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ORIGINAL ARTICLE

Extraction and Preliminary Phytochemical Analysis of Different Extracts of *Cassia fistula* Linn. Pods

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ABSTRACT

Aims and Objectives: The presence of active chemical compounds in natural sources can help to target plants for their therapeutic activities. Here, chemical scrutiny of *Cassia fistula* through phytochemical testing was performed using different solvents and checked for presence of chemical classes present in it. The aim of study was to identify the possible therapeutic components in pods of *C. fistula* (fresh and dried forms) and their concentrations in following two different solvents methanol and distilled water.
Methodology: The crude extracts obtained from the young pods of *C. fistula* in methanol, aqueous extract and fresh juice were subjected to phytochemical testing.
Results: Chemical testing based phyto-constitutional examination confirmed the presence of flavonoids and saponin, tannin and glycoside, alkaloid in fresh juice, both methanolic extracts and aqueous extract respectively. However, negative results were obtained in case of iodine and trace of glycoside in fresh juice and aqueous extract assured the effect of extracting solvent on chemical composition of plant.
Conclusion: The confirmation of phytochemicals in pods of *C. fistula* gave the positive directions for its medicinal uses that may serve as new therapeutic agent in bacterial infection, constipation, pain and diabetes.

INTRODUCTION

In nature plants are the richest and vital source for investigating new therapeutic agents either directly or indirectly. A tragic situation is that the exploration of potential components is still hampered with lack of knowledge and resources. However, lot of efforts can improve the market with potential therapeutic agents derived from plants. The information links from ancient people motivate the medicinal scientist from decades to focus the plants for medicinal components. Traditional medicine with variety of curative potentials, was used extensively from ancient time, indicated the production of secondary metabolites with therapeutic properties [1]. Pharmacologically active chemical constituents, primarily, are identified by simple phytochemical testing in conjunction with standards. Additionally, analytical and pharmacological approaches, later, confirmed the existence and medicinal activity of selected plants [2]. Pharmaceutical industries for their research and development are mainly depends upon these chemical constituents-based libraries, identified from phytochemical testing [3].

Cassia fistula belongs to family Caesalpiniaceae, is also referred as Amulthus, one of chemically richest plant, being used extensively in Ayurvedic system of treatment. Golden rain tree or Indian Laburnum native to Asia, especially to outer Himalaya, is an

ornamental plant of medium size having deciduous colored leaves, yellow pendulous flowers and cylindrical legume fruits [3,4]. The long pods turn black from green upon ripening, however, pulp is sticky brown mucilage with sweet taste and unpleasant smell [5]. Sweetish pulp is enriched with numerous black-brown flat seeds, each segmented by internally fine transverse compartments of pods [6]. Medicinally, extract from different parts possess anti-inflammatory, anti-nociceptive, anti-tussive, anti-fungal, anti-bacterial, hepatoprotective and wound healing effects [3]. Each plant part was reported to have varied chemical composition in different growing phase, with influence of environment to which they exposed. Anthraquinone glycosides, rhein and sennosides were found to be present in almost all parts [7]. However, barbaloin, albuminous starch, gluten, calcium oxalate, different esters and acids, pectin and tannin were specifically present in pod's pulp [8]. Meena Rani et al. in 1998, isolated formyl hydroxy derivative of anthraquinone from the pods of *C. fistula* for the first time [9]. Moreover, seeds contain sufficiently free sugar and amino acids of galactomannan and flowers are enriched in ceryl alcohol, kaempferol and fistulin. In addition to tannin and anthraquinone, root barks also contain phlobaphenes [8]. Extraction, fractionation and evaluation from leaves confirmed the presence of chrysophanil, physician and kaempferol through spectroscopic analysis [10].

The study objective was to identify the presence of chemical components for therapeutic activity of *C. fistula*. The phytochemical estimations of fresh and dried pods extracted *via* different organic solvents were performed using chemical testing. Following the identification, comparative analysis of these chemical constituents that are presence in different solvents in different forms was identified.

MATERIALS & METHODS

Collection of Plant Material

The pods of plants were collected from herbal garden of Hamdard University in its flowering season. Herbarium section of department of Botany, University of Karachi confirmed the plant identification against voucher #G.H.87990. Half of collected pod's sample was allowed to shade dry at ambient temperature for more than 15 days. Following complete drying; pods were coarsely crushed through grinder and then packed in airtight containers to protect it from temperature and humidity.

Extraction of Fresh and Dried Plant Maceration

The crushed fresh pods of 100g were weighed and soaked in 400ml of methanol for more than 2 days at room temperature. After period of soaking, mixture was allowed to filter and concentrate it through drying. Store the extract in dry cool place at 4 ºC.

Solvent Extraction

Soxhlet method was used, for the extraction of dried pods. 20g of sample was packed uniformly in a thimble. Methanol, was used in quantity of 250 ml for the extraction process, until the solvent in extractor become transparent. Collected extract was dried on rotary evaporator at temperature of about 30-40 ºC and the dark brown pasty material was stored in refrigerator at 4 ºC until further use.

