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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL PROPERTIES OF METHANOLIC LEAF EXTRACT OF *JATHROPHA CURCAS LINN.*

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ABSTRACT

Jathropha curcas linn. is highly a medicinal plant. This research project aims or stems at the phytochemical screening and antibacterial properties of the methanolic extract of leaf of *Jathropha curcas Linn.* which has a crude extract of 23.48w/w was tested against some organisms namely; Salmonella typhi, Pseudomon aerognosa, Staphylococcus aereous, Streptococcus pyrogenes, Escherichia coli, Klebsiella pneumonia, Shigella dysentriae. The extraction process is done by reflux method using methanol as the solvent. The phytochemical screening conducted shows that carbohydrate, cardiac glycoside, saponin and alkaloid are highly present, flavonoid and tannin are moderate while phlobatannin, terpenoid, anthraquinone and soluble starch are found to be absent in this methanol extract of the leaves of *Jathropha curcas linn.* The antibacterial test conducted on the organisms showed activities as follows; it was found that majority of the test organisms were found to have some activities on the methanol extract of the dried leaves of *Jathropha curcas Linn* at higher concentration of 400mg/ml and 600mg/ml unlike in the 200mg/ml. Salmonella typhi was show to be the more activity to the methanolic extract in concentration of 200,400, and 600mg/ml while Klebsiella pneumonia, streptococcus pyrogenes, Escherichia coli, Shigella dysentriae, Pseudomon aerognosa were found to show moderately activity in concentration of 200,400 and 600mg/ml and staphylococcus aereous has less activity in concentration of 200mg/ml while it show no activity to 400 and 600mg/ml concentrate.

Keyword: Phytochemical Screening, Methanolic Leaf Extract, *Jatropha Curcas,* Antibacterial properties.

INTRODUCTION

The term medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants are considered as rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants consider as important source of nutrition and as a result of that these plants are recommended for their therapeutic values (Rasool H, 2012). These days the term **"Alternative Medicine"** became very common in western culture, it focus on the idea of using the plants for medicinal purpose. But the current belief that medicines which come in capsules or pills are the only medicines that we can trust and use. Even so most of these pills and capsules we take and use during our daily life came from plants. Medicinal plants frequently used as raw materials for extraction of active ingredients which is used in the synthesis of different drugs. Like in case of laxatives, blood thinners, antibiotics and antimalaria medications, contain ingredients from plants (Rasool H, 2012). Medicinal plants remain feasible source of new compound for the drug development process. *Jatropha curcas* L. is becoming a very useful economic resource both in agriculture, phytomedicine development and development of new lead compounds (Mkoma and Mabiki, 2012). The plant belongs to the family Euphorbiaceae, and the genus *Jatropha* has over 170 species. *Jatropha curcas* L. has over 19 species most of which are toxic due to the presence of phorbol esters (Campa *et al.*, 2010). The plant survives mostly in the tropics and has great plasticity to survive in arid climate. Traditionally, the seed oil has been reported to be used as purgative and for skin diseases, the leaf decoction is used for cough and as disinfectant after birth, the stem sap is used to stop bleeding, while the latex has antimicrobial property. The leaf has been reported to contain flavonoids (apigenin), glycosyl-flavonoids (vitexin and isovitexin), sterols (stigmasterol), sapogenin steroids and terpenes (Campa *et al.*, 2010).

In Indonesia, *J. curcas Linn* has long been used in traditional medicine, because it contains chemical compounds that are antibacterial, fever, anti-inflammatory and inhibitor of bleeding (Hariana A, 2006). *Jatropa curcas* leaves are often used as a medicine for skin infections, the seed is used for constipation, treating cervical cancer, and fungal infections (Zulkifli N, 2005). In some countries, *J. curcas Linn* is used as a drug, such as malaria medicine, in Mali (Henning K, *et al*, 1997) and in Africa as a drug haemostatic (Gubitz G.M, *et al*, 1999), skin infections, diarrhea, and several other diseases caused by microorganisms (kambu K, 1990). *Jatropha curcas Linn* oil is used as medicine

constipation, skin diseases and relieves the pain of rheumatism. In addition, the fruit of *J. curcas Linn* is used directly as medicine constipation and anthelmintic drugs (Gubitz G.M, *et al*, 1999). 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. curcas Linn* exhibits antimicrobial activity (Akinpelu D.A *et al* 2009). The crude stem extracts of *J. curcas Linn* to inhibit the growth of bacteria family Enterobacteriaceae like *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* (Igbinosa O.O, 2009). The ability of the crude stem extracts of *J. curcas Linn* to inhibit the growth of bacteria is an indication of its broad spectrum antimicrobial potential which may be employed in the management of microbial infections.

