

Original Research Article

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Phytochemical Screening and Evaluation of the Effects of Methanolic and Ethanolic Extracts of *Jatropha curcas* and *Chlorophora excelsa* on *Candida albicans* and *Staphylococcus aureus*

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ABSTRACT

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The 80% methanolic and ethanolic extracts of the dried leaves of *Jatropha curcas* were subjected to phytochemical and antimicrobial sensitivity screening. The phytochemical screening for the methanolic extract revealed the presence of alkaloids, tannins, saponins, flavonoids and cardiac glycosides while the ethanolic extract revealed the presence of alkaloids, tannins, saponins, flavonoids, anthraquinones and combined anthraquinones. The phytochemical screening of the methanolic extract of *Chlorophora excelsa* also revealed the presence of the above metabolites found in *J. curcas* excluding the cardiac glycosides whilst the phytochemical screening of the ethanolic extract revealed the presence of alkaloids, tannins, saponins and flavonoids. The antimicrobial activity of both leaf extracts were independently assessed using clinical isolates of *Candida albicans* and *Staphylococcus aureus*. The techniques used were the well-in-agar diffusion and agar dilution methods which showed that the leaves were active against *Staphylococcus aureus* but not against *Candida albicans*.

Introduction

The use of medicinal plants for the treatment of various conditions has persisted amongst African tribes for generations. It could be deemed the oldest form of health care management. Successive generations across the continent still rely chiefly on herbal medicines. The WHO (2005) maintains that traditional medicine serves the health needs of a large portion of the world's population especially in the rural areas of developing countries. In many parts of Africa, herbal medicine still plays a vital role in health care delivery systems especially in remote areas

where clinics and hospitals are sparsely located. In these communities, traditional herbalists operate closer to the people, taking advantage of the biodiversity of plant species in such areas to manage various diseases and ailments (Zuma *et al.*, 2016; Mahamoodally, 2013, Payyappallimana, 2010). The need for more cost effective, easily accessible alternative therapeutic options to currently used antibiotics which are becoming increasingly ineffective against hitherto susceptible bacteria cannot be over-emphasized. Medicinal plants fit the criteria.

Jatropha curcas, commonly known as physic nut, purging nut, Barbados nut or pig nut, has been used in traditional medicine for several generations to treat various health conditions including tumours, fever, jaundice, rheumatism, mouth infections and guinea worm sores (Rampadarath, 2016, Arekemase *et al.*, 2011, Arun *et al.*, 2013). Members of the rural communities of India have been known to use the herb in the treatment of dysentery and colic (Kalimuthu *et al.*, 2010). Its antimicrobial effects have been compared to those of Gentamycin by Dada *et al.*, (2014). *Chlorophora excelsa* is the tree popularly known as 'iroko'; its extracts have been used for wound healing as well as the treatment of cough, fever, backache, toothache, hepatitis and oedema (Udegbunam *et al.*, 2013, Ndenecho, 2009).

This study seeks to evaluate the antimicrobial activity of the 80% methanolic and ethanolic extracts of the dried leaves of *Jatropha curcas* and *Chlorophora excelsa* using the same extraction method, solvent concentration, test bacteria and susceptibility testing methods via agar dilution and agar diffusion techniques.

Materials and Methods

Fresh leaves of *Jatropha curcas* and *Chlorophora excelsa* collected in the month of September from the Botanical garden of the University of Port Harcourt, Rivers State, Nigeria were sorted out and dried at room temperature protected from sunlight. Botanical identification was carried out in the herbarium unit of the Department of Plant Science and Biotechnology, University of Port Harcourt.

Separate samples consisting of 82.9g each of crushed air-dried leaves of *Chlorophora excelsa* were soaked in methanol and ethanol for 72 hours. For *Jatropha curcas*, 85g samples were used. The respective samples

were then filtered using Whatmann no. 1 filter paper and the filtrates (crude extracts) were separately put through a rotary evaporator and concentrated to dryness in a water bath at 40°C to give a constant weight. The presence or absence of alkaloids, tannins, phlobatannins, saponin, flavonoids, anthraquinones (free and combined) and cardiac glycosides were determined according to the methods of Trease and Evans (1989) and Sofowora (2008).

