



BOTANY

PRELIMINARY SCREENING OF ANTIMICROBIAL AND PHYTOCHEMICAL STUDIES OF *JATROPHA GOSSYPIFOLIA* LINN.

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Abstract

The antimicrobial effect of *Jatropha gossypifolia* Linn. (Euphorbiaceae) leaf extract was evaluated on microbial strains like gram-positive species *Staphylococcus* spp., and *Bacillus* spp. and gram-negative species *Escherichia* Spp. and *Pseudomonas* spp. The solvent used for extraction of plant were Petroleum ether, Alcohol, Chloroform. The alcoholic extract of leaves of *Jatropha gossypifolia* shows maximum antimicrobial activity. The *in vitro* antimicrobial valuation was carried out by agar disc diffusion method. The significant antibacterial activity of active extract was compared with standard antibiotic Ampicillin. The samples of leaves were further used for the phytochemical studies. Results of the Phytochemical analysis indicated the presence of phenolic compounds, tannins, lignin, starch grains and saponins. Physico-chemical evaluation includes ash values, extractive values and moisture content while Phytochemical isolations includes saponins, tannins, phenols, cellulose, nitrogen, crude proteins and minerals like calcium, phosphorous and potassium. The antibacterial activities of the leaves were due to the presence of various secondary metabolites.

Keywords: Antimicrobial, Physico-chemical, Phytochemical, *Jatropha gossypifolia*

Introduction

Plants are known to have beneficial the therapeutic effect documented in traditional Indian system of medicine. Much work has been done on ethno medicinal plant in India. Interest in a large number of traditional natural products has increased. Plant derived drugs remains important resource especially in developing countries, to combat serious disease. Approximately 62 – 80% of the world's population still relies on traditional medicines for the treatment of common illness [1, 2]. In fact, plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times [3]. Over 50% of all modern clinical drugs are of natural product origin [4]. And natural products play an important role in drug development programmes in the pharmaceutical industry [5]. There are a few reports on the use of plants in traditional healing by either tribal people or indigenous community [6, 7, 8, 9, 10].

The antimicrobial activity have been screened because of their great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, becomes an ever increasing therapeutic problem [11]. Natural products of higher plants may give a new source of antimicrobial agents. There are many research groups that are now engaged in medicinal plants research [12,

13,14]. The development of drug resistance in human pathogens against commonly used antibiotics has necessitate the search for new antimicrobial substance from other sources. Screening of medicinal plants for antimicrobial activities and phytochemical is important for finding potential new compounds for therapeutic uses.

The present study was carried out on the phytochemical and antibacterial activity of leaf of *Jatropha gossypifolia*, which is popularly known as the "*Jangali yerend*". It is a well known remedy for the treatment of various types of disorders in the ayurvedic and folklore system of medicine in India. Decoction of the bark is used as emmenagogue [15, 16]. Decoction of leaves is used as purgative and stomachic, whereas leaves are employed as febrifuge in intermittent fevers and swollen mammae [15, 16]. Crushed leaves mixed with breast milk are applied on the head to cure infantile diarrhea [17]. The seeds possess drastic purgative, emetic properties and are almost potent in action as those of *Jatropha curcas*. The oil obtained from them is used in indigenous medicine as an external stimulation, application in rheumatism and in paralytic affections, also in skin diseases. The juice of the plant is acrid whereas, the root has a purgative action [18]. Roots employed against leprosy [15].

Material and Methods

Sample collection and authentication

The fresh, mature healthy leaves of *Jatropha gossypifolia* Linn (Euphorbiaceae) were collected from west land of Dhule away from pollution. The plant

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materials were identified using the Flora of Dhule and Nadurbar District [19] at Post graduate Department of Botany, SSVS Sansthas, L.K.Dr.P.R. Ghogrey Science College, Deopur, Dhule-424005 (M.S.) India.

Sample preparation

Fully grown leaves and bark of *J. gossypifolia* were weighed (1kg). The plant samples were shade dried ground and sieved with 2mm rubber sieve to form uniform powder and stored in airtight bottles.

Preparation of extract

The dried plant material was pulverized into fine powder using a grinder (mixer). About 50 g of powdered material was extracted in Soxhlet extraction apparatus with 250 ml of each of the following solvents; petroleum ether, chloroform, and alcohol [20]. The extracts obtained with each solvent were filtered through Whatman filter paper No. 1 and the respective solvents were evaporated (at 40°C) with the help of heating mantle. The sticky greenish-brown substances were obtained and stored in refrigerator for prior to use [21]. Some of the extracts of each solvent were used for the qualitative phytochemical screening for the identification of the various classes of active chemical constituents, using standard prescribed methods [22, 23, 24, 25]. The positive tests were noted as present (+) and absent (-).

Preparation of microorganism

Isolation of bacterial species of gram positive (*Staphylococcus* spp. and *Bacillus* spp.) and gram negative (*Escherichia* spp. and *Pseudomonas* spp.) takes place. The cultures of these bacteria were checked for purity by doing gram staining and biochemical test and they were grown in nutrient broth at 37°C and maintained in nutrient agar slants at 2-8°C. Nutrient agar medium was used as bacterial culture medium in the antibacterial assays.

Selection of reference antibiotic

Reference antibiotic Amphotericin was obtained from authorized medical shop Dhule. The purity of the antibiotic is 99.8%

Dilutions and inoculum preparations

The dried plant extracts of *J. gossypifolia* and antibiotic Amphotericin were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentration of 50, 100mg/ml. The inoculums of *Staphylococcus* spp., *Bacillus* spp., *Escherichia* spp. and *Pseudomonas* spp. were prepared in nutrient broth medium and kept incubation at 37°C for 8 hours. After growth was observed, the cultures are stored in the refrigerator at 2-8°C for analysis.