MATERIALS AND METHOD

Sample and Sampling.

The sample were collected from University of Maiduguri and its vicinity and was identified by Mr. Benesheik of the Department of Biotechnology University of Maiduguri, the fresh leaf were conveyed to Department of chemistry University of Maiduguri where it is allowed to dry at room temperature for 7 days in part four (iv) laboratory of the same department. After drying it was grinded into powdered form using pestle and mortar, it weighed 200g after grinding, hence it is made ready for further use.

EXTRACTION

Procedure

Two hundred grams (200g) of the grinded sample was weighed using and electric weighing balance, the weighed sample was transferred to 5litre refluxing flask, and then 2litres of 95% methanol was poured unto the sample in the reflux flask. The mixture was reflux for 5hours after refluxing the mixture was allowed to cool and then the mixture was filtered, the filtrate is a green viscous liquid it was allowed to dry under hot air oven at 50^0C, after drying it still maintains the green coloration, which is referred to as the crude extract. The crude extract weighed 23.48g then the extract is transferred into a clean dried container, hence ready for further use.

PHYTOCHEMICAL SCREENING

Chemical test were conducted on the methanolic extract using standard procedures to identify the chemical constituents present as described bellow;

TEST FOR CARBONHYDRATE

General Test (Molisch Test)

Few drops of Molisch reagent where added to each of the extract dissolved in distilled water. This was followed by adding 1ml of conc. Tetraoxosulphate (vi) acid (H_2SO_4) by the side of the tube; so that acid will form a layer beneath the aqueous layer. The mixture was then allowed to stand for two minutes and then dilute with 5ml of distilled water. Formation of violet colour at the interphase of the two layers will be a positive test (Trease and Evans, 2002).

Test for Ketoses (Salivanoff's Test)

Few crystals of resorcinol and 2ml of hydrochloric acid were added to a small quantity of each extract and the solution boiled for 5 minutes. A red coloration indicate the presence of ketoses (Vishnoi, 1979).

Test for free reducing sugars (Fehling's test) and test for combined sugars was carried out using procedure as described by (Trease and Evans 2002).

Tests for Soluble Starch

A small quantity of each extract was boiled with 1ml of 5% potassium hydroxide (KOH), cooled and acidified with H_2SO_4. A yellow coloration indicates the presence of soluble starch (Vishnoi, 1979).

Test for free anthraquinones (Borntrager's test) and test for combined anthraquinones (Borntrager's test) was carried out using procedure as described by (Trease and Evans 2002).

Test for Cardiac Glycoside

Salkowski's Test

Each of the plant extract (0.5g) under study will be dissolved in 2ml of chloroform tetraoxosulphate (VI) acid was carefully added by the side of the test tube to form a lower layer. Appearance of a yellow at the interphase indicates for the presence of a steroidal ring (i.e. aglycone portion of cardiac glycoside) or methylated sterols (Silva *et al*, 1998).

Liebermann-Burchard test

Steroidal Nucleus

To (0.5g) of each extract, 2ml of acetic anhydride was added, when dissolved it was cooled well in conc. tetraoxosulphate (VI) acid will be added carefully. Color development form violet to blue or bluish green indicated the presence of a steroidal ring i.e. aglycone of cardiac glycoside (Silva *et al*, 1998).

Test for Terpenoid

A little of the extract was dissolved in ethanol. To it 1ml of acetic anhydride was then be added followed by the addition of conc. H_2SO_4. A color changes from pink to violet show the presence of terpenoids (Silva *et al*, 1998).

Test for Saponins

One gram of each extract was boiled with 5ml of distilled water filtered and filtrate divided into two portions. To the first portion, about 3ml of distilled water was added and then shaken for about 5minutes. Frothing which persist on warming was an evidence for the presence of saponins (Sofora, 1993).

Test for Flavonoid

Shinoda's TEST

Each of the extract (0.5g) to be tested was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of conc. HCL. A pink, orange to purple coloration indicates the presence of flavonoids (Markham, 1982).

Ferric Chloride Test

Each extract was boiled with distilled water and then filtered. To 2ml of the filtrate, few drops of 10% ferric chloride solution were then added. A green violet coloration indicates presence of phenolic hydroxyl group (Trease and Evans 2002).

Lead Ethanoate Test.

