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Research Article

EPICARP OF THE FRUIT OF *LANDOLPHIA OWARIENSIS* IS RICH IN MEDICINAL PHYTOCHEMICALS AND HAS BROAD SPECTRUM ANTIMICROBIAL POTENTIAL

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Abstract

Extracts of the fruit (epicarp) of *Landolphia owariensis* were investigated as part of an ongoing study on the fruits of the medicinal plant. Phytochemical screening showed the plant to have alkaloids, cyanogenic glycosides, saponins, tannins, triterpenes, carbohydrates, anthraquinones and flavonoids (methanol extracts); cyanogenic glycosides, alkaloids,saponins, steroids and triterpenes (ethyl acetate and chloroform);terpenes and steroids (hexane extracts) while steroids were not detected in any extract. Extracts inhibited growth of common disease causing pathogens (Bacteria and fungi): *Staphylococcus aureus, Streptococcus pyogens, Salmonella typhi, Shigella dysenteriae, Klebsiella pneumoniae, Candida albicans, Candida krusei, Candida stellatoidea,* but were ineffective against *Microsporum gypseum, Microsporum spp Candida tropicalis,Pseudomonas aeruginosa,Escherichia coli* and *Trichophyton rubrum*. Results show that ethno-medicinal claims on *Landolphia owariensis* are probably true.

Keywords: *Landolphia owariensis;* epicarp, Phytochemical screening, Antimicrobial Assay.

Introduction

Search for novel antimicrobials, in an eternal battle against disease, is a major area of research in modern science(Dias et al. 2012; Sarker & Nahar 2012). Plants have remained a major recourse for treatment of various ailments in the third world. In Africa, oral tradition has maintained a repertoire of methods and treatments based on herbal medicine(Sofowora 1996). Unfortunately these are gradually fading into oblivion. One demon is that custodians of this knowledge seldom pass these traditions on without very dense superstition and that each emerging younger generation increasingly disconnected from things "traditional", "tribal", or "primitive"is gradually no longer listening. Our quest therefore is to improve on knowledge brought forward by these sentinels of a dying tradition by investigating their claims and hopefully arriving at useful galenicals, scientific knowledge or synthetic drugs.

Landolphia owariensis P. Beauvis an important plant in African traditional medicine; it is a forest climber found throughout West-Central Africa, Southern Africa and Madagascar (Nwaogu et al. 2007; Nwogu et al. 2008). It is known variously as*Vine rubber* (English) *Eso*or *akitipa* (Igbo), *mba*(Yoruba),*Ciwo*(Hausa) and *Ipungwa*(Tiv).

The sweet fruits are consumed in the Southern, North-Central regions of Nigeria and Southern parts of Cameroun. Folkloric uses of the plant which are legion are summarized in Table 1.

The many important uses of *L. owariensis* have, in turn, spawned many scientific investigations; these are summarized in table 2.

Here we investigate phytochemical and antimicrobial activity of the epicarp of the fruit of *L. owariensis;* an area hitherto not investigated.

Table 1. Use of *Landolphia owariensis* P. Beauv in Traditional Medicine

Part Used	Nature of Use/Ailment/Treatment	Preparation	Mode of Administration	Country/Region	Reference
Unripe fruits	fever pains	Aqueous Extract	a steam bath	Côte d' Ivoire	(Bouquet, 1969)
Roots	fever pains	Aqueous Extract	a steam bath	Côte d' Ivoire	(Bouquet, 1969)
Roots	gonorrhea	Soaked In Gin	Oral		(Gill, 1992)
Leaves	purgative	Aqueous Extract	Oral	Côte d' Ivoire/Nigeria	(Burkill, 2004)
Leaves	malaria	Aqueous Extract	Oral	Côte d' Ivoire/ Nigeria	(Burkill, 2004)
Stem bark	vermifuge	Aqueous Extract	Oral	Côte d' Ivoire	(Lewis and Elvin-Lewis, 1977)
Latex	ingredient of arrow poison			Côte d' Ivoire	(Irvine, 1961)
latex	natural preservative				(Anthony, 1995)
latex	vermifuge		Oral/Enema	Equatorial Guinea	(Irvine, 1961)
Leaves, roots, stem bark, seeds	Venereal Infections			Yoruba	(Kayode & Kayode 2008)

