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Research Article

Comparative Studies on the Phytochemistry, Antimicrobial and Anti-Oxidant Activities of Jatropha Species (*J. curcas* L. and *J. gossypifolia* L.) of Odisha

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ABSTRACT

In the recent years Jatropha plant has gained a lot of importance due to its property to produce biodiesel. Another reason is the medicinal properties of some species of Jatropha genus. Both the *J. curcas* and *J. gossypifolia* species having antimicrobial and anti-oxidant activities. The latex, leaf, bark and fruits contain several saponins, alkaloids, tannins, phytosterols, flavonoids and steroids that exhibit wide range of medicinal properties. Commercial exploration for biopharmaceuticals, antimicrobial activity and bioenergy production are some of the prospective future potential of these plants. But the question arises, out of two species of *Jatropha* which one has more efficiency against some skin diseases producing microorganism and anti-oxidant activity. The aim of this study was extraction from leaf of two plant species such as methanolic and petroleum ether extracts and comparative investigation of phytochemical compounds, antimicrobial and anti-oxidant activities. The phytochemical constituent of the dried powdered in organic (methanolic) solvent was carried out. First we studied the phytochemical screening by which we got number of secondary metabolites like flavonoids, glycosides, alkaloids, tannins. After that the antimicrobial activity of the extracts was evaluated and its inhibition was studied by disc diffusion method. The result indicated that out of four extracts of both species petroleum ether extract of *J. gossypifolia* showed more potential against micro-organisms and free radicals than *J. curcas*.

Keywords: *Jatropha curcas*; *Jatropha gossypifolia*; Extract; Microorganisms; Phytochemical; TLC; Antimicrobial and Anti-oxidant.

INTRODUCTION

Antimicrobial agents are substances that kill microorganisms or inhibit their growth. They are widely employed to cure bacterial diseases. In Angiosperm group Euphorbiaceae is one of the largest family, covers about 300 genera containing approximately 7800 species and 5 subfamilies worldwide[1]. All the species are mostly occurring in tropical and subtropical regions. Out of different genera belonging to this family, Jatropha L. belongs to the subfamily Crotonoideae, Jatropha tribe and is contained about 200 species[1]. Genus *Jatropha* with 172 species having significant antimicrobial, antioxidant, and anticancer and pesticidal activities. Out of these *J. curcas* and *J. gossypifolia* are found abundantly in India. *J. curcas* has played a major role in the treatment of various diseases including bacterial and fungal infection. Several species from genus Jatropha such as *Jatropha curcas, Jatropha gossypifolia, and Jatropha elliptica* have been reported for their medicinal uses, biological activities and chemical constituents among others[2]. Antimicrobial agents that reversibly inhibit growth of bacteria called bacteriostatic where as those with irreversible action on bacteria are known as bactericidal[3]. Earlier studies have been done the efficacy of Jatropha species against wide range of different microorganisms[4]. Plant extracts and their products are used in many parts of the world as the active component in herbal medicines[5]. They are used locally in the treatment of infection; many centuries before scientific studies were discovered, there are thousands of species of medicinal plants used globally for the cure of different infections. These plants are used as antimicrobial agents and several works have been carried out by scientists to find out its scientific basis[6]. A great number of antibacterial agents exist for various purposes; some of these are usually in the form of plants. The action of these plants on microorganisms have been found to be due to the presence of certain substances such as alkaloids, glycosides, volatile oils, gums, tannins, steroids, saponins, flavonoids and a host of other chemical compounds referred to as secondary metabolites present in them[7,8]. Several studies have also revealed that all parts of *J. curcas* are used in traditional medicine; including the treatment of mouth disease and wounds[9]. Moreover, many studies have been done to demonstrate that *J. curcas* has anticancer and antitumor properties also. The phytochemical studies of *J. curcas* also have demonstrated the presence of secondary metabolites. The leaf extract of *J. curcas* have displayed potent cytotoxic, antitumor and antimicrobial activities in different assay[10]. The plant *J. gossypifolia* is a bushy gregarious shrub, grown widely almost throughout India.

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Several reports have been demonstrated that *J. gossypifolia* is used in traditional system of medicine for the treatment of various diseases such as bronchitis, diarrhoea, dysentery, piles, gum infection and inflammation itching etc[11]. Earlier studies have been stated that the plant has been used ethonomedically for the treatment of cough, tuberculosis, bacterial infection and cancerous growth[12]. It possesses significant anticancer and pesticidal activity[13]. The stem sap stops bleeding and itching of cuts and scratches[14,15]. Both species of Jatropha are frequently reported as a good source of anti-oxidant components such as phenolics compounds[16]. Regarding chemical compounds, earlier reports have been stated that both the species having alkaloids, flavonoids, phenols, saponins, steroids, tannins and terpenoids were detected in different extracts from different parts of the plants[17]. From the above earlier reports it has been concluded that both the species of Jatropha (*J. curcas* and *J. gossypifolia*) having antimicrobial and anti-oxidant activities. To the best of our knowledge there is no in vitro comparative research work on the basis of antimicrobial and anti-oxidant activities of the leaf extracts of two species. The objective of this study was (1) to investigate the comparative action between *J. curcas* and *J. gossypifolia* against some human skin diseases microorganism (2) to find out which species of *Jatropha* having more free radical scavenging activity (3) to determine the phytochemicals present in both species of *Jatropha*.

MATERIAL AND METHODS

Collection and Identification of plant materials

Plant Materials: (a) *Jatropha curcas* L. (Fam. Euphorbiaceae) (b) *Jatropha gossypifolia* L. (Fam. Euphorbiaceae) Leaves of *J. curcas* L. were collected from Naharkanta village, Rasulgarh, Bhubaneswar while *J. gossypifolia* L. were collected from Utkal University campous, vanivihar, Bhubaneswar. Both species of *Jatropha* were identified by using the Flora of Orissa and also identified by Dr. Kunjabihari Satapathi, Taxonomist and Professor, Department of Botany, Utkal University[18]. The voucher specimens of the plants were deposited in the Herbarium of post graduate department of Botany, Utkal University, Bhubaneswar.

Preparation of plant extract

Fresh leaves of two plants were harvested and washed with distilled water so as to remove dust and foreign particles. These leaves were chopped into small pieces, air dried at room temperature (25 ± 2ºC) and ground (Bajaj mixture) into powder (40–80 mesh). The powdered materials were further used for phytochemical screening and preparation of extracts. For purification of different organic constituent of dried plant material (leaves) reflux method of solvent extraction was used. Solvent series for successive separation was as follows – Petroleum ether → Methanol 1200g of air- dried and coarsely powered plants material were kept in soxhelt extraction unit and exhaustively extracted with petroleum ether (70-90˚C) for 72-96 hrs. To confirm whether the extraction is complete or not, the extraction from the siphoning tube of soxhelt is taken in a watch glass. If no residue remained in the watch glass the extraction was completed, if not the extraction continued. The extract was filtered through a cheese cloth followed by filter paper (Whatman No. 1). The whole filtrate was concentrated under reduced pressure at 50–55 ºC through a rotatory vacuum evaporator. The extracted plant material was then air dried, repacked in soxhelt apparatus and thoroughly extracted with methanol in series for such time maintain again. The crude extract and the friction obtained at every step, and then these were filtered and distilled to evaporate the solvent from the extract.