The clinical isolates of *Candida albicans* and *Staphylococcus aureus* were identified and characterised using morphological, Gram staining and biochemical tests. MacFarland standard (0.5) was used to standardize the test organisms. A stock solution of 500mg/ml, from which concentrations of 250mg/ml, 125mg/ml, 62.5mg/ml, 31.25mg/ml and 15.624mg/ml were made, was prepared for both the methanolic and ethanolic extracts of *Chlorophora excelsa* and *Jatropha curcas*.

A 24-hour old broth culture of the test organisms (standardized inocula) was swabbed on to Mueller–Hinton agar (MHA) for *S. aureus* and Sabouraud Dextrose Agar (SDA) for *Candida albicans* in petri dishes using sterile cotton swabs. A sterile stainless steel 6mm diameter cork borer was used to create uniform wells in the agar. Each of the five different concentrations of the relevant extract was put in the wells and labeled appropriately. The Mueller-Hinton agar seeded with bacteria was incubated at 37°C for 24 hours. Control plates containing sterile distilled water in the wells were made in parallel.

For the Agar dilution test, 1ml of reconstituted extract of the different concentrations were added to different petri dishes having sterile 9ml Mueller-Hinton agar (MHA) for *S. aureus* and Sabouraud Dextrose Agar (SDA) for *C. albicans*. The agar plates

were prepared in duplicates and allowed to set at room temperature. The standardized culture was then aseptically inoculated onto the agar using the spread plate technique. Incubation was at 37°C for 24 hours. Control plates comprising inocula without the extracts were made in parallel (Collins *et al.*, 1995).

Results and Discussion

The methanolic extract of *J. curcas* contained alkaloids, tannins, saponin, flavonoids, combined anthraquinones and cardiac glycoside with high concentrations of tannins. The ethanolic extract contained similar phytochemicals but had a higher concentration of saponins. With *C. excelsa*, the methanolic extract contained alkaloids, tannins, saponin, flavonoid and combined

anthraquinones while the ethanolic extract contained alkaloids, tannins, saponin, flavonoids, combined anthraquinones and cardiac glycoside. The ethanolic extract contained higher concentrations of flavonoids than the methanolic extract (Table 1).

With the susceptibility tests, a clear zone of inhibition indicates antimicrobial activity against the test organism while the absence of this zone of inhibition is indicative of resistance. Neither the ethanolic nor methanolic extracts of either plant affected the growth of *Candida albicans* but zones of inhibition of growth were observed with *Staphylococcus aureus* for both the ethanolic and methanolic extracts of both plants (shown in Tables 2 – 5).

Table.1 Phytochemical screening of methanoilicand ethanolic extracts of *J. curcas* and *C. excelsa*

Phytochemical	<i>Jatropha curcas</i>		<i>Chlorophora excelsa</i>	
	Methanoilic Extract	Ethanoilic Extract	Methanoilic Extract	Ethanoilic Extract
Alkaloids	+	++	+	+
Tannins	+++	++	++	+
Phlobatanins	-	-	-	-
Saponin	++	+++	+	+
Flavonoids	++	+	+	++
Anthraquinones	-	+	++	+
Combined Anthraquinones	+	+	+	+
Cardiac Glycoside				
- Salkowski Test	+	+	-	+
- Liebermann Test	+	-	-	-
- Keller Killiani Test	-	+	-	-

KEY: +++ Highly present, ++ Moderately present, + Present, - Absent

Table.2 Antimicrobial activity of the methanolic and ethanolic extracts of *J. curcas* using the agar dilution method

Concentrations of extract incorporated in MHA for <i>S.aureus</i> and SDA for <i>C.albicans</i> (mg/ml)	Methanolic Extract		Ethanolic Extract	
	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
500.00	-	+	-	+
250.00	-	+	-	+
125.00	-	+	-	+
62.50	+	+	+	+
31.25	+	+	+	+
15.625	+	+	+	+
Control	+	+	+	+