Table 2. Scientific Investigations on *Landolphia owariensis* P. Beauv

Study	Part Studied	Significance	Reference
Isolation and Characterization of Potential Bioactive Compounds (ascorbic acid)	Seed Pulp	stringy seed pulp has strong anti-oxidant effect	(Okonkwo & Osadebe 2013)
Anti-Inflammatory And Analgesic Activities	Leaves	Leaf extracts have anti-inflammatory, analgesic activities and antioxidative activities.	(Owoyele et al. 2001)
Physicochemical Properties of the Seed oil	Seed oil	Low yielding, May not be industrially useful; could be edible	(Akubugwo & Ugbogu 2007)
Phytochemical and antimicrobial activity	Leaves	alkaloids, flavonoids, tannins and saponins are present in leaves, effective against *Staphylococcus* sp., *Proteus* sp., and *E. coli*.	(Nwaogu et al. 2007)
Phytochemical and antimicrobial activity	Leaves and Roots	Similar phytochemicals; cyanogenic glycosides in Root, Leaf extracts show higher inhibitory action	(Nwaogu et al. 2008)
effects of extracts on liver function profile and haemoglobin	Leaves	extract is not hepatotoxic in rats; has haemoglobin	(Nwogu et al. 2008)

concentration		lowering-effect	
comparative study	Leaves	chronic administration of extract is not harmful	(Ilesanmi et al. 2011)

Materials and Methods

Fresh fruits of *Landolphia owariensis* were collected from the wilderness around Adikpo, Benue State, Nigeria (6° 50' 37.8 "N 9°15 '31.1" E). The plant was identified by Mr. Ikyobo John N., Department of Wildlife and Range Management, University of Agriculture, Makurdi, Nigeria. The epicarp was extracted and diced usinga stainless kitchen knife; shade dried at ambient temperature (36±1^{0}C) for two weeks and pulverized using a domestic kitchen blender (Supper master, China). The plant material was stored in black polyethylene bags in vacuumed desiccators at ambient temperatures (36±1^{0}C) until needed for extraction.

Microwave Assisted Extraction of Sample

Microwave Assisted Extraction was carried out using a domestic microwave oven according to methods described by(Ganzler et al. 1986; Ganzler et al. 1990; Carro et al. 1997; Abuın et al. 2000; Young 1995) with modifications. The pulverized plant (100 g) was introduced to extraction vessel[Winchester bottle (2.5 L)]. Hexane, Chloroform, Ethyl Acetate and Methanol (200 mL each) were added sequentially to the plant material and the mixture bombarded with microwaves (70 Watts/Defrost Function) using a modified domestic kitchen microwave (Mio-star, Model 7173.295, Germany) for 30 minutes (3 minute sessions with 15 minute cooling pauses). Pressure build up was vented after every two successive sessions by very slowly unscrewing cap of extraction vessel and agitating very gently. Watman number 1 (size: 24) filter paper was used to filter the extracts which werethen concentrated *in vacuo*at 40 ^{0}C and air dried at ambient temperatures (36±1 ^{0}C). The dried extracts were stored in clean glass bottles at ambient temperatures until required for use. The percentage yield of various extracts was calculated thus:

$$\%\ yield = \frac{weight\ of\ Extract\ (g)}{100\ g} \times 100$$

Phytochemical screening

Phytochemical screening of the plant extracts was carried out as described by (Odebiyi & Sofowora, 1991; Sofowora, 1982; Evans, 2009; Usman, Abdulrahman, & Usman, 2009). Sodium Hydroxide (NaOH) Test was used in detecting presence of flavonoids in the plant extracts. Mayer's, Wagner's and Dragendorff's tests were used in determination of alkaloids. Cardiac glycosides were determined by the KellerKiliani's test, Tannins by Ferric chloride test, Saponins was by the frothing test, carbohydrates by Molisch test, Steroids and triterpenes by Liebermann–Burchardt's test while Anthraquinones was by Bontrager's test.

