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Physico-chemical and Preliminary Phytochemical Study of Seeds of *Datisca cannabina* Linn (Datiscaceae) from Himalaya Region in Pakistan

Ijaz S*, Perveen A, Kousar S and Ghaffar N

Centre of Plant Conservation, University of Karachi, Pakistan

Abstract

The indigenous medicine involves the use of various plant extracts or the bioactive constituents, phytochemical analysis of such plants confirm the presence of different phytochemicals. *Datisca cannabina* Linn belong to family *Datiscaceae*. The whole plant is used in medicines in a mixture forms. The aim of this study was to assess the seeds of *Datisca cannabina* physicochemically and phytochemically. Maximum bioactive compounds (carbohydrate, alkaloids, proteins and aminoacids, phenolic compounds, flavonoids, glycosides fixed oil and terpenoids) were detected in the extract of methanol, chloroform, acetone and water. Presence of several phytochemical compounds showed high therapeutic potential of *Datisca cannabina* and it can take for medicinal purposes after determining the seeds pharmacologically.

Keywords: *Datisca cannabina*; Phytochemical; Physicochemical; Bioactive compounds; *Datiscaceae*

Introduction

Plants comprise the main source of many pharmacologically active compounds. Traditional medication based on plants has a long history. According to fossil records, the human use of plants as medicines may be traced back at least 60,000 years [1]. In modern society despite the development of synthetic drugs plants are the basic source of new healthcare and pharmaceuticals products. According to WHO about 11% of the 252 drugs are entirely originated from plants [2]. In recent years, herbal medication has received considerable attention. Herbal remedies are safer and less harmful than synthetic drugs [3]. It has enormous contribution as an alternative mode of treatment against many diseases worldwide [4]. World Health Organization (WHO) assessed that up to 80% of the total population in underdeveloped and developing countries depend on folk medicine practices for their primary health care needs. For pharmaceutical usage and to cure various ailments phytochemicals should be extract and screen to find out various bioactive compounds. Classical methodologies of chemistry used to analyze unidentified bioactive secondary metabolites from plant source to be discovered [5]. Secondary metabolites are essential to all living organisms. These compounds also give plants their distinctive aromas and help the plants for pollination or spread the seeds by attracting insects and other creatures as well as, protect against biotic or abiotic stresses [6]. Increasing Popularity of herbal medicine for the treatment of various diseases is also a great risk for the survival of plants. *Datisca cannabina* is a medicinally important plant. The plant is distributed in tropical and subtropical western Himalaya, from Kashmir to Nepal, Turkey, Afghanistan, and Pakistan (flora). According to IUCN red list data 1997 and version 3.1 (2001) this species is putted under endangered category. The whole plant is used for the extraction of natural dye as well as used medicinally as diuretic, febrifuge purgative and sedative and laxative.

Methodology

Collection and authentication

Whole plant specimen and seeds of *Datisca cannabina* was collected from Neelum valley Azad Kashmir in the month of September 2016. Plant was identified with the help of available literature flora of Pakistan and voucher specimen was deposited in (KUH) Karachi University Herbarium, Centre for Plant Conservation, University Of Karachi [7].

Drying and grinding

Seeds were gathered from the collected plant and washed with water. After washing it was subjected to shed-drying. When seeds were suitable for grinding, it was grinded into coarse powder by a grinder (Wuhu motor factory, China). Finally, the powder material was stored in a sealed container and kept in a dark, cool and dry place until farther processing.

Extraction from seeds extraction

Air-dried powdered plant material (5 g) were percolated in 100 ml of using 4 different solvents Acetone, methanol, chloroform, and water in a conical flask, and then placed on a rotary shaker for 48 h. After then filtered through Whatmanno. 1 filter paper then placed to complete dry. Extract was used for further tests.

Physico-chemical analysis

Ash values, extractive values under various solvents, water solubility and acid solubility percentage were checked as described by [8]. Phytochemical screening, Alkaloids test [9]. An amount of (25 mg) extract was stirred with few ml of dilute Hcl and filtered. Filtrate was then used for following tests.

Mayer's test: Two drop of Mayer's reagent was added to 2 ml of filtrate, by the side of test tube. A white or creamy precipitate trace the test is positive.

Wagner's test: 2 drop of Wagner's reagent added to 2 ml test tube filtrate, reddish brown precipitate color shows the test is positive.

Tests for protein: The extract (25 mg) is dissolved in 2.5 ml of distilled water and filtered through filter paper then filtrate was used in this test.

***Corresponding author:** Ijaz S, Centre of Plant Cons ervation, University of Karachi, Pakistan, E-mail: shabirijaz@hotmail.com

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A. **Million's test**: Few drop of Million's reagent added in 2 ml of filtrate in a test tube then white precipitate confirms the presence of proteins [10].

Biuret test: One drop of copper sulphate solution with 2 ml filtrate and 1 ml ethanol were taken and added some pellets of KOH. Appearance of pink color with Ethanolic layer conforms the protein is present [11].

Tests for carbohydrate

Benedict's test: The extract (100 mg) was dissolved in 5 ml of water then filtered with the help of filter paper. Filtrate (0.5 ml) and 0.5 ml of Benedict's reagent was mixed then heated on water bath for 2 minutes. A characteristic precipitate confirms the presence of sugar [12].

Fehling test: In a test tube, equal parts of Fehling's solution (A) and (B) previously mixed. In the aqueous extract, a mixture of equal parts of Fehling's solution (A) and (B) previously mixed was added and heated. Formation of red precipitation of cuprous oxide indicates the presence of sugars.

