PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF THE CRUDE, COLD AND ETHANOLIC EXTRACTS OF THE ROOT, STEM BARK AND LEAVES OF JATROPHA CURCAS (PHYSIC NUT OR PURGING NUT)

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Abstract- The root, stem bark and leaves of Jatropha curcas which have some traditional medicinal uses was investigated. Phytochemical screening of the root, stem bark and leaves reveals the presence of Alkaloids, Tannins, Saponins, Resins, Anthraquinone, Glycosides, Cardiac Glycosides, Steroids and Carbohydrates. The antimicrobial screening of the cold, crude and ethanolic extracts carried out on the following organisms; Staphylococcus aureus, Escherichia coli, Salmonella typhi, and Pseudomonas aeruginosa showed that the ethanolic extracts of the stem bark had wider range of activity on these organisms than the crude and cold extracts of the plant. The ethanolic extract of the stem bark inhibited the growth of S. aureus, E,coli, Salmonella typhi and P. aeruginosa at concentration of 40% while the crude and cold extracts of the stem bark had Minimum Inhibition Concentration (MIC) of 50% on the organisms. The leaves extracts had a MIC of 60% on the organisms except P. aeruginosa and E. coli which had a MIC of 50%. The crude and cold extracts of the root had a MIC of 70% on S. aureus, E.coli, S.typhi while the ethanolic extract of the root had a MIC of 60% on P. aeruginosa. The MIC was found to be lowest on the stem bark extracts which indicates mat the stem back may contain the highest concentration of active ingredient.

Keywords- Phytochemical, Antimicrobial, Minimum Inhibitory concentration.

I. INTRODUCTION

The global change that preoccupies the research institution, pharmaceutical companies and academy is that of the search for newer sources of antibiotics. Since many infectious agents are becoming resistant to synthetic drugs (Latha and Namabran, 2006). Infectious diseases are the world major threat to human and account for almost 50,000 deaths everyday (Ahmed and Beg, 2001). The situation has further been complicated with the rapid development of multidrug resistance by microorganisms to the antimicrobial agents available. Even though, pharmaceutical industries have produced a number of new antibiotics in the last three decades, persistence to these drugs by microorganisms has increase (Nascimento et al, 2007). In general bacteria have the genetic, ability to transmit and acquire resistance to drug which are utilized as therapeutic agents (Cohen, 1992) such a fact is cause for concern because of the number of patients in hospitals who have suppressed immunity and due to new bacteria strains which are multi resistant. Consequently, new infections can occur in hospitals resulting in high mortality.

For a long period of time plants have been a valuable source of natural therapies (Nascimento et al, 2007). The use of plant compounds for pharmaceutical purpose has gradually increased across the world especially Africa, Asia and Latin America, according to world health organization medicinal plants would be the best source to obtain a variety of drugs about 80% of individuals from developed countries uses traditional medicine which has compound derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties safely and efficiency (Nascimento et al, 2007). Jatropha curcas is a small tree or shrub with smooth gray or green bark which exudes whitish coloured latex when cut. Normally, it grows between 3-5m in height but can attain a height of upto 8-10 under favourable condition (Grim, 1999). The plant has large green to pale-green alternate to sub-opposite, three to five-lobbed with a spiral phyllotaxis. Fruits are produced in winter and it may produce several crops during the year if soil moisture is good and temperature is sufficiently high. Each inflorescence yields a bunch of approximately ten or more ovoid fruits. Three bi-valved cocci is formed after the seeds mature when the capsule changes from green to yellow, after two to four months (Singh, 2000).

Phytochemicals are bioactive non-nutrient plants components in fruits, vegetables, grains and plant foods that have been likened to reducing the risk of major degenerative diseases.

The use of plant extracts and phytochemicals both with known, antimicrobial properties can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency many plants have been used because of their antimicrobial traits which are due to compounds synthesized in the secondary metabolism of the plants. These products are known by their active substance for instance the phenolic compounds which are part of the essential oils as well as in tannin (Nascimento et al, 2007).

he rapid increase in the antibiotic resistance of microorganisms to the available synthetic drugs and the discomforting side effect of synthetic drugs necessitate the search for an alternative source of treatment more tolerable like herbal drugs. Since

herbal extracts have significant effect on some microorganisms a trial with J. curcas that is readily available comes to mind.