Antimicrobial Activities

Collection of Microbial isolates

The human pathogens; *Staphylococcus aureus; Streptococcus pyogens, Escherichia coli, Salmonella typhi, Shigella dysenteriae, Pseudomonas aeruginosa, Klebsiella pneumoniae, Candida albicans, Candida tropicalis, Candida krusei, Candida stellatoidea, Microsporum gypseum, Microsporum spp and Trichophyton rubrum* used in the study were obtained from the stock culture of the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Kaduna State, Nigeria.

Antimicrobial sensitivity Test

The sensitivity of test organisms to n-Hexane, Chloroform, Ethyl acetate and Methanol extracts of *Landolphia owerensis* fruit epicarp was carried out using the diffusion method described by Ebi and Ofoefule (1997). The extract (0.3 g) was weighed and dissolved in DMSO (10 mL) to obtain a concentration of 30 mg/mL. Mueller Hinton agar

was used as growth medium for bacteria while Sabouraud dextrose agar was used as growth medium for fungi. The media were prepared according to standard procedures and poured into sterile petri dishes, cooled and allowed to solidify. Sterilized media were fed with standard inocula (0.1 mL) of test microbes. Inocula were spread evenly over surface of media by use of a sterile swab. Using a standard cork bearer of 6mm in diameter, a well was cut at the center of each inoculated medium. The standard solution of the extract (0.1 mL) of concentration 20mg/mL was then introduced into each well on the inoculated medium.

The Petri-dishes were allowed to stand for about 30 minutes at room temperature to allow for proper diffusion of extracts to take place. The plates were then incubated at 37^{o}C for 24 hours for the bacteria and at 30^{0}C for 1-7 days for the fungi. The zones of inhibition of growth in millimeters were measured and recorded.

Minimum Inhibitory Concentration (MIC) Test

The broth dilution method described by (Forbes et al. 2007)was used to determine the minimum inhibitory concentration. Muller Hinton and Sabouraud dextrose broth were prepared; 10mL of the broth was dispensed into test tubes and were sterilized at 121^{0}C for 15 minutes and allowed to cool. McFarland turbidity standard scale number 0.5 was prepared to give turbid solution. Normal saline (10 mL) was prepared and dispensed into sterile test tubes; test microbes were inoculated and incubated at 37^{0}C for 6 hours. Dilution of test microbes in normal saline was made until turbidity reached the Mcfarland scale by visual comparison, at this point test microbes had a concentration of about 1.5×10^{8}cpu/mL. Two-fold dilution of the extract in sterile broth was made to obtain the concentrations of 20, 10, 5, 2.5 and 1.25 mg/mL. The initial concentration was obtained by dissolving of the extract (0.3 g) in the sterile broth(10 mL). Standard inoculaof test microbes in normal saline (0.1 mL) were then inoculated into the different concentrations, onto Mueller Hinton and Sabouraud dextrose broth, incubated at 37^{0}C for 24 hours and 30^{0}C for 1-7 days for bacteria and fungi respectively. Thereafter, each test tube of broth was observed for turbidity (growth). The lowest concentration of extract in broth which showed no turbidity was recorded as minimum inhibition concentration.

Minimum bacteria/fungi concentration (MBC/MFC) Test

Minimum bacteria and fungi concentrations were carried out to determine whether test microbes were killed or their growth was inhibited. Mueller Hinton and Sabouraud dextrose broths were prepared using standard procedures(Forbes et al. 2007), poured into sterilized petri dishes and allowed to cool and solidify. Content of minimum inhibition concentration in serial dilution was sub cultured onto the media; bacteria on Mueller Hinton and fungi on Sabouraud dextrose agar. Incubation was made at 37^{0}C for 24 hours and 30^{0}C for 1-7 days respectively. Thereafter, each plate was observed for colony growth. MBC/MFCswere plates with lowest concentration of extract without colony growth.

Results

Phytochemistry

Phytochemical screening of extracts of fruit bark(epicarp) of *Landolphiaowariensis* revealed that it contains alkaloids, cyanogenic glycosides, saponins, tannins, triterpenes and carbohydrates. However, anthraquinones and flavonoids were detected only in methanol extract while steroids were not detected in any of the extracts while terpenes and steroids were the only constituents in detected Hexane extract (Table 3).

