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# COMPARATIVE STUDY OF PHYTOCHEMICAL SCREENING, MINERAL COMPOSITION AND ANTIMICROBIAL CAPACITIES OF *JATROPHA CURCAS* AND *LONGE PEDUNCULATA*

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## ABSTRACT

This study was conducted to investigate and compare the phytochemical screening, mineral composition and antimicrobial capacity of aqueous and organic (ethanol and n-hexane) extracts from dry leaves of *Jatropha curcas* and *Longepedunculata. In vitro* phytochemical screening of all crude extracts from dry leaves of *Jatropha curcas* and *Longepedunculata* was carried out and showed positive results for alkaloid, tannin, flavonoid, saponin, sterol and oxalate while quantitative analysis revealed that important secondary metabolites were slightly abundant in *Jatropha curcas* than *Longepedunculata,* thus; alkaloid 6.4%, tannin 50.0%, flavonoid 16.0%, saponin 40.0%, oxalate 0.01g/100g and alkaloid 5.4%, tannin 50.0%, flavonoid 14.6%, saponin 40.0%, oxalate 0.0092g/100g respectively, but phytate was found to be relatively

**Introduction:**

*Jatrophacurcas* or phyoic nut is a drought resistant monoecious large shrub or small tree 5m to 8m tall, it is a species of flowering plant in small surge family of *Euphorbiaceca* belonging to the genus Jatropha which consists of over 170 species, producing oil containing seeds. Its leaves appear with a petiole 3-20cm long and a blade broadly ovate in outline usually shallowly 5-lobes (Heller, 1996). *Jatropha curcas* originates from neighbouring parts of Central America, Caribbean where the species was already used by the Mayas. Jatropha was likely distributed by Portuguese ships via the cape to other countries in Africa and Asia long time ago and is



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abundant in *Longepedunculata*than *Jatrophacurcas* with 10.8g/100g and 9.8g/100g respectively. These different phytochemicals are shown to perform different biological activities in humans and animals. Antimicrobial activity of the extracts (aqueous ethanol, n-hexane) against some selected bacteria prepared at 200mg/ml using agar disc diffusion method showed that *Bacillus subtilis* exhibit the highest level of susceptibility to antimicrobial effect of *Jatropha curcas* while *Bacillus subtilis* and *Staphilococcus aureus* had the highest level of susceptibility to antimicrobial effect of *Longepedunculata*. Hence, they are therefore recommended for pharmaceutical and therapeutic purposes.

***Keywords**: Jatropha curcas, Longepedunculata, phytochemical, antimicrobial.*

Now naturalized throughout the tropics and subtropics (Swallow, 2007). Throughout the tropics, different part of *Jatropha curcas* have been used for various purposes which range from being used as a love fence (hedge) to prevent animal from entering gardens, roads and houses to medicinal application, *Jatropha curcas* is used for the treatment of various human and veterinary ailments. Traditionally,it is used for treating dysentery and diarrhea. It also serves as anti-bacterial, antibiotic, antimalarial and it cures leprosy. It expels internal parasites (Muller, 2010). Biologically active substances from Jatropha seeds have been reported with activities ranging from anti-inflammatory effect (Muhammad *et. al*., 2018); haemagglutination (Cano,Asseleih and Plumbley, 2017); insecticidal (AbdoulHabou*et. al*., 2014); molluscidal effect (Angaye,*et. al*., 2014) and skin irritation (Reddy Prasad, 2012). The seed oil and oil rich seeds are used as a purgative and to expel internal parasites, their application leads to strong irritation of the gastrointestinal tract and in some cases poisoning (Tomomatsu, 2007). The use of *Jatrophacurcas* for the production of bio-fuel is novel. However, the primary use of *Jatropha curcas* seeds in Egypt is for oil extraction which is a good alternate to biofuel and has proven success either independently or by mixing the diesel to operate farm machinery (Martin, 1985). In South-

West Nigeria, *Jatrophacurcas*is known as 'Ewe lapalapa', 'Noixde' in French, 'Ainidazugu' in Hausa, 'Wuluida' in Igbo, Castor oil in Chinese, Curcas, Barbados nut, pig nut, physic nut, castor oil, purging nut, fig nut, wild oil nut in English and general name (Mayeux, 1985).

## Materials and Methods

### Collection of plant materials

*Leaves of Jatrophacurcas*(Ewe lapalapa) were locally sourced from 'Osunte' in Offa Local Government Area, Kwara State while leaves of *Securidaca longipedunculata* were collected from Ondo town in Ondo State, Nigeria.

### Phytochemical investigation

### Sample preparation

The leaves of *Jatropha curcas* and *Securidaca longipedunculata* were washed with distilled water and dried separately under shade at room temperature for four weeks. The dried leaves were grind into fine powdered using laboratory mortar and pestle.

