



Evaluation of antibacterial activity of *Jatropha curcus* seed oil

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ABSTRACT

This study investigated the antibacterial activities of hexane extracts of *Jatropha curcus* essential oil on the microorganisms *Staphylococcus aureus*, *E.coli*, *Klebsiella*, *Streptomyces griseus* and *Serratia marcescens* by agar diffusion method. Phytochemical screening of the extracts was also determined. Phytochemical screening revealed the presence of steroids, tannins, flavonoids, alkaloids, while phenol and reducing sugars were absent. The hexane extracts of *J. curcus* seed oil showed highest inhibition of 33 mm against *Staphylococcus aureus*, *Klebsiella pneumonia*, followed by *Escherichia coli* (31 mm), *Streptomyces griseus* (22 mm) and the least inhibition was recorded in *Serratia marcescens* (18mm). No inhibition was observed in the negative control. The results show the seed oil extract of *J. curcus* can be effectively used as potential candidate for controlling microbes and can be considered eco-friendly.

Keywords: *Jatropha curcus*, *Staphylococcus aureus*, *E. coli*, *Klebsiella*, *Streptomyces griseus* and *Serratia marcescens*

INTRODUCTION

Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in developing countries. The intractable problems of antimicrobial resistance have led to the resurgence of interest in herbal products as sources of noble compound to suppress or possibly eradicate the ever-increasing problems of emergence of newer diseases though to be brought under control (Wurochekker *et al.* 2008). Also, the search for new antibacterial drugs of natural origin is urgently needed in the light of growing cases of microbial resistance to the available synthetic antibiotics (Kalimuthu *et al.* 2010; Krishnaiah *et al.* 2009). Priyanka and Mahesh (2014) investigated the potentiality of *Aegle marmelos* as an effective antimicrobial agent. Plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Their role is two fold in the development of new drugs. They may become the base for the development of a medicine, a natural blue print for the development of new drugs or a phytomedicine to be used for the treatment of diseases.

Jatrophas species belong to the family Euphorbiaceae and are used in traditional medicine to cure ailments in Africa, Asia and Latin America (Burkill, 1994). *Jatrophas curcas* is commonly called physic nut, purging nut or pig nut. Previous studies have reported that the plant exhibits bioactive activities for fever, mouth infections, jaundice, guinea worm, sores and joint rheumatism (Oliver-Bever, 2000). Aiyelaagbe (2001) reported the anti-parasitic activity of the sap and crushed leaves of *J.curcas*. The water extract of the branches also strongly inhibited HIV induced cytopathic effects with low cytotoxicity. Previous works have shown that many Jatrophas species possess antimicrobial activities (Aiyelaagbe, 2001).

This tropical plant has received extensive attention of many scientists in view of its great economic importance, medicinal significance and for its seed oil as commercial source of fuel (Grimm, 1996; Heller, 1996; Henning, 2000). *J. curcas* is widely used in traditional medicine in many countries to cure various ailments such as skin infections, diarrhea, gonorrhoea, fever and several other diseases caused by microorganisms (Burkill, 1994; Kambu, 2012). Previous studies have reported that *J. curcas* exhibits antimicrobial activity (Aiyelaagbe *et al.* 2007; Akinpelu *et al.* 2009).

Previous works have shown that many Jatropha species possess antimicrobial activity (Aiyelaagbe *et al.* 2000; Aiyelaagbe, 2001). Several studies have confirmed the antimicrobial efficacy of different Jatropha species; however, there is insufficient information regarding the antimicrobial activities of *J. curcas* Linn. Kaushiki and Mahesh (2013) investigated and reported the larvicidal activity of Jatropha seed oil against *Aedes aegypti*.

The search for new antibacterial drugs of natural origin is urgently needed in the light of growing cases of microbial resistance to the available synthetic antibiotics (Iwu *et al.* 1999; Wurochekker *et al.* 2008; Krishnaiah *et al.* 2009).

Much of the previous work on *J. curcus* as a medicinal plant has focused on the leaf extracts. Limited information is available regarding antimicrobial activity of *J. curcus* oil; therefore, present study was carried out to investigate antimicrobial activity of essential of *J.curcus* against various pathogenic bacterial species. Preliminary photochemical studies of these extracts are also undertaken to find out bioactive compounds having antimicrobial activity of essential activity of *J. curcus* as

part of the exploration for new and novel bio-active compounds. In this paper, the antibacterial potency of hexane oil extract of the seed of *J. curcus* was investigated.