Sterility Test of the plant leaf extract

Each of the above extract (methanolic and petroleum ether extract) were tested for growth and contaminants. This was carried out by inoculating 1ml of each of them on nutrient agar and incubated at 37˚C for 24 hrs. The plates were observed for growth. No growth in the extracts after incubation indicated that the extracts were sterile. The extract was then assessed for antimicrobial activity.

Collection and Maintenance of test organisms

The test organisms were selected based on their availability, although the choice attempted selection of medically important pathogenic bacteria and fungi. Hence 9 bacteria and 5 fungi strains were used to test; the organisms were collected from Institute of Microbial Technology (IMTECH), Chandigarh. The organisms were *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Enterococcus faecalis, Micrococcus luteus, Pseudomonas aeruginosa, Streptococcus mitis, Staphylococcus aureus, Salmonella typhi* and five fungal strains were *Candida albicans, Candida tropicalis, Candida glabrata, Cryptococcus marinus, Aspergillus niger*. The microorganisms were maintained on nutrient agar slant, Potato Dextrose Agar (PDA) medium respectively and stored in the refrigerator at 4˚C. Both the bacteria and fungi strains were subcultered on the fresh media in regular intervals.

Preliminary phytochemical screening of the methanolic extract of J. curcas and J. gossypifolia

The methods described were used to test for the presence of saponins, alkaloids, tannins and flavonoids. Lieberman Burchad reaction method was used to test for steroids.

Testing for Saponins (Frothing test)

Methanolic extract (0.5g) was mixed with water in the test tube. Foaming which persisted on warming was taken as an evidence for the presence of saponins.

Testing for Tannins ($FeCl_3$ test)

Methanolic extract (0.5g) was stirred with distilled water and then filtered. Few drops of $Fecl_3$ reagent was added to the filtrate. Blue-black or blue-green colouration or precipitation was taken as an indication of the presence of phenolics and tannins.

Testing for Alkaloids (Mayer's test)

Methanolic extract (0.5g) was stirred with 5ml of 1% HCl on a steam bath. The solution obtained was filtered and 1ml of treated with a few drops of Mayer's reagent. The turbidity of the extract filtrates on addition of Mayer's reagent which was taken as evidence of the presence of alkaloids in the extract.

Testing for Flavonoids (Specific test)

Methanolic extract (0.5g) was taken in 5 ml of alcohol and treated with few drops of HCl and 0.5g magnesium cunnings and observed for formation of pink colour. Few drops of the extract was taken in a test tube with a small amount of water and shaken vigorously for one minute.

Testing for Steroids (Libermann- Burchard's test)

Methanolic extract (0.5g) was added with 5 drops of acetic anhydride and then a drop of conc. H_2SO_4. The mixture was steamed for 1 hour and neutralized with sodium hydroxide (NaOH), followed by the addition of 2ml chloroform. The appearance of a blue –green colour indicated the presence of steroid.

Determination of total Phenolic content

The concentrations of total phenolic content in the crude extracts were expressed as micrograms of pyrocatechol equivalents (PEs), determined with FCR according to the method of Slinkard and Singleton[19]. Briefly, 1mg of sample or standard (2.5–50 mg/L gallic acid) plus 150µl of diluted Folin–Ciocalteu reagent (1:4 reagents: water) was placed in each well of a 96-good plate, and incubated at room temperature for 3 min. Following an addition of 300µl of sodium carbonate (2:3, saturated sodium carbonate: water) and a further incubation for 2 h at room temperature, the absorbance was read at 765 nm using a spectrophotometer. The phenolic compound content was determined as gallic acid equivalents using the linear equation based on the calibration curve: $C = (c \cdot V)/m$, where, C =total content of phenolic compounds (mg/g plant extract in GAE), c = concentration of gallic acid obtained from calibration curve (mg/ml), V =the volume of the sample solution (ml), m= weight of the sample (g). All tests were conducted in triplicate.

Determination of total flavonoid content (TFC)

TFC of the leaf extract was determined using the spectrophotometric with slight modification[20]. Briefly, 1mg of extract solution and standard (quercetin) at different concentrations were taken in test tubes. 3.0 ml of methanol followed by 200µl of 10% aluminum chloride solution was added into the test tubes. Two hundred microliters of 1M potassium acetate solution was added to the mixtures in the test tubes. Furthermore, each reaction test tube was then immediately diluted with 5.6 ml of distilled water and mixed to incubate for 30 min at room temperature to complete reaction. The absorbance of pink colored solution was noted at 415 nm using a spectrophotometer against blank methanol. TFC of the extract was expressed as quercetin equivalents (QE) after calculation using the following equation: $C= (c \times V)/m$, where, C =total flavonoid contents, mg/g plant extract in QE, c = concentration of quercetin obtained from calibration curve (mg/ml), V =the volume of the sample solution (ml), m= weight of the sample (g). All tests were conducted in triplicate.

Bacterial strains

Microbial cultures were collected from Institute of Microbial Technology (IMTECH), Chandigarh and used for antimicrobial screening, which includes five gram negative (-ve) bacteria such as *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi* and four gram (+ve) bacteria such as *Enterococcus faecalis, Micrococcus luteus, Staphylococcus aureus*, , *Streptococcus mitis* and five fungal strains which includes *Candida albicans, Candida tropicalis, Candida glabrata, Cryptococcus marinus, Aspergillus niger*. Microbial cultures were grown on Muller-Hilton (MH) agar (Hi Media) at 37˚C for 12-14hrs. They were maintained at 4˚C in the laboratory.

Antibacterial screening by disc diffusion technique

The antimicrobial test involves the measurement of the relative potency of the activity of compounds by determining the amount of required to produce a stipulated effect on a suitable organism under standard condition. The antibacterial screening of extracts was carried out by determining the zone of inhibition using disc diffusion method[21]. The test microbial strains were taken from the broth cultures with inoculating test tube containing 10ml of sterile nutrient broth. These test tubes were inoculated at 37˚C for 24hrs and referred until the turbidity was equal to 0.5 McFarland standards[22]. Cotton swab was then used to inoculate the test tube suspension onto the surface of Muller Hinton agar plate and the uniformly swabbed plates were then allowed to dry. On the dry inoculated surfaces were placed disks prepared as follows. Sterilized Whatman paper disks (6 mm in diameter) were prepared by placing 0.5 ml of the desired solution (1, 2 and 3 mg/disk) of the extract on (6 mm diameter) disks in 0.01- or 0.02ml increments and allowing the disks to dry at 40ºC after each application. Ampoxicillin+ Clavulinic acid (AC) (20+10) (30µg/disc) and ciprofloxacin CF (25 µg/disc) were used as standards. The same was done for negative control (ethanol). The controls were prepared by using the same solvents employed to dissolve the extracts. The disks containing plant extract were placed with blunt-nosed thumb forceps on the inoculated plates at equidistance in a circle. These plates were kept for 4–6 hrs at a low temperature (<8ºC) to allow for diffusion of the extract from the disk into the medium. The plates were incubated at 37ºC for 24hrs. The experiment was conducted in triplicates. Antimicrobial activity was determined by a measurement of the inhibition zone diameter (mm) around each test organism.

