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Broad Spectrum Antimicrobial Activity of Extracts of *Jatropha curcas*

Egharevba Henry Omoregie* and Kunle Oluyemisi Folashade
Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research & Development (NIPRD), Idu Industrial Layout, Idu, P.M.B. 21 Garki, Abuja, Nigeria.

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ABSTRACT

Jatropha curcas L. leaf and stem were extracted successively with hexane, ethylacetate, methanol and aqueous methanol. The extracts were tested *in vitro* for activity against standard strains microorganisms and clinical isolates. The zones of inhibition, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined. The organisms exhibited different degree of susceptibility to the inhibitory activity of the crude extracts. The zones of inhibition, MIC and MBC/MFC ranged from 14-37 mm, 1.25-10 mg/ml and 2.5-20 mg/ml for the susceptible organisms, respectively. The methanol extract was the most active and exhibited good activity against most food pathogens like *Escherichia coli, Staphylococcus aureus, Salmonella typhimurium, Shigella dysenteriae, Psuedomonas aerugunosa, P. flourescenses, Klebsiella pneumonia* and *K. ozaenae*. The highest activity was an MIC of 1.25 mg/ml and MBC of 2.5mg/ml. The activities observed could be due to the presence of some of the secondary metabolites like tannins, alkaloids, sterols, glycosides, saponins, terpenes and flavonoids which have been reported from the plant by other workers.

INTRODUCTION

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 (Saetae and Suntornsuk, 2010; 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). The stem bark also contains stigmasterol, tannins (37%), lectin (curcin or toxalbumin) which are responsible for the agglutination of red blood cells, and other cyanogenic compounds that give blue colour, as well as alkaloids such as jatrophine or jatropham and atherospermidine (Thomas et al., 2008; Campa et al., 2010; Gupta et al., 2011). The latex from the stem also contains tannins (10%), hydrocyanic acid, toxalbumine and jatrophine. The fruit/seed is rich in fat/oil, which is used as biofuel in many countries. It also contain trypsin inhibitors, phytic acid (12%), saponins and curcin (Saetae and Suntornsuk, 2010; Campa *et al.*, 2010). Some other compounds which have been reported from the plant include tetradecyl-(*E*)-ferulate, 3-O-(*Z*)-coumaroyl oleanolic acid, heudelotinone, epi-isojatrogrossidentadione, 2-alpha- hydroxy- epiisojatrogrossidione, 2- methyanthraquinone, lathyrane and podocarpane (Ravindranath *et al.*, 2004). The toxic varieties contain the phorbol esters, which is a tumor promoter and activate protein kinase C (PKC). Some derivatives of phorbol esters have been reported to have antimicrobial, antitumor, molluscicidal and insecticidal properties (Saetae and Suntornsuk, 2010). The plant has a long history of use in Africa (Sofowora, 2008). In Nigeria, the leaf and stem decoction is used singly or in combination with other recipes for the treatment of skin diseases, and stomach disorder. However, very little work has been done on the Nigeria species. The reported antimicrobial work on the local species covers very few microbes. This work intends to reveal the broad-spectrum antimicrobial potential of the plant found in North-Central Nigeria.

* *Corresponding Author*
Phone number: +234 805 155 9005
E-mail: omoregieegharevba@yahoo.com

MATERIALS AND METHODS

All the solvents and reagents used in the study were of Analar grade and were sourced from Zayo-Sigma Abuja, Nigeria.

Collection and Extraction of Plant Material

The was plant collected on the 28th of April 2009 from Chaza Suleja, Nigeria and identified by the Taxonomist in the Department of Medicinal Plant Research and Traditional Medicine of the National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria.

The stem and leaf were rinsed with clean water and air-dried for two weeks, and then pulverized using a mechanical grinder. The pulverized plant was kept in an air-tight cellophane bag until required.

Test Organisms

The organisms used include standard strains, *Staphylococcus aureus* NCTC 6571, *Bacillucsubtilis* NCTC 8236, *Eschericia coli NCTC 10418, Pseudomonas aeruginosa* NCTC 6750, *Salmonella typhimurium ATCC 9184, Klebsiella pneumonia* ATCC 10031 and *Staphylococcus aureus* ATCC 13704, and clinical isolates; *Staphylococcus aureus,* Methicilin Resistant *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus faecalis, Corynebacterium ulcerans, Listeria monocytogenes, Bacillus subtilis, Bacillus cereus, Escherichia coli, Klebsiella pneumonia, Klebsiall aozaenae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas flourescense, Salmonella typhimurium, Shigella dysenteriae, Aspergillus fumigates, Candida albicans, Microsporum gypseum* and *Trichophyto nrubrum.*

Preparation of extracts

200g of the pulverized plant was macerated successively in Hexane, ethylacetate, methanol and aqoues methanol for 24 hrs each. The extracts were then filtered under vacuum and the filtrates concentrated at 46°C using a rotary evaporator. The methanol concentrate was evaporated to dryness on a water bath. For aqueous-methanol extract (70% methanol in water), the concentrate was freeze-dried using a table-top freeze-dryer. The extracts were stored in airtight sample bottles and kept in a desiccator until required.