METHODOLOGY

Seed collection and Oil extraction

The seeds of *Jatropha curcas* were collected from GKVK, Bangalore. The seeds were ground, using a mechanical grinder, and defatted in a soxhlet apparatus, using hexane (at 20–30°C). The extracted lipid was obtained by filtrating the solvent lipid contained to get rid of the solid from solvent before the hexane was removed using distillation apparatus at 40°C. Extracted seed oil was stored in freezer at 4 °C for subsequent physicochemical analysis.

Phytochemical analysis

The extracts were subjected to qualitative phytochemical tests for plant secondary metabolites; alkaloids, flavonoids, saponins, tannins, steroid and reducing sugars according to the method described by Harborne (1998).

Test Microorganisms

The test microorganisms used in this study were *Staphylococcus aureus*, *E.coli*, *Klebsiella*, *Streptomyces griseus* and *Serratia*. The organisms were obtained from Microbiology Laboratory, Maharani's college of science Bangalore.

Antimicrobial susceptibility Testing

This was done using the agar diffusion method of Boakye-yiadon (1979). The test organisms were separately inoculated on nutrient agar plates for *Staphylococcus aureus*, *E.coli*, *Klebsiella*, *Streptomyces griseus* and *Serratia* and spread uniformly using spread plate method. Holes of 4mm diameter were punctured on the agar medium using a sterile cork-borer and cut agar discs were aseptically and carefully removed with sterile forceps. A sterile Pasteur pipette was used to introduce different concentration of the *Jatropha curcas* seed extract into the wells or holes bored on the surface of the agar medium containing the cultures. Using a micropipette, 25µl of each extracts were added onto each well on all plates. 25µl of Streptomycin solution (1µg/ml) was used as positive control and water was used as negative control. The plates were allowed to stand for one hour at room temperature to allow for

diffusion of the substrates to proceed before the growth of the organisms commenced. The plates were finally incubated at 24 hours for 37°C. The presence of zone of inhibition around the hole containing the extracts indicates the antimicrobial activity against the test organisms. Antimicrobial activity was expressed in terms of diameter of zones of inhibition (mm).

RESULTS AND DISCUSSION

Phytochemical analysis

The qualitative phytochemical analysis indicated the presence of active constituents in seed oil extract. The qualitative estimation of the phytochemicals conducted on *J. curcas* extracts revealed the presence of alkaloids, saponins, flavonoids, tannins and steroid (Table 1). The active principles of many drugs found in plant are secondary metabolites. These compounds are known to be biologically active and therefore aid the larvicidal activity of *J. curcas* (Kaushiki and Mahesh, 2013). Fortunately, these compounds exert antibacterial activity through different mechanisms (Rabe, 2000)

Table: 1 Phytochemical analysis of Hexane extract of *Jatropha curcas* seed

SN	Phytochemicals	Present /Absence
1	Tannins	+
2	Saponins	+
3	Phenols	-
4	Alkaloids	+
5	Flavonoids	+
6	Steroids	+
7	Reducing sugars	-

The presence of various compounds viz., saponins, steroids, alkaloids, phenolic groups and flavonoids in *J. curcas* is known to be biologically active and therefore aid the antimicrobial activities of *J. curcas* (Fasola and Egunyomi, 2005). 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 (Haslam *et al.* 1989).

Tannins have been found to form irreversible complexes with proline rich protein (Shimada, 2006) resulting in the inhibition of cell protein synthesis. Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of

inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003). These observations therefore support the use of *J. curcas* in herbal cure remedies. Li and Wang (2003) reviewed the bio-logical activities of tannins and observed that tannins have anticancer activity and can be used in cancer prevention, thus suggesting that *J. curcas* has potential as a source of important bioactive molecules for the treatment and prevention of cancer. The presence of tannins in *J. curcas* supports the traditional medicinal use of this plant in the treatment of different ailments.

Saponin was found to be present in *J. curcas* extracts and has supported the usefulness of this plant in managing inflammation. This finding was in agreement with previous works of El Diwani *et al.* 2009, who reported the presence of saponins in *Jatropha curcas* leaf. Different parts of *J. curcas* contain the toxic alkaloids curcin and phorbol ester which prevent animals from feeding on it. Just *et al.* (1998) revealed the inhibitory effect of saponins on inflamed cells. Saponin was found to be present in *J. curcas* extracts and has supported the usefulness of this plant in managing inflammation. Steroidal compounds present in *J. curcas* extracts are of importance and interest due to their relationship with various anabolic hormones including sex hormones (Okwu, 2001). Quinlan *et al.* (2000) worked on steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates. Hence, the presence of these compounds in *J. curcas* supports the observed antimicrobial activities.

