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EVALUATION OF PHARMACOGNOSTIC, ANTIOXIDANT, ANALGESIC AND ANTIMICROBIAL ACTIVITIES OF *Jatropha curcas* LEAVES

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ABSTRACT
Pain, microbial infections and oxidative stress are managed using synthetic drugs, which are associated with many undesirable effects. Herbal medicines form an alternative therapy to synthetic drugs since they possess fewer side effects. The study was aimed to evaluate the pharmacognostic, antioxidant, antimicrobial and analgesic activities of Jatropha curcas leaves. Chemomicroscopic, phytochemical and antimicrobial evaluations were carried out using the standard methods. The anti-oxidative activities of the methanol extract were determined by means of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Acetic acid induced writhing test in mice were used to evaluate the analgesic effect. Chemo-microscopical examination of powdered Jatropha curcas leaves revealed the presence of cellulose, tannins, starch, lignin, calcium oxalate, suberin, aleurone grain and mucilage but calcium carbonate was absent. Flavonoid, alkaloids, tannins, carbohydrate, glycoside and phenols were detected in both aqueous and methanolic extracts. The extract demonstrated dose-dependent varying degrees of anti-oxidative efficacy in the radical scavenging assays. For the DPPH assay, IC_{50} values of 0.606 µg/mL was recorded. Antimicrobial activities of methanol extract of Jatropha curcas leaves showed inhibition on all the tested clinical isolates Staphylococcus aureus, Escherichia coli, Candida albicans and Aspergillus fumigatus at 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml and recorded respective MIC and MBC/MFC values of 12.5 mg/ml and 25 mg/ml against all the tested isolates. The extract and standard significantly decreased the number of writhes caused by acetic acid. The effects observed at 20 mg/kg more than that of 40mg/kg and 80 mg/kg of the extract. The extract was found to possess constituents that may be associated with its analgesic and antimicrobial effects observed at doses tested.
Keywords: Antimicrobial, Antioxidant, Chemomicroscopic, phytochemical, Pharmacognostic,

INTRODUCTION

Plant secondary have been attracting much interest as natural alternatives to synthetic drugs (Ibrahim, 2016). However, herbal medicine is not much developed when compared to conventional system of medicine, mainly because of the lack of proper scientific standardization in this field (Ibrahim, 2016). Various challenges such as poor drug solubility, stability, adsorption and high toxicity affect the use of synthetic medicines as therapeutic agents (Ibrahim, 2016). In addition, some of these drugs are expensive and generally unavailable to citizens of developing countries, especially those residing in the rural areas (Sule *et al.*, 2011). Development of resistant against antibiotics and also the side effects associated with them is urging people not to use synthetic antibiotics (Ibrahim, 2016). Therefore, there is a constant and urgent need to develop new antimicrobial drugs for the treatment of infectious disease from medicinal plant (David, 2009; Ibrahim, 2016).

Until recently, research and development efforts have provided new drugs in time to treat bacteria that developed resistance to older antibiotics (WHO, 2012). A global public health disaster appears likely as effective supply of antibiotics have continues to be exhausted due to bacterial antibiotic resistance (WHO, 2012). Substitutes from the natural source to the antibiotics are becoming the prime need of the society in the present and in future (Ibrahim, 2016).

Emergence of multi-drug resistant bacteria and the infectious diseases caused by them are serious global problems (Albuquerque *et al.,* 2007; Ibrahim, 2016). Thus, there is an urgent need for novel antimicrobials and/or new approaches to combat these problems (Liu *et al.,* 2000; Ibrahim, 2016). Antibiotics that work today may not work tomorrow. Therefore, drug synergism between known antimicrobial agents and bioactive plant extracts is a novel concept and has been recently reported (Ibrahim, 2016).

Therefore, combination therapy is often profitable for patients with serious infections caused by drug-resistant pathogens (Davis *et al.,* 2003; Ibrahim, 2016).

