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Comparative Phytochemical and Antimicrobial Studies of Leaf Extracts of Three species of *Jatropha*

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Abstract – The increasing microbial drug resistance coupled with the high cost and side effects of antibiotics have necessitated concerted efforts in promoting alternative to synthetic antibiotics. Jatropha species is one of the natural sources of antimicrobial agents. The ethanolic and aqueous leaf extracts of three species of Jatropha were screened against selected pathogenic microorganisms using agar well diffusion method while phytochemical components were determined by spectrophotometric method. Aqueous and ethanolic leaf extracts of Jatropha gossypifolia exhibited antimicrobial activities with the zones of inhibition ranging from 13 mm to 18 mm against Escherichia coli and 13 mm to 16 mm against Neisseria gonorrhea. Both the ethanolic and aqueous extracts of Jatropha gossypifolia exhibited zones of inhibition between 9 mm and 10 mm against Candida albicans. The minimum inhibitory concentration (MIC) of the leaf extracts was between 3000-5000 µg/ml for the bacteria strains and 2000 µg/ml for the fungus. Phytochemical analysis of the extracts revealed the presence of tannin, saponin, flavonoids, phytate and phenol in higher concentrations in the aqueous and ethanolic extracts of J. gossypifolia. Result indicated that out of the six extracts of the three species, ethanolic leaf extract of J. gossypifolia exhibited more potential against the tested microorganisms.

Keywords: Jatropha species, leaf extracts, pathogenic microorganisms, phytochemicals and spectrophotometric

1.0 Introduction

Medicinal plants are important sources of potentially useful new compounds for the development of chemotherapeutic agents (Vital and Rivera, 2009). Emergence of pathogenic microorganisms that are multi resistant to major classes of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs. In addition, high cost and adverse side effects that are commonly associated with popular synthetic antibiotics are major burning global issues in treating infectious diseases (Ogundare, 2007). As a result, there is an urgent and continuous need of exploration and development of cheaper, effective new plant based drugs with better bioactive potential and least side effects. Hence, recent attention has been paid to biologically active extracts and compounds from plant species used in herbal medicines (Matsuse *et al*., 1999; Vital and Rivera, 2009).

Jatopha species belong to the family Euphorbiaceae with about 172 species within the genus. The species have significantly antimicrobial, antioxidants, anticancer and pesticidal activities (Ashish *et al*., 2016). *Jatopha* species have being used in traditional medicine to cure ailments in Africa (Ayelaagbe, 2007). The predominant *Jatropha* species in Nigeria are *Jatropha curcas* L., *J. gossypyfolia* L. and *J. multifida* L. The phytochemicals chemical analysis of *Jatropha* leaves in Nigeria shows that the leaves contain saponin, tannin, glycoside, steroid, alkaloid and flavonoid. These compounds are known to be biologically active and therefore aid the antimicrobial activities of plants (Ogundare, 2007).

Earlier studies have confirmed the efficacy of the extracts from the different parts of the three species of *Jatropha* (*J. curcas*, *J. gossypifolia* and *J. multifida*) against wide range of different microorganisms (Ayelaagbe, 2007; Ogundare, 2007; Ashish *et al*., 2016). However the comparative antimicrobial and phytochemical studies of the three species had rarely been reported. Thus, this study aimed at comparative phytochemical analysis and antimicrobial activities of *J. curcas, J. gossypifolia and J. multifida* against *Escherichia coli, Staphylococcus aureus, Neisseria gonorrhea and Candida albicans*.

2.0 Materials and methods

2.1 Collection and identification of plant materials

The leaves of *Jatropha curcas*, *Jatropha gossypifolia* and *Jatropha multifida* were collected from different locations in Ede and Osogbo, Osun State, Nigeria. The identification of the three species was confirmed at the Herbarium unit of the Department of Botany, Obafemi Awolowo University, Ile Ife.

2.2 Collection and maintenance of test organisms

Pure clinical isolates of *Escherichia coli*, *Staphylococcus aureus*, *Neisseria gonorrhea* and *Candida albicans* were collected from the Microbiology Unit of the Science Laboratory Technology Department, Federal Polytechnic, Ede, Osun State, Nigeria.