Procedure for performing the Disc Diffusion test

The required amount of Petri plates is prepared and autoclaved at 121°C for 15 minutes. And they were allowed to cool under Laminar air flow. Aseptically transfer about 20 ml of media into each sterile Petri dishes and allowed to solidify. 1 ml inoculum suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. The readily prepared sterile discs were loaded with different concentrations of about 50, 100mg/ml of plant extract of *J. gossypifolia* and antibiotic amphotericin into each separate disc of about 100µl. The paper diffused discs were placed on the medium suitably apart and the plate were incubated at 5°C for 1 hour to permit good diffusion and then transferred to an incubator at 37°C for 24 hours. The antibacterial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader (mm) [26].

Chemical analysis

Histochemical tests were performed on fresh plant materials according to the method of Johansen [27] and Guerin *et al.* [28]. The moisture content was determined by heating the drug at 105°C to a constant weight and calculating the loss of weight. The extract of drug samples were prepared by using solvents and total acid insoluble and acid soluble ash content obtained. [29].

Two gm of each of the plant samples were weighed and taken in a previously weighed vitrosil silica crucible, to which added 2 drops of the mixture of H₂SO₄: HNO₃ (2:1). Then it was heated on the hot plate for about 30 minutes, till the sample was sufficiently charred and turns black. After this, replace the lid of the crucible and keep it in muffle furnace. The temperature allowed to rise up to 600°C and kept it constant for 2 hours. The crucible was removed on cooling and 50 ml of 5 N HCl was added to the ash in crucible. The mixture was heated for 30 minutes in hot water. Then it was allowed to cool and filtered through Whatman filter paper No. 42 and volume was made up to 100 ml with deionized water. This solution was used for mineral analysis. Calcium (Ca) content was determined by A.O.A.C. [30] method. Phosphorus (P) content was estimated by colorimetric method [31]. Potassium (K) content was determined on a flame photometer (model Mediflame-127) as suggested by Jackson [32].

Nitrogen (N) content in dry plant material was estimated by micro-Kjeldal method [33] and crude protein content was expressed as N x 6.25. The amount of Tannins by Folin-Denis Method, saponins, cellulose and phenols were estimated following Sadasivam and Manickam [34].

Results and Discussion

Antibacterial efficacy

Results obtained for the antibacterial tests performed on different solvent extracts of *Jatropha gossypifolia* are presented (Table 1). Among the extracts tested, Alcohol extracts showed broader spectrum of activity, being active to both Gram-positive and Gram-negative organisms compared to chloroform and petroleum ether. Activities of the various extracts were comparable to those of standard antibacterial agent ampicillin as control. Of all the bacteria tested the Gram-positive were slightly more susceptible to the

extracts than the Gram-negative bacteria. The differences in the observed activities of the various extracts may be due to varying degree of solubility of the active constituents in the four solvents used. It has been documented that different solvents have diverse solubility capacities for different phytochemical constituents [35]. The alcohol extract at 50mg/ml for example, 18 mm was recorded as diameter zone of inhibition against *Staphylococcus* spp. The least activity 4 mm against same microorganism at 100mg/ml was recorded by petroleum ether extracts.

Table 1. Antibacterial efficacy of different solvent extracts of *Jatropha gossypifolia* leaf

Sr. No.	Microorganism	Concentration (mg/ml)	Zone of inhibition (mm)			
			Petroleum ether	Chloroform	Alcohol	Ampicillin (40 µg/ml)
1.	<i>Escherichia</i> spp. (Gram - ve)	50	08	10	11	14
		100	06	08	08	
2.	<i>Pseudomonas</i> spp. (Gram - ve)	50	06	07	12	15
		100	05	05	09	
3.	<i>Staphylococcus</i> spp. (Gram + ve)	50	05	08	18	23
		100	04	07	15	
4.	<i>Bacillus</i> spp. (Gram + ve)	50	08	09	12	20
		100	06	08	11	

Table 2: Histochemical tests – *Jatropha gossypifolia* leaf

Test	alkaloids	phenolic compounds	tannins	lignin	starch grains	saponins	flavanoids
Result	-	+	+	+	+	+	-

Note: + present; - absent

Table 3. Physico-Chemical evaluation of *J. gossypifolia*

Physical evaluation (%)		Chemical evaluation (%)	
Parameter	Leaves	Constituents	Leaves
Moisture content	4.41	Saponins	03
Extractive values		Tannins	57
a) Petroleum Ether	2.10	Phenols	0.53
b) Alcohol	4.96	Cellulose	03
c) Methanol	5.3	Nitrogen	1.2
e) Water	29.2	Crude protein	7.5
Ash values		Calcium	1.36
a) Total ash	15.10	Phosphorus	0.77
b) A.I.A.	1.15	Potassium	2.83
c) A.S.A.	13.95		

Histology

Histological results indicate presence of phenolic compounds, tannins, lignin, starch grains, saponins, and absence of alkaloids and flavonoids (Table 2).

Physio-chemical characters

The physio-chemical characters summarised in Table 3.

Phytochemical evaluation

The preliminary studies revealed presence of various phytochemicals viz saponin, tannins, phenols, cellulose, nitrogen, Crude protein, Calcium, Phosphorus, Potassium. The value obtained for various phytochemicals in drug sample are presented in Table 2.

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