Another secondary metabolite compound observed in the extract of *J. curcas* was alkaloid. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines (Nobori *et al.* 1994). Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications (Kam and Liew, 2002). The results of the present study confirm the presence of alkaloids in *J. curcas*. Igbinsosa *et al.* (2009) and Akinpelu *et al.* (2009) observed the presence of alkaloids in *J. curcas* stem bark and leaves extracts respectively. These compounds have been

associated with medicinal uses for centuries and were reported as the most efficient, therapeutically significant plant substance and exert antibacterial activity through different mechanisms (Njoku and Akumefula, 2007; Rabe and Shimada, 2000).

Flavonoids, another constituent of *J. curcas* extracts exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties (Hodek *et al.* 2002). The results of the present study confirm the flavonoids presence in the *J. curcas*. Different parts of *J. curcas* contain the toxic alkaloids curcin and phorbol ester which prevent animals from feeding on it. Hence, the presence of these compounds in *J. curcas* corroborates the antimicrobial activities observed. It is concluded that *J. curcas* stem bark could be a potential source of active antimicrobial agents, and a detailed assessment of its in vivo potencies and toxicological profile is ongoing.

Steroidal compounds present in *J. curcas* extracts are of importance and interest due to their relationship with various anabolic hormones including sex hormones (Okwu, 2001). Quinlan *et al.* (2000) worked on steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates. Neumann *et al.* (2004) also confirmed the antiviral property of steroids. The results of the present study confirm the steroids presence in the *J. curcas*.

Different parts of *J. curcas* contain the toxic alkaloids which prevent animals from feeding on it. Hence, the presence of these compounds in *J. curcas* corroborates the larvicidal activities observed. It is concluded that *J. curcas* could be a potential source of active Larvicidal agents, and a detailed assessment of its in vivo potencies and toxicological profile is ongoing.

Using qualitative analysis, Igbinsosa *et al.* (2009) and Akinpelu *et al.* (2009) observed the presence of the same compounds in *J. curcas* extracts respectively.

These phytochemicals are known to support bioactive activities in medicinal plant and therefore aid the antimicrobial activity of *J. curcas*. These compounds have been associated with medicinal uses for centuries and were reported as the most efficient, therapeutically significant plant substance (Nobori *et al.* 1994).

Antimicrobial activity

The extract of the seed tested showed varying degree of antibacterial activities against the test bacterial species (Table 2). The antibacterial activities of the hexane extracts compared favorably with standard antibiotics (streptomycin) and have appeared to be broad spectrum as its activities were independent on gram reaction.

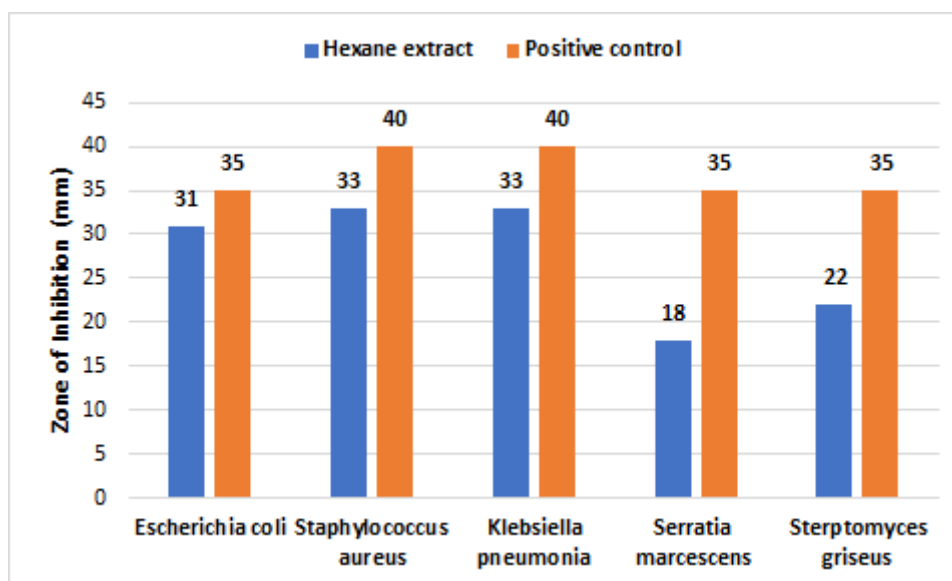
The screening of the extract of *Jatropha curcas* showed highest inhibition of 33 mm against *Klebsiella pneumonia* and *Staphylococcus aureus*(33mm) followed by *Escherichia coli* (31 mm), *Sterptomyces griseus* (22 mm) and the least inhibition was recorded in *Serratia marcescens* (18mm) (Table 2, Graph 1).