Jatropha curcas is called physic nut or Purging nut in English, *fula* in Fulfulde, *binidazugu* in Hausa*, lapalapa* in Yoruba*, etookpa* in Efik and *oru-ebo* in Edo (Ibrahim, 2016). *J. curcas* is the commonest species in Nigeria, mainly grown for bio-diesel because of its high oil content (48%). It is a shrub or small tree with smooth grey bark, which exudes whitish coloured watery latex when cut. *Jatropha curcas* is a medicinal plant that belongs to the family Euphorbiaceae and has a long history of cultivation in tropical America, Africa, and Asia (Ravindranath *et al*., 2004; Ibrahim, 2016). *J. curcas* is the commonest species in Nigeria, mainly grown for bio-diesel, biogas and bioethanol because of its high oil content (48%) (Ibrahim, 2016). *Jatropha curcas L*. is becoming a very useful economic resource both in agriculture, phytomedicine development and development of new lead compounds (Saetae and Suntornsuk, 2010; Mkoma and Mabiki, 2012; Ibrahim, 2016). *Jatropha curcas* grows well particularly in the Northern part of Nigeria and can be used to provide cheap source of antibiotic and disinfectant due to its antimicrobial activities that have been reported (Igbinosa, *et al*., 2009). Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in developing countries (WHO, 2002). Previous studies have reported that *J.* c*urcas* exhibits antimicrobial activity (Ekundayo *et al*., 2011). The search for new analgesic, antioxidant and antimicrobial drugs of natural origin is urgently needed in the light of growing cases of microbial resistance to the available synthetic antibiotics.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Jatropha curcas leaves were collected from local farms in Rimin gata kwarin adiya, Kumbotso Local Government Area, Kano State, Nigeria. The plant was identified and authenticated in the herbarium of the Plant Biology Department of Bayero University, Kano, Kano State, Nigeria and a voucher specimen BUKHAN60 was deposited.

Chemo-microscopic Studies of the Powdered *Jatropha curcas* leaves

Powdered *Jatropha curcas* leaves were analysed to detect the presence of cell wall materials and cell inclusions, finely ground samples of plants were cleared in a test tube containing 70% chloral hydrate solution. These were then boiled in a water bath for about thirty minutes to remove obscuring materials. The cleared samples were mounted with dilute glycerol onto a microscope slides. Using various detecting reagents the presence of cell wall materials and cell inclusions were detected in accordance to WHO (2011) guidelines.

Cell wall Materials

Test for Cellulose

Two millilitres of iodinated zinc chloride were added to the powdered sample and allowed to stand for a few minutes and thus observed under a microscope. The reagent stained cellulose cell wall blue to blue- violet (WHO, 2011).

Test for Lignin

The powdered plant materials were moistened on a slide with a small volume of phloroglucinol and allowed to stand for about two minutes or until almost dry (WHO, 2011). A drop of hydrochloric acid were added and viewed under a microscope. Pink stained or cherry red were observed, for the presence of lignin.

Test for Suberized or Cuticular cell walls

Two millilitres of Sudan red were added to the cleared powdered sample and allowed to stand for few minutes and observed under a microscope (WHO, 2011). Orange red or red colour was observed presence of suberin or cutin on the cell.

Test for Gum and Mucilage

To a small portion of the cleared powdered sample of the plants, a drop of ruthenium red were added (WHO, 2011). Appearance of pink coloration was considered positive for gums and mucilage.

Cell Inclusions/ Cell Contents

Test for Starch grains

To a small portion of the cleared powdered sample of the plant materials, N/50 iodine was added (WHO, 2011). Appearance of blue-black or reddish-blue coloration on some grains was considered positive for starch.

Test for Calcium oxalates and Calcium Carbonates

To a small portion of the cleared powdered sample of the plant, HCl was added, dissolution of crystals in the powdered drug without effervescence was considered positive for calcium oxalate while slow dissolution with effervescence was considered positive for calcium carbonate (WHO, 2011).

Inulin

A drop of 1-naphthol and that of sulphuric acid was added to the powdered sample and viewed under the microscope. Spherical aggregations of crystals of Inulin turned brownish red and dissolved (WHO, 2011).

Test for Tannins

To a small portion of the cleared powdered sample of the plant, 5% ferric chloride solution was added. Appearance of greenish black colour was considered as positive for tannins (WHO, 2011).