II. METHODOLOGY

The specimens of the plant (root, stem bark and leaves) of J. curcas were collected from Maiduguri bye pass in Bauchi State and Kuru Jos, Plateau State Nigeria in May, 2012.

The test organisms used in this study were S. typhi, P. aeruginosa, E. coli and S. aureus. The organisms were collected from the Abubakar Tafawa Balewa Teaching Hospital Laboratory and were stored on a nutrient agar slant in screw capped bottles in a refrigerator until required.

Preparation of Plant Sample
The fresh sample were collected in a clean container, sorted and washed with tap water to remove all dirt and debris.

Extraction of Bioactive Ingredients from the Plant
Plant active ingredients were obtained by different extraction methods described below:

Cold Extraction Method.
The procedure used in this research work for cold extraction was that of Omoregbe and Osaghae (1997). This was obtained by soaking 40g of the grinded (sieved) sample in 350ml of distilled H_2O in a sterile conical flask and left to stand with occasional string for 24hours at room temperature.

Crude Extraction Method.
The extraction method described by Effriam (2001) for cold and hot water and ethanol extraction was adopted. The fresh plant sample collected after proper washing was crushed & grounded where necessary. Ethanol was used as a solvent due to it's ability to dissolve and produce more of the components from the plant.

Confirmatory Identification of Organisms
The test organisms were identified by both morphological characteristics and biochemical techniques as described by Cheesbraigh, (2010).

Antibacterial Susceptibility Testing
The method described by Chung (1990) for cup plate technique was used. Nutrient agar was prepared according to the manufacturer's instructions, sterilized and allowed to cool to about 45°C. The pure cultures of the tests organism were introduced on the surface of the various media plate. Holes of about 4mm in diameter were made on nutrient agar in the plate using a sterile cork borer. 0.5ml of the extracts were introduced into the holes at different concentration and given a pre-incubation time of 30 minutes before incubating at 37°C for 24 hours. Observation was done after wards for zone of inhibition.

Determination of the Minimum Inhibitory Concentration (MIC)
This was determined by adopting the tube dilution method of Fuest and Frobister (1978) and Onawunmi et al (1989). In this method, the lowest concentration inhibiting growth of Micro organisms was taken as the MIC.

III. RESULT

Below are the results of phytochemical and antimicrobial screening of the crude, cold and ethanolic extractions of the root, stem bark and leaves of J. curcas including the Minimum Inhibition Concentration (MIC).

All metabolites screened in this research work were present except flavonoids which was found to be absent as shown in table 1. The presence of these metabolites may be due to the medicinal value of J. curcas. The zone of inhibition of the extracts on the microorganisms shows that the stem bark has the highest zone of inhibition on the various organisms tested (see table 2). This may be attributed to the fact that during dry season the plant shed its leaves to control transpiration and all nutrients and active components are transported from the root to the stem bark and there they are retain. This account for the effectiveness of the stem barks on the test organisms.

Table 3 and 4 shows the MIC in percentage of the crude and cold extracts of the root on the organisms tested. The result showed that the MIC obtained on all organisms was 70%. The ethanolic extract of the root shows a MIC of 70% on S. aureus, E. coli and S. typhi except P. aeruginosa which had a MIC of 60% (see table 5). The MIC in percentage of the crude and cold extracts of the stem bark on the organisms tested shows that the organisms has a MIC of 50% as shown in table 6 and 7 while table 8 which is the ethanolic extract of the stem bark shows a MIC of 40%. Table 9 which is the crude extract of the leaves show a MIC of 60% on S. aureus, E. coli and S. typhi except P.aeruginosa which had a MIC of 50%. The cold extract of the leaves shows a MIC of 70% on S. aureus, E. coli and S. typhi except P. aeruginosa which had a MIC of 60% as shown in table 10. The ethanolic extract of the leaves shows a MIC OF 60% on S. aureus, and E. coli and a MIC of 50% on S. aureus and P.aeruginosa (see table 11). Figure i-iv shows the effect of different methods of extraction of various tree parts on zone of inhibition on each of the organisms (see table 2 for more details).