Table 3: Phytochemical screening for various extracts of *Landolphia owariensis*

	Phytochemical Tests								
Extract	**Anthra quinones**	**Alkaloids**	**Flavonoids**	**Cyanogenic Glycosides**	**Saponins**	**Steroids**	**Tannins**	**Terpenes**	**Carbo hydrates**
N-Hexane	-	-	-	-	-	+	-	+	-
Chloroform	-	+++	-	+	+	+	-	+	-
Ethyl acetate	-	+++	-	+	+	-	-	+	+
Methanol	+	+++	+	+	+	-	+	+	+

+ = Present, - = Not present

Antimicrobial activity

Chloroform, ethyl acetate, methanol and hexane extracts of *Landolphia owariensis* fruit bark showed antimicrobial activities against *S. aureus, S. pyogenes, S. typhi, S. dysenteriae, K. pneumonia, C. albicans, C. krusei* and *C. stellatoidea.* No activity was shown by the extracts on *E. coli, P. aerogenosa, C. tropicalis, M. gypseum, Microsporum spp.* and *T. rubrum* (Table 4). Results also showed that ethyl acetate extract had highest zones of inhibition while hexane extract had smallest diameters of zones of inhibition.

Table 4: Anti-microbial activities and Diameter of Zone of inhibition (DZI) of ***Landoiphia owariensis*** fruitbark extracts

Microbes	Anti-microbial activity/ Zone of inhibition (mm)			
	Hexane	Chloroform	E/Acetate	Methanol
S. aureus	+/18	+/20	+/23	+/21
S. pyogenes	+/18	+/21	+/25	+/20
E. coli	-/0	-/0	-/0	-/0
S. typhi	+/17	+/22	+/27	+/20
S. dysenteriae	+/19	+/20	+/24	+/
P. aeruginosa	-/0	-/0	-/0	-/0
K. pneumonia	+/18	+/24	+/28	+/22
C. albicans	+/16	+/24	+/27	+/20
C. tropicalis	-/0	-/0	-/0	-/0
C. krusei	+/17	+/25	+/26	+/22
C. stellatoidea	+/18	+/24	+/28	+/20
M. gypseum	-/0	-/0	-/0	-/0
M. spp.	-/0	-/0	-/0	-/0
T. rubrum	-/0	-/0	-/0	-/0

Key: S = Sensitive, R = Resistant

Table 5: Minimum inhibition (MIC) and Bactericidal/Fungicidal concentrations (MBC/MFC) of *L. owariensis* fruit bark extracts against test microbes (mg/mL)

Microbes	MIC/[MBC/MFC] (mg/mL)			
	Hexane	Chloroform	E/Acetate	Methanol
S. aureus	2.5/20	5/5	5/5	5/20
S. pyogenes	2.5/20	5/5	5/5	5/20
E. coli				
S. typhi	2.5/20	5/2.5	5/5	5/20
S. dysenteriae	2.5/20	5/2.5	5/5	5/20
P. aeruginosa				
K. pneumonia	2.5/20	5/2.5	2.5/2.5	5/10
C. albicans	2.5/20	5/2.5	2.5/5	5/20
C. tropicalis				
C. krusei	2.5/20	5/2.5	5/5	5/10
C. stellatoidea	2.5/20	5/2.5	5/2.5	5/20
M. gypseum				
M. spp.				
T. rubrum				

Results of inhibition of bacterial growth by the extracts showed that effect of extracts is dose dependent since no activity was observed at very low concentrations (Table 5) and indicated that higher doses of extracts would be more effective in treatment of bacterial and fungal infections. Furthermore, extracts had minimum inhibition concentrations of 5mg/mL (chloroform and methanol), 10mg/mL (hexane) while ethyl acetate ranged between 2.5mg/mL and 5mg/mL; implying ethyl acetate extract was more potent even at lower concentrations. Minimum bactericidal concentrations of extracts were 20mg/mL (hexane), 10 mg/mL to 20 mg/mL (chloroform), 10 mg/mL to 20 mg/mL (methanol),and(5 mg/mL to 10 mg/mL) ethyl acetate. Minimum fungicidal concentrations of extracts were 20mg/mL (hexane), 10mg/mL (chloroform), while those of ethyl acetate and methanol ranged between 5mg/mL to 10mg/mL and 10mg/mL to 20mg/mL respectively. The results show that ethyl acetate extract would be more effective treating bacterial and fungal infections