The hexane extracts of *J. curcas* compared favourably with the standard antibiotics (Streptomycin) on all the pathogenic bacteria except *Serratia marcescens* (18mm) and *Sterptomyces griseus* (22mm). The results also showed that the hexane extract of *J. curcas* seed oil had similar antibacterial activity compared to its positive control i.e. streptomycin (Table 2, Graph1). This could be as a result of its relatively high percentage extract recovery. This is in agreement with the findings of Srinivasan *et al.* (2001), who reported that different solvents have different extraction capacities and different spectrum of solubility for the phyto-constituents which are known to be biologically active. The inhibitory activity of plant extract is also largely dependent on the concentration, parts of the plant used and the microbes tested Kalimuthu *et al.* (2010). Although it has been stated that aqueous extracts of plant generally showed little or no antibacterial activities (Koduru *et al.* 2006; Nyembo *et al.* 2012), the results of the present investigation proved otherwise.

Table: 2 Antibacterial activities profile of hexane extract of oil of *J. curcas*

Name of organism	Hexane extract(mm)	Positive control
<i>Escherichia coli</i>	31	35
<i>Staphylococcus aureus</i>	33	40
<i>Klebsiella pneumonia</i>	33	40
<i>Serratia marcescens</i>	18	35
<i>Sterptomyces griseus</i>	22	35

*Zone of inhibition measured in millimeters (mm)



Graph 1: Graphical representation of antimicrobial activity of *Jatropha curcas* against selected pathogens

The results suggests that hexane extract could be very effective in treating gastrointestinal tract infections, skin infections as well as other food poisoning from *E. coli* and *Staphylococcus* species. *S. aureus* is a pyogenic bacterium known to play significant role in invasive skin diseases including superficial and deep follicular lesions and other nosocomial infections (Reuben *et al.*, 2008; Adamu *et al.* 2009). *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 (Usman *et al.* 2007). The susceptibility of *E.coli* to hexane extract of *Jatropha* seed oil strengthens the suggestion that it could be useful for treating gastroenteritis, food poisoning and urinary tract infections. The hexane extract also showed activity against *Klebsiella pneumonia*, suggesting its usefulness in respiratory tract infection. The hexane extract was generally medium in activity relative to the methanol. Hence it may not be the best extraction solvent for anti-infective preparations (Kubmarawa *et al.* 2007)

The results presented above indicated that seed extract from *Jatropha curcas* exhibited antimicrobial properties, thus justifying scientifically their traditional used as medicinal plants. The study suggested that the extract contain phytochemicals such as steroids, flavonoids etc which are active against those strains of organisms tested as earlier reported by Sofowora (1993) that plants traditionally claimed useful in treating wounds should show antimicrobial activities.

This suggests that the plant activity may reside in the averagely polar to polar compounds like the phenols, polyphenols, and other fairly polar compounds like flavonoids (apigenin and vitexin), alkaloids (jatrophine or jatropham) (Fig. 1), etc. Where the level of the toxic phorbol esters are considered high and not fit for consumption, formulations may be used externally for fungi and bacteria skin infections. Therefore, the antimicrobial action of the plant used was not surprising. In this study, antibacterial test of the seed extract of *Jatropha curcas* showed that the plant exhibited broad spectrum of activities by inhibiting the growth of five bacteria used (Table 2, Graph1).

DISCUSSION

Phytochemicals confer pharmacological activities on natural products derived from plants. Many drugs in common use in modern medicine today were isolated and purified from plants. These natural products have been found to possess a range of activities, which include antimicrobial and antioxidant (Olajire and Azeez, 2011; Upadhyay, 2011). This study, thus investigates the phytochemical composition and antimicrobial activities of the aqueous extract of *Jatropha curcas* Linn leaves.