Minimum Inhibition concentration (MIC)

Minimum inhibition concentration is considered the gold standard for deciding the susceptibility of organisms to judge the performance of all other method of susceptibility testing. The MIC is defined as the minimum concentration of the agent that inhibits visible growth of an organism after overnight (16-18hrs) incubation. The MIC of the crude extracts was determined by micro dilution method using serially diluted (2 folds) plant extract. The methanolic and petroleum ether extract were dissolved in mixture of 6% dimethyl formamide and then diluted to highest concentration (150µg/ml). Subsequently two-fold serial dilutions were made in concentration range of various concentrations of 0.0–25, 0.0–50, 0.0–75, 0.0–100, 0.0–125, and 0.0–150 µg/ml respectively. Equal volume of each extract and nutrient broth was mixed in a test tube. Specifically, 0.1 ml of standardized inoculum ($1–2 \times 10^7$ cfu/ml) was added in each tube. The tubes were incubated aerobically at 37ºC for 18–24 hrs. Two control tubes were

maintained for each test batch. These included antibiotic control (tube containing extract and growth media without inoculum) and organism control (tube containing the growth medium, saline and the inoculum). Control was chosen using 1ml broth, 1ml of solvent and then adding 50µl inoculums without the extract. The lowest concentration (highest dilution) of the extract that produced no visible bacterial growth (no turbidity) when compared with the control tubes were regarded as MIC. Contents of each tube containing skin infected pathogens under test were treated with different concentration of each extract for 24hrs. The content of each tube were then sub cultured on nutrient agar plates by adding 10µl of the inoculums and incubated at 37˚C for determination of MIC. The solvent control tubes were also observed for any inhibitory action and found to leave no zone of inhibition[23].

Antifungal Study

The extract was screened for their antifungal activity using food poison method[24]. For this experiment Potato dextrose agar was used as a culture medium. The methanol and petroleum extracts were dissolved in 6% dimethyl formamide (DMF, S.D. Chemicals), which was previously tested for antifungal activity against all test fungi and found to have no anti-fungal activity. For this, the required concentration of extract (10% sample solution) was taken in a sterilized pipette in a sterilized petriplate and then 15 ml of medium was poured into the petriplate to mix well and allowed to solidify. Inoculation was done at the center of each plate with 5 mm of mycelium block for each fungus. The mycelium block was prepared with the help of cork-borer from the growing area of a 5-7 days old culture of the test fungi on PDA. The blocks were placed at the center of each petriplate in an inverted position to get greater contact of the mycelium with the culture medium. The inoculated plates were incubated at (25± 2ºC). The experiment was repeated three times. Proper control (PDA without extract) was also maintained. The diameters of fungal colonies were measured after 5-7days of incubation. The average of three measurements was taken as colony diameter of the fungus in millimeters. Sabouraud Dextrose Agar (SDA, Hi Media lab) with DMF without extract served as control sets through the study. Petroleum ether extract was solubilised in a mixture of DMF and surfactant 2% Sodium Dodecyl Sulphate (SDS, S.D. fine chemicals) to make 1mg/ml solution and finally sterilized by filtration using 0.45mm Millipore filters. An attempt was made to compare antifungal activity of *J. curcas* and *J. gossypifolia* leaf extracts with broad spectrum antifungal agent fluconazole (Hi Media Lab.) as standard effective against the selective human fungal pathogens.

TLC Study

Thin layer chromatography profile of petroleum ether extract of *J. gossypifolia* was carried out on pre coated silica gel plate (Merck 60F254) as stationary phase and solvent acetic acid: n-hexane (15; 85), acetone: n-hexane (15:85). Sample of petroleum ether extract was spotted on silica gel plate with the help of capillary tube. The spotted silica gel plate was kept inside the glass chamber containing solvent system of different ratio according to the polarity of chromatograms present in the extract. In each case the solvent system was allowed to run to a distance of 10cm from the point of application of the extract in the plates. The time required for development of chromatogram varied from 20-25 mins. After completion of the run the plates were removed from the closed chamber and dried in air. The plates were then view under iodine chamber. The colour of the spots developed and their RF values were measured.

Anti-oxidant Activity Study

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk of chronic diseases including cancer and heart diseases. The main characteristic of an antioxidant is its ability to trap free radicals and oxygen species which are present in biological systems from a wide variety of sources.

DPPH Assay

The free radical activities of both the species of Jatropha were assessed with DPPH stable scavenger assay[25]. Different concentration (100, 200, 300 and 400 µg/ml) of extracts were prepared in the ethanol. 4mg DPPH in 100ml was prepared and 150µl of this solution was added to 3.0 ml of extract solution in methanol at different concentration of extract. Thirty minutes later, the absorbance was measured at 517nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations was used as standard. The degree of DPPH-purple decolourization to DPPH –yellow indicated the scavenging efficiency of the extract. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

Measurement of radical scavenging activity (%)

$$\text{DPPH Scavenged (\%)} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Where (A) control is the absorbance of the control reaction and (A) test is the absorbance in the presence of the sample of the extracts.

IC_{50} value of the extract

The antioxidant activity of the extracts was based on the screening of the extract measurements (triplicate), the inhibition concentration IC_{50} value was determined from the plotted graph of scavenging activity versus the concentration of extract (using linear regression analysis), which is defined as the amount of antioxidant necessary to reduce the initial radical concentration by 50%. The IC_{50} value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50 % and used as standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity or lower IC_{50} value indicates the higher scavenging effect[26].

Statistical analysis

The resultant zones (clear) or suppression (diffuse) around the discs were measured in mm. The antibacterial activity of oil and plant extracts were indicated by clear zones of growth inhibition. All the experiments were conducted in triplicate and the data are presented as mean values ± standard deviation.