Discussion

Preliminary phytochemical screening results of hexane, chloroform, ethylacetate and methanol extracts of fruit bark (epicarp) of *L. owariensis* indicated presence of alkaloids, glycosides, Saponins, tannins, terpenes and carbohydrates(Table 3). Presence of these phytochemicals had earlier been reported in leaves and roots of the plant (Nwaogu et al. 2007; Burkill 2000). Presence of these phytochemicals in the fruit bark (epicarp) of the plant are responsible for its medicinal value (Sofowora 1996; Edeoga et al. 2005). Presence of flavonoids shows fruit bark of *L. owariensis* contains may also possess anti-oxidative properties (Owoyele et al. 2001; Cook & Samman 1996). Many plants containing flavonoids have been shown to have diuretic, laxative, anti-spasmodic, anti-hypertensive, anti-inflammatory, anti-microbial and anti-oxidant actions (Kubmarawa & Ajoku 2007). Anti-oxidant property of flavonoids has been employed in protection against cancer and other degenerative diseases (Lee 1999; Lee & Shibamoto 2002); tannins would confer wound healing properties(Osadebe& Ukwueze 2004)and anti-bacterial properties (Banso & Adeyemo 2007). Saponins have been reported as anti-fungal agents however presence of Saponins, tannins and alkaloids in the fruit bark of *L. owariensis* is suggestive of anti-nutritional tendency that can cause haemolysis, nutrient malabsorption and abnormal hematopoiesis(Balogun& Akinloye 2012). Alkaloids and saponins have been employed dietetically in the management of headache associated with hypertension (Mensah 2013). Cardiac glycosides are used for the treatment of congestive heart failure and cardiac arrhythmia (Mensah 2013).

Flavonoids have been implicated in numerous studies to have, among a host of other medicinal properties, diuretic, laxative, antispasmodic, anti-hypertensive and anti-inflammatory, antioxidant and antiradical properties (Macdonald et al. 2010; Abdelrazig 2013; Asgary & Naderi 1999; Nogueira & Lopes 2011; Peterson & Dwyer 1998; Tor-Anyiin & Anyam 2013);tannins promote wound healing among other numerous medicinal implications(Liu et al. 2014; Lim et al. 2006; Tan et al. 1991);cyanogenic glycosides release hydrogen cyanide but have proven to be useful when used in therapeutic doses in inhibiting or preventing the growth and spread of tumors or malignant cells (Seigler 1991; Jones 1972; Conn 1969; Conn 1981).

Extracts of *Landolphia owariensis* fruit bark (epicarp) inhibited the growth of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi*, *Shigella dysenteriae*, *Klebsiella pneumoniae*, *Candida albicans*, *Candida krusie* and *Candida stellatoidea* corroborating previous study by (Galadima, Kabiru, & Garba, 2010), that methanolic extract of leaves of the plant were able to inhibit growth of *Staphylococcus aureus*; that ethanolic extract of the leaves inhibits growth of *Salmonella typhi* (Nwaogu et al. 2007). Finally leaves and roots of *Landolphia owariensis* are used for treating venereal disease; inhibition of *Staphylococcocus aureus* by the extracts agrees with traditional use of leaves and roots.

Conclusion

Phytochemical screening of fruit bark extracts of *Landolphia oweriensis* indicated presence of important secondary metabolites. Antimicrobial studies showed that the extracts could inhibit growth of several microorganisms (*Staphylococcus aureus*, *Streptococcus pyogenes, salmonella typhi, Shigella dysenteriae, Klebsiella pneumoniae, Candida albicans, Candida krusie* and *Candida stellatoidea*). Use of *Landolphia owariensis* for treatment of various ailments in African traditional medicine is supported by findings of this study.

References

Abdelrazig, S., 2013. Isolation, Characterization and Biological Activity of Flavonoids from (Vitex doniana). Available at: http://41.67.53.40/handle/123456789/4112 [Accessed April 9, 2015].

Abuın, M., Carro, A. & Lorenzo, R., 2000. Experimental design of a microwave-assisted extraction–derivatization method for the analysis of methylmercury. *Journal of Chromatography A*. Available at: http://www.sciencedirect.com/science/article/pii/S0021967300002107 [Accessed April 12, 2015].

