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## Analysis of the Phytochemical Content and Anti-microbial Activity of *Jatropha gossypifolia* L.

Ruchi Seth\* and Renu Sarin

Laboratory of Bioactive and Natural Products, Department of Botany, University of Rajasthan, Jaipur, India

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### ABSTRACT

The importance of plant is well known. Life and its growth cannot be imagined without plants. They not only produce food for survival but also create healthy environment and eco-friendly atmosphere to live. *Jatropha gossypifolia* Linn. well known plant of family Euphorbiaceae is used as a therapeutic agent. The leaf decoction of this plant is used for bathing wounds, sores, sprains, rash and bewitchment. In the present investigation the leaves of *J. gossypifolia* L. were screened for antibacterial activity by extracting them successively in various solvents such as Petroleum ether, Benzene, Chloroform, Acetone, Alcohol and Water. Crude powder of leaves was screened for the presence of chemically active compounds by standard methods and for antibacterial activity by zone of inhibition. The results revealed the presence of saponin, tannin, flavonoid, reducing sugars, cardiac glycosides, terpenoids, triterpenoids, steroids, xanthoprotein, starch. The medicinal values of this plant could be attributed to the presence of one or more of the detected metabolites.

**Key words:** Phytochemicals, Euphorbiaceae, Antibacterial activity, Inhibition zone, *Jatropha gossypifolia*.

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### INTRODUCTION

India possesses a variety of medicinal plants and it is one of the richest countries in the world in regard to genetic resources of medicinal plants. India exhibits a wide range in topography and climate, which bears varietal emporium of vegetation and floristic composition. Moreover, the agro-climatic conditions are favorable for introduction and domestication of new exotic plant varieties [1]. Since time immemorial, man has used various parts of plants in the treatment and prevention of various ailments [2]. In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [3]. Thus, it is anticipated that phytochemicals with

adequate antibacterial efficacy will be used for the treatment of bacterial infections [4] in near future.

Historically plants have provided a good source of anti-infective agents with compounds which are highly effective instruments in the fight against microbial infections. Infectious diseases are the leading cause of death world-wide. Phytochemicals derived from plants have shown great promise in the treatment of obstinate infectious diseases. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Now a day's antibiotic resistance has become a global concern [5] as the clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens [6]. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind [7]. Therefore, researchers are increasingly turning their attention to folk medicine and looking for new leads day by day to develop better drugs against microbial infections [8].

*Jatropha gossypifolia* Linn. belongs to the family Euphorbiaceae and the order, "Geraniale". A number of other species of *Jatropha* like *Jatropha curcas*, *Jatropha multifida*, *Jatropha podagrica* are also known. The common name for *J. gossypifolia* is bellyache bush, pignut or fignut, and in Yoruba land it is commonly known as "Lapalapa" [9]. It grows wild in different parts of India. The plant is known to possess various medicinal and pesticidal properties. *J. gossypifolia* is commonly known as Belly ache bush. It is a bushy, gregarious shrub, up to 1.8m, 3-5 lobed, approximately 20 cm long and wide, with leaves having a long petiole, covered with glandular hairs. The seed are greenish capsule-like seeds. The leaf stalks are covered with coarse dark brown hairs and the young leaves are sticky. It has thin, often greenish bark, which exudes copious amount of watery sap when cut. The fruits are three-celled with one seed per cell. *J. gossypifolia* is widely cultivated as ornamental plant. It is the common red species planted around houses. It is also planted to control the soil erosion along the slopes. It prefers arid environment.

*J. gossypifolia* is used as a therapeutic agent in different ways. The leaf decoction of this is used for bathing wounds [10]. The leaf bath is used for sores, sprains, rash and bewitchment in Latin America and the Caribbean [11, 12]; the poultices are used for sores and pain in Trinidad [11]. The stem sap stops bleeding and itching of cuts and scratches [11, 13]. In Southern Nigeria, the extract from fresh leaf applied with crushed leaf is routinely used by herbalists and local people to stop bleeding from the skin and nose. The young stem of the plant is used as toothbrush as well as to clean the tongue in the treatment of thrush. The tuber of the plant grinded into a paste is locally used in the treatment of hemorrhoids in Nigeria.

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. The aim of this study is to investigate the antimicrobial activity of *J. gossypifolia* leaves extracts as well as to identify the active constituents.