Preparation of Stock Concentration of Extract for Antimicrobial Screening

A test stock concentration of 10 mg/ml for aqueous methanol, methanol and ethylacetate extracts were prepared by dissolving 0.1 g of each extract in 10 mls of distilled water in separate test tubes. For the hexane extract a concentration of 20 mg/ml was prepared by dispersing 0.2 g in 10mls of DMSO. The positive control drugs were sparfloxacin (0.2 mg/ml), erythromycin (0.5 mg/ml) and flouconazole (0.5 mg/ml), obtained from Zayo-Sigma Abuja Nigeria.

Antimicrobial activity

Well diffusion method described by Hugo and Russel (1992) was used to determine microbial susceptibility. Determination of zones of inhibition, minimum inhibitory concentration and minimum bactericidal and fungicidal concentrations were carried out using thewell diffusion method, agar dilution and broth dilution methods respectively as described by Egharevba and co-workers (Egharevba *et al.*, 2010).

RESULTS

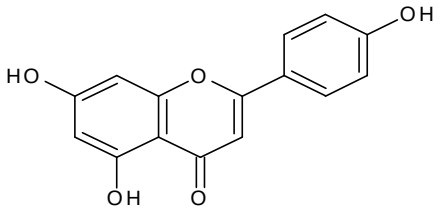
The results as shown in Tables 1 and 2 below. The zones of inhibition of the organisms by the extracts are shown in Table 1, while the minimum inhibitory concentration (MIC) and the minimum bactericidal/fungicidal concentration (MBC/MFC) are shown in Table 2.

DISCUSSION

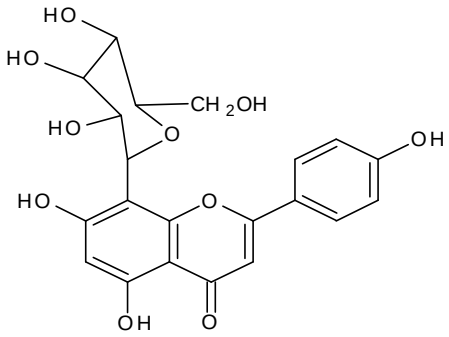
The extracts exhibited selective activities against the various pathogens. The methanol extract was generally the most active. This may be attributed to the presence of soluble phenolic and polyphenolic compounds (Igbinosa *et al.* 2009). Only the methanol and aqueous-methanol extracts showed activity against *Candida albicans* ATCC 10231. Kubmarawa (2007) and Gupta (2010) earlier reported the susceptibility of *C. albicans* and *B. subtilis* to extract of *J. curcas*. This result differs from that obtained by Igbinosa (2009) and Saetae (2010), which reported a broad antifungi activity from the stem and seed extracts, respectively (Kubmarawa*et al.*, 2007; Igbinosa*et al.*, 2009; Saetae and Suntornsuk, 2010). However, the clinical isolate of *C. albicans* was resistant to the aqueous-methanol extract in this study. The methanol, ethylacetate and hexane extracts showed varied activities against the clinical isolate of *Candida albicans*. This observed selectivity in susceptibility between the typed strains and clinical isolate may be due to development of strain resistance. The most susceptible organism to the methanol extract appeared to be *E. coli* NCTC 10418 and *S. aureus* NCTC 6571. In the ethylacetate extract, the most susceptible organisms were *Proteus mirabilis* and *vulgaris*. These organisms had an MIC of 1.25 mg/ml (Table 2). This suggests that methanol extract could be very effective in treating gastrointestinal tract infections, skin infections as well as other food poisoning from *E. coli* and *Staphylococcus* species, while the ethylacetate extract could be useful in managing urinary tract infections due to *Proteus* bacteria (Kubmarawa *et al.*, 2007). *S. aureus* is a pyogenic bacterium known to play significant role in invasive skin diseases including superficial and deep follicular lesions and other nosocomial infections (Reuben *et al*., 2008; Adamu *et al*., 2009). *E. coli* has been reported to be the commonest cause of urinary tract infection and accounts for about 90% of first urinary tract infection in young women (Brooks *et al.*, 2002 and Usman *et al.*, 2007). The susceptibility of *Salmonella typhimurium, Shigella dysenteriae, Psuedomonas aerugunosa* and *P. flourescenses* to methanol

extract strengthens the suggestion that it could be useful for gastroenteritis, food poisoning and urinary tract infections. The methanol extract also showed activity against *Klebsiella pneumonia* and *Klebsiella ozaenae*, suggesting its usefulness in respiratory tract infection. The hexane extract was generally medium in activity relative to the methanol. Hence it may not be the best extraction solvent for anti-infective preparations.