Table 1: Phytochemical Screening of the Root, Stem Bark and Leaves of Jatropha Curcas

Phytochemical	Root	Stem bark	Leaves
Alkaloids	+	+	+
Tannins	+	+	+

Saponnins	+	+	+
Flavonoids	-	-	-
Resins	+	+	+
Anthraquinone	+	+	+
Glycosides	+	+	+
Cardiac Glycosides	+	+	+
Carbohydrates	+	+	+
Steroids	+	+	+

KEY: + = Present - = Absent

Table 2: Diameter of Zone of Inhibition in Millimeter (MM)

	Root			Stem Bark			Leaves		
Test Organisms	Crude	Cold	Ethanolic	Crude	Cold	Ethanolic	Crude	Cold	Ethanolic
S. aureus	X	X	5	13	14	17	8	10	12
E. coli	10	7	12	14	13	16	11	7	8
S. typhi	X	8	8	15	12	15	12	X	10
P. aeruginosa	8	X	12	13	14	16	8	12	10

KEY: X = No Inhibition

Table 3: MIC of the Crude Extracts (Root)

	Concentrations in Percentage (%)							
Test Organisms	100	80	70	60	50	40	20	10
S. aureus	--	--	--	++ *	++	++	++	++
E. coli	--	--	--	++ *	++	++	++	++
S. typhi	--	--	--	++ *	++	++	++	++
P. aeruginosa	--	--	--	++ *	++	++	++	++

KEY: * = Minimum Inhibitory Concentration, -- = No growth, ++ = Growth

Table 4: MIC of the Cold Extracts (Root)

	Concentrations in Percentage (%)							
Test Organisms	100	80	70	60	50	40	20	10
S. aureus	--	--	-- *	++	++	++	++	++
E. coli	--	--	-- *	++	++	++	++	++
S. typhi	--	--	-- *	++	++	++	++	++
P. aeruginosa	--	--	-- *	++	++	++	++	++

KEY: * = Minimum Inhibitory Concentration, -- = No growth, ++ = Growth

Table 5: MIC of the Ethanolic Extracts (Root)

	Concentrations in Percentage (%)							
Test Organisms	100	80	70	60	50	40	20	10
S. aureus	--	--	-- *	++	++	++	++	++
E. coli	--	--	-- *	++	++	++	++	++
S. typhi	--	--	-- *	++	++	++	++	++
P. aeruginosa	--	--	--	-- *	++	++	++	++

KEY: * = Minimum Inhibitory Concentration, -- = No growth, ++ = Growth

Table 6: MIC of the Crude Extracts (Stem Bark)

	Concentrations in Percentage (%)							
Test Organisms	100	80	70	60	50	40	20	10
S. aureus	--	--	--	--	-- *	++	++	++
E. coli	--	--	--	--	-- *	++	++	++
S. typhi	--	--	--	--	-- *	++	++	++
P. aeruginosa	--	--	--	--	-- *	++	++	++

KEY: * = Minimum Inhibitory Concentration, -- = No growth, ++ = Growth

Table 7: MIC of the Cold Extracts (Stem Bark)

	Concentrations in Percentage (%)							
Test Organisms	100	80	70	60	50	40	20	10
S. aureus	--	--	--	--	-- *	++	++	++
E. coli	--	--	--	--	-- *	++	++	++
S. typhi	--	--	--	--	-- *	++	++	++
P. aeruginosa	--	--	--	--	-- *	++	++	++

KEY: * = Minimum Inhibitory Concentration, -- = No growth, ++ = Growth

Table 8: MIC of the Ethanolic Extracts (Stem Bark)

	Concentrations in Percentage (%)							
Test Organisms	100	80	70	60	50	40	20	10
S. aureus	--	--	--	--	--	-- *	++	++
E. coli	--	--	--	--	--	-- *	++	++
S. typhi	--	--	--	--	--	-- *	++	++
P. aeruginosa	--	--	--	--	--	-- *	++	++

KEY: * = Minimum Inhibitory Concentration, -- = No growth, ++ = Growth

Table 9: MIC of the Crude Extracts (Leaves)

	CONCENTRATIONS IN PERCENTAGE (%)							
Test Organisms	100	80	70	60	50	40	20	10
S. aureus	--	--	--	--	++	++	++	++
E. coli	--	--	--	--	++	++	++	++
S. typhi	--	--	--	--	++	++	++	++
P. aeruginosa	--	--	--	--	-- *	++	++	++

KEY: * = Minimum Inhibitory Concentration, -- = No growth, ++ = Growth

Table 10: MIC of the Cold Extracts (Leaves)

