Research Article

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ALCOHOLIC EXTRACTION AND PHYTO-CHEMICAL EVALUATION OF CHAKRAMARDA SEEDS (*CASSIA TORA* LINN.)

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ABSTRACT

Chakramarda (*Cassia tora*. Linn*)* is well known drug for its kustaghna, dadrughna karma as per literature available in Ayurvedic classics. Chakramarda taila is anubhuta yoga, where seeds of Chakramarda are used for preparation of taila. It is used for external application in Dadru Kushta. Phyto-chemical study helps to identify active constituents which are responsible for bringing out drug action. It also provides preliminary information on the quality of the drug. The alcoholic extract of Chakramarda seeds were collected from coarse powder of Chakramarda seeds by using of Soxhlet extraction. Alcoholic extract of Chakramarda was then subjected to phytochemical screening to test for presence of metabolites such as alkaloids flavonoids, phenol, tannins, saponins, sugar, glycosides, steroids, Carbohydrates, glycosides, carboxylic acid, Resin and anthraquinone, which were qualitatively analyzed. This study would provide preliminary scientific evidence for Chakramarda as potent drug, because of Chakramarda seeds have more active principles like alkaloids flavonoids, phenol, tannins, saponins, sugar, glycosides, steroids, Carbohydrates, glycosides, carboxylic acid, Resin and anthraquinone. Hence phyto-chemical study of Chakramarda seeds is essential in order to evaluate active constituents responsible for its medicinal actions.

Keywords: Phyto-chemical evaluation, Chakramarda seeds, Active constituents, *Cassia tora* Linn.

INTRODUCTION

In recent years skin diseases have gained more importance and attention by medical science as well as public. Skin diseases accounts for prevalence rate 10-20% of all consultation in general practice[1]. Herbal plants are rich in a wide variety of secondary metabolites, which have anti-fungal, anti -bacterial properties[2] and are used throughout the history of human beings either in the form of plant extracts or pure compounds against various diseases[3].

The medicinal herbal plants are beneficial for therapeutic as well as for curing of human diseases because of the presence of phytochemical constituents[4]. Phytochemicals are naturally occurring in the medicinal plants, leaves, seeds and roots that have defense mechanism and safeguard from numerous diseases. Phytochemicals are primary and secondary compounds. The primary metabolite like chlorophyll, amino acids, nucleotides[5], simple carbohydrates or membrane lipids, play predictable roles in photosynthesis, respiration, solute transport, translocation, nutrient assimilation and differentiation[6]. Secondary metabolites are synthesized by the plants as part of the defense system of the plant [7]. Chakramarda (*Cassia tora*. Linn) is well-known traditional medicinal plant, also called as ring worm plant which possesses kushtaghna, kandughna and dadrughna properties[8]. It is useful in gulma, kasa, krimi and swasa. It improves the metabolic rate of the body, thus helps in maintaining it in healthy limits. It also nourishes the skin and useful in skin infection specially ring worm infestation. Bavaprakasha, Madanapala and kaiyadeva nigantu mention Chakramarda to possess krimighna properties. The present study was designed to investigate the phytochemical properties of ethanol extracts of seeds of Chakramarda (*Cassia tora*. Linn). Phytochemical screening refers to the extraction, screening and identification of the medically active substances found in plants. The separation of useful components of herbal drugs and tissues using particular solvents through standard procedure are defined as extraction. Soxhlet extraction[9] is a general and well-established technique, widely used for extraction of compounds.

MATERIALS AND METHODS

Collection of plant material

The seeds of Chakramarda were collected from Mysuru and was cleaned, dried in the shade. After drying, seeds were ground into coarse powder using blender and powder was transferred into airtight containers with proper labeling for future use.

Authentication of the drug

The authentication of seed of Chakramarda was done in the Department of Dravyaguna, Shri Dharmasthala Manjunatheshwara College of Ayurveda and hospital, Hassan.

Preparation of alcoholic extract of seed of Chakramarda

Requirements

a) Instrument: Soxhlet apparatus
b) Seeds of Chakramarda coarse powder - 35gm
c) Ethanol - 350ml

d) Cotton and filter paper

Procedure for alcoholic extract of seeds of Chakramarda

35 grams of coarse powder of Chakramarda seeds (*Cassia tora* Linn) was placed inside a thimble made from thick filter paper which was loaded into the main chamber of the Soxhlet extractor. 350ml of Ethanol as extraction solvent was taken in a distillation flask and Soxhlet extractor was placed on the flask. The Soxhlet was then equipped with a condenser. The solvent was heated to reflux. The solvent vapour traveled up a distillation arm, and flooded into the chamber housing the thimble of sample. The condenser ensured that any solvent vapour cools and drips back down into the chamber housing the solid material.

