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Original Article

Comparative study of in-vitro antimicrobial activity of *Jatropha gossypifolia* l. (Euphorbiaceae) stem and leaf extract

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ABSTRACT

The antibacterial activity of crude chloroform, methanolic and aqueous extracts of *Jatropha gossypifolia* L stem and leaves was investigated using disc agar diffusion against clinical isolates of bacteria consisting of *Bacillus subtilis*, *Escheria coli* and *Pseudomonas putida*. *Jatropha gossypifolia* L well known plant of family Euphorbiaceae is used as a therapeutic agent. Crude powder of stem and leaves screened for antibacterial activity by zone of inhibition. The extracts exhibited broad spectrum antibacterial activities against the microorganisms. The chloroform stem extract exhibited highest zone of inhibition against *Escheria coli* (12±0.07mm) among bacteria. Both methanol and chloroform leaves extract showed better results over the aqueous extract. The methanolic leaves extract exhibited highest zone of inhibition against *Pseudomonas putida* (10.9±0.02mm) and chloroform extract exhibited highest zone of inhibition against bacteria *Escheria coli* (10.8±0.04mm). Activity Index of chloroform crude extracts of stem is maximum (0.73) for *E. coli* and chloroform crude extracts of leaves for *E. coli is maximum* (0.658). The findings in this study provide the basis for further study on the plants with the aim of isolating and identifying the active substances. The plants could also be standardized to develop cheap, culturally acceptable herbal medicines.

Key words: Antibacterial, Inhibition zone, Activity Index, Extract

INTRODUCTION

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients in developing countries [1]. Drugs from higher plants continue to occupy an important niche in modern medicine. Medicinal plants constitute the dominant part of drug substances. There are some 1250 Ayurvedic medicinal plants which go into formulating therapeutic preparation as per Ayurvedic and other traditional systems [2]. If folklore medicinal herbs are added to these the total number of plants with medicinal applications used throughout India will exceed 2000. Therefore, India has often been referred to as the Medicinal Garden of the World. Medicinal plants are good source of natural novel antimicrobial compounds that can be employed in controlling some infections in human and animals [3]. In recent era, more emphasis is placed on evaluation of herbal active principles in the control and treatment of various infectious diseases. Isolation and identification of active compounds from medicinal plants permits synthesis of newer drug with reduced toxicity in body and resistance amongst bacteria [4]. Approximately 62 – 80% of the world's population still relies on traditional medicines for the treatment of common illness [5, 6]. Over 50% of all modern clinical drugs are of natural product origin [7]. And natural products play on important role in drug development programs in the pharmaceutical industry [8]. The use of plants to heal diseases, including infectious one, has been extensively applied by people. *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. 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.

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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. The present study was planned to investigate and assess the antimicrobial activity of methanol and chloroform, water extracts of *Jatropha gossypifolia* leaf and stem against selected microorganisms. The aim of the investigation was also to find out the effective extract content in different solvent system upon bacterial growth compared to the commercial antibiotics. Plant's ethnopharmacological applications are well known, but much of the information is empirical and lacking in scientific validation.

MATERIALS AND METHODS

Collection and identification of plant material: Stem and 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. Stem and fresh leaves of *J. gossypifolia* were harvested and washed with distilled water so as to remove dust and other foreign particles. The Stem and leaves were then left on a clean surface to dry well. The Stem and leaves were air-dried under shade. 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.

Preparation of plant extracts: Fifty grams air-dried and coarsely powdered plant material was kept in Soxhlet extraction unit and exhaustively extracted with 80% methanol at 60° C for twenty four hours and same procedure is applied for chloroform and water. Chloroform and methanol used were of analytical grade. The separated extracted were then filtered through Whatmann No. 1 filter paper and evaporated under reduced pressure using rotary evaporator.

Culture and maintenance of bacterial strains: In vitro antimicrobial activity of various plant extracts was examined using *Escherichia coli*, *Pseudomonas putida* and *Bacillus subtilis* as test organisms. Pure culture of *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922) and *Pseudomonas putida* (ATCC 27853) were obtained from S. M. S. Medical College and Hospital, Jaipur. 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 24 hours of transferring.

