Available online on 15.02.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Extraction, Qualitative and Quantitative Determination of Secondary Metabolites of *Corchorus Olitorius*

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ABSTRACT

Jute (Corchorus spp.) leaf has long been used as a remedy in many cultures. Jute leaf products, which include the leaf juice, fried leaf and some time whole green leaf are used, among other reasons, as laxatives, in creams for skin care, and as a treatment for a wide range of diseases, respectively. The heterogeneous nature of jute leaf products may contribute to the diverse biological and therapeutic activities that have been observed. The aim of the present study is to examine *Corchorus olitorius* whole plant for phytochemical profile. Qualitative analysis of various phytochemical constituents and quantitative analysis of total flavonoids were determined by the well-known test protocol available in the literature. The present study provides evidence that successive solvent extracts of *Corchorus olitorius* contains medicinally important bioactive compounds and this justifies the use of plant species as traditional medicine for treatment of various diseases. Quantitative analysis of flavonoids was carried out by aluminium chloride method. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids, fixed oil and fats. The total flavonoids content of whole plant extracts was found to be chloroform- 0.505, ethyl acetate-1.300, methanol-2.050, aqueous-1.785 mg/100mg.The present study concluded that the crude extract of *Corchorus olitorius* is a potential source of various activates and this justifies its use in folkloric medicine.

Keywords: *Corchorus olitorius,* Qualitative, Quantitative analysis, Total flavonoids

Article Info: Received 13 Jan 2019; Review Completed 05 Feb 2019; Accepted 09 Feb 2019; Available online 15 Feb 2019

Cite this article as:

Patil DK, Jain AP, Extraction, Qualitative and Quantitative Determination of Secondary Metabolites of *Corchorus Olitorius*, Journal of Drug Delivery and Therapeutics. 2019; 9(1-s):252-255 **DOI: http://dx.doi.org/10.22270/jddt.v9i1-s.2340**

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INTRODUCTION

Corchorus olitorius (Linn.) belong to the family Tiliaceae and commonly known as "Jute or Jew Mallow". It is disturbed throughout the tropical region of Africa and Asia [1] and is considered as a common vegetable in Egypt, the Philippines, Australia, Senegal and Thailand [2], as a common weed for India, Afghanistan, Nepal, Kenya and Turkey and as a principle weed for Sudan [3]. Its tender leaves and shoots, rich in proteins and minerals servers as a main dietary source for proteins and are used in ethnic soup preparation in several tropical countries [4]. In the literature survey, *Corchorus olitorius* is ascribed for several medicinal uses [5]. Its leaves have been reported to be used for the treatment of pain, gonorrhoea, chronic cystitis and tumors [6]. The dried young leaves are diuretic, demulcent, tonic and slightly febrifuge; infusion of dried leaves is used to restore appetite and strength [7]. Its seeds are purgative and have been reported to exhibit estrogenic activity [8] as well as effective in cardiac diseases due to presence of appreciable amounts of active cardiac principles in particular Olitoriside, which showed equivalent effect to strophanthin with chronic cardiac patients [9]. The stem acts as a main source of jute fiber [10]. In addition to this, it is an ingredient in lotions, facial and hand creams and hair tonics [11]. Moreover, pharmacologically C. olitorius possesses a diverse biological activities which includes, antioxidant [12], anti-tumor [13], hypoglycemic [14], antimicrobial [15], anti-inflammatory, analgesic [7], antiobesity [16], gastroprotective [17] and wound healing effects [18]. Further more extensive phytochemical investigation showed presences of cardiac glycosides: coroloside, veticoside, erysimoside, helveticoside, corchoroside A, corchoroside, B, strophanthidol, evonoside, strophanthidin, olitor and chorchorusoside A-E from the seeds [19]; triterpenes, intones, steroids, acidic polysaccharide rich in uronic acid and consisting of rhamnose, glucose, galacturonic acid and glucuronic from the leaves [20] while as root contains corosin, β-sitosterol and triterpene [21]. The medicinal properties of plants are due to some chemical substances that produce certain definite physiological action on the human body. These non-nutritive components are called phytochemicals. The qualitative analysis as well as quantification of phytochemicals of a medicinal plant is regarded as vital step in any kind of medicinal plant research. Phytochemical processes have been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals usually employing chromatographic techniques [22]. Quantification

usually employs the use of gravimetric and spectroscopic methods with several advanced approaches now available [23]. Extensive effort have now been channelled towards screening of plants for more active and effective new drugs to eliminate diseases which have strains of pathogenic organism that resist the effect of drug in use today [22]. Based on the many ethnomedicinal values of this plant, it is becomes imperative to determine the active ingredients present in different parts of the plant as well as their composition.