RESULTS AND DISCUSSIONS

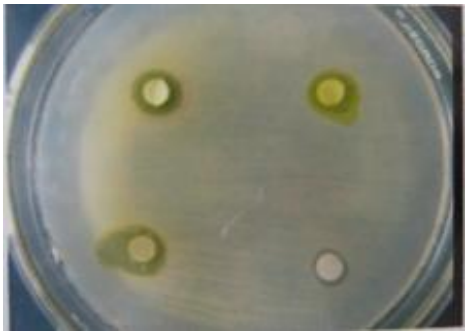

Figure 1: Zone of inhibition against *E. coli*

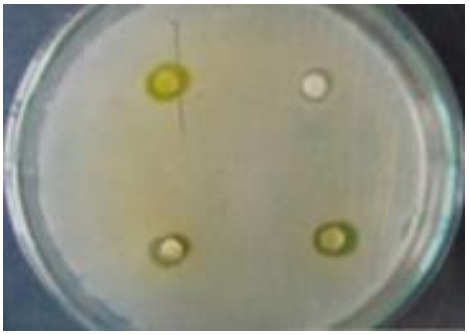

Figure 2: Zone of inhibition against *M.luteus*

Figure 3: Zone of inhibition against *S. aureus*

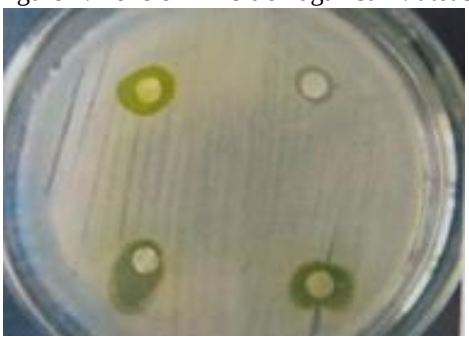

Figure 4: Zone of inhibition against *S.typhi*

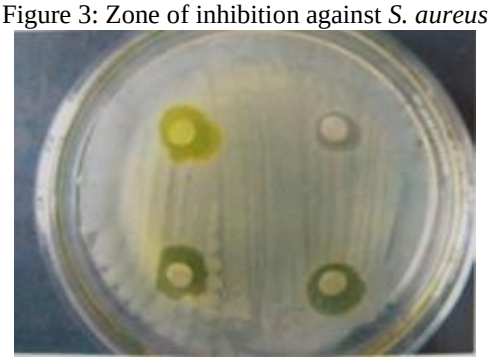

Figure 5: Zone of inhibition against *P. mirabilis*

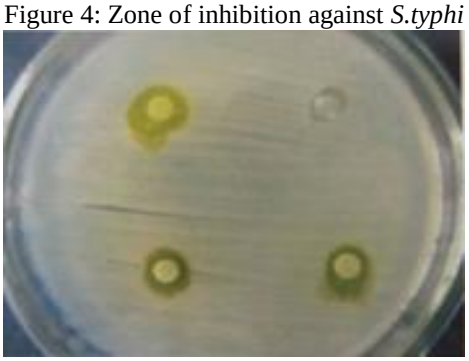

Figure 6: Zone of inhibition against *K. pneumoniae*

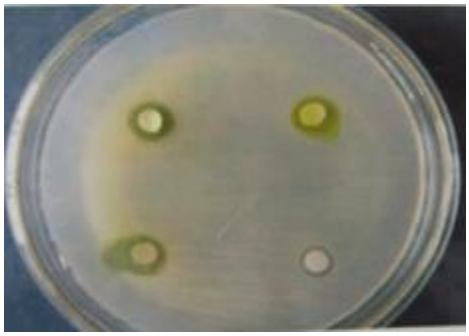

Figure 7: Zone of inhibition against *P.aeruginosa*

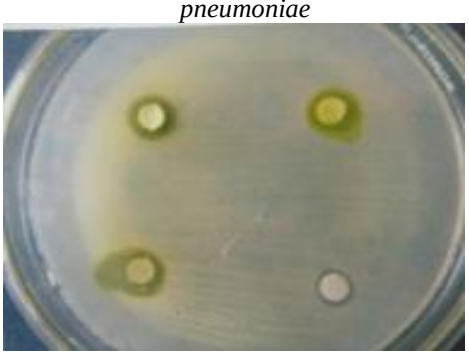

Figure 8: Zone of inhibition against *E. faecalis*

Figure 9: Zone of inhibition against *S. mitis* Hexane:Acetone (85:15)

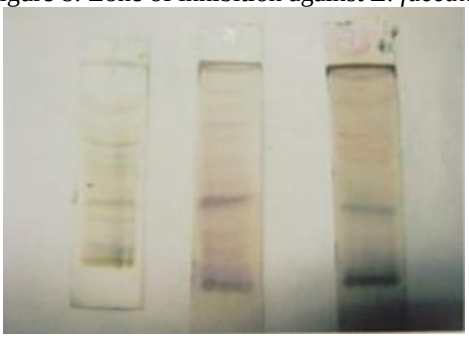

Figure 10: TLC plate of *J. gossipifolia* petrolium ether leaf extract n-

Phytochemical Screening

The major phytochemicals of pharmaceutical interest present in the methanolic leaf extract of *J. curcas* revealed the presence of saponins, tannins, alkaloids and flavonoids while that of *J. gossypifolia* contained terpenoids, reducing sugar. The petroleum ether extract of *J. gossypifolia* revealed the presence of saponins, tannins, alkaloids and flavonoids, steroid and terpenoids while in case of *J. curcas* contained only alkaloids, flavonoids and terpenoids. Out of all phytochemicals only alkaloids and tannins compounds are common in methanolic extract of

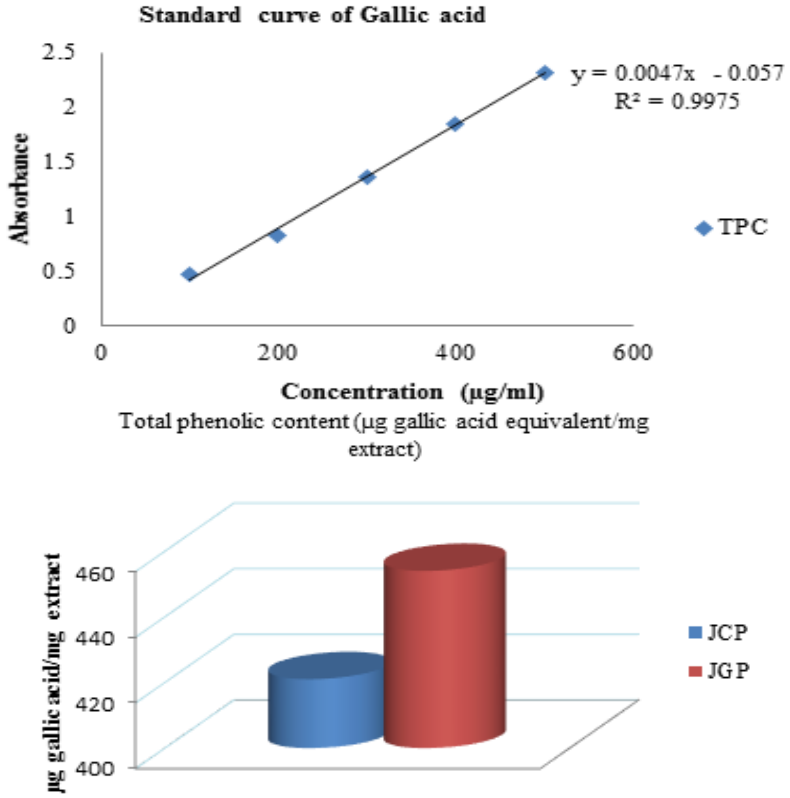

Figure 11: Comparative study of total phenolic content of both petroleum extract of *J. curcas* and *J. gossypifolia* leaf extracts.