A small quantity of the each extract will be dissolved in water and filtered. To 5ml of each of the filtrate, 3ml of lead ethanoate solution will then be added. Appearance of a buff-colored precipitate indicates the presence of flavonoids (Brain and Turner, 1975).

Sodium Hydroxide Test

A small quantity of the extract each was dissolved in water and filtered; to this 2ml of the 10% aqueous sodium hydroxide was added to produce a yellow coloration. Change in color from yellow to colorless on addition of dilute hydrochloric acid indicate the presence of flavonoids (Trease and Evans, 2002).

Test for Tannins

Ferric Chloride Test

Each extract was boiled with distilled water and then filtered. To 2ml of the filtrate, few drops of 10% ferric chloride solution were then added. A green

violet coloration indicates presence of phenolic hydroxyl group (Trease and Evans 2002).

Lead Ethanoate Test

A small quantity of the each extract will be dissolved in water and filtered. To 5ml of each of the filtrate, 3ml of lead ethanoate solution will then be added. Appearance of a buff-colored precipitate indicates the presence of flavonoids (Brain and Turner, 1975).

TEST FOR PHLOBATANNINS.

Small amount of each extract was boiled with distilled water and then filter the filtrate was further boiled with 1% aqueous HCl. The appearance of red precipitate shows the presence of phlobatannins (Trease and Evans, 2002).

TEST FOR ALKALOIDS

Preliminary test for alkaloid

Each of the extract (0.5g) was stirred with 5ml of 1% aqueous HCl on water bath and then filter, 3ml will be taken and divided equally into 3 portions in a test tube. To the first portion few drops of Drogendroff's reagent were added; the occurrence of orange-red precipitates was taken as positive. To the second 1ml Mayer's reagent was added and appearance of buff-colored precipitate indicates the presence of alkaloids and to the last, 1ml, few drops of Wagner's reagent will be added and a dark-brown precipitate will indicate the presence of alkaloids (Brain and Tuner 1975).

ANTIBACTERIAL ACTIVITY TEST

Preparation of Culture Media (Nutrient Agar and Peptone Water)

28g of nutrient agar powder was weighed using weighing balance. The powder was distilled in 100ml of distilled water, the solution was mixed up homogeneously until the crystal are completely dissolved, using auto clave tap, the cover was tightly raped to avoid spillage when autoclaving. The solution was loaded inside a basin, and then put into the autoclave machine. The temperature and pressure gauge was set and the machine was tight in all the opposite direction, finally it was plugged to a light source for stabilization. The machine was kept on and allowed to work at 121 degree Celsius for 15minutes at 151bs (pounds pressure). The machine was kept on and allowed to cool for one hour, gently remove the cap and the media. The media was allowed to cool and store in refrigerator for further uses.

INNOCULATION

The sterile nutrient agar plate was loaded inside a drier to drain excess water of condensation using a sterilized wire loop; I obtain a discrete colony of the young cultured isolation (bacteria) of respective, 5 gram negative and 2 gram positive. The isolates are inoculated into 1ml of a sterile peptone water, allowed to stayed for a minute, the components are transferred to the nutrient agar plate by a pour plate method and swan to the entire surface, drain off the excess fluid and allow the plate right side up.

MINIMUM INHIBITION CONCENTRATION VALUE (MIC)

These are the concentration set to determine the drugs or extract concentration in mg/ml.

METHOD

Sterile test tubes are arranged in a test tube rack, 2ml of peptone water was poured into all the tubes and 1loopfull of the isolates was added. Add 2ml of the extract into the 1st tube and titrate across the test tube and discard the tubes, repeat all this to all the isolates and incubate at 37^{0}C for 18-24 hours.

READING

Reading was taken by the physical examination, notice where turbidity starts to show bacterial growth. This is termed as T, where the tube is termed as C, the minimum inhibition concentration value (MIC) is taken from the first clear tube. Read and record each from all the tubes.

