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Original Article

Phytochemical analysis and identification of flavonoid "quercetin" from *Jatropha gossypifolia* l. (euphorbiaceae) leaf extract

Vidhya Kumari*, Nitu Kumari, Mohan Lal Kuri, Krishna Kishori Verma and Anu Shrivastav

Bio-Technology Laboratory, Department of Botany, University of Rajasthan, Jaipur, 302004. India.

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ABSTRACT

Medicinal plants are the most exclusive source of life saving drugs for the majority of the world's population. *Jatropha gossypifolia* Linn. well known plant of family Euphorbiaceae is used as a therapeutic agent. In the present investigation, maximum percent extractive value was calculated for methanol extractive (24.0%). Preliminary phytochemical screening was performed as per standardized procedure of the various phytoconstituents in methanol, chloroform and water extract of *J. gossypifolia* L. leaves. Crude leaves extract in different solvent exhibited the presence of secondary metabolites such as flavonoids, alkaloids, cardiac glycosides, tannins and saponins. Flavonoid "quercetin" was isolated from leaves. The Rf value of test sample coincided with the Rf value of standard quercetin.The medicinal values of this plant could be attributed due to the presence of one or more of the detected metabolites.

Key words: Phytoconstituents, Euphorbiaceae, *Jatropha gossypifolia*, Flavonoid, Metabolites

Address for Correspondence: Dr. Vidhya Kumari, Research Scholar, Department of Botany University of Rajasthan, Jaipur, India; Email: vidya15345@gmail.com

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INTRODUCTION

Plants have potent biochemicals and have components of phytomedicine. Man is able to obtain from them a marvelous assortment of industrial chemicals. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. that is any part of the plant may contain active components [1].

Primary metabolites are mainly used as industrial raw materials, food or food additives. Many primary metabolites lie in their impact as precursors pharmacologically active metabolites of pharmaceutical compounds such as antipsychotic drugs [2, 3, 4, 5].The induction of secondary metabolism is linked to particular environmental conditions or developmental stages. These metabolites have common origin up to some extent and then are diverted to different pathways. However, all are derived from primary product of photosynthesis the glucose, and have a strong inter-relation between both the two primary and secondary metabolites.Tremendous and intensive efforts have been made to discover new antimicrobial compounds from natural products. Systematic screening of them may result in the discovery of novel effective compounds [6].

Flavonoids, including the anthocyanins, flavonols and flavones, are among the most intensely studied secondary products with over 6,000 known compounds [7]. Many of them play important roles as flower and fruit pigments, UV protectants, signaling molecules between plants and microbes, and regulators of auxin transport [8, 9]. The flavonoids are also thought to have antioxidant, anti-allergenic, and anti-inflammatory effects, thus contributing to human health [10, 11]. Chemically they are water soluble phenolic compounds forming a skeleton of three rings system, called flavone nucleus, with two side aromatic rings and a central oxygenated heterocyclic. They are synthesized from phenyl propanoid and acetate derived precursors. Considerable quantities of flavonoids are consumed daily in our vegetable diet, so adverse biological effects on man are not particularly intense. Indeed, there is growing belief that some flavonoids are particularly beneficial, acting as antioxidants and giving protection against cardiovascular diseases, certain form of cancer and it is claimed, and age related degeneration of cell components [12]. Their polyphenolic nature enables them to scavenge injurious free radical such as superoxide and hydroxyl radicals. Quercetin is a flavonoid widely distributed in nature. *Jatropha gossypifolia* (Bellyache bush) is an erect perennial shrub with a shallow root system. Field studies have shown that plants can live longer than 10 years, with anecdotal evidence suggesting life spans greater than 20 years. The shrub generally grows to around 2m in height, although it can reach up to 4m under favorable conditions. Extracts from the various components of the plant, including the leaves, stems, bark, seeds and roots, have been used to treat a number of human ailments ranging from anemia, vertigo, worms, leprosy, dysphonic, urinary complaints, ulcers, itches, snake bite and venereal disease. Study indicates that *Jatropha gossypifolia* L. extracts enhance glucose uptake in cultured myotubes and adipocytes and also improving glucose tolerance in an *in vivo* model [13]. The neuropharmacological action of the methanol extract of the leaves of *J. gossypifolia* was evaluated [14]. Similar results were observed in the methanolic extract from fruits [15]. *J. gossypifolia* leaf extract, by oral route, altered the major hormones involved in estrous cycle regulation, indicating its antifertility effect on mice [16]. Evaluating other parameters (estrogenic and early abortifacient activities) the anti-infertility effect of the extract was once more demonstrated later [17].