Preparation of Plant extracts

The *Jatropha curcas* leaves were cleaned, air dried and ground to coarse powder using grinding machine. The powder was stored in air tight containers for further use. Two hundred grams (200 g) each of the powdered leaves was soaked into 2 L of methanol and distilled water respectively.

The mixtures were allowed to stand for 3 days at room temperature (28 ±2ºC) with hourly agitations. The extract was sieved through a muslin cloth, filtered through a Whatman (No.1) filter paper, poured unto a clean evaporating dish and placed on a water bath at 50ºC until all the solvent evaporated.

Qualitative Phytochemical screening of Methanol and Aqueous extract of *Jatropha curcas* leaves

Jatropha curcas leaf extracts were subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the methods described below.

Tests for carbohydrates

Molish's (General) Test for Carbohydrates: To 1 ml of the filtrate, 1 ml of Molish's reagent was added in a test tube, followed by 1 ml of concentrated sulphuric acid down the test tube to form a lower layer. A reddish colour at the interfacial ring indicates the presence of carbohydrate (Evans, 2009).

Tests for Saponins

Frothing test: About 10ml of distilled water was added to a portion of the extract and was shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 mins. A honeycomb froth that persists for 10-15 mins indicates presence of saponins (Evans, 2009).

Test for Flavonoids

Shinoda Test: A portion of the extract was dissolved in 1-2 ml of 50% methanol in the presence of heated metallic magnesium chips and a few drops of concentrated hydrochloric acid were added. Appearance of red color indicates the presence of flavonoids (Evan, 2009).

Test for Alkaloids

Wagner's Test: Few drops of Wagner's reagent was added into a portion of the extract, whitish precipitate indicates the presence of alkaloids (Evans, 2009).

Test for Steroids and Triterpenes

Liebermann-Burchard's test:

Equal volumes of acetic acid anhydride was added to the portion of the extract and mixed gently. Concentrated sulphuric acid (1 ml) was added down the side of the test tube to form a lower layer. A colour change observed immediately and later indicates the presence of steroid and triterpenes. Red, pink or purple colour indicates the presence of triterpenes while blue or blue green indicates steroids (Evans, 2009).

Test for Cardiac Glycosides

Kella-killiani's test:

A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Interphase for purple-brown ring was carefully observed, this indicates the presence of deoxy sugars and pale green colour in the upper acetic acid layer indicates the presence of cardiac glycosides (Evans, 2009).

Test for Tannins

Ferric chloride test:

Exactly 3-5 drops of ferric chloride solution was added to the portion of the extract. A greenish black precipitate indicates the presence of condensed tannins while hydrolysable tannins give a blue or brownish blue precipitates (Evans, 2009).

Test for Anthraquinones

Borntrager's test:

Exactly 5ml of chloroform was added to the portion of the extract in a dry test tube and shaken for at least 5mins. This was filtered, and the filtrate shaken with equal volume of 10% ammonium solution, bright pink colour in the aqueous upper layer indicates the presence of free anthraquinones (Evans, 2009).

Antioxidant activity Procedure

The antioxidant activity of the *Jatropha curcas* methanolic leaf extract was measured in terms of radical scavenging ability, using a stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the modified method adopted from (Sani and Dailami, 2015). 200µl of 100µM methanol solution of DPPH were added to 100µL of various concentrations of the sample fractions in methanol (1000, 500, 250, 125, 62.5, 31.25, 15.63 and 7.8µg/ml) and made to react in dark for 30mins time at room temperature. Absorbance of the blank, test and control were recorded at 517 nm. The experiment was performed in triplicate and scavenging activity was calculated by using the following formula and expressed as percentage of inhibition.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

The concentration corresponding to the 50%inhibition (IC_{50}) was determined using probit analysis by means of SPSS 16.0 software. The IC_{50}values obtained are compared with that of ascorbic acid as a standard antioxidant.

Microbiological analysis

According to Gram staining technique, isolates were cultured on numerous selective and differential media to find out their color, colony morphology and ability of fermentation (Karzan *et al.,* 2017).