MATERIAL AND METHOD

Plant material

Whole plant material of *Corchorus olitorius* were collected from ruler area of Nagpur (Maharashtra) in the month of August, 2017.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SigmaAldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction procedure

Defatting of plant material

Powdered plant material of *Corchorus olitorius* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place

Extraction

50gm of dried plant material were exhaustively extracted with different solvent using maceration method for 48 hrs. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts.

Qualitative phytochemical tests

Phytochemical examinations were carried out for all the extracts as per the standard methods.

1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's test: Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's test: Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's test: Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

4. Legal's test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

5. Detection of saponins

Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

6. Detection of phytosterols

Salkowski's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

7. Detection of phenols

Ferric Chloride test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

8. Detection of tannins

Gelatin test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

9. Detection of flavonoids

Alkaline Reagent test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

10. Detection of proteins and aminoacids

Xanthoproteic test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Ninhydrin test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

11. Detection of diterpenes

Copper acetate test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes [24-26].

Estimation of total flavonoid Content

Principle: Determination of total flavonoids content was based on aluminium chloride method [27].

Preparation of standard: 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.

Preparation of extract: 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of flavonoids.

Procedure: 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extracts or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

RESULTS AND DISCUSSION

The crude extracts so obtained after the maceration process, extracts was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from sample using chloroform, ethyl acetate, methanol and water as solvents are depicted in the Table 1.

Table 1: % Yield of plant material

S. No.	Solvents	*Corchorus olitorius*
1	Pet ether	0.8%
2.	Chloroform	3.2%
3.	Ethyl acetate	4.6%
4.	Methanol	8.2%
5.	Aqueous	7.1%

A small portion of the dried extracts were subjected to the phytochemical test using standard methods to test for alkaloids, glycosides, tannins, saponins, flavonoids and steroids separately for extracts of all samples. The outcomes of the results are discussed separately in the table 2.

Table 2: Phytochemical screening of extracts *Corchorus olitorius*

Constituents	Chloroform	Ethyl acetate	Methanol	aqueous
Alkaloids				
Dragendroff's test	-ve	-ve	-ve	-ve
Hager's test	-ve	-ve	-ve	-ve
Glycosides				
Legal's test	-ve	-ve	-ve	-ve
Flavonoids				
Lead acetate	+ve	+ve	+ve	+ve
Alkaline test	+ve	+ve	+ve	-ve
Phenolics				
$Fecl_3$	-ve	-ve	+ve	+ve
ProteinsAnd Amino acids				
Xanthoproteic test	+ve	-ve	-ve	+ve
Carbohydrates				
Fehling's test	-ve	-ve	+ve	+ve
Saponins				
Foam test	-ve	-ve	+ve	+ve
Diterpins				
Copper acetate test	+ve	+ve	+ve	+ve

From the results obtained it is clear that the *Corchorus olitorius* plant shows the presence of alkaloids, glycosides, saponins, tannins, flavonoids, amino acid, terpenoids were found present in whole parts when extracted with different solvents using maceration extraction procedure. The phytochemical analysis of *Corchorus olitorius* plant indicates the presence of phenols and flavonoids present in sufficiently enough quantity according to preliminary phytochemical analysis. Phenolic and flavonoids are the phytochemical that are present in ethyl acetate, methanol and water.

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: **$Y=0.040X + 0.009$, $R^2=0.999$**, where X is the quercetin equivalent (QE) and Y is the absorbance. The results are given in Table 3.

Table 3: Estimation of total flavonoids content of *Corchorus olitorius*

S. No.	Extracts	Total flavonoids content (mg/ 100 mg of dried extract)
1	**Chloroform**	0.505
2	**Ethyl acetate**	1.300
3	**Methanol**	2.050
4	**Aqueous**	1.785

CONCLUSION

The present study concluded that this medicinal plant viz. *Corchorus olitorius* is a promising source of various activities and may be efficient as preventive agents in the pathogenesis of some diseases. However, the strength of the existing data is not enough to suggest a reasonable mode of action. Further phytochemical studies are also required to isolate and characterize active ingredients that are responsible for its antioxidant activity and to explore the existence of synergism if any, among the compounds.

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