Sterilization procedure: In order to avoid any type of contamination and cross contamination by the test organisms the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. UV light was switched on one hour before working in the Laminar Hood. Petridishes and other glass wares were sterilized by autoclaving at a temperature of 121ºC and a pressure of 15 lbs./sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

Preparation of the test plates: The bacterial suspension was immediately transferred to the sterilized petridishes. The petridishes were rotated several times clockwise and anticlockwise to assure homogenous distribution of the test organisms on the media.

Preparation of discs: Three types of discs were used for antimicrobial screening.

Preparation of Sample discs: The sterilized Whatmann No.1 filter paper disc (6 mm) were impregnated with 1mg /ml of extracts dried and placed aseptically on seeded plates with the help of a sterile forceps.

Standard discs: The standard discs (6 mm) impregnated with antibiotics amoxicillin tri-hydrate (1000µg /ml) was used as positive control. Amoxicillin tri-hydrate discs were prepared using the same procedure employed for test samples and used as the reference standard. Standard discs were used as positive control to ensure the activity of standard antibiotic against the test organisms as well as for comparison of the response produced by the known antimicrobial agent with that of the test samples.

Blank discs: Negative controls were prepared using the same solvents employed to dissolve the test samples. The negative controls were used to ensure that the residual solvent (left over the discs even after air-drying) and the filter paper were not active themselves.

Diffusion and incubation: The sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in

the agar plates pre-inoculated with test bacteria. The plates were then inverted and kept in an incubator at 37 ºC for 24 hours.

Determination of antimicrobial activity by the zone of inhibition: The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which give clear zone of inhibitions. After incubation, the Antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale. The experiment was carried out in triplicate and the average zones of inhibition in millimeter were calculated.

Determination of activity index: The activity index of extract was calculated according to Arya *et al.,* 2010[9]

RESULTS

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 crude extract from *Jatropha gossypifolia* stem were tested against the three bacterial cultures *Bacillus subtilis, Escheria coli, Pseudomonas putida.* Among crude solvent extract of stem , chloroform extract of *Jatropha gossypifolia* showed more pronounced antimicrobial activity in comparison to other extracts (Table:1). The chloroform stem extract exhibited highest zone of inhibition against *Escheria coli* (12±0.07mm) (Fig.2). However, none of them were more or equal to the control (Table: 1). The methanolic and aqueous extracts of *Jatropha gossypifolia* were also found to be effective against pathogenic strains tested. This shows that more active ingredient may less dissolve in methanolic and aqueous extract but more dissolve in chloroform extract. It may be because of the stem extract of *Jatropha gossypifolia* was effective in controlling *E.coli* but is less effective against *B. subtilis and P. putida*(Fig 1)(Table:1). The extract from *Jatropha gossypifolia* leaves were tested against the same three bacterial cultures *Bacillus subtilis, Escheria coli, Pseudomonas putida.* In this, both methanol and chloroform extract showed better results over the aqueous extract. The aqueous extract shows zone of inhibition for *Bacillus subtilis, Escheria coli, Pseudomonas putida* this shows that some active ingredient may be dissolve in aqueous extract. The chloroform extract exhibited highest zone of inhibition against bacteria *Escheria coli* (10.8±0.04mm) and methanolic extract exhibited highest zone of inhibition against *Pseudomonas putida* (10.9±0.02mm). *Jatropha gossypifolia* leaves extract showed less activity against *Bacillus subtilis* campare to *Escheria coli, Pseudomonas putida* (Table: 1 and Fig 3). Chloroform, aqueous and methanolic extracts posses the response for all three bacterial cultures (Table 1). Activity Index of chloroform crude extracts of *Jatropha gossypifolia* stem is maximum (0.73) for *E. coli* (Table.2 and Fig. 4) and chloroform crude extracts of *Jatropha gossypifolia* leaves for *E. coli is maximum* (0.658) (Table 2 and Fig. 4).