Qualitative Phytochemical Analysis

The presence of different pharmacologically active metabolites was identified using standard phytochemical testing procedures [11-13].

Test for Protein Identification

Millon's test

A French scientist designed a color test specifically for identification of phenolic amino acid; tyrosine. Tyrosine is an amino acid present nearly in all proteins, thus, test is normally used to check the presence of protein. 2ml Millon's reagent was mixed with extract resulted in white precipitates that then turned to reddish brown confirmed the presence of tyrosine.

Ninhydrin test

Ruhemann's complex based deep blue coloration upon coupling and boiling of 0.2% 2ml Ninhydrin's reagent with small quantities of extracts indicated the presence of free amines, amino acids and protein.

Test for carbohydrates Identification

The extracts of fresh and dried pods were allowed to divide into four portions for following testing and confirmations.

Fehling's test

Reducing and non-reducing characteristics of carbohydrates can be detected in presence of Fehling solution A and B. The equal ratio of two reagents was mixed together with the testing sample, followed by its boiling. Time dependent oxidation resulted in formation of brick red precipitate that confirmed the presence of reducing sugars in given sample.

Benedict's test

Additionally, Benedict's reagent is also used for the detection of reducing sugars through identification of aldoketal functional groups. Reddish brown precipitates indicated the presence of carbohydrates when extracts were mixed and boiled with 2ml of Benedict's reagent.

Molisch's test

Molisch's test is a very sensitive chemical test used to confirm the presence of carbohydrates through concentrated acid base dehydration resulted in the formation of a colored complex. Test confirmed the presence of carbohydrate when sample extracts was treated in presence of 2ml of Molisch's reagent with subsequent shaking, followed by addition of concentrated sulfuric acid along the wall, resulted in formation of a violet ring at the interphase.

Iodine test

The presence of starch and starchy components in crude extracts can be detected by using 2ml of iodine solution. The tri-iodide anion forms a complex with starch present in sample and gives an intense or dark blue coloration. The color intensity is directly related to the available concentration of starch in extracts.

Test for Phenols and Tannins Identification

2ml (2%) diluted solution of ferric chloride was mixed with filtered and dried sample upon which appearance of blackish-green color that turns to olive green coloration indicated the presence of phenols and tannins.

Test for Flavonoids Identification

Alkaline reagent test

A sharp yellow coloration upon alkalization of crude extract with 2% solution of sodium hydroxide indicated the existence of flavonoids. This yellow appearance changed to colorless with drop wise addition of dilute acid, further confirmed the presence of flavonoidal constituents.

Test for Saponins Identification

Forthing test

Pasty crude extract was diluted with 5ml of distilled water and continuous vigorous shaking for about half a minute. The resultant was permitted to stagnant for more than 30 min

showed formation of stable foams as indicative of saponins presence in the sample.

Test for Glycosides Identification

Salkowski's test

2ml of concentrated sulfuric acid was added to the mixture of extract and chloroform. Concentrated sulfuric acid was added to form a lower layer and help to show a reddish-brown color at interface with the indication for the presence of steroidal nucleus.

Keller-Kilani test

Glacial acetic acid in volume of 2ml with few drops of 2% solution of ferric chloride was used to confirm the presence of cardiac glycosides in extract. 2ml concentrated sulfuric acid when added to the mixture of extract and reagents formed a brown ring at interface of two liquids as indication of positive results

Test for steroid Identification

Liebermann's test

Mixture of chloroform and acetic acid (in ratio of 1:1) in ice bath was mixed with tested sample. Addition of concentrated sulfuric acid to the mixture produced violet to green coloration confirmed presence steroidal nucleus.

Test for Terpenoids Identification

A positive test for the presence of terpenoids was confirmed on appearance of grayish coloration when extracts were heated with 2ml of chloroform and 2ml of concentrated sulfuric acid.

Test for Alkaloids Identification

Dilute HCl of 1% strength when mixed with crude extract in presence of Mayer's And Wagner's reagents showed turbidity upon heating. Cloudiness appeared in response to the precipitation of alkaloids confirmed the positive results.

RESULTS

The phytochemical screening of the four different types of extract from *C. fistula* pods is presented in Table **1**.

Table 1: Phytochemical analysis of *Cassia fistula* pods.

Phytochemical	Test	Methnolic extract of fresh pods	Methnolic extract of dry pods	Aqueous extract	Fresh juice
carbohydrates	Fehling's test	++	+	+++	+++
	Benedict's test	++	+++	-	-
	Molisch's test	+	-	-	-
Alkaloids	Mayer's reagent	++	+++	++	-
	Dragendorff's reagent	-	-	++	+
	Wagner's reagent	-	-	-	-
Tannin	With Ferric chloride	+++	+	++	+++
Glycoside	Keller-kiliani test	+++	+++	++	+
	Salkowski's test	-	-	-	-
Protein and amino acid	Ninhydrin test	++	++	++	++-
	Millon's test	-	-	-	-
Flovonoids	Alkaline reagent	+	++	++	+++
Saponins	Form test	+++	++	+++	+++
Steroids	Libermann burched	+	++	+	+
Iodine	Iodine solution	-	-	-	+
Terpenoids	With chloroform and H_2SO_4	++	++	+	+++