Most parts of *J. curcas* plant is reported to be toxic (Kumar and Sharma, 2008), probably explaining the antimicrobial activity shown in the present study. Hexane seed extract of *J. curcas* contains flavonoids,

phenols, tannins and alkaloids (Table 1). This agrees with reports by Oseni and Alphonse (2011) and Oskoueian *et al.* (2011). Flavonoids are secondary metabolites, which are the most common group of polyphenolic compounds that are found ubiquitously in plants (Upadhyay, 2011). They have been reported to exhibit activity against gram-positive bacteria (Meyer *et al.* 1997) and Streptococcus mutants (He *et al.* 2006).

Phenols or phenolics are widely distributed organic compounds in the plant kingdom, which help in defence against predators and pathogens; they have been reported to be active against a wide range of organisms (Upadhyay, 2011). Tannins are polyphenolic compounds used for tanning or colouring leather (Gajendiran and Mahadevan, 1990) and possess sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecules. Tannins have been reported to possess activity against *A. naeslundii*s and Methicillin Resistant *S. aureus* (MRSA) as well as gram-negative rods, *Klebsiella* spp., *Escherichia coli* and *Enterobacter* spp. (Min *et al.* 2008). Alkaloids are heterocyclic secondary plant metabolites containing basic nitrogen atoms and act as local anaesthetic and stimulant. Common examples are caffeine, nicotine, morphine and the antimalarial drug quinine (Upadhyay, 2011).

However, most of the toxic components are contained in the seeds and include saponins, lectins (curcin), phytates, protease inhibitors, curcalonic acid and phorbol esters (Kumar and Sharma, 2008). Alkaloid present in the seed of *Jatropha* such as phorbol esters, particularly. Previous studies have reported the antimicrobial activity and medicinal importance of *Jatropha curcas* plant parts (Igbiosa *et al.* 2009; Sharma *et al.* 2010; Arekemase, 2011; Narayani *et al.* 2012; Oloyede *et al.* 2012; Rachana *et al.* 2012; Omoregie and Folashade, 2013). Its antimicrobial activity has been attributed to the presence of certain phytochemicals which include saponins, tannins, alkaloids and glycosides (Arekemase, 2011). The aqueous extract of *Jatropha curcas* leaf inhibited the growth of most test organisms, with the exception of *Candida* sp.

Earlier, Arekemase (2011) reported that the latex of *J. curcas* inhibited the growth of *Candida albicans* at higher concentration of the latex, while the aqueous extract of *J. curcas* roots failed to inhibit the growth of *Candida albicans* at concentrations tested. He further reported that the aqueous root extract of *J. curcas* exhibited

antimicrobial activity against a number of microbes including, *Neisseria gonorrhoea*, *Escherichia coli*, *Staph. aureus*, *Pseudomonas aeruginosa* and *Aspergillus flavus*. Similarly, Igbiosa *et al.* (2009) reported the antimicrobial activity of stem bark aqueous extract of *J. curcas* against a wide range of bacterial isolates excluding *Klebsiella pneumonia*. In another study by Narayani *et al.* (2012), no antimicrobial activity against *E. coli*, *Proteus* sp., *S. aureus* and *P. aeruginosa* was observed from aqueous leaf extract of *J. curcas*. However, Oloyede *et al.* (2012) reported that aqueous extracts of *J. curcas* (up to a concentration of 500mg/ml) showed antimicrobial activity against *K. pneumonia*, *E. coli* and *P. aeruginosa*.

The disparities in the different reports may be attributable to differences in extract preparation and concentrations, and as well as strain differences. Microbial antibiotics sensitivity patterns have been reported to be strain-dependent within a given species (Kwon and Lu, 2007). For example, certain strains of *Staphylococcus aureus* are resistant to methicillin (Methicillin-resistant *Staphylococcus aureus*, MRSA), whereas some are not.

The hexane extract of *Jatropha* oil exhibited antimicrobial activity against organisms that were resistant to standard antibiotics like streptomycin (Table 2). The Hexane extract was active against the Gram negative bacteria used while the Gram-positive bacterium studied (*Staphylococcus aureus*) was resistant to the extract. This study has substantiated earlier reports of the presence of phytochemicals and the antimicrobial potentials of hexane solvent extract of *Jatropha curcas* against pathogenic bacteria have also been demonstrated. In conclusion, our findings showed that the plant *J. curcas* exhibits antibiotic activity against the important microbes. These results could encourage the search for new active natural compounds offering an alternative to synthetic antibiotic from other medicinal plants. It is concluded that *J. curcas* seed oil could be a potential source of active antimicrobial agents. However, there is need to conduct toxicological assessment of the bark and leaves to ascertain their safety on human.

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