Akubugwo, I. & Ugbogu, A., 2007. Physicochemical studies on oils from five selected Nigerian plant seeds. *Pakistan Journal of Nutrition*. Available at: http://docsdrive.com/pdfs/ansinet/pjn/2007/75-78.pdf [Accessed April 9, 2015].

Asgary, S. & Naderi, G., 1999. Anti-oxidant effect of flavonoids on hemoglobin glycosylation. *Pharmaceutica Acta …*. Available at: http://www.sciencedirect.com/science/article/pii/S0031686598000259 [Accessed April 16, 2015].

Balogun, E. & Akinloye, D., 2012. Biochemical Effects of Methanolic Extract of Morinda Morindoides And Morinda lucida Leaveson Lipid Profile, Bilirubin and Some Marker Enzymes. Available at: http://www.ejmanager.com/mnstemps/71/71-1330453627.pdf [Accessed April 18, 2015].

Banso, A. & Adeyemo, S., 2007. Evaluation of antibacterial properties of tannins isolated from

Dichrostachys cinerea. *African Journal of Biotechnology*. Available at: http://www.ajol.info/index.php/ajb/article/view/57787 [Accessed April 18, 2015].

Burkill, H.M., 2000. *Useful Plants of West Tropical Africa* 2nd ed., London: Royal Botanic Gardens, Kew.

Carro, N., Garcia, C. & Cela, R., 1997. Optimisation and comparison of two microwave-assisted extraction procedures of terpenic compounds in Vitis vinifera must samples. *SPECTROSCOPY-OTTAWA-*. Available at: http://www.cem.de/documents/pdf/RE/RE042.PDF [Accessed April 12, 2015].

Conn, E., 1969. Cyanogenic glycosides. *Journal of Agricultural and Food Chemistry*. Available at: http://pubs.acs.org/doi/abs/10.1021/jf60163a014 [Accessed April 20, 2015].

Conn, E., 1981. Cyanogenic glycosides. *The Biochemistry of Plants: A Comprehensive Treatise*. Available at: http://scholar.google.com/scholar?q=cyanogenic+glycosides&btnG=&hl=en&as_sdt=0%2C5#1 [Accessed April 20, 2015].

Cook, N. & Samman, S., 1996. Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources. *The Journal of nutritional biochemistry*. Available at: http://www.sciencedirect.com/science/article/pii/S0955286395001689 [Accessed April 16, 2015].

Dias, D.A., Urban, S. & Roessner, U., 2012. A historical overview of natural products in drug discovery. *Metabolites*, 2(2), pp.303–36. Available at: http://www.mdpi.com/2218-1989/2/2/303/htm [Accessed December 16, 2014].

Edeoga, H., Okwu, D. & Mbaebie, B., 2005. Phytochemical constituents of some Nigerian medicinal plants. *African journal of biotechnology*. Available at: http://www.ajol.info/index.php/ajb/article/view/15167 [Accessed April 9, 2015].

Evans, W.C., 2009. *Trease and Evans' Pharmacognosy*, Elsevier Health Sciences. Available at: https://books.google.com/books?id=zYbz4ezpR_kC&pgis=1 [Accessed April 13, 2015].

Forbes, B.A. et al., 2007. *Bailey & Scott's diagnostic microbiology*, Elsevier Mosby. Available at: https://books.google.com/books?id=MIRqAAAAMAAJ&pgis=1 [Accessed April 13, 2015].

GALADIMA, A., KABIRU, A. & GARBA, Z., PHYTOCHEMICAL, ANTIMICROBIAL AND HEAVY METALS STUDIES OF LANDOLPHIA OWARIENSIS LEAVES EXTRACT. *abu.edu.ng*. Available at: http://www.abu.edu.ng/publications/2012-08-30-131445_5321.pdf [Accessed April 9, 2015].

Ganzler, K., Salgo, A. & Valkó, K., 1986. Microwave extraction: A novel sample preparation method for chromatography. *Journal of Chromatography A*. Available at: http://www.sciencedirect.com/science/article/pii/S0021967301947144 [Accessed April 12, 2015].

