Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 7 [4] April 2018 : 01-06

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Phytochemical Screening And Antimicrobial Activities of *Jatropha Curcas* L. (Euphorbiaceae) Leaves Extract

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ABSTRACT
Plants are considered to be the oldest source of pharmacologically active compounds and have provided humankind with many medically useful compounds. To evaluate the phytochemical and antimicrobial activities of different extracts of Jatropha curcas L. leaves, the experiment were designed. Determination of the presence of phytochemicals in the crude plants extracts such as methanol, chloroform and water extract by standard methods. Disc diffusion method was used to detect the antimicrobial sensitivity and for activity index. The results revealed the presence of alkaloids, flavonoid, cardiac glycosides, saponins, steroids and tannin. Antibacterial activity of Jatropha curcas showed varied degree of zone of inhibition against the tested bacterial pathogens. Methanolic leaves extract showed maximum effect on Escheria coli (11±0.06mm) whereas chloroform leaf extract showed the maximum effect on P. putida (10.0±0.04mm). Methanolic leaf extract gave highest activity index (0.67) on Escheria coli while on the same organism chloroform extract gave nearly least. The inhibitory effect of the extract of Jatropha curcas against pathogenic bacterial strains can introduce the plant as a potential candidate for drug development. The role of Jatropha curcas in medicinal uses should be taken into consideration as it shows promising future in the pharmaceutical field.
***KEY WORDS**: Jatropha curcas, Antibacterial, Phytochemical. Inhibition zone, Activity Index*

Received 12.02.2018 Revised 02.04.2018 Accepted 26.04.2018

INTRODUCTION

Plants derivatives have made a large contribution to human health as they have been used as source of preliminary compound of drugs. Widespread usages of drugs have led to the development of pathogen resistance, hence, urging research of new drugs for the treatment of diseases. The plant extracts and their products are used in many parts of the world as the active principles in herbal remedies and locally in the treatment of infectious diseases [1]. The use of plants to heal diseases, including infectious one, has been extensively applied by people. Active compound present in the medicinal plants provide the bountiful resource of the pharmaceutical, cosmetics and food industries, and recently in agriculture for pest control [2].

Antimicrobial agents are substances that kill microorganisms or inhibit growth of the microorganisms. They are widely employed to cure bacterial diseases. Antimicrobial agents disrupt microbial processes or structures that differ from those of the host. They may damage pathogens by hampering cell wall synthesis, inhibiting microbial protein and nucleic acid synthesis, disrupting microbial membrane structure and function, or blocking metabolic pathways through inhibition of key enzymes [3, 4, 5].

"*Jatropha*", derived from the Greek words *"jatros"* [meaning: doctor] and *"trophe"* [meaning: food], is a plant which is well known for its folklore use. It is also known as physic nut (family Euphorbiaceae), classified as a large shrub or a small perennial tree able to reach a height between three and ten meters [6]. This plant is widespread in tropical and subtropical regions of Southeast Africa, Central and Latin America, Asia and India. *Jatropha curcas* L. is a species that is able to grow in dry and hot conditions, where many species do not survive [7, 8]. It has played a major role in the treatment of various diseases including bacterial and fungal infections. Apart from their traditional uses, *Jatropha* gained importance from the view point of biotechnology in recent times [9].

Data from the literature reveal the great potential of plants for therapeutic treatment, in spite of the fact that they have not been completely investigated. Therefore, more studies need to be conducted to identify

their uses against the wide range of pathogenic microorganisms as well as search for new compounds. The objectives of this study were to investigate the effectiveness of *Jatropha curcas* plant against some selected human pathogens which are known to cause diseases and to compare the extent of antimicrobial properties of the different extracts of *Jatropha curcas*, hence determining the most active extract of the plant.

MATERIALS AND METHODS:

The study was carried out at Department of Botany, University of Rajasthan, Jaipur, Rajasthan (India) in the year 2014.