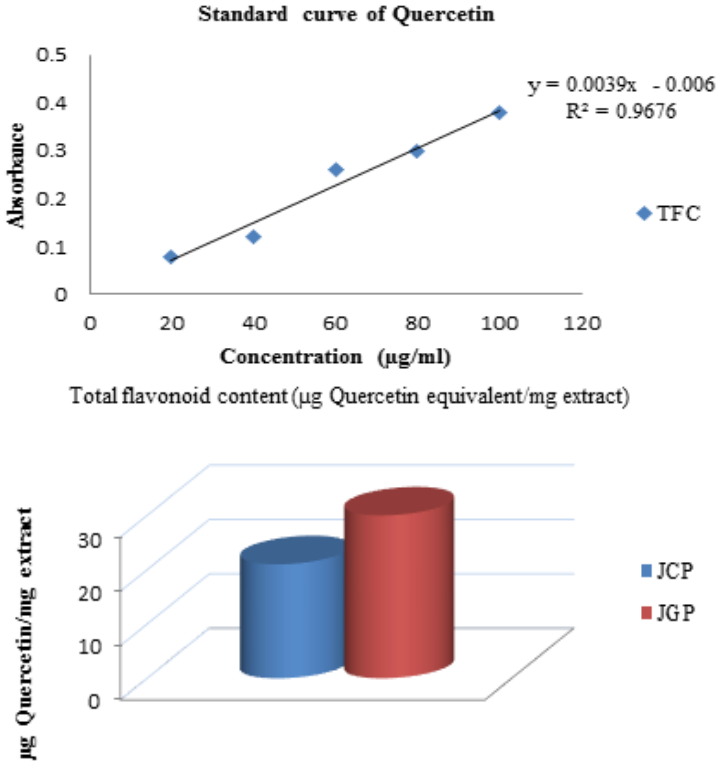

Figure 12: Comparative study of total flavonoid content of both petroleum extract of *J. curcas* and *J. gossypifolia* leaf extracts.

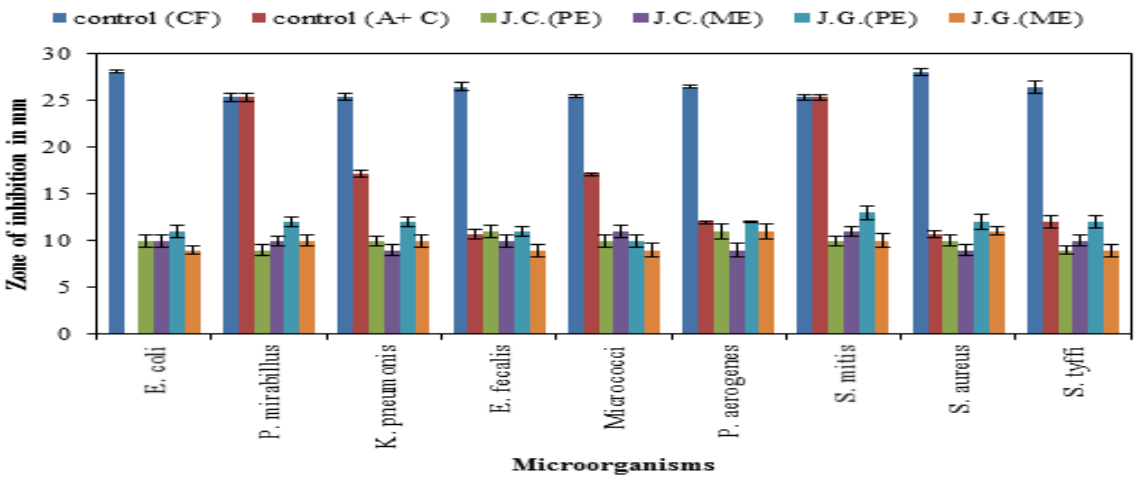

Figure 13: Comparison among four extracts of two *Jatropha* species at 100µg/ml with standard antibiotics against selected human pathogenic bacteria.

Table 1: Phytochemicals present in the leaf extract of Jatropha species

Phytochemicals	Methanolic Extract		Petroleum ether Extract	
	J. curcas	*J. gossypifolia*	*J. curcas*	*J. gossypifolia*
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	-	-	+
Tannins	+	-	-	+
Steroids	-	-	-	+
Resins	-	-	-	-
Terpenoids	-	+	+	+

+ = Present, – = Absent

Table 2: The MIC of the petroleum ether extracts of *J. gossypifolia* plant against bacteria tested by two-fold serial dilution assays.

Microorganisms	MIC (µg/ml) of *J. gossypifolia* P.E extract	MIC of Ampoxilin (µg/ml)
Gram negative bacteria		
Proteus mirabilis	≥ 50	≥ 8
Klebsiella pneumoniae	≥ 75	≥ 8
Escherichia coli	≥ 100	≥ 16
Salmonella typhi	≥ 50	≥ 8
Pseudomonas aeruginosa	≥ 100	≥ 16
Gram positive bacteria		
Enterococcus faecalis	≥ 50	≥ 8
Streptococcus mitis	≥ 25	≥ 4
Micrococcus luteus	≥ 50	≥ 8
Staphylococcus aureus	≥ 25	≥ 4

MIC = Minimum inhibition concentration

both the species which are report in table -1 and this result agreed with the previous report by[27].

Determination of total Phenolic content

The total phenolic content is expressed in terms of gallic acid. Both the petroleum extract of *J. curcas* and *J. gossypifolia* leaf obtained in compararative with gallic acid as control. 1 mg of both the petroleum ether leaf extracts was taken for experiment. Both the results are shown in figure-1 (A) and (B). The petroleum ether extract of *J. gossypifolia* showed more total phenolic content (442 mg/ml) in comparison to PE. extract of *J. curcas* (418mg/ml).

Determination of total flavonoid content (TFC)

The total flavonoid content of petroleum ether extract of both the Jatropha species were determined by quercetin as control. Both the extract having good amount of flavonoid content. In comparison to petroleum ether extract of *J. curcas* P.E. extract *J gossypifolia* leaf extract showed more flavonoid content.