The chamber containing the sample slowly filled with warm solvent. When the Soxhlet chamber was almost full, the chamber got automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle was allowed to repeat 4 times. After extraction, the Ethanol was removed and it was later dried over water bath. The final product obtained was 2.67 gram of alcoholic extract of Chakramarda. It was solid in nature. This extract was used for phytochemical screening. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use.

Table 1: Weight of alcoholic extract of seeds of Chakramarda

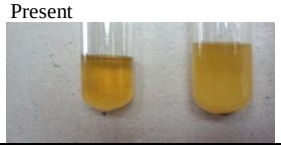
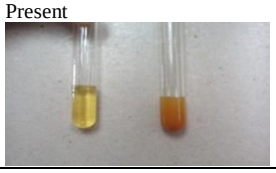
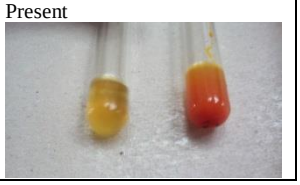
Extraction	Weight
Weight of empty bottle	28.82 g
Weight of bottle with extract	31.49 g
Weight of extract	2.67 g

Phytochemical analysis[10]

Solid residue of the alcoholic extract of Chakramarda was then subjected to phytochemical screening to test for presence of metabolites such as alkaloids flavonoids, phenol, tannins, saponins, sugar, glycosides, steroids, Carbohydrates, glycosides, carboxylic acid, Resin and anthraquinone, which were qualitatively analyzed.

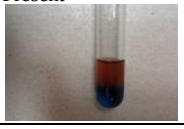
Small amount of extract was stirred with a few ml of dilute HCl, shake well and filtered. In three clean dry sterile test tubes 3ml each of filtrate was taken. The filtrate was tested with various reagents such as Mayer's, Dragendorff's, Wagner's and Hager's reagent.

Table 2: Test for Alkaloids[11]

Test	Procedure	Observation	Results
Mayer's test	3ml filtrate was taken in a test tube and added few drops of Mayer's reagent.	Cream precipitate was observed, indicates presence of alkaloids	Present 
Dragendorff's reagent	3ml filtrate was taken in a test tube and few drops of Dragendorff's reagent was added.	Orange brown precipitate was observed, indicates presence of alkaloids	Present 
Wagner's test	3ml filtrate was taken in a test tube and few drops of Wagner's reagent was added	Reddish brown precipitate was observed, indicates presence of alkaloids	Present 

Small amount of extract was stirred with a few ml of dilute HCl, shake well and filtered. 3ml of filtrate was taken in clean dry sterile two test tubes separately. The filtrate was treated with Fehling's reagent and Benedict's reagent separately to ensure for presence of carbohydrates.

Table 3: Test for carbohydrates[12]

Test	Procedure	Observation	Results
Fehling's test	Small portion of the extract was treated with Fehling's solution I and II and then heated on water bath.	Brick red color precipitate was not found indicating absence of carbohydrates.	Absent
Benedict's test	Small portion of the extract was treated with Benedicts' reagent. Boiled on water bath.	Reddish brown precipitate was observed which indicates presence of carbohydrates.	Present 

The extract was dissolved in ethanol and then subjected to the following tests.

Table 4: Test for flavonoids[13]

Test	Procedure	Observation	Results
Shinoda's test	To the alcoholic solution of extract , a small piece of magnesium was added along with few drops of concentrated HCl and heated on water bath	Colour change of the solution to Magenta was not observed, indicates the absence of flavonoids.	Absent 
Ferric chloride test	To the small quantity of alcoholic solution of extract, few drops of neutral ferric chloride was added.	Colour changed to blackish red color indicates the presence of flavonoids.	Positive 

Small amount of extract was dissolved in 5ml of chloroform separately. Then this chloroform layer was subjected to Salkowski test and Liebermann - Burchard test.