Collection and identification of plant material

Leaves of *Jatropha curcas* L. (family Euphorbiaceae) were collected from University of Rajasthan campus Jaipur, Rajasthan, India. A Voucher specimen of the plant has been deposited as RUBL211369 (*Jatropha curcas*) in the Herbarium, Department of Botany, University of Rajasthan for further references. Leaves of *Jatropha curcas* were harvested and washed with distilled water so as to remove dust and other foreign particles then left on a clean surface to dry well. Then air-dried under shade. After this, 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 Whatman's No. 1 filter paper and evaporated under reduced pressure using rotary evaporator.

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 [10-13]. 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.

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 Watmann 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[14]

$$\text{Activity Index} = \frac{\text{Mean of the inhibition zone (mm) of the extract}}{\text{Inhibition zone (mm) of the standard antibiotic drug}}$$

RESULT

Preliminary phytochemical screening was performed as per standardized procedure of the various phytoconstituents in methanol, chloroform and water extract. The leaves extract of *Jatropha curcas* in chloroform exhibited the presence of alkaloides, cardiac glycosides and steroids. Methanolic extract exhibited the presence of all the secondary metabolites tested but water extract showed the presence of alkaloids, saponine and tannins (Table. 1).

Jatropha curcas methanolic leaves extract showed maximum effect on *Escheria coli* (11±0.06mm) (Fig. B) followed by *P. putida* (8.5±0.02mm) (Fig. C) whereas chloroform leaf extract showed the maximum effect on *P. putida* (10.0±0.04mm) (Fig. C) followed by *E. coli* (7.78 ±0.03mm) (Table 2) (Fig. B). Both the extract showed minimum reactivity against *Bacillus subtilis* (Table. 2) (Fig. B).

Methanolic leaf extract gave highest activity index (0.67) on *Escheria coli* while on the same organism chloroform extract gave nearly least (Table 3). Here comes the role of active ingredient that is extracted in different solvents.

Table 1: Preliminary Phytochemical analysis of *Jatropha curcus* L. (Leaves) with different solvents

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

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

Table 2: Antimicrobial activity of crude extracts in organic solvents of *Jatropha curcas* L on the basis of inhibition zone (IZ).

Tested strains	Extracts of plant part assayed				
	Leaves				
Bacteria	Chloroform	Methanol	Aqueous	PC	NC
B. s.	7.1± 0.02	7.2 ± 0.01	0± 0	15.5 ± 0.02	0± 0
E. c.	7.78±0.03	11.0± 0.06	0 ± 0	16.4 ± 0.04	0± 0
P. p.	10.0 ±0.04	8.5±0.02	10.0± 0.0	18.2 ± 0.0	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 (mm).*

Table 3: Activity Index of crude extracts in organic solvents of *Jatropha curcus* on the basis of inhibition zone (IZ).

Tested strains	Plant part assayed		
	Leaves		
Bacteria	Chloroform	Methanol	Aqueous
B. s.	0.46	0.47	0.0
E. c.	0.469	0.67	0.0
P. p.	0.55	0.47	0.55