2.3 Preparation of the leaf extracts

Ethanolic and aqueous leaf extracts of the three species of *Jatropha* were prepared according to the procedure of Olajire and Azeez (2011). Portions (30 g) each of the powdered samples were weighed into a clean conical flask into which 300 ml of distilled water and ethanol were added respectively. The mixtures were allowed to stand for 48 hours at room temperature. The filtrations were then carried out using muslin cloth and the filtrates were taken as the extracts. The extracts were then evaporated using rotatory evaporator.

3.0 Phytochemical study

3.1 Determination of Tannin

Total tannin content of the leaf extracts of the three species was determined according to the method of Padmaja (1989). An aliquot (0.1 ml) of each extract was mixed with 7.5 ml of distilled water, 0.5 ml of Folin-Denis reagent, and one ml of 35% sodium carbonate solution, and diluted to 10 ml with distilled water. The mixture was shaken well and allowed to stay at room temperature for 30 minutes, after which the absorbance was measured at 760 nm. A blank was prepared with water instead of the extract. Tannin content was expressed as tannic acid equivalent in mg/g of extract dry weight. The experiment was done in triplicates.

3.2 Determination of phytate

Phytate was quantified according to Lorenz *et al*. (2007). A portion (2 g) of each extract was weighed into a 250 ml conical flask, 100 ml of 2% concentrated HCl was added, it was allowed to soaked for 3 hrs and was then filtered. 50 ml of the filtrate was pipetted into a 250 ml beaker; 100 ml of distilled water was added to improve acidity. 10 ml of 0.3% ammonium thiocyanate solution was added as indicator. It was titrated with standard iron 111 chloride ($FeCl_3$) solution which contains 0.00195 g iron/ml until a brownish yellow colour appear and persist for 5 minutes.

3.3 Determination of phenol

The total phenol content of the leaf extracts of the three species of *Jatropha* was determined by using Folin-Ciocalteus method as modified by Olajire and Azeez (2011). Each extract (0.1 ml) was added to 10 ml of deionized distilled water and 2.5 ml of 0.2 N Folin-Ciocalteu's phenol reagent. The mixture was allowed to stand for five minutes at room temperature before adding 2 ml of sodium carbonate. The absorbance was measured at 780 nm after ten minutes using quercetin as standard for calibration curve. This assay was carried out in triplicate.

3.4 Testing for saponin

Total saponin content of the leaf extract of the three species of *Jatropha* was determined by the method described by Makkar *et al*. (2007). An aliquot **(**0.25 ml) of each extract was mixed with 0.25 ml vanillin reagent (8% vanillin in ethanol) and 2.5 ml of 72% aqueous H_2SO_4 in test tubes. The test tubes mixtures were heated in a water-bath at 60° C for 10 mins, cooled in ice for 4 mins and then, allowed to acclimatize to room temperature. Subsequently, the absorbance was measured at 544 nm. Diosgenin was used as a standard and the results obtained were expressed as mg diosgenin equivalent per g of extract dry weight. The experiment was done in triplicates.

3.5 Testing for total flavonoid

Total flavonoid content of the leaf extract of the three species of *Jatropha* was determined using $AlCl_3$ method as reported by Kale *et al*. (2010). An alliquot (0.5 ml) of each extract was dispensed into test tube, followed by 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The mixture was shaken and allowed to stay at room temperature for 30 minutes, after which the absorbance was measured at 514 nm. Total flavonoid content was expressed as mg flavonoid/mg quercetin. This assay was carried out in triplicate.