Ganzler, K., Szinai, I. & Salgo, A., 1990. Effective sample preparation method for extracting biologically active compounds from different matrices by a microwave technique. *Journal of Chromatography A*. Available at: http://www.sciencedirect.com/science/article/pii/0021967390851099 [Accessed April 12, 2015].

Ilesanmi, F., Balogun, E. & Ilesanmi, O., 2011. Comparative effect of chronic administration of chloroquine phosphate and methanol extract of Landolphia owariensis on the activities of rat Enzymes. Available at: http://imsear.li.mahidol.ac.th/handle/123456789/150852 [Accessed April 9, 2015].

Jones, D., 1972. Cyanogenic glycosides and their function. *Phytochemical ecology*. Available at: http://scholar.google.com/scholar?q=cyanogenic+glycosides&btnG=&hl=en&as_sdt=0%2C5#3 [Accessed April 20, 2015].

Kayode, J. & Kayode, G., 2008. Ethnomedicinal survey of botanicals used in treating sexually transmitted diseases in Ekiti State, Nigeria. *Ethnobotanical leaflets*. Available at: http://opensiuc.lib.siu.edu/cgi/viewcontent.cgi?article=1041&context=ebl [Accessed April 9, 2015].

Kubmarawa, D. & Ajoku, G., 2007. Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. *African Journal of …*. Available at: http://www.ajol.info/index.php/ajb/article/view/57755 [Accessed April 9, 2015].

Lee, K. & Shibamoto, T., 2002. Analysis of volatile components isolated from Hawaiian green coffee beans (Coffea arabica L.). *Flavour and fragrance journal*. Available at: http://onlinelibrary.wiley.com/doi/10.1002/ffj.1067/full [Accessed April 18, 2015].

Lee, K.H., 1999. Anticancer drug design based on plant-derived natural products. *Journal of Biomedical Science*, 6(4), pp.236–250. Available at: http://www.ncbi.nlm.nih.gov/pubmed/16889228.

Lim, S., Darah, I. & Jain, K., 2006. Antimicrobial activities of tannins extracted from Rhizophora apiculata barks. *Journal of Tropical Forest Science*. Available at: http://www.researchgate.net/publication/228371501_Antimicrobial_activities_of_tannins_extracted_from_Rhizophora_apiculata_barks [Accessed April 9, 2015].

Liu, C. et al., 2014. Rhubarb Tannins Extract Inhibits the Expression of Aquaporins 2 and 3 in

Magnesium Sulphate-Induced Diarrhoea Model. *BioMed research international*. Available at: http://www.hindawi.com/journals/bmri/2014/619465/abs/ [Accessed April 9, 2015].

Macdonald, I., Oludare, A. & Olabiyi, A., 2010. Phytotoxic and anti-microbial activities of flavonoids in Ocimum gratissimum. *Life Sci J*. Available at: http://www.sciencepub.net/life/life0703/06_2743life0703_45_48.pdf [Accessed April 9, 2015].

Mensah, J., 2013. Nutritional, phytochemical and antimicrobial properties of two wild aromatic vegetables from Edo State. *Journal of Natural …*. Available at: http://scholarsresearchlibrary.com/JNPPR-vol3-iss1/JNPPR-2013-3-1-8-14.pdf [Accessed April 18, 2015].

Nogueira, C.R. & Lopes, L.M.X., 2011. Antiplasmodial Natural Products. *Molecules*, 16(3), pp.2146–2190. Available at: http://www.mdpi.com/1420-3049/16/3/2146/.

Nwaogu, L. et al., 2008. A comparative study of the antimicrobial properties of the ethanolic extracts of Landolphia owariensis leaf and root. *African Journal of Biotechnology*, 7(4), pp.368–372. Available at: http://www.ajol.info/index.php/ajb/article/view/58431 [Accessed April 9, 2015].

Nwaogu, L. et al., 2007. Phytochemical and antimicrobial activity of ethanolic extract of Landolphia owariensis leaf. *African Journal of …*, 6(7), pp.890–893. Available at: http://www.ajol.info/index.php/ajb/article/download/56939/45343 [Accessed April 9, 2015].