Isolation of Bacteria Species

The specimens were cultured on sterile blood agar, chocolate agar and Mac-conkey agar plates at 37°C for 24 h in an incubator. Discrete colonies were picked based on their morphology and further sub-cultured on blood agar and chocolate agar to obtain pure strains. The isolated colonies were Gram stained and based on their Gram reactions were inoculated on different selective media — mannitol salt agar, cetrimide agar, eosin methylene blue agar. Different biochemical tests were conducted (catalase, coagulase, and oxidase tests). All the isolates that grew on selected agar media were then placed on nutrient agar and chocolate agar slants and maintained in a refrigerator at 4°C (Cheesbrough, 2006).

Identification and Characterization of Test Organism using Rapid Test Kits

Identification and characterization of the bacteria was carried out using Microgen Identification Kit (XYZ). The test was performed according to the manufacturer's specifications (API biomerieux). It was performed by adding saline suspension of the test organisms to each of the wells, and appropriate wells (1, 2, 3 and 9) were overlaid with sterile paraffin oil. After overnight incubation (18-24 hours) at 37°C, suitable reagents such as Nitrate A and B, Kovacs, Typtophan deaminase (TDA), Voges-proskauer (VPI and II) were added to wells 8, 10 and 12 for additional tests and colour changes of the different tests recorded. The results were converted into four to eight digits codes depending on the organisms being tested and interpreted using the Microgen Identification Software Package (MID-60) (Sylvester, 2016).

Antimicrobial Susceptibility Test

Preparation of Extract Concentration

This was carried out according to the method described by Srinivasan *et al.,* (2009). Stock solution of the plant extracts were prepared by adding 0.2 g of each crude plant extract in 1ml dimethyl sulphuroxide (DMSO). From each of the stock solutions, 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml concentrations were prepared using Two-fold serial dilution method (Srinivasan *et al.,* 2009).

Standardization of bacterial Inoculum

Using inoculum loop, over-night grown agar culture (bacteria and fungi) were transferred into a test tube containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard as described by the National committee for clinical laboratory standard (NCCLS, 2008).

Susceptibility Test of Bacterial and Fungal isolates to Different Concentrations of the Extracts

The antimicrobial activity of *Jatropha curcas* leaf crude extracts against *Staphylococcus aureus, Escherichia coli, Candida albicans,* and *Aspergillus fumigatus* were evaluated using agar well diffusion method of susceptibility test (Srinivasan *et. al.,* 2009*)*. Mueller-Hinton agar and Sabouraud Dextrose agar plates were inoculated with 0.1ml of standardized inoculum of each bacterium (in triplicates) using 0.1ml pipette and spread uniformly with sterile swab sticks. Three wells of 6mm size were made with sterile cork borer (6 mm) into the inoculated agar plates. Using micropipette, 0.1ml volume of the various concentrations; 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml each of the crude extracts were dispensed into wells of inoculated plates. DMSO was used as negative control. Commercially available standard antibiotic, ampicillin and fluconazole were used as positive control parallel with the extract. The prepared plates were then left at room temperature (37 ºC) for 10 minutes, allowing the diffusion of the extracts into the incubation at 37 ºC for 24 hrs in an incubator. The diameter of inhibition zones (DIZ) were measured and expressed.

Determination of Minimum Inhibitory Concentration (MIC)

The method used was the tube dilution method (Adesokan *et al*., 2007). Thus, the plant extracts were serially diluted from 200 mg/ml solution to obtain varying concentration. The concentrations were; 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml. Doubling dilutions of the extract were incorporated in Muller Hinton broth (Oxoid, UK) and Sabouraud Dextrose broth, and then inoculated with 0.1ml each of standardized suspension of the test organisms into the various test tube containing varying concentrations. Another set of test tubes containing only Mueller Hinton broth were used as negative control, and another test tube containing Mueller Hinton broth and test organisms were used as positive control. All the test tubes and controls were then incubated at 37 ^{o}C for 24hrs. After incubation period, the presence or absence of growth on each tube was observed. A loop full from each tube was further sub cultured onto nutrient agar to confirm whether the bacterial growth was inhibited.