Antibacterial Activity

In the experiment, it was observed that the petroleum ether and methanolic extracts of *J. curcas* and *J. gossypifolia* leaves were subjected to antibacterial activity in term of zone of inhibition as reported in Fig-1. Among the four extracts of two species of *Jatropha*, the petroleum ether extract of *J. gossypifolia* showed maximum activity against *Streptococcus mitis* (i.e.13±1.0mm), while against *Proteus mirabilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Salmonella typhi* showed nearly about 12±1.0mm. On the other hand, the methanolic extract of this plant showed maximum inhibition zone against *Enterococcus faecalis* and *Pseudomonas aeruginosa* (i.e. 11±0.0mm) and maximum effect was shown against *Salmonella typhi* and *Proteus mirabilis*. The methanolic extract of *J. curacs* showed maximum zone of inhibition against *Micrococcus luteus* and *Streptococcus mitis* (i.e. 11±0.0mm) and minimum effect against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* (i.e. 9±1.0mm). The activities of all these extracts are comparable with that of standard antibiotic disc taken into consideration to know which extract show optimum inhibitory activity against a particular bacterial strain (i.e. 16±1.0mm) Ciprofloxacin and Ampoxilin at a concentration of 5µg/ml showed maximum zone of

Table 3: Antifungal activity of *J. curcas* and *J. gossypifolia* leaf extracts

Microorganisms	Conc.(mg/mm)	Zone of restriction in mm				
		1	2	3	4	5
Candida albicans	50	12±0.38	10±0.43	15±0.33	12±0.27	17.4±0.17
Candida tropicalis	50	11±0.15	11±0.26	12±0.20	10±0.66	19.3±0.36
Candida glabrata	50	10±0.52	11±0.42	12±0.33	11±0.19	18.33±0.15
Cryptococcus marinus	50	11±0.62	10±0.90	11±0.82	10±0.74	26.4±0.17
Aspergillus niger	50	11±0.63	10±0.66	12±0.55	11±0.72	18.4±0.15

Table 4: The TLC Chromatogram of petroleum ether extract of *J. gossypifolia* revealed 10 spots in n-hexane: acetone (85:15).

No. of spots	RF	Colour
1	0.49	Greenish yellow
2	0.54	Greenish
3	0.57	Pink
4	0.62	Green
5	0.67	Bluish
6	0.71	Red
7	0.73	Yellowish green
8	0.84	Violet
9	0.89	Brick red
10	0.91	Greenish yellow

Table 5: The TLC Chromatogram of petroleum ether extract of *J. gossypifolia* revealed 6 spots in n-hexane: acetic acid (85:15).

No. of spots	RF	Colour
1	0.32	Pink
2	0.42	Yellowish
3	0.57	Bluish
4	0.62	Brick red
5	0.72	Orange
6	0.79	Dark green

inhibition against *Pseudomonas aerogenosa* and *Escherichia coli* respectively.

The petroleum ether extract of *J. gossypifolia* showing highest antibacterial activity was subjected for evaluation of minimum inhibition conc. (MIC) value which is represented in table-2. The petroleum ether extract of *J. gossypifolia* showed most potential MIC value 250µg/ml against *E. coli, S. typhi* and *E. faecalis*. J.C (PE), J.C (ME), J.G (PE), J.G (ME) stand for *Jatropha curcas*, Petroleum ether, *Jatropha curcas* methanol, *Jatropha gossypifolia* petroleum ether, *Jatropha gossypifolia* methanolic extracts. CF, A+C stands for Ciprofloxacin, Ampoxilin + Clavulanic acid respectively. Values showed significant difference from those of solvent control at P<0.001

Minimum inhibitory concentration

The Minimum inhibitory concentrations of *J. gossypifolia* leaf extract for different bacterial strains ranged from 25 to 100 µg/ml. The MIC value is higher in case of Gram negative bacteria (start at 25) than Gram positive bacteria (start at 50). Out of five grams positive and four gram negative bacteria *Streptococcus mitis, Staphylococcus aureus* showed good MIC value with petroleum ether extract of *J. gossypifolia*. *Proteus mirabilis* and *Salmonella typhi Pseudomonas* of the five gram negative bacteria showed good MIC value than the others with petroleum ether extract of *J. gossypifolia.*

Antifungal Study

The antifungal activity of *J. curcas* and *J. gossypifolia* extract was done by disc diffusion method. The antifungal activity of two *Jatropha* species extracts against some skin diseases infected fungal strains like *Candida albicans*, *Candida tropicalis*, *Candida crusu*, *Cryptococcus marinus* and *Aspergillus niger* which are shown in table-3. The petroleum ether extract of *J. gossypifolia* leaf showed maximum zone of inhibition against *Candida albicans* (15mm) and *Candida tropicalis*, *Candida crusu*, *Aspergillus niger* (nearly about 12mm). The methanolic extract of same plant extract exhibited maximum zone of inhibition against *Candida albicans* and *Candida tropicalis*. *Cryptococcus marinus* affected less and showed minimum inhibition zone (nearly about 10mm). The petroleum extract of *J. curcas* showed maximum inhibition zone against *Candida albicans* (12mm) while *Candida crusu* showed minimum inhibition zone against the same extract. The methanolic extract of this species showed zone of inhibition against the five fungal strains in between the ranges of 10-12mm. This result suggested that leaf extract from the two *Jatropha species* possessed antifungal activity, since the growth of the test fungus was inhibited[28]. Petroleum ether extract of *J. curcas*; 2. Methanolic extract of *J. curcas*; 3. Petroleum ether extract of *J. gossypifolia*; 4. Methanolic extract of *J. gossypifolia*; 5. Fluconazole as control. All values are mean ± standard deviation of three determinations. Values showed significant difference from those of solvent control at $P \leq 0.001$.

TLC Study

The result of TLC analysis on petroleum ether extract of *J. gossypifolia* along with the colour of spots on the iodine chamber was observed (table-4 and table-5). The various concentration of solvent system like n-hexane, acetone showed a no. of distinguished spots which indicated that better separation occurs in that solvent system. Accordingly, the solvent system would be chosen for further isolation of phytoconstituents of petroleum ether extract subjected to column chromatography separation. This result suggested that leaf extract of both species of *Jatropha* have a no. of secondary metabolites which agree with the report[29].

Antioxidant Activity

The antioxidant activity of the plant extracts and the standards were assessed on the basis of the radical scavenging effect of the stable DPPH free radical. In this experiment the most potent activity among the two plants were to be observed according to the ascorbic acid control (Fig-4,5,6) on the basis of IC_{50} value, hence it has been