Table.3 Antimicrobial activity of the methanolic and ethanolic extracts of *C. excelsa* using the agar dilution method

Concentrations of extract incorporated in MHA for <i>S.aureus</i> and SDA for <i>C.albicans</i> (mg/ml)	Methanolic Extract		Ethanolic Extract	
	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
500.00	-	+	-	+
250.00	-	+	-	+
125.00	-	+	-	+
62.50	+	+	+	+
31.25	+	+	+	+
15.625	+	+	+	+
Control	+	+	+	+

KEY: +: Growth -: No Growth

Table.4 Diameter of zones of inhibition (mm) in susceptibility test of the methanolic and ethanolic extracts of *J. curcas* using the well in agar diffusion method

Concentrations of extract incorporated in MHA for <i>S.aureus</i> and SDA for <i>C.albicans</i> (mg/ml)	Methanolic Extract		Ethanolic Extract	
	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
500.00	11 ± 0.1	-	13 ± 0.2	-
250.00	10 ± 0.2	-	14 ± 0.1	-
125.00	10 ± 0.1	-	12 ± 0.2	-
62.50	-	-	-	-
31.25	-	-	-	-
15.625	-	-	-	-
Control	-	-	-	-

Table.5 Diameter of zones of inhibition (mm) in susceptibility test of the methanolic and ethanolic extracts of *C. excelsa* using the well-in-agar diffusion method

Concentrations of extract incorporated in MHA for <i>S.aureus</i> and SDA for <i>C.albicans</i> (mg/ml)	Methanolic Extract		Ethanolic Extract	
	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>	<i>Staphylococcus aureus</i>	<i>Candida albicans</i>
500.00	10± 0.1	-	11 ± 0.1	-
250.00	10 ± 0.2	-	10± 0.2	-
125.00	10 ± 0.1	-	10± 0.1	-
62.50	-	-	-	-
31.25	-	-	-	-
15.625	-	-	-	-
Control	-	-	-	-

KEY: No zone of inhibition

The methanolic and ethanolic extracts of the leaves of both plants had inhibitory effects on *Staphylococcus aureus* at concentrations of 125mg/ml and higher but had no effect on the growth of *Candida albicans*. At lower concentrations (less than 125mg/ml), there was no inhibitory effect on either microorganism. This is similar to the results obtained by Igbinsa *et al.*, (2009) and Rampadarath *et al.*, (2016) who indicated that the extracts of *Jatropha curcas* inhibited the growth of *Staphylococcus aureus*. A study by Chime *et al.*, (2011) also confirms the inhibitory effect of the methanolic extract of *C. excelsa* against *S. aureus*. Similarly, Padayachee and Odhav (2013) agree that the growth of *Staphylococcus* sp. is inhibited by extracts of *C. excelsa*. These findings are somewhat in contrast to the conclusions drawn by Arun *et al.*, (2013) who inferred that neither the methanolic nor ethanolic extracts of the latex of *J. curcas* affected the growth of *S. aureus*. Arekemase *et al.*, (2011) on the other hand, found extracts of *J. curcas* to be effective against both *S. aureus* and *C.albicans*. Clearly, the inhibitory effects of the plant extract are largely dependent on the concentration, parts of the plant used and the microorganisms involved (Kalimuthu *et al.*, 2010).

Based on the inhibitory effects of the ethanolic and methanolic extracts of the leaves of *Jatropha curcas* and *Chlorophora excelsa* in this study, both plants can be used in the treatment of ailments caused by *Staphylococcus aureus* provided there are no side effects.

It is concluded that Methanol and ethanol are efficient in extracting alkaloids, tannins, saponins, flavonoids and anthraquinones present in the dried leaves of both *Jatropha curcas* and *Chlorophora excelsa*. This study indicates that the methanolic and ethanolic extracts of *Jatropha curcas* and *Chlorophora excelsa* were active against *Staphylococcus aureus* but inactive against *Candida albicans*.