DISCUSSION

Data from the literature as well as our results reveal the great potential of plants for therapeutic treatment, in spite of the fact that they have not been completely investigated. Once extracted, and before being used in new therapeutic treatments, they should have their toxicity tested *in vivo*. Therefore, our results revealed the importance of plant extracts when associated with antibiotics, to control bacteria, which are becoming a threat to human health. *Jatropha gossypifolia* leaves are used for intermittent fevers, carbuncles, eczema, itches and soreson the tongues of babies, swollen mammae, stomach ache, and veneral disease [10]. Its leaf extract has been used as an anticoagulant for biochemical and haematological analyses [11]. It has been reported that a number of flavonoids possess anti-inflammatory activities [12]. It is used ethnomedically for the treatment of various disease conditions such as cough, tuberculosis; bacterial infections and cancerous growths [13]. The seeds possess drastic purgative, emetic properties and are almost potent in action as those of *Jatropha curcas*. Methanol extracts of bark of the plant are potent antimicrobial and anti-inflammatory agent [14, 15]. However, the stem latex has been shown to possess coagulant activity and its mechanism of action as haemostatic agent found to be by precipitation of coagulant factors [11]. The latex of *Jatropha gossypifolia* is used for ringworm. While the roots, and young shoots are for bronchitis and cough. The most susceptible organism to the methanol extract appeared to be *E. coli* and *S. aureus*. *E. coli* has been reported to be the commonest cause of urinary tract infection and accounts for about 90% of first urinary tract infection in young women [16, 17]. In the ethyl acetate extract, the most susceptible organisms were *Proteus mirabilis* and *vulgaris*. This suggests that methanol extract could be very effective in treating gastrointestinal tract infections, skin infections as well as other food poisoning from *E. coli* and *Staphylococcus* species, while the ethyl acetate extract could be useful in managing urinary tract infections due to *Proteus* bacteria [18]. Our results also show the similarity. The literature, as well as, our results reveal leaves and

stem of *Jatropha 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 for pharmacological activities before its commercialization for the benefit of human beings.

CONCLUSION

The methanolic, chloroform and aqueous extract of *Jatropha gossypifolia* were also found to be effective against pathogenic strains tested therefore It is hoped will create new avenues for exploiting these chemicals by the pharmaceutical industry to develop chemotherapeutic agents.

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TABLE 1: ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACTS IN ORGANIC SOLVENTS OF *JATROPHA GOSSYPIFOLIA* ON THE BASIS OF INHIBITION ZONE (IZ).

Tested strains	Extracts of plant part assayed							
	Stem			Leaf			Control	
Bacteria	Chloro-form	Methanol	Aqueous	Chloro-form	Methanol	Aqueous	PC	NC
B. s.	9.2± 0.01	8.5 ± 0.04	7.3 ± 0.06	7.2 ± 0.01	6.0 ±0.03	6.7± 0.05	15.5 ± 0.02	-
E. c.	12.0± 0.07	9.8 ± 0.02	7.4 ± 0.03	10.8 ±0.04	9.5 ±0.02	6.8±0.03	16.4 ± 0.04	-
P.p.	7.0 ±0.03	8.3±0.05	9.5 ± 0.02	8.0 ±0.06	10.9 ±0.02	8.2±0.01	18.2 ± 0.0	-

Abbreviations: *B. s. = Bacillus subtilis, , E.c.= Escheria coli, P.p. =Pseudomonas putida,* PC =Positive Control (Amoxycillin tri-hydrate) and NC = Negative Control; Diameter of inhibition zone (mm) including the diameter of disc (6mm) values are mean (±SD); IZ= Inhibition zone.

TABLE 2: ACTIVITY INDEX OF CRUDE EXTRACTS IN ORGANIC SOLVENTS OF *JATROPHA GOSSYPIFOLIA* ON THE BASIS OF INHIBITION ZONE (IZ).

Tested strains	plant part assayed					
	Stem			Leaf		
Bacteria	Chloroform	Methanol	Aqueous	Chloroform	Methanol	Aqueous
B. s.	0.59	0.548	0.47	0.46	0.387	0.43
E. c.	0.73	0.597	0.45	0.658	0.58	0.41
P. p.	0.38	0.46	0.52	0.44	0.59	0.45

Abbreviations: *B. s. = Bacillus subtilis, E.c. = Escheria coli, P.p. =Pseudomonas putida*

Figure: Effect of *Jatropha gossypifolia* extracts on bacterial strains: *Pseudomonas putida, Escheria coli, Bacillus subtilis*