RESULTS AND DISCUSSION

Percentage yield of extract: this is calculated using the formula below;

$$\text{Percentage yield} = \frac{\text{weight of crude extract}}{\text{Weight of leave powdered used}} \times 100$$

Where;
Weight of crude extract =23.48g
Weight of leave powdered used = 200g

$$\text{Hence; \% yield} = \frac{23.48 \times 100}{200}$$

$$= 0.1174 \text{ w/w}$$

PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITIES

TABLE 1: PHYTOCHEMICAL SCREENING RESULTS OF METHANOLIC EXTRACT OF LEAF OF *JATHROPHA CURCAS LINN.*

s/n	Phytochemicals	Results
1	Test for carbonhydrate	
i.	General test- molisch test	+
ii.	Test for monosaccharide Barfoed	Nil
iii.	Test for free reducing sugar (fehlings)	+
iv.	Test for combined reducing sugar	+
v.	Test for ketoses	+
2.	Test for soluble starch	-
3i.	Test for free anthraquinones	-
ii.	Test for combined anthraquinone	-
4.	Test for cardiac glycoside	
i.	Salkowski's test	+
ii.	Lieberman Burchard	+
5.	Test for terpenoid	-
6.	Test for saponins	+
7.	Test for flavonoid	
i.	Shinoda's test	+
ii.	Ferric chloride test	+
iii.	Lead acetate test	-
iv.	Sodium test	+
8.	Test for tannins	
i.	Ferric chloride test	+
ii.	Lead acetate	-
9.	Test for phlobatannin	-
10.	Test for alkaloid	
i.	Dragendroff's reagent	+
ii.	Mayers reagent	+

Keys:

+ = present

- = absent

TABLE 2: ANTIBACTERIAL ACTIVITY RESULT OF METHANOLI LEAF EXTRACT OF *JATHROPHA CURCAS LINN.*

	Concentration in mg/ml / Zone of inhibition in (mm)		
Organism	**200mg/ml**	**400mg/ml**	**600mg/ml**
Salmonella typhi	+10 +08 +07	+11 +11 +10	+11 +13
Pseudomonas	+09 +09 +07	+10 +08	+12
aeruginosa	+06 00 00	+07	+10 +09
Staphylococcus aureus	+07 +06 +06	00 00	+08
Streptococcus	+09 +08 +07	00	00 00
pyrogenes	+07 +07 +06	+10 +07	00
Escherichia coli	+08 +07 +07	+06	+16 +12
Klebsiella pneumoniae		+11 +11 + 10	+10
Shigella dysenteriae		+07 +06 +	+13 +11
		06	+10
		+10 +11 +10	+09 +08
			+08
			+12 +10
			+09

Keys:
+ = Zone of inhibition in (mm)
00 = No zone of inhibition in (mm).

Table one (1) shows the result of the phytochemical screening of the methanol leaf extract of *Jathropha curcas Linn*. The result shows that carbohydrate, cardiac glycoside, saponin and alkaloid are highly present in the dried methanol extract of *jathropha curcas Linn*. Flavonoid and tannin are moderately present while phlobatannin, terpenoid, anthraquinone and soluble starch are found absent in the methanol extract of the dried leaf of *Jathropha curcas Linn*. These classes of compound are known to show curative activity against several pathogens and is therefore not surprising that the plant is used traditionally to cure a cascade of illnesses.

Table two (2) above shows the result of antibacterial activity of leaf extract of *Jathropha curcas Linn.* In this experiment, it was found that majority of the test organisms were found to have some activity to the methanol extract of the dried leaf of *Jathropha curcas Linn* at higher concentration of 400mg/ml and 600mg/ml unlike in the 200mg/ml with the zone of inhibition in (mm) as shown on table 2 above. Salmonella typhi show more activity to the methanolic extract in concentration of 200,400, and 600mg/ml with zone of inhibition in (mm) as shown on table 2 above, while Klebsiella pneumonia, streptococcus pyrogenes,

Escherichia coli, Shigella dysentriae, Pseudomon aerognosa were found to show moderately activity to concentration of 200,400 and 600mg/ml and staphylococcus aureus has least activity in 200mg/ml while it show no activity to 400 and 600mg/ml concentrate with zone of inhibition in (mm) as shown on table 2 above.

CONCLUSION AND RECOMMENDATION

Base on the results obtained it can be concluded that the methanolic leaf extract of *Jathropha Curcas Linn* showed the presence these secondary metabolites; carbohydrate, cardiac glycoside, saponin and alkaloid are highly present while the remaining metabolites are moderately present and some absent. The extract showed antibacterial activities in which Salmonella typhi showed much activity to the methanol extract while staphylococcus aureus least in low concentration and no activity in higher concentration and the remaining test organisms showed moderate activity. The plant metabolites which are present in the extract are known to show curative activity against several pathogens and are therefore not surprising that the plant is used traditionally to cure a cascade of illnesses. Because of the high activity the methanol extract showed on salmonella typhi it can be recommended for the treatment of typhoid, malaria and other diseases which are caused by the organisms which the extract showed activity on.

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