Table 5: Test for phyto steroid[14]

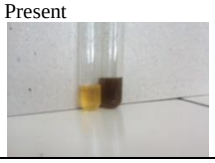
Test	Procedure	Observation	Results
Salkowski test	To 1ml of the above prepared chloroform solutions, few drops of conc. H2SO4 was added.	Solution colour changed to Cherry Red indicates the presence of phyto sterols.	Present 
Liebermann - Burchard test	The above chloroform solution was treated with few drops of conc. H2SO4 followed by 1ml of acetic anhydride solution.	Solution colour changed to Green color indicates the presence of phytosterols.	Present 

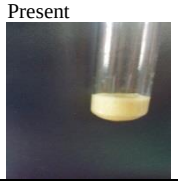
A small amount of the extract was hydrolyzed with hydrochloric acid for one hour on a water bath and hydrolysate was subjected to;

Table 6: Test for Glycosides[15]

Test	Procedure	Observation	Results
Borntrager's test	Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added.	Colour change of the solution to Pink color was not observed which indicates the absence of glycosides	Absent

Table 7: Test for Phenol, Tannins, Saponin, Coumarins, Resin and Quinine[16]

Test	Procedure	Observation	Results
Test for phenol	Extract was treated with dilute solution of ferric chloride.	Colour of the solution changed to Violet colour indicates the presence of phenolic compounds.	Present 
Test for Tannins	To the extract, a few drops of dilute solution of ferric chloride was added.	Colour of the solution changed to dark blue shows the presence of Tannins	Present 
Test for Saponin	To a few mg of extract, distilled water was added and shaken.	Forth formation was observed, indicates the presence of saponin	Present 
Test for Coumarins	To the extract, a few drops of 2N sodium hydroxide solution was added.	Colour of the solution did not turn to Dark yellow indicate the absence of coumarins.	Absent

Test for carboxylic acid	Extract was dissolved in water and treated with sodium bicarbonate.	Brisk effervescence was observed, indicate the presence of carboxylic acid	Present 
Test for Resin	A few mg of the sample was mixed with water and acetone	Solution turned to Turbid, indicates the presence of resin	Present 
Test for quinine	A few mg of the sample was treated with 5% of sodium hydroxide.	Deep colouration like purple or red indicates the presence of Quinine	Present 

The alcoholic extract of seeds of Chakramarda showed more phytochemical constituent's i.e., alkaloids flavonoids, phenol, tannins, saponins, sugar, glycosides, steroids, Carbohydrates, glycosides, carboxylic acid, Resin and anthraquinone are either present or absent in these plants and the results were summarized in Tables 2-7.

DISCUSSION

Phytochemical screening on medicinal plant play an important in the detection of bioactive principles which is a new source of therapeutically and industrially valuable compounds that may lead to drugs discovery and development[17]. Extraction is the vital step to extract the desired chemical components from the plant materials using polar and non-polar solvents[18]. Ethanol was selected as the extraction solvent for this phytochemical analysis study. These parameters provide preliminary information on the quality of the drug. Alcohol provides a particularly effective way of maximizing the bioavailability of the active principles extracted from the plant. Ethanol is a molecule with both a polar and a non-polar end. Many taste molecules are polar whereas most aroma molecules are non-polar, and the ethanol can be used to extract both groups of compounds. The advantages of conventional Soxhlet extraction include the displacement of transfer equilibrium by repeatedly bringing fresh solvent into contact with the solid matrix, maintaining a relatively high extraction temperature with heat from the distillation flask and no filtration requirement after leaching. Soxhlet method is very simple and cheap. Hence Soxhlet apparatus was used for extraction. Phytochemical analysis conducted on the alcoholic extract of seeds of Chakramarda revealed the presence of constituents which are known to exhibit medicinal activities. Analysis of the plant extracts revealed the presence of alkaloids flavonoids, phenol, tannins, saponins, sugar, glycosides, steroids, Carbohydrates, glycosides, carboxylic acid, Resin and anthraquinone. But Glycosides and Coumarins were absent.

CONCLUSION

Phyto-chemical study helps to identify active constituents which are responsible for bringing out drug action. It also provides preliminary information on the quality of the drug. This study would provide preliminary scientific evidence for Chakramarda as potent drug, because of Chakramarda seeds have more active principles like alkaloids flavonoids, phenol, tannins, saponins, sugar, glycosides, steroids, Carbohydrates, glycosides, carboxylic acid, Resin and anthraquinone. Hence phyto-chemical study of Chakramarda seeds is essential in order to evaluate active constituents responsible for its medicinal actions and the manufacturing of new drugs.

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