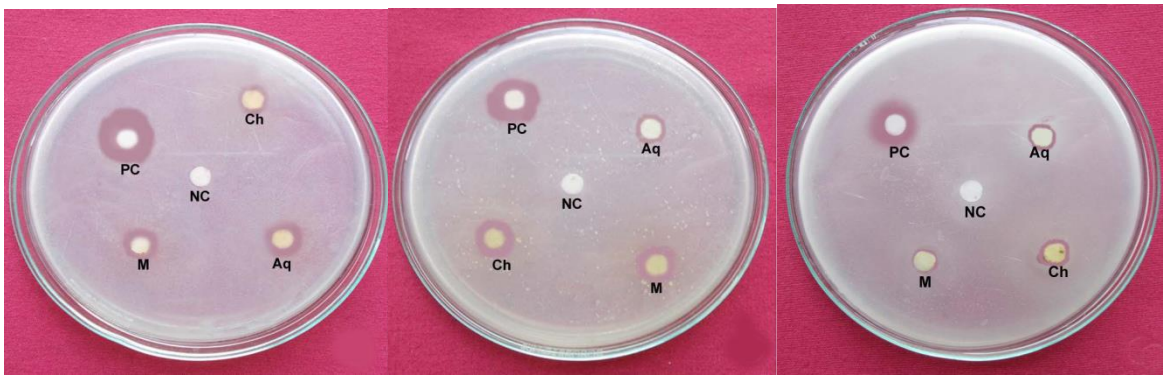

Figure: 1 **Figure: 2** **Figure: 3**

Figure1: **Effect of *Jatropha gossypifolia* stem extracts on *Pseudomonas putida***
Figure 2: Effect of *Jatropha gossypifolia* stem extracts on *Escheria coli*
Figure 3: Effect of *Jatropha gossypifolia* leaves extracts on *Bacillus subtilis*
Abbreviation: Ch =Chloroform extract, M= Methanol extract, Aq = Aqueous extract, PC = Positive Control (Amoxycillin tri-hydrate), NC = Negative Control

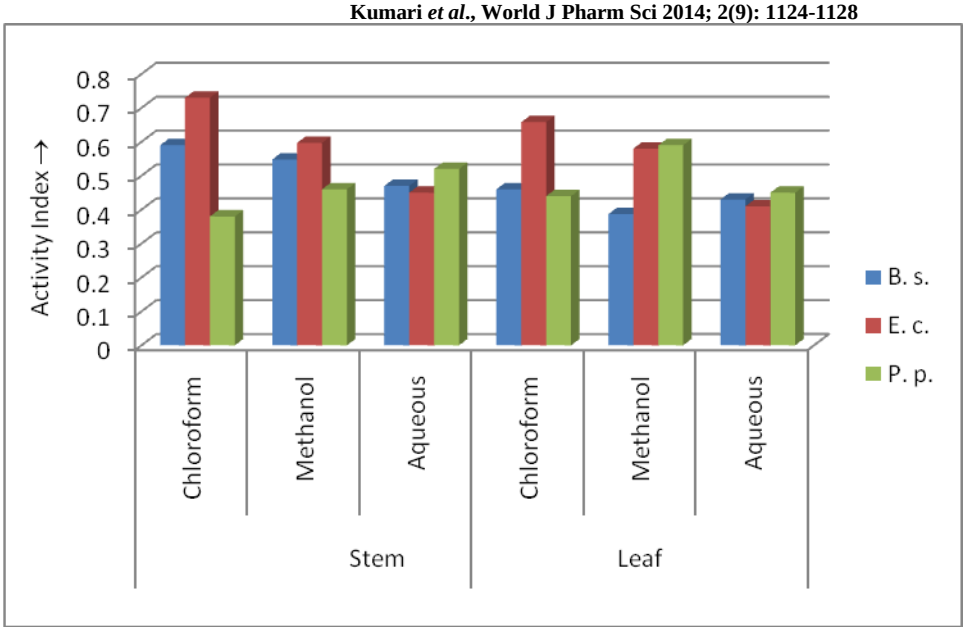

Fig: 4 Activity Index of crude extracts in organic solvents of *Jatropha gossypifolia* on the basis of inhibition zone (IZ)

Abbreviations: *B. s. = Bacillus subtilis, E.c. = Escheria coli, P.p. =Pseudomonas putida*

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