In the present study qualitative methods has been done to understand the metabolic composition of the study material by using various known standard chemical methods for different secondary metabolites. The precise identification of flavonoids was done following TLC (thin layer chromatography) method.

MATERIALS AND METHODS

Collection and Identification of Plant Material: Leaves of *Jatropha gossypifolia* L. (family Euphorbiaceae) were collected from University of Rajasthan campus Jaipur, Rajasthan, India in August 2012. A Voucher specimen of the plant has been deposited as RUBL211370 (*Jatropha gossypifolia* L.) in the Herbarium, Department of Botany, University of Rajasthan for further references. Fresh leaves of *J. gossypifolia* were harvested and washed with distilled water so as to remove dust and other foreign particles. The leaves were left on a clean surface to dry well then air-dried under shade. Dried material was grinded to fine powder using an electric grinder and stored in air tight bottles. The Powdered material was used further, for phytochemical screening and preparation of extracts.

Fifty grams air-dried and coarsely powdered plant material was kept in Soxhlet extraction unit and exhaustively extracted with 80% methanol at 40° C for twenty four hours and same procedure is applied for chloroform and water. Chloroform and

methanol used were of analytical grade. The separated extract were then filtered through Whatman's No. 1 filter paper and evaporated under reduced pressure using rotary evaporator. The dried extracts were weighed and their percentage in terms of the dry weight of the plant material was estimated by the following formula and were given in (Table1).

Qualitative Phytochemical Analysis: Preliminary phytochemical analysis of the crude powder of the leaves collected was determined according to the standard procedures to identify the constituents as described [18, 19, 20, 21]. The qualitative methods are used for identification of secondary metabolites in any plant. They indicates the presence or absence of any metabolite. Foam test for saponins, Salkowski and Liebermann-Burchard test for terpenoids, $FeCl_3$ test for tannins, Keller-Killiani test for cardiac glycosides and ammonia test for detection of flavonoids were performed to identify the constituents present in the extracts of plant leaves.

The present study was undertaken for the isolation and identification of quercetin from the leaves sample of the plant. The identification of the quercetin present in the test samples were carried out by calculating the retention factor (Rf) for the particular compound. The Rf value is the distance traveled by the compound divided by the distance traveled by the solvent.

$$Rf = \frac{\text{distance traveled by compound}}{\text{distance traveled by solvent}}$$

Extraction Procedure: 50 grams of dried plant leaves sample was soxhlet extracted in 80% methanol (100 ml/gm dry weight) on a water bath for 24 hrs [22]. Each of the extracts was concentrated and re-concentrated in petroleum ether (40°-60°C) (fraction-I), ethyl ether (fraction-II) and ethyl acetate (fraction-III) in succession. Each of the steps was repeated three times to ensure complete extraction in each case. Fraction I was rejected since it was rich in fatty substances whereas fraction II was analyzed for the free flavonoids in each of the samples. Fraction III of each of the test samples was hydrolyzed by refluxing with 7% H_2SO_4 (10 ml/gm residue) for 5 hours. The mixture was filtered and the filtrate extracted with ethyl acetate in a separating funnel. The ethyl acetate layer was washed with distilled water till neutrality and dried *in vacuo*. The residues were taken up in small volumes of ethanol separately and then subjected to various tests for quercetin.

Preparation of TLC Plates: The glass plates (20 x 20 cm) coated with silica gel 'G' (0.2-0.3 mm thick by suspending 6 gm/15 ml distilled water) (E-Merck, India). The coated plates were dried at room temperature. The dried plates were activated at 100^0 C for 30 minutes in an oven and cooled at room temperature.

Preparation of Standard Solution: Quercetin standard were prepared as 0.01% solution in methanol.

Chromatographic Procedure: The methanolic soxhlet extracted leaves samples were loaded on TLC plates. 0.1 gm of dried extract was dissolved in methanol for loading the TLC plates, with the help of capillaries (Top-Tech Biomedical, 90mm in length). Spots were applied for standard and extract from the study material at an equal distance.