## MATERIALS AND METHODS

### *Collection and Identification of Plant Material*

Leaves of *Jatropha gossypifolia* L. (family Euphorbiaceae) were collected from World Arboretum, Jaipur, Rajasthan, India in August 2008. A Voucher specimen of the plant has been deposited 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 then left on a clean surface to dry well. The leaves were air-dried under shade for 10-15 days. Then the 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.

### **Preliminary Phytochemical Analysis**

Qualitative phytochemical analysis of the crude powder of the leaves collected was determined according to the standard procedures to identify the constituents as described by [14-17]. Foam test for saponins. Salkowski and Liebermann-Burchard test for terpenoids and triterpenoids. FeCl<sub>3</sub> test for tannins. Keller-Killiani test for cardiac glycosides. Fehling's test for reducing sugars, xanthoproteic test for proteins, iodine test for starch and ammonia test for detection of flavonoids were performed to identify the constituents present in the extracts of the leaves of the plant.

### **Culture and maintenance of Bacterial Strains**

*In vitro* antimicrobial activity of various plant extracts was examined using *Escherichia coli* and *Bacillus subtilis* as test organisms. Pure cultures of the test organisms namely: *Escherichia coli* (MTCC1562) and *Bacillus subtilis* (MTCC441) were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacterial cultures were revived in Nutrient Broth medium and incubated at 37°C for 48 hours. Each bacterial culture was further maintained at 37°C on nutrient agar slants and nutrient broth after every 48 hours of transferring.

### **Preparation of plant extracts**

For partial purification of different organic constituents of dried plant material (leaves) reflux method of solvent extraction was used [18]. Solvent series for successive separation was as follows:

Petroleum ether → Benzene → Chloroform → Acetone → Alcohol → Water

Forty grams air-dried and coarsely powdered plant material was kept in Soxhlet extraction unit and exhaustively extracted with petroleum ether (60-80° C) for twelve hours. The extracted plant material was then air-dried, repacked in the soxhelt apparatus and exhaustively extracted with next solvent in the series for twelve hours again. The crude extracts and fractions obtained at every step including aqueous fraction were filtered and evaporated under reduced pressure using rotary evaporator. The dried extracts and fractions were weighed and their percentage in terms of the dry weight of the plant material was estimated by the following formula and given in (Table-1)

$$\text{Percent Extractive} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plants material}} \times 100$$

### **Media Preparation and Antibacterial Activity**

The disc diffusion method was adopted to assess the antibacterial activity of the prepared extracts [19]. 0.6 ml of standardized bacterial stock suspensions (10<sup>8</sup>-10<sup>9</sup>) CFU/ml was used as inoculum. 5mm sterile Whatmann No.1 filter paper discs were soaked into the dissolved crude extracts for a minimum of two hours and dried. Blank discs were impregnated with tetracyclin (10µg/disc) and used in each plate as positive control. By use of a sterile forcep, six seeded discs of the plant extracts were placed equidistantly onto each of the inoculated plates. One extra disc for positive control was placed at the middle of plate. The plates were then incubated in the upright position at 37° C for 24 hours. The experiment was carried out in three replicates/three

times for each extract against each of the test organism. After incubation antibacterial activities were determined by measuring the diameters of zone of growth inhibition in mm. The average of the diameters of zones of growth inhibitions for the treatments was tabulated as shown in table 3.

## RESULT AND DISCUSSION

Plants are of great importance to the health of individuals and communities from time immemorial. Plant kingdom provides a tremendous reservoir of various phytochemicals with potential therapeutic properties. The major phytochemicals of interest are alkaloids, tannins, flavonoids, phenolic compounds, steroidal saponins (saponins), however, other diverse groups of naturally occurring phytochemicals such as unsaturated sterols, triterpenoids, essential oils are also present. These phytochemicals play important role in herbivore deterrence due to astringency or they may act as phytoalexins, killing bacteria that the plant recognizes as a threat [20]. Plant extracts are used to treat numerous human diseases [21] and have prominent effect on the animal system, important therapeutic properties and antimicrobial activities against various pathogens [19, 22, 23]. Plants can function as sources of anti-cancer agents [24].

Percent extractive value of various fractions of partially purified hot extracts is depicted (Table-1). Maximum percent extractive value was observed for aqueous fraction.