### Extraction procedure for dry leaf powder samples

The ethanol extract of *Jatropha curcas* and *Securidaca longipedunculata* were prepared by soaking 50g of the powdered samples into 100ml of ethanol in two separate beakers for 24hours. While the aqueous extracts were prepared by soaking 50g of powdered samples into 100ml of distilled water in a beakers for 48hours at room temperature. The n-hexane extracts were prepared by soaking 50g of powdered samples into 100ml of n-hexane for 24hours at room temperature in a beaker. The extracts were filtered with filter paper and the filtrates was subjected to water bath under reduced pressure for total evaporation of the solvent used for the extraction.

### Qualitative screening of the phytochemical in the plant investigated

Ethanolic and aqueous extracts of *Jatropha curcas* and *Securidaca longipedunculata* were used to perform the phytochemical screening using standard methods for the following;

a. **Test for alkaloid (Wagner's Reagent)**

   A fraction of the extracts were treated with 3-5drops of Wagner's reagent and formation of reddish brown precipitate was produced

which indicates the presence of alkaloid in the plants (Sofowora, 1993).

b. **Test for flavonoid (Alkaline reagent test)**
2ml of the extract was heated with few drops of 20% of sodium hydroxide (NaOH) solution, formation of intense yellow colour which in addition of dilute hydrochloride acid turnscolourless which showed that flavonoids are present in the extract (Trease and Evans, 1989).

c. **Test for Phenol (Ferric Chloride test)**
A fraction of the extracts was treated with 5% aqueous ferric chloride, formation of deep blue or black colour shows the presence of phenol in the plants (Trease and Evans, 1989).

d. **Test for Saponin (Foam test)**
25ml of extracts was added to 6ml of water in a test tube and shook vigorously. The mixture was observed for the formation of persistent foam showing the presence of saponins (Sofowora, 1993).

e. **Testfor sterol (Liebermann-Burchard test)**
1ml of the extract was treated with drops of chloroform, acetic anhydride and conc. H2SO4 and formation of dark pink or red colour was observed indicating the presence of sterol (AOAC, 1990).

f. **Test for Tannin (Braymer's test)**
2ml of extract was treated with 10% alcoholic Ferric Chloride solution and formation of blue or greenish solution was observed indicating the presence of tannin (Trease and Evans, 1989).

g. **Test for Oxalate**
2 to 3ml of the extracts were added to few drops of glacial ethanolic acid. A greenish black colouration was observed which shows that oxalate is present (AOAC, 1990).

**Quantitative determination of the chemical constituents**

Quantitative analysis for phytochemicals in the extracts of the plants were carried out using procedures from literature with little modifications, Oxalate (Kayode, 2011), Flavonoid (Boham, 1994), Saponin (Obadoni, 2011), Alkaloid (Harbone, 1999), Phytates and Tannin (Joselyn, 1970).

**Standardization of inoculation**

0.1ml of 1% barium chloride was added to 9.9ml of 1% of sulphuric acid to give 1 McFarland standard, it was then reconstituted to another 10ml of

sterile distilled water to make 0.5ml of McFarland standard. The broth culture of the test organism was then compared in terms of turbidity of 0.5ml Mcfarland. A loopful of the standard culture was used for antimicrobial activity (Moody *etal.*, 2006).

i. **Test for the organism**

The test organism used for the antimicrobial assay of *Jatrophacurcas*and *Securidacalongipedunculata*includes *Staphylococcus aureus, Proteus, Escherichia coli, Bacillus subtilis*.

ii. **Confirmation and identification of organism**

The identity of organisms collected were confirmed by sub-culturing each of the isolate in different selective and differential medium such as brilliant agar, eosin methyl-blue agar and MacConkey agar and was further characterized with the use of different biochemical test which include Gram stain, catalase, Coagulase, Oxidase, indole, motility and the characteristic with a standard taxa (Cheesbruogh, 2006).

iii. **Screening of extract for antimicrobial activity using well method**

Agar well diffusion method was employed to test for antimicrobial activities of the plants. 1g of Aqueous was reconstituted in 5ml of sterile distilled water to make 20% concentration and was vortered for homogeneity. The broth culture of the test organism was compared to the turbidity of 0.5% McFarland standard, 3 drops of standardized cultured was transferred into a sterile petridish, freshly prepared cooled tempered sterile molten Muller Hilton agar was added to petri dish that contained standard organism, it was rocked gently and allowed to set at room temperature. A sterile cock borer was used to make 2 well of 6mm diameter on the solidified agar, a drop of 0.1ml of each extracts of aqueous, ethanolic and n-hexane was introduced into the well and was labelled respectively, it was incubated at 37$^{o}$C for 24hours, control Agar plate were made in parallel and included (OVC) Organism Viability Control, (MSC) Medium Sterility Control (Olafimihan, 2003).