Determination of Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

The MBC and MFC were determined by collecting 1ml of broth culture from the tubes used for the MIC determination and sub culturing into fresh solid nutrient agar plates. The plates were incubated at 37 ^{0}C for 24 h. The least concentration that did not show any growth after incubation was regarded as the MBC and MFC (Adesokan *et al*., 2007).

Analgesic studies of *Jatropha curcas* leaves

Acetic acid induced writhing in mice

Acetic acid induced writhing method described by (Koster *et al.,* 1959) was adopted for evaluation of analgesic activity. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, turning of trunk (Mishra *et al.,* 2011). 30 Swiss albino mice of both sexes were divided into five groups, 1 and 5 served as negative control (distilled water 10 ml/kg) and positive control (Diclofenac 20 mg/kg), while groups 3, 4, and 5 received 250 mg/kg, 500 mg/kg, and 1000 mg/kg of the extract. All administrations were per oral.

Sixty minutes after treatment, the mice received 0.6% acetic acid (10ml/kg) interperitoneally to induce pain. 5minutes after acetic acid injection, the animals were observed and number of writhes by each mouse was counted for 15minutes. Percentage inhibition was calculated using the following formular (Mishra *et al.,* 2011).

$$\% \text{ inhibition} = \frac{\text{Average number of writhes (control)} - \text{Average number of writhes (test)}}{\text{Average number of writhes (control)}}$$

RESULTS

Chemo-microscopical examination of powdered *Jatropha curcas* leaves revealed the presence of cellulose, tannins, starch, lignin, calcium oxalate, suberin, aleurone grain and mucilage but calcium carbonate was absent (Table 1).

Table 1: Chemomicroscopical studies of *Jatropha curcas* powdered leaves

Constituents	Inference
Starch	+
Gum and Mucilage	+
Cellulose cell walls	+
Lignin	+
Aleurone grain	+
Calcium oxalate crystals	+
Calcium carbonate	-
Suberized/Cuticular cell wall	+
Inulin	+

Keys: + = present, - = absent

Flavonoid, alkaloids, tannins, carbohydrate, glycoside and phenols were detected in both aqueous and methanolic extracts while saponins, steroids, triterpenes and anthraquinones were detected in aqueous extract but absent in methanolic extract (Table 2).

Table 2: Phytochemical screening of the Leaf extracts of *Jatropha curcas*

Phytochemical	Inference	
	Methanolic	Aqueous
Alkaloid	+	+
Flavonoid	+	+
Saponins	-	+
Cardiac glycoside	+	+
Tannins	+	+
Steroid	-	+
Triterpenes	-	+
Phenol	+	+
Anthraquinones	-	+
Carbohydrate	+	+

Keys: + = present, - = absent

Jatropha curcas leaf extract displayed appreciable DPPH free radical scavenging activity. However, the scavenging activity was lower than that of ascorbic acid (Table 3).

Table 3: Antioxidant activities of Methanolic extract of *Jatropha curcas* leaves

Analyte	**Concentration (µg/mL) / % Inhibition**							
	1000	**500**	**250**	**125**	**62.5**	**31.25**	**15.6**	**7.8**
Extract	67.0	78.2	84.0	86.5	86.8	85.7	83.8	66.17
Ascorbic acid	91.5	88.5	89.6	89.4	89.2	82.8	46.6	38.7

The trend of the inhibition of DPPH radical by the extract was concentration dependent with respect to the IC_{50} values (concentration of the extract to cause 50% inhibition), the DPPH radical scavenging ability of the extract showed the following trend methanol extract> Ascorbic acid. The IC_{50} values of the extract of *Jatropha curcas* leaves against DPPH free radical was 0.606 µg/ml. The high IC_{50} values obtained from this study which showed that extract had higher antioxidants activities against DPPH radicals than the standard ascorbic acid (Table 4).

Table 4: Antioxidant Activities of Methanolic extract of *Jatropha curcas* leaves

Sample	IC_{50} (µg/mL)
Extract	0.606
Ascorbic acid	8.781

The extract significantly decreased the number of writhes caused by acetic acid in a dose independent manner as shown in Table 5. The effects observed at 20 mg/kg more than that of 40 and 80 mg/kg of the extract. The extract and standard drug significantly decrease the number of writhes caused by acetic acid.