4.0 Antimicrobial activities of leaf extracts of three species of *Jatropha* on test microorganisms

The microbial properties of the leaf extracts were determined using agar well diffusion method as described by Cheesbrough (2004). Nutrient agar was used for the bacteria isolates while Potato Dextrose Agar (PDA) was used for the fungus. An aliquot (0.1 ml) of the test isolate was spread on the agar plate using glass spread. A sterile stainless steel cork borer was used to make holes (8 mm in diameter) on the medium which has been seeded with the test organism. An aliquot (0.1 ml) of each extract was dropped into the well. The procedure was repeated for all the extracts with different organism. Distilled water, ethanol and standard antibiotics (Metronidazole and fluconazole at1 mg/ml) were used as the control. The experiments were done in triplicates. The bacteria plates were incubated at 37°C and the zones of inhibition was measured after for 24 hrs after while the fungal plates were left at room temperature and the zones of inhibition was measured after three days.

4.1 Determination of minimum inhibitory concentration (mic) of leaf extracts of Jatropha species

The MIC was determined by using the method of Akinpelu and Kolawole (2004). The concentrations of the extracts used for the MIC were 10000 µg/ml, 5000 µg/ml and 2,000 µg/ml. Two fold dilution of each of crude extracts was prepared and 2 ml of different concentrations of the solution was added to 18 ml of sterilized molten nutrient agar at 40°c to give 10000 µg/ml final concentrations. The procedure was repeated to give concentration of 5000 µg/ml and 2000 µg/ml. The media were then poured into sterile petridishes and were allowed to set. The media plates were streaked with 17 hours old culture, and the plates were incubated at 37 °C for 24 hrs for bacteria and 27 °C for 3 days for fungi. The same procedure was repeated for antibiotics where metronidazole was used as antibacterial agent and fluconazole was used as antifungal agent. After incubation, the plates were examined for the presence or absence of growth and the MIC was taken as lowest concentration that prevented growth of the test organism.

5.0 Statistical Analysis

The data obtained from the experiments were subjected to two way Analyses of Variance (ANOVA) using the Statistical Package for Social Sciences (SPSS 20) while the mean differences were compared using pairwise comparisons (Steel and Torrie, 1984).

6.0 Result and discussion

The comparative phytochemical analysis of the aqueous and ethanolic leaf extracts of the three species of *Jatropha* is presented in Figure 1. The phytochemical studies revealed that the six extracts contained tannin, phytates, flavonoids, saponins and phenols in different concentrations. The concentration of tannin (mg/kg) was the highest in the extracts (2426 – 4027 mg/kg) except the aqueous leaf extract of *J. curcas*

which has saponin as the highest quantified phytochemicals (Fig. 1). The least of the quantified phytochemicals in the six extracts was phytate (51.26–81.83 mg/kg). In the three species of *Jatropha*, the ethanolic leaf extracts had higher phytochemicals than the aqueous extracts (Fig. 1). The ethanolic extract of *J. gossypifolia* had the highest concentration of tannin (4027.5 mg/kg), this was followed by the ethanolic leaf extract of *J. multifida* while the ethanolic leaf extract of *J. curcas* has the least concentration of tannin (Fig. 1).