iv. **Determination of Minimum Inhibitory Concentration using paper Disk Method**

0.9g, 0.8g, 0.7g, 0.6g, 0.5g, 0.4g, 0.3g, 0.2g, 0.1g of plant extracts of aqueous, ethanolic and n-hexane were accurately weighed respectively on a weighing balance and dissolved in 2ml of sterile distilled water while ethnolic and n-hexane solvents were dissolved in 2ml of DMSO to make a concentration of 200mg/ml, 180mg/ml,

60mg/ml, 40mg/ml, 20mg/ml respectively. The sterile paper disk was soaked in each extract and allowed to air-dry. Molten mullerhitton agar was poured into a sterile petridish and allowed to solidify at room temperature test organisms. The organisms were standard as described for antimicrobial assay. Each plate was then streaked with a loopful of standardized sensitive test organism (Organism that were sensitive to the plate extract during determination of antimicrobial activity) each paper disk of the extract of both aqueous, ethanolic and n-hexane extracts at different concentration was placed on a nutrient agar that contain the standardized organism, control agar plate were made in parallel include Organism Viability Control (OVC), Medium Sterility Control (MSC) and Extract Sterility Control (ESC). The plate was incubated at 37$^{o}$C for 24hours (Fawole, 2003).

## Mineral Composition

20g of each sample were ashed in a muriffle furnace set at 450$^{o}$C for 3hours, the ashes were cooled and digested using 0.1m hydrochloric acid, the digest was filtered into a 100ml volumetric flask while the filtrate was made up to the mark 100ml. the filtrate was used for mineral determination using Atomic Absorption Spectrometer (Fawole, 2003).

## Results and discussion

Phytochemical constituent of the plants studied was investigated for the following metabolites: alkaloids, flavonoid, phenol, saponin, tannin, sterol and oxalate. Qualitative screening of phytochemical constituents of *Jatrophacurcas* L and *Securidaca longipedunculata* using aqueous extract indicates the presence of alkaloid, phenol, sterol and absence of flavonoid, saponin, tannin and oxalate while the ethanolic extract indicate the presence of alkaloid, flavonoid, tannin, oxalate, sterol and absence in phenol and saponin as shown in table 1 below.

**Table 1**: Qualitative phytochemical screening of ethanol and aqueous extracts of *Jatropha curcas* and *Securidaca longipedunculata*

| Inference | Extracts | Phytochemicals | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Alkaloids | Flavonoids | Phenol | Saponin | Tannin | Oxalate | Sterol |
| Ethanol Extract | *Jatropha curcas* | ++ | ++ | -- | -- | ++ | ++ | ++ |
| | *Securidaca longipedunculata* | ++ | ++ | ++ | ++ | -- | -- | ++ |
| Aqueous Extract | *Jatrophacurcas* | ++ | -- | ++ | -- | -- | -- | ++ |
| | *Securidaca longipedunculata* | -- | -- | ++ | ++ | -- | -- | ++ |


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++ = Represent Positive (Present), -- = Represent Negative (Absence)

Quantitative analysis of phytochemical in the plant indicate that it contain these phytochemicals in varying amount in the leaf extracts. The phytochemical with the highest quality was Tannin follow by Saponin, flavonoid, phytate, alkaloid, oxalate respectively as shown in table 2 below. Phytochemicals are non-nutritive chemical that have disease preventive properties which are considered to be beneficial to human health. The presence of bioactive ingredients and the quantitative determination of chemical constituent of the plants studied shows that the leaves are rich in alkaloid, tannin, flavonoid,phytate and saponin.
Alkaloids have a wide range of pharmacological properties including antimalarial', antiasthma, anticancer properties. The presence of flavonoidshelp to prevent platelets sickness and platelets aggregation. The presence of tannin possess physiological astringent and haemostatic properties which hasten wound healing and ameliorated inflammed mucus membrane. Saponin are active as expectorant and is very useful in the treatment of upper respiratory track inflammations: it also have anti-diabetic and anti-fungi properties (Olafimihan, 2003).

Table 2: Quantitative estimation of Phytochemical from *Jatropha curcas and Securidaca longipedunculata*

| S/No. | Chemical group | Values in %g/100g for *Jatropha curcas* | Values in %g/100g *Securidaca longipedunculata* |
|---|---|---|---|
| 1 | Oxalate | 0.013 | 0.0092 |
| 2 | Flavonoid | 16 | 14.6 |
| 3 | Saponin | 40 | 40 |
| 4 | Tannin | 5.0 | 50 |
| 5 | Alkaloid | 6.4 | 5.42 |
| 6 | Phytate | 9.82% | 10.8 |

% = Percentage g = Gram

## Antimicrobial activity

Antimicrobial activities of aqueous, ethanol, n-hexane extracts of *Jatrophacurcas* and *Securidacalongipedunculata*was investigatedat various concentration against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichiacoli* and *proteus* in 200mg/ml.