- Phenolic compounds

A. Ferric chloride

The extract (1 g) was stirred with 10 ml of water and to this; few drops of 5% ferric chloride solution was added. Presence of dark green color confirms phenolic compounds [13].

B. Lead acetate

Extract (0.5 g) was dissolved with few ml of distilled water then was added 10% lead acetate solution 3 ml. A bulky white precipitation conforms the test is positive [14].

X. Flavonoids

Aqueous filtrate 1 ml was treated with 2 ml 10% ammonia then 1 ml concentrated H_2SO_4 was added. Yellow color confirms the presence of flavonoids.

Tests for glycosides

Borentrager's test: In 2 ml filtrate 3 ml chloroform was mixed, and then 1 ml of 10% solution of ammonia was added. Development of Pink coloration indicates the presence of glycosides [14].

Test for fixed oils

A. Spot test: Powder of seeds samples was pressed between two filter papers. Oil spot on the paper shows the presence of fixed oils [15].

B. Terpenoids: An amount of (250 mg) extract was mixed with 1 ml chloroform then was added 1.5 ml of H_2SO_4. Development of brownish red color conform the presence of terpenoids [16].

X. Test for Saponin: The extract (25 mg) was dissolved in 10 ml autoclave distilled water, then shake continuously for 20 min. appearance of 2 cm layer of foam conform the presence of saponins.

Results and Discussion

Discussion

The plant species *Datisca cannabina* L commonly known as hemp is attractive bushy plants herbaceous plants, with alternate and pinnate leaves, cultivated for their ornamental foliage and readily propagated by seeds or cuttings. They are only non-woody plants, which host nitrogen-fixing bacteria in their roots. The plant yields a bitter tasting purgative juice; the roots contain the alkaloid Datiscine and give a yellow dye (flora of Pakistan) prepared natural pigments [17]. Thermal degradation of the pigments was determined by thermogravimetric analysis. High char yields were found for all pigments. In many areas of world the whole plant traditionally used as a diuretic, expectorant, and mild laxative [18]. The leaves and flowering stems are bitter, diuretic, febrifuge and purgative. The root is used as a sedative in the treatment of rheumatism. It is also applied to carious teeth [19]. There is only two species of *Datisca,* both have been chemically investigated and medicinally important compounds are reported in huge quantity. The main free amino acid in *D. cannabina* nodules was arginine [7]. Arginine in the body changes into nitric oxide which is a powerful neurotransmitter helps vessels relaxation, blood flow and also improves circulation [20]. Aerial parts of *D. cannabina* contained hentricontane, alpha -amyrin, erythrodiol, oleanolic acid, and beta -sitosterol, and beta -sitosterol glucoside. Datisdirin is a valuable drug, showed activity against the ureases enzyme. Urease is directly involved in the formation of stones and contributes to the ammonia, pathogenesis of urolithiasis, hepatic coma, pyelonephritis, hepatic encephalopathy, urinary catheter encrustation reported flavonoid datisdirin, along with eight known compounds tectochrysine, sideroxyline, cearoin, ursolic acid, arjunolic acid; corosolic acid, erythrodiol and oleanolic acid were isolated from the ethyl acetate fraction of *D. cannabina* [21,22]. Seeds of *Datisca cannabina* were examined physic-chemically and phytochemically. Our study shown presence of various bioactive compounds in the seeds of *Datisca cannabina* by using acetone, chloroform, methanol, water as solvents. Presence of many compounds revealed the medicinal value of *Datisca cannabina* seeds. Alkaloids, Flavonoids, phenoliccompound, terpenoids, carbohydrate, glycosides, protein and fixed oil were present in different solvents but saponins were present in low amount only in water extract. Amino acid and Protein not detected by Million's reagent as well as in in Biuret test in acetone extract while present in all

1.	Physical state of ash	fine powder
2.	Colour of ash	black
3.	% of loss on drying	10%
4.	% of ash content	8%
5.	Water soluble ash	30%
6.	Water insoluble ash	70%
7.	Acid soluble ash (Nitric acid)	32%
8.	Acid insoluble ash (Nitric acid)	68%
9.	Acetone soluble extractive value	6%
10.	Chloroform soluble extractive value	8%
11.	Methanol soluble extractive value	7%
12.	Water soluble extractive value	8.4%

Table 1: Physio-chemical analysis of seeds of *Dastisca cannabina*.

S.NO	Phytochemical test	Acetone	Chloroform	Methanol	Water
1.	**Alkaloid** Wagner's reagent Mayer's reagent	+ +	+ +	++ ++	+++ +++
2.	**Carbohydrate** Benedict's test Fehling test	+ +	+ +	+ ++	+ ++
3.	**Protein and amino acid** Biuret Test Millions test	_ _	++ ++	++ ++	+ ++
4.	**Phenolic compounds** Lead acetate Ferric chloride test	+ +	+ +	++ ++	++ ++
5.	**Flavonoids**	+	+	++	++
6.	**Glycoside** **Salkow`s test** Borntrager's test	+ +	++ +	++ +	_ +
7.	**Fixed oil** Spot test	+	+	+	+
8.	**Terpenoids**	+	+	++	++
9.	**Saponin** Foam test	_	_	_	+

Table 2: Preliminary Screening of major Phytochemicals of *Dastisca cannabina*.

other extracts. Glycosides detected in all extracts but found absent in water. All the phytochemical screening results are represented in Table 1. For seeds of *Datisca cannabina,* high extractive value was found in water with 8.4% w/w, second solvent is chloroform extractive value was 8% while extractive value in methanol and acetone was 7% and 8% respectively. Ash value was 15.6% all physico-chemical parameter results are presented in Table 2.

Conflicts of Interest

The authors have not declared any conflict of interests.

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