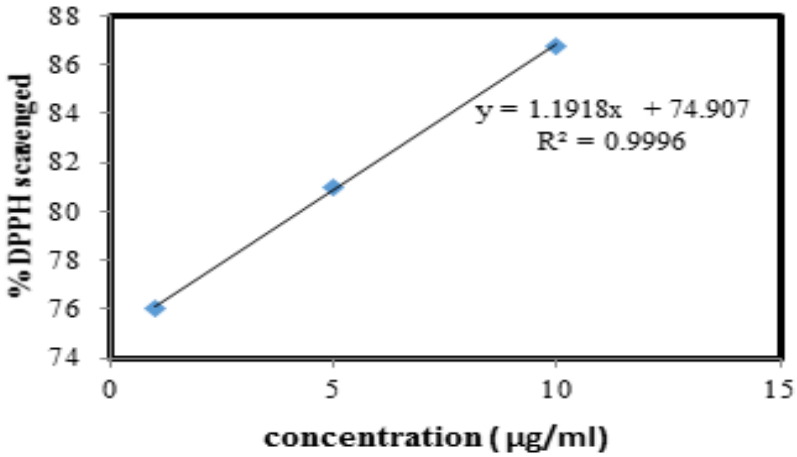

Figure 14: Free radical activity of Ascorbic acid on DPPH

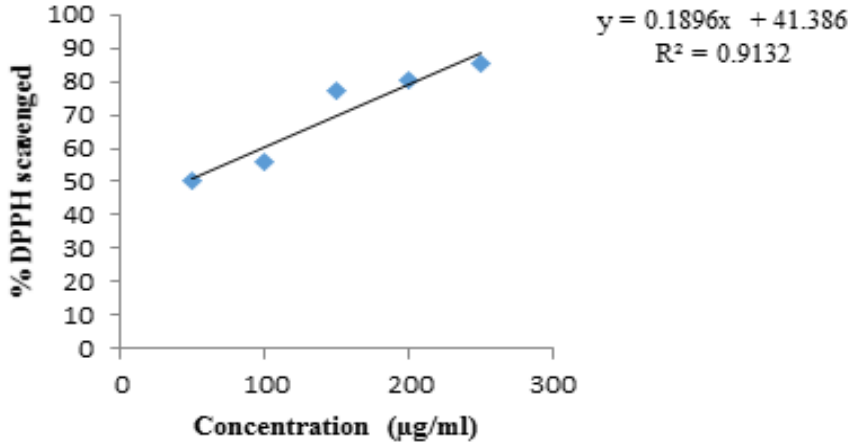

Figure 15: Free radical scavenging activity of methanolic extract of leaves of *J. curcas* on DPPH.

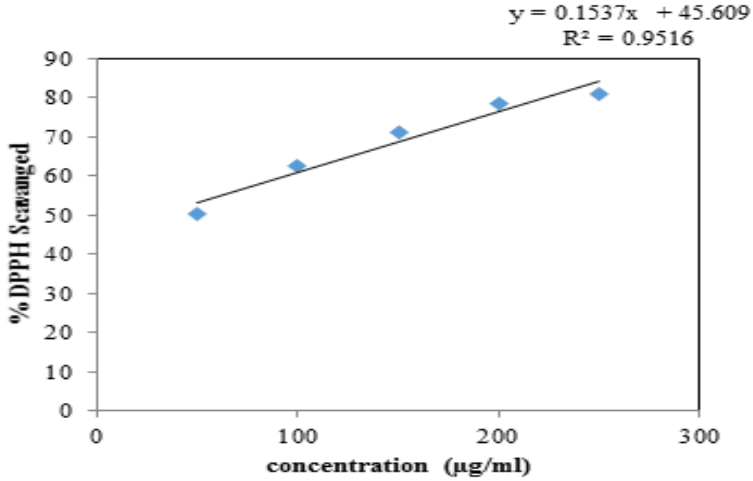

Figure 16: Free radicals scavenging activity of methanolic extract of leaves of *Jatropha gossypifolia* on DPPH.

screened for antioxidant activity using scavenging of various radical by bleaching of P- NDA and deoxyriobose method, superoxide radical by alkaline DMSO method and hydrogen peroxide method. Among the two *Jatropha* species of methanolic extracts and one standard was tested for invitro antioxidant activity using the DPPH method. The crude methanolic extracts of *J. gossypifolia* showed potent antioxidant activity with the IC_{50} value 34.10µg/ml (table-6) while *J. curcas* showed less antioxidant activity with IC_{50} value 45.6 µg/ml (table-7). However, none of the extracts were found to be more active than the standard ascorbic acid, Which IC_{50} value found to be higher. This result suggested that leaf extract of both species of *Jatropha* have antioxidant activity and *J. gossypifolia*

species showed potent antioxidant activity than the *J. curcas* which agreed with the report[30].

DISCUSSION

Phytochemical Screening

Presence of secondary metabolites in the plant extract plays a key role in the pharmacological action (important for removal of oxidative stress) of any parts of plant. This study was conducted to evaluate the comparative approach about the presence of phytochemical compounds present in both the species leaf extract of Jatropha plant. Both flavonoids and alkaloid were present in petroleum ether and methanolic extracts of *J. curcas* and *J. gossypifolia* plants. These screened results were consistent with the previously described partial studies[31.] In this study we comparatively studied the total phenolic and total flavonoid content of both the petroleum extract of *J. curcas* and *J. gossypifolia* by taking gallic and quercetin as controls. Both cases petroleum ether extract of *J. gossypifolia* showed more potential activity in comparison to *J. curcas* leaf extract.

Antibacterial activity

Plants have been continuously screened from the ancient periods whether they contain any important source of drug and compounds with therapeutic activity[32]. There were so many research work conducted for the antimicrobial activity of both the species of Jatropha and found both having good antimicrobial activity against number of skin disease causing micro organisms[33,34]. But in this research work, we evaluated the comparative study between both the species of Jatropha to find out more efficient among them against some microorganisms. In this study both disk diffusion technique and the mean zone of inhibition methods were applied for the comparative analysis. Like previous research conducted on different medicinal plants, the antimicrobial activity would be generally expected that a much greater number would be active against gram positive than gram negative bacteria. In our research also showed same results but in comparative study petroleum ether extract of *J. gossypifolia* showed more efficient anti-microbial action against the both skin disease causing Gram +ve and Gram –ve bacteria than the *J. curcas* leaf extracts. Apart from this the low MIC values for bacteria are an indication of high efficacy of antimicrobial activity while high MIC value for bacteria are an indication of low potential activity. In this study among the four extracts of two species of Jatropha the petroleum ether extract of *Jatropha gossypifolia* showed low MIC value against some gram negative bacteria than gram positive bacteria and almost good potential against other Gram +ve and Gram – ve bacteria.

Antifungal activity

Almost all higher plant leaf extracts have been reported to exhibit antifungal activity under laboratory condition. On the basis of fungal mycelia growth inhibition showed the leaf extract had a very promising inhibitory effect on fungal strains. Previously so many researches were conducted to evaluate the antifungal activity of both *J. curcas* and *J. gossypifolia*. In this study we comparatively evaluated the most efficient activity between the two species of Jatropha against five fungal species. The results presented above indicated that out of four extracts of two species, petroleum extract of *J. gossypifolia* plant exhibited more efficacies against all five fungal strains. All the crude extracts like alkaloid, flavonoids, steroid and tannin were found in petroleum ether extract of *J. gossypifolia* plant and all these biochemical constituents having antifungal activity[35].

Anti-oxidant activity:

In this study, to evaluate the antioxidant activity of the plant extracts DPPH stable free radical method was investigated against positive control ascorbic acid. It is a sensitive method to determine the scavenging effect of the extract[36]. On the basis of decolourization with respect to number of electron captured, the more anti-oxidant occurred in the extract, the more DPPH reduction occurs. Previously number of researchers described about the anti-oxidant activity of both Jatropha species. But in this study showed that IC_{50} value of petroleum ether extract of *J. gossypifolia* 34.10 µg/ml which was less than *J. curcas* IC_{50} value 45.60 µg/ml. The quantification of anti-oxidant is made by calculating the IC_{50} value. The lower IC_{50} value more free radical scavenging activity. So in comparison to petroleum ether extract of both species *J. gossypifolia* showed more free radical scavenging activity.