The aqueous extract exhibited significant inhibitory effect on Bacillus subtilis as shown by increase in zone of inhibition as the concentration of extract increase. Table 3 shows the inhibitory effect of increasing doses of the extracts and control of test organisms. Table 4 shows the effect of standard antibiotic on the test organism. The lowest concentration of the extract that inhibited growth of test organisms was 20g/ml, the inhibition of test organisms by the extracts was done dependent with the lowest concentration being 20mg/ml. this was the Minimum Inhibitory Concentration (MIC) of the extract for *Bacillus substilis* as indicated below.

Table 3: Inhibitory effect of increasing doses of the extracts and control of test organisms.

| Solvent/Dose | Extracts | Test organisms | | | | | |
|---|---|---|---|---|---|---|---|
| | | Bacillus subtilis | *Staphylococcusaureus* | E. coli | Proteus | Control | Ciprofloxacin |
| Ethanol Extract | *Jatropha curcas* | 11mm | -- | -- | -- | -- | 12mm |
| | *Securidaca longipedunculata* | 10mm | -- | -- | -- | -- | 14mm |
| n-hexane | *Jatropha curcas* | -- | -- | -- | -- | -- | 12mm |
| | *Securidaca longipedunculata* | -- | 7mm | -- | -- | -- | 16mm |
| Aqueous Extract | *Jatropha curcas* | 8mm | -- | -- | -- | -- | 15mm |
| | *Securidaca longipedunculata* | -- | -- | -- | -- | -- | 14mm |
| Dose (ml) | | 0.5ml | 0.5ml | 0.5ml | 0.5ml | 0.5ml | 0.5ml |

Key: - = No activity
Mg/ml = Milligram/milliliter
Minimum inhibitory concentration of sample extracts prepared at various concentration in Mg/ml against bacillus subtilis

Table 4: Inhibited growth of test organisms was 20g/ml

| Extract | Zone of inhibition (mm) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Conc. mg/ml | 180 | 160 | 140 | 120 | 100 | 80 | 60 | 40 | 20 |
| Aqueous | 9mm | 6mm* | - | - | - | - | - | - | - |
| Ethanol | 5mm* | - | - | - | - | - | - | - | - |
| n-hexane | - | - | - | - | - | - | - | - | - |
| Control | | | | | | | | | |
| Ciprofloxacin | 14mm | 10mm | 8mm | 4mm | | | | | |

Key: - = No activity mm = millimeter
Mg/ml = milligram/mill * = minimum inhibitory concentration


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From table 5 below, the result indicate the highest level micro-element in *Jatrophacurcas* leaves was potassium (k) and Calcium (Ca). The presence of these could also contribute to various nutraceutical properties displayed by *Jatrophacurcas* leaf. Potassium has been implicated as the principal cation in intracellular fluid, regulation of osmotic pressure conduction of nerva impulse, the presence of iron shows the useful in prevention of anemia, the presence of calcium and magnesium are important in the formation of bones and teeth as a cofactor of enzyme and a component of ATP, DNA, RNA and cell membrane, manganese perform various important functions in human like the formation of hemoglobin, growth and sexual maturation and the recommended daily allowance (RDA) value are added for comparism

Table 5: Mineral composition

| Elements | Concentration in Mg/kg | RDA (%RDA) |
|---|---|---|
| **Manganese (Mn)** | 4.31 | 2.3 (1.9) |
| **Zinc (Zn)** | 0.92 | 12 (7.66) |
| **Lead (Pb)** | 0.05 | 250 (2.0) |
| **Cadmium (Cd)** | ND | 30 - |
| **Iron (Fe)** | 2.83 | 20 (1.42) |
| **Magnesium (Mg)** | 5.23 | 350 (1.5) |
| **Calcium (Ca)** | 18.60 | 3500 (5.31) |
| **Sodium (Na)** | 6.28 | 2300 (2.73) |
| **Potassium (K)** | 30.50 | 3500 (8.7) |

Key: ND = Not detected
Mg/kg = Milligram/kilogram

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## Conclusion and Recommendation

Summarily, it is noted that *Jatrophacurcas* contains some important bioactive components with pronounced antibacterial activities and also some mineral composition, hence, can be used as therapeutic and medicinal agents in prevention and treatment of wide spectrum of ailments.

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