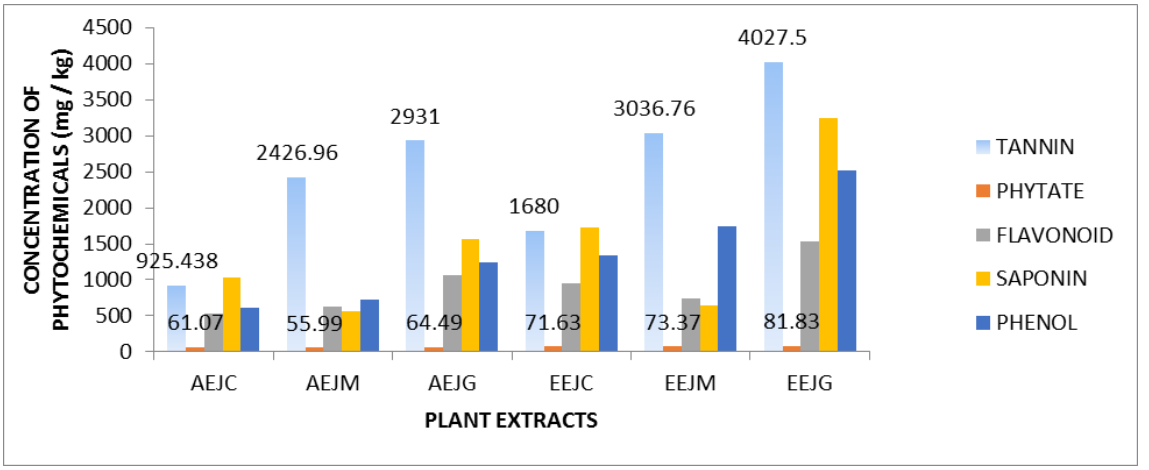

Figure 1: Phytochemical constituents (mg/kg) of leaf extracts of three species of *Jatropha*

AEJC = Aqueous leaf Extract of *J. curcas*, AEJM = Aqueous leaf Extract of *J. multifida*, AEJG = Aqueous leaf Extract of *J. gossypifolia*, EEJC = Ethanolic leaf Extract of *J. curcas*, EEJM = Ethanolic leaf Extract of *J.multifida* and EEJG = Ethanolic leaf Extract of *J. gossypifolia*

The aqueous leaf extract of *J. gossypifolia* has the highest concentration of phytate (81.73 mg/kg). However concentration of phytate in the aqueous extract of *J. multifida*, was not significantly different from the concentration of phytate ($p = 0.02$) in the aqueous leaf extract of *J. curcas*. Similarly, the concentrations of phytate in the ethanolic extracts followed the same trend.

Also, the aqueous leaf extract of *J. gossypifolia* has the highest concentration of saponin and phenol while the aqueous leaf extract of *J. curcas* has the least concentrations of the two phytochemicals. Similar trend was observed in the ethanolic leaf extracts of the three species as regard the concentration of saponin and phenol. Consequently, the aqueous leaf extracts of *J. gossypifolia* has the highest concentration of flavonoid followed by aqueous leaf extracts of *J. multifida* while the aqueous leaf extracts of *J. curcas* has the least concentration of flavonoid. Similar trend was observed in the concentration of flavonoids in the ethanolic leaf extracts of the three species.

The result of the antimicrobial study is presented in Table 1. Among the six aqueous and leaf extracts of *Jatropha*, the ethanolic extracts of *J. gossypifolia* and *J. multifida* exhibited maximum antibacterial activity against *E. coli* with 18±0.98 mm and 17±1.07 mm zones of inhibition respectively. These were not significantly different from the zone of inhibition ($p = 0.01$) exhibited by the standard antibiotics (metronidazole) 18±1.2 mm. The aqueous extracts *J. gosypifolia* and *J. multifida* showed 10±0.07 mm and 13±0.14 mm respectively. However both aqueous and ethanolic extracts of *J. curcas* did not show antibacterial activity against *E. coli* as there were no zones of inhibition.

The ethanolic leaf extract of *J. gossypifolia* that showed highest antimicrobial activity was subjected for evaluation of Minimum Inhibitory Concentration (MIC) which ranged from 3000-5000 µg/ml for all the

strains of bacteria (Table 2). This was significantly higher than the MIC ($p = 0.01$) of the standard antibiotics (metronidazole). The MIC against *C. albicans* ranges between 2000-3000 µg/ml.

Table 1: Antimicrobial Activities of leaf extracts of three species of *Jatropha* showing Zone of inhibitions (mm) against selected microorganisms

TREATMENTS	***E. coli*** **(ZI in mm)**	***S. aureus*** **(ZI in mm)**	***N. gonorrhea*** **(ZI in mm)**	***C. albicans*** **(ZI in mm)**
AEJC	0	12.34 ± 0.14^{c}	10.34 ± 0.07^{d}	0
AEJM	13 ± 0.14^{b}	8.67 ± 0.18^{e}	5.34 ± 0.5^{f}	7.67 ± 0.1^{d}
AEJG	10 ± 0.07^{c}	10.34 ± 0.1^{d}	13.67 ± 0.17^{c}	8.34 ± 0.02^{b}
EEJC	0	14.34 ± 0.07^{b}	11.00 ± 0.05	0
EEJM	17 ± 1.07^{a}	9.34 ± 0.12^{e}	9.34 ± 0.01^{e}	8.67 ± 0.20^{c}
EEJG	18 ± 0.98^{a}	16.34 ± 0.07^{a}	16.00 ± 0.01^{b}	10.34 ± 0.1^{b}
M/F	18 ± 1.2^{a}	0	25.67 ± 2.12^{b}	20.67 ± 1.98^{a}
AC	NA	NA	NA	NA
EC	6 ± 0.60^{d}	5.00 ± 0.16^{f}	5.00 ± 0.17	6.67 ± 0.10^{e}