	Concentrations in Percentage (%)							
Test Organisms	100	80	70	60	50	40	20	10
S. aureus	--	--	--	++	++	++	++	++
E. coli	--	--	--	++	++	++	++	++
S. typhi	--	--	--	++	++	++	++	++

P. aeruginosa	--	--	--	-- *	++	++	++	++

KEY: * = Minimum Inhibitory Concentration, -- = No growth, ++ = Growth

Table 11: MIC of the Ethanolic Extracts (Leaves)

	Concentrations in Percentage (%)							
Test Organisms	100	80	70	60	50	40	20	10
S. aureus	--	--	--	--	--	++	++	++
E. coli	--	--	--	--	--	++	++	++
S. typhi	--	--	--	--	-- *	++	++	++
P. aeruginosa	--	--	--	--	-- *	++	++	++

KEY: * = Minimum Inhibitory Concentration, -- = No growth, ++ = Growth

IV. DISCUSSION

The presence of phytochemical (i.e. bioactive substance) have been reported by Sriniwassan el al (2001) to confer resistance to plants against bacteria, fungi and pest. This thereby, explains the antibacterial activities demonstrated by the plant extract used in these work. The organisms used were positively identified and confirmed by the various biochemical test carried out as described by (Cheesbrough, 2010).
The phytochemical screening of the root, stem bark and leaves of Jatropha curcas , revealed the presence of saponnins, tannins, alkaloids, glycosides, resins, steroids, * anthroquinone, cardiac glycosides in all the plant parts studied as show in Table I. The presence of saponins, tannins, alkaloids in all the plant parts studied (root, stem bark, and leaves) calls for an in depth study on the plant. The metabolites are of various pharmacological importance. Many saponins, alkaloids and their aglycones have been reported by Hostettmann et al (1995) to have varied uses as antiulcerogenic, anti-inflammatory, iibrinolytic, analgesic and anti-gum infection. The presence of saponins, glycosides, tannins in the plant could be responsible for the traditional use of J. curcas in the relaxation of muscles, treatment of wounds, boils, cold and insecticides as reported by Hostettman (1995).

The result on the study of the antibacterial property of the extracts of the root, stem bark and leaves of J. curcas plant indicates that the samples posses inhibitory substances against bacterial colonization. The result showed that the ethanolic extracts are more active than the crude and cold extracts as seen in table 2 and figure i-iv. This agreed with the results of Villegas (1997) which he confirmed to be due to the presence of some very active bioactive substances. The MIC result presented in Table 3-11 showed that the ethanolic extract of the stem bark inhibited and fully prevented the growth of S. aureus, S.typhi, and P, aeruginosa at a concentration of 40% as recorded in table 8. This might be due to the high content of antibacterial constituent in the stem bark and the ethanol as the best solvent for extraction as reported by Owunubi (1998). Meanwhile, the ethanolic extract gave greater zone of inhibition compared to crude and water extract. This could be attributed to the fact that ethanol tends to penetrates the different parts of the plant thereby extracting more of f the bioactive substance which confer sensitivity to microorganisms. The activity of the ethanolic extract could not be said to be due to residual ethanol since proper and adequate evaporation of the ethanol was carried out.

In related development, it was observed that most of the bacteria are sensitive to the crude extract. This shows that the concentration of the active ingredient has not been altered hence it was only crushed to obtained the juice from the extract as can be seen from figure i-iv and agreed with the findings of Omoregbe and Osaghae (1997). The water extract also inhibited the growth of the organism to some extent. One wonders why water without any dissolving chemicals could perform wonderfully well. This might be attributed to the fact that the rate of absorption of the water extract by the cell membrane of the different organisms is very high. Furthermore, cold water extraction does not alter or destroy the delicate structure and balance of the photochemical and nutritional component as stated by Walker (1996).

CONCLUSION

Jatropha curcas can be used in therapeutic preparations. The ethanolic extracts of the stem bark is highly favoured in this regard and have proved to be more efficacious than the root and leaves as seen in this in -vitro studies. These results agree with the claims by our medicinal practitioners that the plant can be used in the treatment of fever, wounds, diarrhea, gastrointestinal troubles, tooth infections, boils and other disease caused by the test organism.

RECOMMENDATION

Further research work on the combine parts of the plant (root, stem bark and leaves) to widen the scope of this research work is recommended.

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