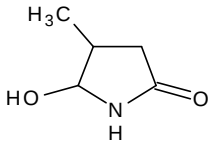
This suggests that the plant activity may reside in the averagely polar to polar compounds like the phenols, polyphenols, and other fairly polar compounds like flavonoids (apigenin and vitexin), alkaloids (jatrophine or jatropham) (Fig. 1), etc. Where the level of the toxic phorbol esters are considered high and not fit for consumption, formulations may be used externally for fungi and bacteria skin infections.



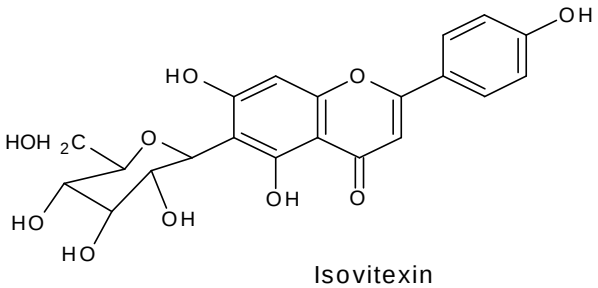
Apigenin



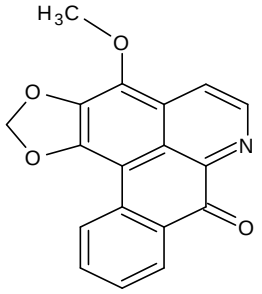
Vitexin



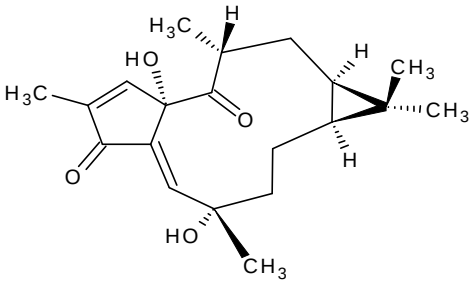
Jatropham



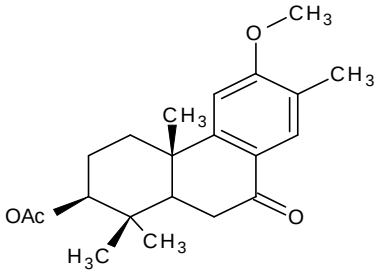
Isovitexin



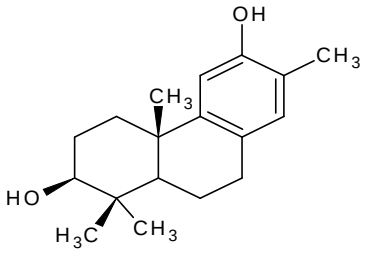
Atherospermidine



15-*epi*-(4*E*)-jatrogrossidentadione



3b -acetoxy-12-methoxy-13-methyl-podocarpa-8,11,13-trien-7-one



3b ,12-dihydroxy-13-methylpodocarpane-8,10,13-triene

Fig. 1: Some compounds from *Jatropha species.*

Table. 1: Zone of Inhibition.

TEST ORGANISM	STRAINS	ZONE OF INHIBITION (mm)						
		J. curcas Extract				Control drugs		
		Am	M	E	H	Sp	Er	Fl
Staphylococcus aureus	NCTC 6571	21	30	22	19	29	22	-
Bacillus subtilis	NCTC 8236	19	22	27	21	20	22	-
Escherichia coli	NCTC 10418	17	37	20	20	22	24	-
Pseudomonas aeruginosa	NCTC 6750	22	29	0	0	24	0	-
Salmonella typhimurium	ATCC 9184	20	29	22	17	25	27	-
Klebsiella pneumoniae	ATCC 10031	0	27	22	0	25	29	-
Staphylococcus aureus	ATCC 13704	21	28	24	20	20	27	-
Candida albicans	ATCC 10231	19	24	0	0	0	0	22
Staphylococcus aureus	Isolate	25	30	27	17	20	21	-
Methicilin Resistant Staph. aureua	Isolate	22	23	24	0	0	27	-
Streptococcus pyogenes	Isolate	0	25	27	14	20	26	-
Streptococcus faecalis	Isolate	20	27	0	17	24	29	-
Corynebacterium ulcerans	Isolate	22	26	27	0	25	30	-
Listeria monocytogenes	Isolate	22	28	26	14	25	24	-
Bacillus subtilis	Isolate	24	25	0	14	20	25	-
Bacillus cereus	Isolate	0	27	0	17	24	26	-
Escherichia coli	Isolate	22	24	27	0	27	20	-
Klebsiella pneumoniae	Isolate	19	22	0	14	26	19	-
Klebsiella ozaenae	Isolate	20	22	30	15	24	18	-
Proteus mirabilis	Isolate	0	20	31	0	22	20	-
Proteus vulgaris	Isolate	0	25	29	0	0	24	-
Pseudomonas aeruginosa	Isolate	0	22	0	0	19	22	-
Pseudomonas flourescenses	Isolate	19	23	0	14	0	24	-
Salmonella typhimurium	Isolate	19	27	29	19	20	22	-
Shigella dysenteriae	Isolate	20	25	0	14	20	20	-
Aspergillus flavus	Isolate	0	0	0	0	-	-	27
Aspergillus fumigatus	Isolate	0	0	0	0	-	-	23
Candida albicans	Isolate	0	17	19	14	-	-	24
Microsporum gypseum	Isolate	0	0	0	0	-	-	20
Trichophyton rubrum	Isolate	0	0	0	0	-	-	24