CONCLUSION

From the above study it can be concluded that both the species of *Jatropha* display antimicrobial and antioxidant activity. It has further confirmed that the plant extracts could be used for the treatment of various skin infections. The result lend acceptance to the folkloric use of this plant in treating microbial infection and showed that both the species of *Jatropha* could be exploited for new patent antibiotics. It was also found that many other secondary metabolites are present in the leaf extracts of the both species. In comparative study of four extract both the species of Jatropha, petroleum extract of *J. gossypifolia* showed more potential activity of antimicrobial, antioxidant and phytochemical constituents in comparison to *J. curcas* leaf extract.

REFERENCES

1. Webster GL. “Classification of the Euphorbiaceae,” *Annals of the Missouri Botanical Garden*. 1994; 81: 3–143.
2. Sabandar CW, Ahmat N, Jaafar FM, Sahidin I. “Medicinal property, Phytochemistry and pharmacology of several *Jatropha* species (Euphorbiaceae): a review,” *Phytochemistry*. 2013; 85: 7–29.
3. Rajesh B, Rattan LI. Essentials of Medical Microbiology. 4th Edition Jaypee Brothers Medical Publishers (P) Ltd. New Delhi. 2008; pp 500.
4. Das B, Laxminarayana K, Krishnaiah M, Srinivas Y, Raju TV. Multidione, a novel diterpenoid from *Jatropha multifida*. Tetrahedron Lett. 2009; 50(34):4885-4887.

5. Haslam E, Lilley TH, Cai Y, Mangoloto D. Traditional Herbal Medicine--the Role of Polyphenols, Planta Med. 1989; 55(1): 1-8.
6. Omotayo AE. Antimicrobial Activity of some Antimicrobial plants. Proceeding of Nigerian Society for Microbiology. 1998; 36: 69-72.
7. Kochlar SL. Tropical crops. In: A Test book of economic Botany. Macmillan Publisher Ltd, London and Basingstoke. 1986; 21-25.
8. Oyagade JO, Awotoye OO, Adewunmi JT, Thrope HT. Antimicrobial activities of some Nigerian medicinal plants. Screening for Antimicrobial Activity. Bioscience Research Communication. 1999; 11: 193-197.
9. Kumar A, Sharma S. An evaluation of multipurpose oil seed crop for industrial uses (*Jatropha curcas* L.): A review. Ind. Crops Prod. 2008; 28: 1–10.
10. Thomas OO. Re- examination of the antimicrobial activities of *Xylopiaaethiopica, Crcica Papaya, Ocimimum gratissimum* and *Jatropha curcas.* Fitoterapia60. 1989; 147-161.
11. Bhagat R, Misar AV, Ambavade SD, Kulkarni DK. HPTLC analysis and anti-inflammatory activity of *Jatropha gossypifolia* L. root in mice and Wistar rats. International Journal of Pharmacological Research. 2013; 3(1): 13-17.
12. Aiyelaagbe OO, Adesogan K, Ekundayo O, James BG. Antibacterial diterpenoids from Jatropha podagrica Hook. Phytochemistry. 2007; 68: 2420 – 2425.
13. Hartwell JL. Plants used against cancer, a survey Lloydia. 1969; 32: 153-205.
14. Morton JF. A Survey of Medicinal Plants: Curacao. Economic Botany. 1968a; 22: 87-102.
15. Morton JF. Caribbean and Latin America folk medicine and its influence in the United States. Quarterly Journal of Crude Drug Research. 1980b; 18b: 57-75.
16. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem J. 1999; 47: 3954–3962.
17. Zhang XP, Zhang M L, Su XH, Huo CH, Gu YC, Shi QW. "Chemical constituents of the plants from genus Jatropha," *Chemistry and Biodiversity*. 2009; vol. 6(12): 2166– 2183.
18. Saxena HO, Brahman M. The flora of Orissa. Council of Scientific & Industrial Research (India) Regional Research Laboratory (Bhubaneswar, India). 1994.
19. Harborne JB. Phytochemical Methods: a guide to modern Techniques of plant analysis. Chapman and Hall London. 1973; 161.
20. Slinkard K, Singleton VL. Total phenol analyses: automation and comparison with manual methods. Am. J. Enol. Viticult. 1977; 28: 49–55.
21. Kumaran A, Karunakaran RJ. In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. LWT - Food Sci. Technol. 2007; 40: 344–352.
22. Bauer AW, Kirby WMM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standard disc method. American J. Clinical Pathol. 1966; 45: 493-496.
23. Perez C, Paul M, Bazerque P. An Antibiotic assay by the agar good diffusion method. Acta. Bio. Med. Exp. 1990; 15: 113-115.
24. Aiyelaagbe OO, Adesogan EK, Ekundayo O, Adeniyi BA. The antimicrobial activity of roots of *Jatropha podagrica* Hook. Phyto Res. 2000; 14: 60-62.
25. Mishra M, Tiwari SN. Toxicity of *Polyalthia longifolia* against fungal pathogens of rice. Indian Phytopath. 1992; 45: 56–61.
26. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. 1995; 28:25–30.
27. Chew AL, Jessica JJA, Sasidharan S. Antioxidant and antibacterial activity of different parts of *Leucas aspera*. Asian Pac. J. Trop. Biomed. 2012; 2: 176–180.
28. Fagbenro- Beyioku AF, Oyibo WA, Anuforom BC. Disinfectant/Antiparasitic Activities of *Jatropha curcas*. East African Medical Journal. 1998; 75: 508-511.
29. Anonymous Quantity standards of Indian Medicinal plants ICMR. 2005; 2:151.
30. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958; 181: 1199-1200.
31. Girija S, Nazeema TH. Characterarization of the active antiproliferative principles of *J. curcas* and *J. gossypifolia* on Hela cell lines. Int. J. Pharmacy and Pharmaceutical Sciences. 2013; 5(2).
32. Rosy BA, Joseph H, Rosalie. Phytochemical, Pharmacognostical, antimicrobial activity of *Indigofera spalathoids* Vahl. (Fabaceae). Int. J. Biol. Technol. 2010; 1: 12–15.
33. Arekemase MO, Kayode RMO, Ajiboye AE. Antimicrobial Activity and Phytochemical Analysis of *Jatropha Curcas* Plant against Some Selected Microorganisms. International Journal of Biology. 2011; 3: 52-59.
34. Bapat UC, Mhapsekar DR. Study of antimicrobial activity and phytochemical evaluation of *Jatropha gossypifolia, Sapium sebiferum, Kirganelia reticulata, Phylanthus fraternus* and *Pedilanthus tithymaloides*. Int. J. Pharmaceutical Sciences and Research. 2014; Vol5 (11): 4933-41.
35. Sule WF, Okonko IO, Omo-Ogun S, Nwanze JC, Ojezele MO, Ojezele OJ, Alli JA, Soyemi ET, Olaonipekun TO. Phytochemical properties and in vitro antifungal activity of *Senna alata* Linn. crude stem bark extract. J. Med. Plants. Res. 2011; 5: 176–183.
36. Suresh PK, Sucheta S, Sudarshana VD, Selvamani P, Latha S. Antioxidant activity in some selected Indian medicinal plants. Afr. J. Biotechnol. 2008; 7: 1826–1828