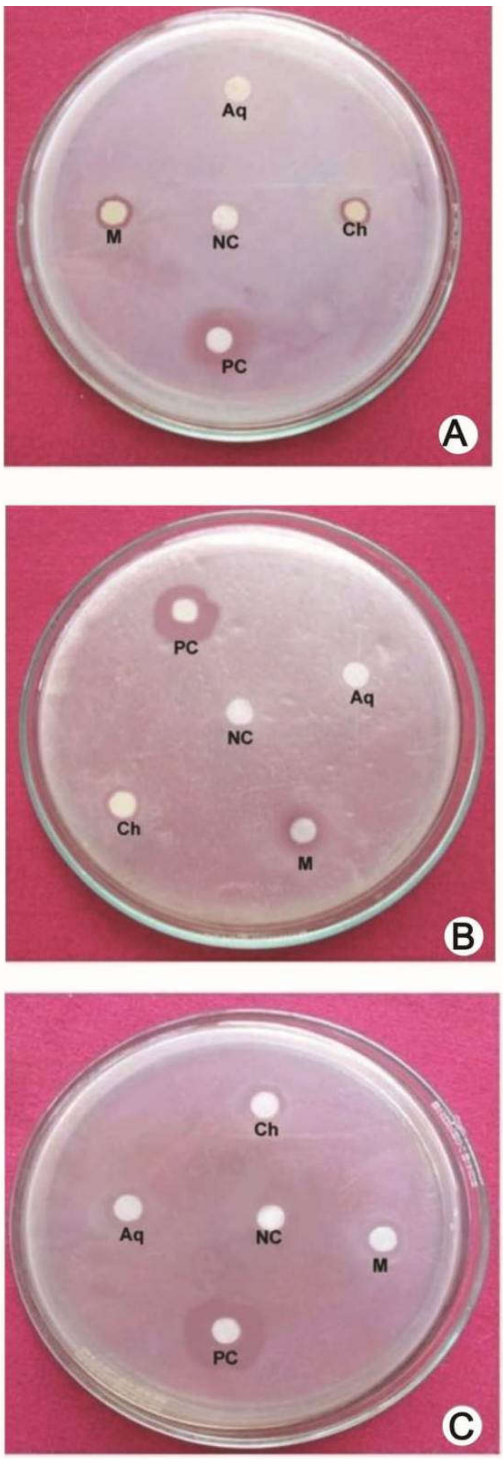

Figure A: **Effect of *Jatropha curcus* leaves extracts on *Bacillus subtilis***
Figure B: Effect of *Jatropha curcus* leaves extracts on *Escheria coli*
Figure C: Effect of *Jatropha curcus* leaves extracts on *Pseudomonas putida*
Abbreviations:
Ch = Chloroform extract, M=Methanol extract, Aq = Aqueous extract,
PC= Positive Control (Amoxycillin tri-hydrate), *NC = Negative* Control

DISCUSSION

Jatropha curcas is a versatile plant having potential uses in the medicinal field. These medicinal uses of different plant had been intensively investigated and studied by several researchers. The plant contains mixtures of different chemical compounds that may act individually, additively or in synergy. Solvents used for extraction played an important role in the extraction of the phytochemicals. This could be due to

the higher presence of biologically active metabolites. Literature data are available on the composition and biomedical applications of *Jatropha curcas* L. leaves and the identified compounds include cyclic triterpenes, alkaloids, and flavonoids [15]. Steroids present in *Jatropha curcas* leaves extract are of great importance and interest due to their relationship with various anabolic hormones including sex hormones. Steroids extract from some medicinal plants exhibits antibacterial activities on some bacterial isolates [16].Steroids have an anti-inflammatory effect [17, 18]. The results of our study confirm the steroids presence in the chloroform and methanolic extracts of *Jatropha curcas.*

The leaves were used as remedy for malaria, rheumatic, and muscular pains [19, 20]. In vivo studies on antihyperglycemic activity of methanolic extract of leaves of *Jatropha curcas*. L were also reported [21]. The most susceptible organism to the methanol extract appeared to be *E. coli* and *S. aureus*. 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 [22]. *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 [23, 24]. The results of our study are similar to those of Akinpelu *et al.,* (2009) who reported strong inhibitory activity against *S. aureus* and *E. coli* in the methanolic extract of the leaves of *Jatropha curcas* [25].

Plant phenolics constitute one of major groups of compounds acting as primary antioxidant or free radical terminators [26-30]. Therefore, our results revealed the importance of plant extracts when associated with antibiotics, to control resistant bacteria, which are becoming a threat to human health. Furthermore, in a few cases, these plant extracts were active against antibiotic resistant bacteria under very low concentration, thus minimizing the possible toxic effects.

CONCLUSION:

The role of *Jatropha curcas* in medicinal uses should be taken into consideration as it shows promising future in the pharmaceutical field. The economics of making and marketing for these products should be further explored and encouraged.

ACKNOWLEDGEMENTS

The authors are thankful to Head, Department of Botany, University of Rajasthan, Jaipur for providing necessary facilities to carry out the study. I am not getting any fellowship or financial aid. Grateful acknowledgement is to the supervisor for their sincere suggestions and moral support during research work.

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CITATION OF THE ARTICLE

V Kumari, S Gupta, N Kumari, A Shrivastav and K K Verma. Phytochemical Screening And Antimicrobial Activities of *Jatropha Curcas* L. (Euphorbiaceae) Leaves Extract. Bull. Env. Pharmacol. Life Sci., Vol 7 [5] April 2018 : 01-06