Nwogu, L., Igwe, C. & Emejulu, A., 2008. Effects of Landolphia owariensis leaf extract on the liver function profile and haemoglobin concentration of albino rats. *African Journal of Biochemistry …*. Available at: http://www.researchgate.net/profile/Chidi_Igwe/publication/242177192_Effects_of_Landolphia_owariensis_leaf_extract_on_the_liver_function_profile_and_haemoglobin_concentration_of_albino_rats/links/0deec529def559b2a2000000.pdf [Accessed April 9, 2015].

Odebiyi, O.O. & Sofowora, E.A., 1991. Phytochemical screening of Nigerian medicinal plants II. *Lloydia The Journal Of Natural Products*, 41(3), pp.234–246. Available at: http://www.scopus.com/inward/record.url?eid=2-s2.0-0026355037&partnerID=40&md5=31098e8c7eb4e1d22a94c8db72f30e12.

Okonkwo, T. & Osadebe, P., 2013. Isolation and Characterization of Potential Bioactive Compounds from Landolphia owariensis P. Beauv Stringy Seed Pulp. *… Journal of Applied Research in Natural …*. Available at: http://www.ijarnp.org/index.php/ijarnp/article/view/220 [Accessed April 9, 2015].

Osadebe, P. & Ukwueze, S., 2004. A comparative study of the phytochemical and anti-microbial properties of the Eastern Nigerian specie of African Mistletoe (Loranthus micranthus) sourced from. *Bio-research*. Available at: http://www.ajol.info/index.php/br/article/view/28537 [Accessed April 18, 2015].

Owoyele, B. et al., 2001. Anti-inflammatory and analgesic activities of leaf extracts of Landolphia owariensis. *African Journal of …*. Available at: http://www.ajol.info/index.php/ajbr/article/download/53896/42445 [Accessed April 9, 2015].

Peterson, J. & Dwyer, J., 1998. Flavonoids: dietary occurrence and biochemical activity. *Nutrition Research*. Available at: http://www.sciencedirect.com/science/article/pii/S0271531798001699 [Accessed April 16, 2015].

Sarker, S.D. & Nahar, L., 2012. An introduction to natural products isolation S. D. Sarker & L. Nahar, eds. *Natural Products Isolation*, 864, pp.1–25. Available at: http://hdl.handle.net/2086/6048.

Seigler, D., 1991. Cyanide and cyanogenic glycosides. *GS Rosenthal & MR Berenbaum*. Available at: http://books.google.com/books?hl=en&lr=&id=9CnVtPsFAQAC&oi=fnd&pg=PA35&dq=cyanogenic+glycosides&ots=JDyfI0PYPW&sig=Jm-I0vggYilEfRwXmCBC2_otdKI [Accessed April 20, 2015].

Sofowora, A., 1982. *Medicinal plants and traditional medicine in Africa.*, Available at: http://www.cabdirect.org/abstracts/19842012621.html [Accessed April 9, 2015].

Sofowora, A., 1996. Research on medicinal plants and traditional medicine in Africa. *The Journal of Alternative and Complementary …*. Available at: http://online.liebertpub.com/doi/pdfplus/10.1089/acm.1996.2.365 [Accessed April 9, 2015].

Tan, G.T. et al., 1991. Evaluation of natural products as inhibitors of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase. *Journal of Natural Products*, 54(1), pp.143–154. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1710653.

Tor-Anyiin, T.A. & Anyam, J.V., 2013. Phytochemical evaluation and antibacterial activity : A comparison of various extracts from some Nigerian trees. *Peak Journal of Medicinal Plant Research*, 1(2), pp.13–18. Available at: https://www.peakjournals.org/sub-journals-PJMPR.htm.

Usman, H., Abdulrahman, F. & Usman, A., 2009. Qualitative Phytochemical Screening and In Vitro Antimicrobial Effects of Methanol Stem Bark Extract of Ficus Thonningii (Moraceae). *African journal of traditional complementary and alternative medicines AJTCAM African Networks on Ethnomedicines*, 6(3), pp.289–295.

Young, J., 1995. Microwave-assisted extraction of the fungal metabolite ergosterol and total fatty acids. *Journal of Agricultural and Food Chemistry*. Available at: http://pubs.acs.org/doi/abs/10.1021/jf00059a025 [Accessed April 12, 2015].