The given results showed presence of pharmacological active components in the methanolic extract of dry pods (MeCFD), methanolic extract of fresh pods (MeCFF), aqueous extract (AqCF) and fresh juice of pods (FCF) were prepared using Soxhlet apparatus, maceration and mechanical juicer respectively. In Table **1**, showed results of phyto-analysis such as carbohydrates were highly present in the AqCF and FCF, compared to both methanolic extracts by Fehling test. The alkaloid content was top number in the MeCFF> MeCFD> AqCF but absent in the fresh juice. Test for glycoside revealed the negative results in the aqueous extract and fresh juice in contrast to methanolic extract of the both types by Keller-Kiliani test that gave positive result. However, remaining two tests also showed the similar results. For protein and amino acid identification, the Millon's test gave no results in the all four types of the extract. While, Ninhydrin test exhibited the presence of the protein and amino acid in all extract. Flavonoid occurrence was detected by alkaline reagent with the lesser extent in the MeCFF, AqCF and high to moderate in the MeCFD and fresh juice respectively. Iodine content was absent in all above extract. The steroids were only found in the methanolic extract m whereas, terpenoids were identified in all the extract.

DISCUSSION

Plants are enriched source of medicinal agents through their secondary metabolites. These phytochemicals were synthesized in diverse chemical structures with varied therapeutic activities. Generally, glycosides exhibited laxative and purgative bioactivities [14]. However, saponins are active as anti-diabetes and antimicrobial [14]. Carbohydrates and proteins are effective components of plants may have positive effects in hypoglycemia, edema and malnutrition [16]. Moreover, bacterial infection, headache due to hypertension and diabetes mellitus are the conditions, currently, treated with alkaloids, tannins and flavonoid based naturally derived compounds, respectively [17, 18].

The screening of active constituents from hot water and methanolic extracts of fresh and dry pods of *Cassia fistula* through chemicals testing revealed the existence of carbohydrates as major component. Moreover, proteins, saponins and terpenoids were also found to be the obvious components of plant pods in all extracts. The plant is already studies for its phytochemicals and related effects through different techniques, extensively. An age and solvent related correlation between the existences of constituents was established for the first time to point out the form of pod selection for higher concentrations.

Phytochemical testing with the aqueous extract and fresh juice of *Cassia fistula* pods confirmed the higher concentration of reducing sugars, tannins, flavonoids, terpenoids and saponins in both samples. Flavonoids are the main plant metabolites, in addition to antioxidant activity [19], have also reported for analgesic, anti-inflammatory and anticancer effects [20]. However, marked anti-inflammatory & cytotoxic and antibacterial effects in association with terpenoids and saponins with different doses was also reported [21]. Tannins interfere the protein biosynthesis and used. Thus, predicting the potential antioxidant activity of methanolic extract, in comparison to all other extracts, has already confirmed by a study [22]. However, moderate concentrations of alkaloids and proteins are present in aqueous extract that are even lesser in juice, demonstrated least to no activity against pain, spasm and bacterial infections. Mainly, these three properties are extensively reported as the function of alkaloids and proteins [23-25], thus, low levels in *Cassia fistula* pod's juice marked it as inactive. While, negligible quantities of steroidal glycosides were found and showed no association with

management of hypertension [26]. Levels of proteins remain same in all extracts regardless of solvent and form of pods. Contrary, methanolic extracts of fresh and dry pods through phytochemical screening confirmed the presence of alkaloids and glycosides, notably. Comparatively, tannins and saponins are more pronounced in fresh extract than dried form. However, carbohydrate and alkaloids are more in dried extract of plant pods.

Conclusively, least flavonoids in methanolic extract of fresh pod marked the extract as inappropriate for antioxidant activity. Reasonably higher concentration of glycoside in methanolic fresh pod extract indicated its future prospect towards antibacterial and hypotensive activities. However, alkaloids in juice are less than methanolic pod extracts but more than aqueous extract assured the application of methanolic extract in treatment of pain, spasm and bacterial pathogenesis preferably [24]. The flavonoid and terpenoids were much more in fresh juice comparatively other extracts. Thus, juice form and methanolic extract was found to be most appropriate for maximum extraction of active constituents.

CONCLUSION

The phytochemical active metabolites of the natural origin, such as young pods of the *C. fistula* are seemed to be abundant source of pharmacological active agents. A number of studies, confirmed the presence of these phytochemical for medicinal as well as physiological activities to the plants investigated for treatment of various diseases. The extracts of the *C. fistula* could be seen as a good source for effective medicine. Many of these constituents revealed as results of screening to improve the health status and may be considered as vital for healthy life in near future. Traditionally, it is strongly recommended as medicinal practice of *C. fistula* and also suggested that further research on isolation, purification and characterization of the active component responsible for pharmacological action of this plant. Moreover, the additional studies are encouraged to find the appropriate mechanism of action of these extracts.

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