The spotted plates were dried and saturated in prepared solvent system. For chromatography the run solvents used were (Benzene: Glacial acetic acid: water 125: 72: 3). Chromatography chamber was saturated by the vapors of the run solvent kept for 1 hour before running the chromatogram. Loaded TLC plates were then developed by dipping them in to the saturated chromatographic chamber, where solvent was allowed to rise by capillary action to more than two-thirds of the glass plate length.

The developed plates were air dried. After drying, the plates were then placed in a chamber saturated with ammonia vapors' to observe the color of spots (quercetin deep yellow) and plates were also placed in a chamber saturated with I_2(Iodine) vapors' to observe the color of spots (yellow brown). The developed plates were sprayed with 5% ethanolic ferric chloride solution to observe the color of the spots. Rf values were calculated for isolated samples and compared with coinciding standard.

RESULT

Percent extractive of different extracts of *Jatropha gossypifolia* is depicted (Table-1). In *Jatropha gossypifolia* leaves, maximum percent extractive value was observed for methanol extractive (24.0%).

Preliminary phytochemical screening was performed as per standardized procedure of the various phytoconstituents in methanol, chloroform and water extract. Leaves extract of *Jatropha gossypifolia* in chloroform exhibited the presence of all the secondary metabolites tested except flavonoids. While the methanolic extract exhibited the presence of alkaloids, flavonoid, cardiac

glycosides and saponins. The water extract showed the presence of alkaloids, flavonoids, saponins and tannins (Table 2).

The developed plates were when sprayed with 5% ethanolic ferric chloride solution it showed spots which coincided with that of the reference quercetin (bluish grey) when plates were placed in a chamber saturated with ammonia vapors, it showed deep yellow color of standard quercetin. The Rf value of test samples coincided with the Rf value of standard quercetin (Rf value 0.82) (Table 3). The Rf value matched with that of the standard quercetin in the plant materials hence there is no doubt about the presence of the quercetin in the plant samples.

DISCUSSION

All the tested phytochemicals were present in dried plant material as shown in (Table: 2). Plant possesses various biological properties such as anti-allergic, molluscicidal, and insect repellent activity [15, 23, 24]. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers [25]. Aqueous decoctions of *J. gossypifolia* are used as antidiabetic in Colombia and the Dominican Republic. Besides, in India ethanolic extracts applied in single or multiple doses to a diabetic rat's model showed a reduction in glucose levels [24, 26]. Steroids extract from some medicinal plants exhibits antibacterial activities on some bacterial isolates [27]. Steroids have anti-inflammatory effects [28, 29, 30]. The results of our study confirm the steroids presence in the chloroform extracts of *Jatropha gossypifolia* leaves.

Flavonoids, another constituent of leaves extract exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties [31]. Glycosides, flavonoids, tannins have hypoglycemic activities [32]. The results of the present study confirm the quercetin presence in the methanolic extracts of *Jatropha gossypifolia*. Since quercetin has anti-inflammatory, antioxidant and anticancer properties, isolation and extraction of this compound medicinally important.

CONCLUSION

Preliminary phytochemical screening provides information about the presence of the phytoconstituents in the extracts. This study supports the traditional use of *Jatropha gossypifolia* for the treatment of various infectious diseases in different regions of the world and may serve as a good source of novel bioactive compounds. Results obtained show the great interest in plant pharmaceuticals.

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Table 1: Percent extractive of different extracts of *Jatropha gossypifolia* leaves

S. No.	Solvent	Percent extractive value of *Jatropha gossypifolia* leaves
1	Methanol	24.00%
2	Chloroform	9.8%
3	Aqueous	20.56%

Table 2: Preliminary Phytochemical analysis of *Jatropha gossypifolia* L. leaves with different solvents

Phytochemical test	*Jatropha* leaves extract in different solvent		
	Chloroform	Methanol	Aqueous
Alkaloids	+	+	+
Flavonoids (Ammonia test)	_	+	+
Cardiac glycosides (Keller Killiani test)	+	+	_
Saponins (Foam test)	+	+	+
Tannins ($FeCl_3$ test)	+	_	+
Steroids (Salkowski test)	+	_	_

+ indicates the presence of the constituent; -indicates the absence of the constituent

Table 3: Rf values of the spots obtained on TLC plates with extract of *Jatropha gossypifolia* along with standard quercetin

S. No.	Rf value of *Jatropha gossypifolia* Extract	Rf value of Standard
1	9.8/12 = 0.816	9.9/12=0.82
2	9.9/12 = 0.825	
3	9.9/12= 0.825	

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