AEJC = Aqueous leaf Extract of *J. curcas*, AEJM = Aqueous leaf Extract of *J. multifida*, AEJG = Aqueous leaf Extract of *J. gossypifolia*, EEJC = Ethanolic leaf Extract of *J. curcas*, EEJM = Ethanolic leaf Extract of *J. multifida*, EEJG = Ethanolic leaf Extract of *J.*gossypifolia, ZI = Zone of inhibition, M/F = metronidazole or fluconazole, AC = Aqueous control, EC = Ethanol control and NA = Not Active, Values in a column followed by the same letter are not significantly different at $p > 0.05$

Table 2: Minimum Inhibitory Concentration (µg/ml) of Leaf Extracts of the Three Species of *Jatropha* Against the Test Microorganisms

TREATMENTS	***Escherichia coli*** (MIC in µg/ml)	***Staphylococcus aureus*** (MIC in µg/ml)	***Neisseria gonorrhea*** (MIC in µg/ml)	***Candida albi*** (MIC in µg/m
AEJC	5000	NA	3000	NA
AEJM	5000	5000	5000	2000
AEJG	NA	5000	3000	3000
EEJC	5000	5000	5000	NA
EEJM	5000	5000	5000	5000
EEJG	NA	5000	5000	5000
M/F	1000	1000	1000	1000

AEJC – Aqueous leaf Extract of *J. curcas*, AEJM- Aqueous leaf Extract of *J. multifida*, AEJG- Aqueous leaf Extract of *J. gossypifolia*, EEJC- Ethanolic leaf Extract of *J. curcas*, EEJM- Ethanolic leaf Extract of *J.multifida*, EEJG- Ethanolic leaf Extract of *J.*gossypifolia, ZI-Zone of inhibition, M/F- metronidazole or fluconazole, AC- Aqueous control, EC-Ethanol control and NA- Not Active

The result of the phytochemical study indicated the presence of phenols, tannin, phytate, saponin and flavonoid. The phytochemicals are more concentrated in the leaf extract of both *J. gossypifolia* than *J. multifida* and *J. curcas*. This is partially in line with the discovery of Ashish *et al*. (2016) who discovered that phytochemicals are more concentrated in *J. gossypifolia* than *J. curcas*.

The result of the antimicrobial activities revealed that *S. auerus* was sensitive to both the aqueous and etanolic extracts of *Jatropha* with the zones of inhibition ranging from 9-16 mm. This corroborated the findings of Ayelaagbe (2007) who reported that *S. aureus* was the most sensitive to aqueous and ethanolic root extract of *J. curcas*. The ethanolic leaf extract of the three species demonstrated higher antimicrobial activities as indicated by the zones of inhibition which ranged 9 – 18 mm. This is in line with the report of

Igbinosa and Igbinosa (2009) who recorded 8 -20 zones of inhibition with ethanolic stem extract of *J. curcas*.

7.0 Conclusion and Recommendations

The results of this study confirm that the leaf extracts of the three species have antibacterial and antifungal activities. This supports their use in wound dressing, cuts and treatment of skin infections. In comparative study of six extracts of the three species of *Jatropha*, ethanolic leaf extract of *J. gossypifolia* showed more potential antimicrobial activity and phytochemical constituents in comparison to *J. curcas* and *J. multifida* leaf extracts. However, an *in vivo* antimicrobial and toxicological analysis of the extract is recommended to justify its oral administrations in human.

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