References

- Arekemase, M. O., Kayode, R. M. O. and Ajiboye, A. E. (2011) Antimicrobial Activity and Phytochemical Analysis of *J. curcas* Plant against some Selected Microorganisms. International Journal of Biology 3(3): 52 – 59.
- Arun, K., Bharwaj, A. and Ruchika, G. (2013) A Comparative Study of Antibacterial Activity of Leaves and Latex of *Jatropha curcas* L. International Journal of Pharmacy and Pharmacology 4(4): 190 – 194.

- Chime, C. C., Okorie, I. A., Ekanem, E. J. and Kagbu, J. A. (2011) Analysis and Remediation of Faecal Coliform Bacteria Pollution in Selected Surface Water Bodies of Enugu State, Nigeria. *World Academy of Science, Engineering and Technology* 56: 1565 – 1570.
- Collins, C. H.; Lynes, P. M. and Grange, J. M. (1995) *Microbiological Methods*. 7th Ed. Butterworth-Heinemann Ltd., United Kingdom.
- Dada, E. O., Ekundayo, F. O. and Makanjuola, O. O. (2014) Antibacterial Activities of *Jatropha curcas* Coliforms isolated from Surface Waters in Akure, Nigeria. *International Journal of Biomedical Science* 10(1): 25 – 30.
- Igbinosa, O. O., Igbinosa, E. O. and Aiyegoro, O. A. (2009) Antimicrobial Activity and Phytochemical Screening of Stem and Bark Extracts from *Jatropha curcas*. *African Journal of Pharmacy and Pharmacology* 3(2): 058 – 062.
- Kalimuthu, K., Vijayakumar, S. and Senthilkumar, R. (2010) Antimicrobial Activity of Biodiesel Plant, *Jatropha curcas*. *International Journal of Pharmacy and Pharmacology* 1(3): 1- 5.
- Mahamoodally, M. F. (2013) Traditional Medicines in Africa: An Appraisal of Ten Potent African Medicinal Plants. *Evidence-based Complementary and Alternative Medicines* 2013: Article ID 617459.
- Ndenecho, E. N. (2009) Herbarium and resources for the development of ethnopharmacology in Mount Cameroon region. *African Journal of Pharmacy and Pharmacology* 3(3): 78 – 86.
- Padayachee, T. and Odhav, B. (2013) Antimicrobial activity of plant phenols from *Chlorophora excelsa* and *Virgillia oroboides*. *African Journal of Biotechnology* 12(17): 2254 – 2261..
- Payyappallimana, U. (2010) Role of Traditional Medicine in Primary Healthcare: An Overview of Perspectives and Challenges. *Yokohama Journal of Social Sciences* 14(6): 57 – 77.
- Rampadarath, S., Puchova, D. and Jeewon, R. (2016) *Jatropha curcas* L.: Phytochemical, Antimicrobial and Larvicidal Properties. *Asian Pacific Journal of Tropical Biomedicine* 6(10): 858 – 865.
- Sofowora, A. (2008) *Medicinal plants and traditional medicine in Africa*. 3rd Ed. Spectrum Books, Ibadan.
- Trease, G.E. and Evans, W. C. (1989) *Pharmacognosy*. 13th Ed. Bailliere Tindall, London, UK. pp.345 – 346, 535 – 536, 772 – 773.
- Udegbunam, S. O., Nnaji, T. O., Udegbunam, R. I., Okafor, J. C. and Agbo, I. (2013) Evaluation of Herbal Ointment Formulation of *Milicia excelsa* (Welw.) C. C. Berg. for Wound Healing. *African Journal of Biotechnology* 12(21): 3351 – 3359.
- World Health Organisation (2005) *General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine*. WHO, Geneva.
- Zuma, T., Wight, D., Roachat, T. and Moshabela, M. (2016) Role of Traditional Health Practitioners in Rural Kwazulu-Natal, South Africa: Generic or Mode Specific. *BMC Complementary and Alternative Medicine*, 16(1): 1 – 13.

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