Table 5: Effect of methanol leaf extract of *Jatropha curcas* on Acetic Acid Induced writhing in mice

Treatment	Dose (mg/kg)	Mean No. of Writhes ± SEM	Inhibition (%)
Distilled water	10ml/kg	46.0±0.33	-
Diclofenac	(10)	12.0±0.34	73.9
Extract	(20)	16.0±0.43	65.2
Extract	(40)	20.0±0.49	56.5
Extract	(80)	23.0±0.65	50.0

Antimicrobial activities of methanol extract of *Jatropha curcas* leaves showed inhibition on all the tested clinical isolates of *Staphylococcus aureus, Escherichia coli, Candida albicans* and *Aspergillus fumigatus* at 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml (Table 6). The MIC and MBC/MFC of the *Jatropha curcas* leaf extract recorded respective values of 12.5 mg/ml and 25 mg/ml against *Staphylococcus aureus* and *Escherichia coli, Candida albicans* and *Aspergillus fumigatus.*

Table 6: Antimicrobial activity of methanol extract of *Jatropha curcas* leaves

Clinical isolates	Concentration (mg/ml)/Diameter zone of inhibition (mm)						MIC	MBC/MFC
	200	100	50	25	CPR/FLC	DMSO		
S. aureus	14	12	10	08	32	06	12.5	25
E. coli	15	13	10	09	34	06	12.5	25
S. fumigatus	13	11	10	08	30	06	12.5	25
C. albicans	16	14	11	09	34	06	12.5	25

DISCUSSION

The chemo-microscopic features are most valuable in the identification of powdered drug as their identification is largely based on the form, the presence or absence of certain cell types and cell inclusions. These are very important diagnostic pharmacognostic parameters for the identification and authentication of crude drugs especially in powdered plants (Chanda, 2011).

Preliminary phytochemical screening of *Jatropha curcas* leaf extracts (aqueous and methanol) revealed the presence of some important Phytochemical constituents such as carbohydrate, alkaloids, tannins, flavonoids, steroids, phenols and glycosides which have numerous functions. Crude, pure and isolated alkaloids and their synthetic derivatives have been used as analgesic, antispasmodic and bactericidal agents (Okwu and Okwu, 2004). Flavonoids have been shown to provide antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic and vasodilatory activity (Alan and Miller, 1996). Flavonoid also has immense antioxidant and anti-inflam-matory activity because of its ability to scavenge hydroxyl radicals, super oxide anions and lipid peroxy radicals (Okwu, 2004; Okwu and Josiah, 2006). Tanins have been used in the treatment of wounds especially those emanating from varicose ulcers and hemorrhoids (Njoku and Akumufula, 2007) and is able to stop bleeding during circumcision (Edeoga *et al.,* 2005). Steroids have been reported to have antibacterial and aphrodisiac properties. The presence of steroids in the extracts could support the antibacterial and aphrodisiac properties reported in the literature (Cushine and Lamb, 2005). Steroids are very important compounds due to their relationships with sex hormones and are useful in the treatment of sexual dysfunction (Oyedeni and Afolayan, 2011). This makes *Jatropha curcas* leaves to produce calming effect upon nervous system and there by useful in the treatment of insomnia, anxiety and similar disorders (Robins, 2001). The phytochemical constituents especially the secondary metabolites could be useful as guide to chemotaxonomic markers (Jonathan and Tom, 2008) that will aid in chemo taxonomical classification system and further phylogenetic studies in the euphorbiaceae family.

