absorbance reading of different samples obtained by various extraction methods of *Cassia* species were taken in which *C. siamea* was found to have maximum absorbance of 0.52 which was shown in the sample obtained by maceration method as in fig 1. The absorbance of the 20 drug samples have been taken in the series of Decoction, Maceration, Percolation and Soxhlation method each method having five drug samples viz. CFP, CFM, CFC, CS, CA and their respective reading as given in table 2.

The investigation reveals that seed of *Cassia siamea* followed by *Cassia angustifolia* possess maximum concentration of anthraquinone glycoside and the above seed contain high potential of anthraquinone as a chemical marker which can be utilized in pharmaceutical industry for different pharmacological activity. Further steps should be taken for the isolation and characterization of anthraquinone glycoside in the near future.

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Table 1 Different method of extraction and solvents

S. No	Method	Solvent	Vol. of solvent(ml)	Time consumed (hr)	Temp(^{0}C)
1	Decoction	D.W	600	6	96-98^{0}C
2	Maceration	70% ethanol	600	700	Room temp
3	Percolation	70% ethanol	2000	18	Room temp
4	Soxhlation	70% ethanol	300	312	90^{0}C

Table 2 Absorbance of various cassia species by UV spectrometer

S. No	Sample	Decoction	Maceration	Percolation	Soxhlation
1	CFP	0.270	0.292	0.266	0.258
2	CFM	0.258	0.278	0.252	0.245
3	CFC	0.266	0.285	0.261	0.257
4	CS	0.246	0.520	0.453	0.36
5	CA	0.244	0.266	0.362	0.281

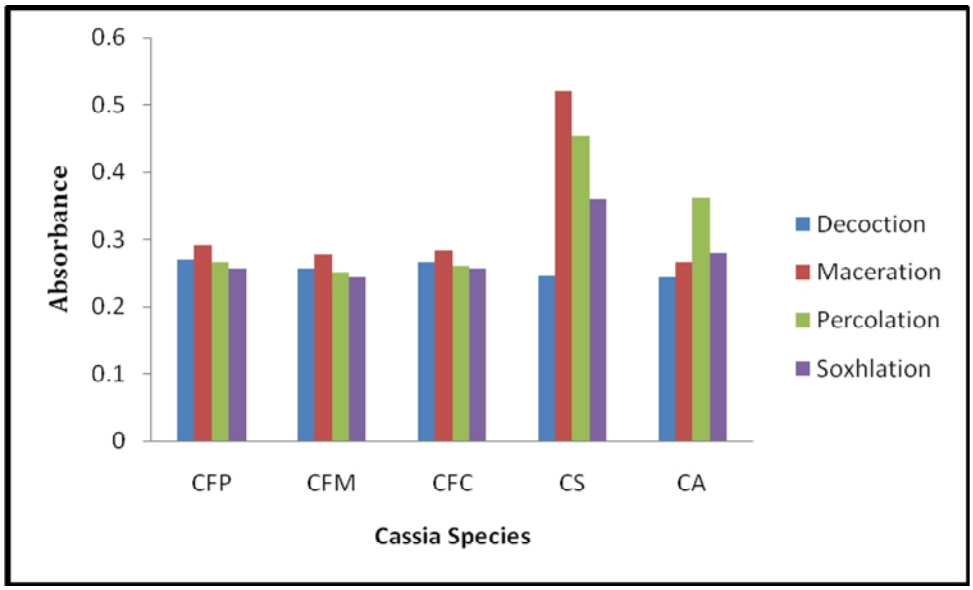

Fig 1 Quantitative analysis of anthraquinone by UV spectrometer

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