Am = 70% aqueous methanol extract*;* M= *methanol* extract*;* E= *ethylacetate* extract*;* H= *hexane* extract Sp= Sparfloxacin; Er = Erythromycin; Fl = Flouconazole.

Table. 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)/Minimum Fungicidal Concentration (MFC).

S/N	TEST ORGANISM	STRAINS	*J. curcas* Extracts							
			MIC				MBC//MFC			
			Am	M	E	H	Am	M	E	H
1	*Staphylococcus aureus*	NCTC 6571	2.5	1.25	2.5	10	10	2.5	10	20
2	*Bacillus subtilis*	NCTC 8236	5	2.5	2.5	5	10	5	5	20
3	*Escherichia coli*	NCTC 10418	5	1.25	2.5	5	10	5	10	20
4	*Pseudomonas aeruginosa*	NCTC 6750	2.5	2.5	-	-	10	10	-	-
5	*Salmonella typhimurium*	ATCC 9184	2.5	2.5	2.5	10	10	5	10	20
6	*Klebsiella pneumoniae*	ATCC 10031	-	2.5	2.5	-	-	5	10	-
7	*Staphylococcus aureus*	ATCC 13704	2.5	2.5	2.5	5	10	5	5	20
8	*Candida albicans*	ATCC 10231	5	2.5	-	-	10	10	-	-
9	*Staphylococcus aureus*	Isolate	2.5	2.5	2.5	10	5	2.5	5	20
10	*Methicilin Resistant Staph. aureus*	Isolate	2.5	2.5	2.5		10	5	10	-
11	*Streptococcus pyogenes*	Isolate	-	2.5	2.5	10	-	5	5	20
12	*Streptococcus faecalis*	Isolate	2.5	2.5		10	10	5		20
13	*Corynebacterium ulcerans*	Isolate	2.5	2.5	2.5	-	10	5	5	-
14	*Listeria monocytogenes*	Isolate	2.5	2.5	2.5	10	10	5	5	20
15	*Bacillus subtilis*	Isolate	2.5	2.5	-	10	10	5	-	20
16	*Bacillus cereus*	Isolate	-	2.5	-	10	-	5	-	20
17	*Escherichia coli*	Isolate	2.5	2.5	2.5		10	10	5	
18	*Klebsiella pneumoniae*	Isolate	2.5	2.5		10	10	10	-	20
19	*Klebsiella ozaenae*	Isolate	2.5	2.5	2.5	10	10	10	5	20
20	*Proteus mirabilis*	Isolate	-	2.5	1.25	-	-	10	2.5	-
21	*Proteus vulgaris*	Isolate	-	2.5	1.25	-	-	5	2.5	-
22	*Pseudomonas aeruginosa*	Isolate	-	2.5	-	-	-	10	-	-
23	*Pseudomonas flourescenses*	Isolate	-	2.5	-	10	-	10	-	20
24	*Salmonella typhimurium*	Isolate	2.5	2.5	2.5	10	10	5	5	20
25	*Shigella dysenteriae*	Isolate	2.5	2.5	-	10	10	5	-	20
26	*Aspergillus flavus*	Isolate	-	-	-	-	-	-	-	
27	*Aspergillus fumigatus*	Isolate	-	-	-	-	-	-	-	
28	*Candida albicans*	Isolate	-	5	5	10	-	10	10	20
29	*Microsporum gypseum*	Isolate	-	-	-	-	-	-	-	-
30	*Trichophyton rubrum*	Isolate	-	-	-	-	-	-	-	-

Am = 70% aqueous methanol extract*;* M= *methanol* extract; E= *ethylacetate* extract*;* H= *hexane* extract.

CONCLUSION

This study supports the traditional use of *Jatropha curcas* for the treatment of various infectious diseases in different regions of the world, and may serve as a good source of novel bioactive compounds. The study also shows that the Nigerian plant may be good as an antibacterial recipe but may not be very useful as an antifungal agent.

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