**Table 1: Percent Extractive of Different Partially Purified Fractions of *J.gossypifolia* Leaves Extract**

S.No.	Solvent	Percent extractive value of <i>J.gossypifolia</i> Leaves
1.	Petroleum ether fraction	4.9%
2.	Benzene fraction	1.72%
3.	Chloroform fraction	2.37%
4.	Acetone fraction	1.4%
5.	Ethanol fraction	4.5%
6.	Methanol fraction	3.37%
7.	Aqueous fraction	6.65%

All the tested phytochemicals were found to be present in dried plant material (leaves) as shown in (Table-2). The presence of flavonoids and tannins in the leaves is likely to be responsible for the free radical scavenging activity. 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]. These findings give credence to the traditional medicinal application of the leaves as remedies for sores, rash and bewitchment, internal and external wounds and infections. Flavonoids have been referred to as nature's biological response modifiers because of strong experimental evidence of their ability to modify the body's reaction to allergies, virus and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anticancer activity. Cardiac are used in the treatment of congestive heart failure and cardiac arrhythmia. They are also, used to strengthen a weakened heart and allow it to function more efficiently [26]. Steroids anti-inflammatory effects [27-29]. Glycosides, flavonoids, tannins have hypoglycemic activities [30]. Saponins possess hypocholesterolemic and antidiabetic properties [31]. The terpenoids have also been shown to decrease blood sugar level in animal studies [32]. Steroids and

triterpenoids showed the analgesic properties [33, 34]. The steroids and saponins are responsible for central nervous system activities [35].

**Table 2: Qualitative Phytochemical Screening of *J. gossypifolia* Leaves**

S.No.	Test	Presence/Absence
1.	Terpenoid (Salkowski test)	+
2.	Steroids (Salkowski test)	+
2.	Saponin (Foam test)	+
3.	Flavonoid (Ammonia test)	+
4.	Triterpenoid (Liebermann-Burchard test)	+
5.	Tannin (FeCl <sub>3</sub> test)	+
6.	Cardiac glycosides (Keller-Killiani test)	+
7.	Reducing sugars (Fehling's test)	+
8.	Proteins (Xanthoproteic test)	+
9.	Starch (Iodine test)	+

+ indicates the presence of the constituent

The results of antimicrobial activity test of different solvents extracts of *J. gossypifolia* are shown in (Table-3). *J. gossypifolia* exhibited considerable antibacterial activity against both test organisms. The maximum efficacy was exhibited by Benzene fraction whereas aqueous fraction has shown minimum antibacterial activity against both cultures. Petroleum ether fraction has shown no antibacterial potential against both test organisms. Acetone and Ethanol fractions have shown mediocre activities against *E. coli*. Chloroform fraction was found to be inactive against *E. coli*. While, in case of *B. subtilis* Chloroform and Methanol fractions have shown mediocre activities. Acetone and Ethanol fractions have shown no efficacies against *B. subtilis*.

**Table 3: Antibacterial activity of different extracts of *J. gossypifolia***

S. No.	Plant Extracts	Zone of inhibition in mm	
		<i>Escherichia coli</i>	<i>Bacillus subtilis</i>
1	Tetracycline	14.04 ± 0.03	12.11 ± 0.04
2	Petroleum ether fraction	0 ± 0	0 ± 0
3	Benzene fraction	13.05 ± 0.02	10.01 ± 0.01
4	Chloroform fraction	0 ± 0	8.05 ± 0.03
5	Acetone fraction	8.04 ± 0.01	0 ± 0
6	Ethanol fraction	7.07 ± 0.01	0 ± 0
7	Methanol fraction	0 ± 0	7.06 ± 0.03
8	Aqueous fraction	4.08 ± 0.02	2.04 ± 0.02

\*All the values are mean ± standard deviation of three determinations



**Figure 1: Zone of Inhibition are the mean values of triplicate readings; PE- Petroleum ether fraction; Be- Benzene fraction; Ch- Chloroform fraction; Ac- Acetone fraction; Et- Ethanol fraction; Me- Methanol fraction; Aq- Aqueous fraction; Std- Standard Tetracycline**

### CONCLUSION

Results reveal leaves of *J. gossypifolia* have quite a number of chemical constituents, which may be responsible for many pharmacological activities. Further work is required to investigate the extracts of leaves of *J. gossypifolia* for various pharmacological activities before its commercialization for the benefit of human beings.

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