The extract exhibited analgesic potency. It significantly inhibited the abdominal constriction induced by acetic acid the mice. Acetic acid causes an increase in peritoneal fluids of PGF2 and PGF2α involving in part, peritoneal receptors and is very sensitive method of screening analgesic effect of compound (Mailafiya, 2014). The observed effect of the extract in this study suggested that postaglandins may be involved in the action of the extract (Mailafiya, 2014), the ability of the extract at 80 mg/kg and 20 mg/kg to produce comparable result to Diclofenac group in the study indicated moderate level of analgesic activity (Table 5). Acetic acid-induced writhing is among the sensitive methods for evaluating potential analgesic drugs or compounds that act peripherally. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, or turning of trunk (Mishra *et al.,* 2011). This model of response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathway. The acetic acid induced writhing model has been associated with increased level of prostaglandins (PGE2 and PGF2α) in peritoneal fluids as well as lipoxygenase products; this enhances inflammatory pain by increasing capillary permeability (Lakshman *et al.,* 2006; Khan *et al.,* 2010). The mechanism, by which any substance that inhibits these writhings causes analgesia, will be preferably by inhibition of prostaglandin synthesis, which is a peripheral mechanism of pain inhibition (Somachit and Shahid, 2003). The data presented suggests that the methanol extract of *Jatropha curcus* leaves possess peripheral analgesic property. The extract at all doses was shown to have analgesic property as evidenced in the model used. The reduction in the number of writhes caused by the extract, suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of prostaglandins and other endogenous substances.

The methanol extract of *J. curcas* inhibited *S. aureus, S. fumigatus, C. albicans* and *E. coli* with diameters of zone of inhibition of 14, 13, 16 and 15 mm respectively. The methanol extract of *Jatropha curcas* exhibited the highest activity against *S. aureus* and *E. coli* with MIC and MBC/MFC of 12.5 and 25 mg/ml respectively. Thus, the methanolic leaf extract showed a broad spectrum of antimicrobial activity against the test organisms. Falodun *et al.* (2003) had previously investigated antibacterial and antifungal activity of the petroleum ether, butanolic and ethanolic extracts of the of *J. curcus* against *E. coli*, *Klebsiella pneumoniae*, *S. aureus*, *P. aeruginosa* and *Bacillus subtilis*. Antimicrobial activity of medicinal plants has been linked with the presence of these chemical substances in medicinal plants (Cowan, 1999). The antibacterial activity of *J. curcas* may be due to the presence of these chemical substances in the leaves (Falodun *et al.,* 2006). The methanolic extract of *J. curcas* tested in this study showed moderate antibacterial activity against *S. aureus* and *E. coli*.

The results of this study were similar to those of Akinpelu *et al.* (2014) who reported strong inhibitory activity against *S. aureus* and *E. coli* in the methanolic extract of the root and leaves of *J. curcas*. Aiyelaagbe *et al.* (2007) reported the antimicrobial activities of some secondary metabolites from the leaves and root extracts of the plant against some microorganisms associated with sexually transmitted diseases. In this study, the methanolic leaf extract showed moderate antibacterial activity against *S. aureus* and *E. coli*. While the differences in results may be due to the varying concentrations of active substances in the root, stem bark and leaves, it may also be due to the different concentrations used (Igbinosa *et al.,* 2009). However, the actual concentration of the extract used in agar well diffusion method employed by Igbinosa *et al.* (2009) may be difficult to determine as the stated concentration is nominal and does not represent the actual amount of substance present in the volume of extract dispensed into agar well. The work of Kisangau *et al.* (2007) showed the possibility of discrepancy in the results of antibacterial activity tested by agar well diffusion method and the paper disc diffusion method. Kisangau *et al.* (2007) found no activity in the water extract of *J. curcas* when agar well was used but strong inhibitory activity of the extract was seen in the disc diffusion method. The effect of these two methods of antibacterial activity screening of plant extracts may require further study. A major goal of the antimicrobial screening of medicinal plants is to find substances with novel mechanism of action against drug resistant strains.

CONCLUSION

The pharmacognostic standards for the powder of *Jatropha curcas* leaves established and these data could be used as a diagnostic tool for the standardization, identification of this medicinal plant and hence inclusion into the pharmacopoeia for official use. The extract was found to possess considerable antioxidant and analgesic properties at doses tested. This partly justifies the claim for the traditional use of the plant in the treatment of toothache and oxidative stress. The results of the study showed that methanol extract of *Jatropha curcas*were active against *Staphylococcus aureus, Escherichia coli, A. fumigatus* and *Candida albicans*.

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