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EVALUATION OF THREE MEDICINAL PLANT EXTRACTS AGAINST *PLASMODIUM FALCIPARUM* AND SELECTED MICROGANISMS

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Abstract

Background: A great revival of scientific interests in drug discovery has been witnessed in recent years from medicinal plants for health maintenance. The aim of this work was to investigate three Nigerian medicinal plants collected in Nigeria for their *in vitro* antiplasmodial and antimicrobial **activities.**
Materials and Methods: Extracts obtained from parts of *Persea americana*, *Jatropha podagrica* and *Picralima nitida* and their fractions were evaluated for *in vitro* antiprotozoal and antimicrobial activity.
Result: The methanol extract of *P. nitida* demonstrated activity against chloroquine-sensitive and chloroquine-resistant *P. falciparum* clones with IC_{50} values of 6.3 and 6.0 µg/mL, respectively. Methanol and chloroform extracts of *P. americana* seed showed antifungal activity against *Cryptococcus neoformans* IC_{50} less than 8 and 8.211 µg/mL respectively. Finally, the petroleum ether extract of *P. americana* had activity against methicillin-resistant *Staphylococcus aureus* (MRSA) with an IC_{50} value of 8.7 µg/mL.
Conclusion: The study revealed the antibacterial and antiplasmodial activities of the plants extracts at the tested concentrations.

Keywords: Antifungal, Antibacterial, *Persea americana*, *Picralima nitida*, *Jatropha podagrica*, *Plasmodium falciparum*

Introduction

Parasitic diseases such as malaria have a high mortality rate having a significant impact in developing countries and affecting several hundred millions of people worldwide. Malaria is one of the most important parasitic diseases in the world and is a major global health problem affecting over one hundred countries with disease prevalence escalating at an alarming rate, particularly in the last two decades. Rapid development of resistance by *Plasmodium falciparum* to the conventional drugs such as chloroquine necessitates the search for new antimalarials (Iwu et al., 1994; Wolf, 2002; Guerin et al., 2002; Fournet and Munoz, 2002). Malaria, a devastating infectious disease caused by highly adaptable protozoan parasites of the genus Plasmodium, has impacted on humans for more than 4000 years, causing illness and an estimated 1.5–2.5 million deaths each year. Malaria is endemic throughout the tropics, especially in sub-Saharan Africa and the developing world, threatening about 40 % of the world's population. Although four Plasmodium parasite species can infect humans, *Plasmodium falciparum* causes the majority of illnesses and deaths. Severe malaria, defined as acute malaria with major signs of organ dysfunction or high levels of parasitemia, predominantly affects children and pregnant women (Piece and Miller, 2009; Rosenthal, 2008; White, 2008).

Chemotherapy is still at the forefront in the fight against malaria due to the unavailability of effective vaccines. Numerous drugs have been developed for the treatment of uncomplicated malaria, for example, mefloquine, primaquine, quinidine, proguanil (Genton, 2008; Vekemans and Ballon, 2005).There is still need to search for newer and novel antimalarial agents from natural products via ethnopharmacological approach.
Similarly, an increasing number of multidrug-resistant microbial pathogens have become a serious problem particularly during the last decade and provide the impetus for the search and discovery of novel antibacterial and antifungal agents active against these pathogens (Liu and Balasubramanian, 2001).

Picralima nitida is a tree or shrub belonging to the family Apocynaceae. It is widely distributed in high deciduous forest of West and Central Africa. It is employed in African traditional medicine as antipyretic, antimalarial, aphrodisiac and for GIT disorders (Wosu and Ibe, 1989).
Jatropha podagrica Hook is a shrub grown in West Africa gardens for its showy red flowers. It is known locally in south western Nigeria as *lapalapa funfun*. *Jatropha* species are found in Africa, Asia and Latin America where they are used in folk medicine to treat various diseases including parasitic skin infections, hepatitis and sexually transmitted diseases (Aiyelaagbe et al., 2002; Sanni et al., 1988; Schmeda-Hirschmann et al., 1992). Various medicinal and pesticidal properties including antibacterial (pneumonia), antitumor and insect antifeedant have been attributed to this plant (Odebiyi, 1980). Phytochemicals in different parts of the plants includes flavonoids, steroids, alkaloids and diterpenoids have been isolated and characterized (Aiyelaagbe et al., 2007, 1998; Das and Venkatail, 2006).

Persea americana Mill commonly known as 'avocado pear' is a medium-sized, single-stemmed, terrestrial, erect, perennial, deciduous, evergreen tree of 15 – 20 m in height. The leaves and other morphological parts of *P. americana* possess medicinal properties, and are widely used in traditional medicines of many African countries as antitussive, antimicrobial, antidiabetic, antiparasitic, anti allergic, antihypertensive, analgesic and anti-inflammatory remedies (Adeyemi et al., 2002; Antia et al., 2005; Adeboye et al., 1999; Owolabi et al., 2005).
In this study, extracts of different parts of *P. americana* were evaluated for the first time for antifungal and antibacterial activities. The methanol extract and fractions of *P. nitida* against two strains of *Plasmodium falciparum* were determined.

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Materials and Methods

Plant materials

All Plant material (Table 1) was collected from Ovia North east, Owan east and Benin City, all in Edo State, Nigeria between January and June, 2013. They were identified and authenticated by Mr. Ugbogu O. A. and Shasanya O. S. of the Forest Research Institute of Nigeria (FRIN), Ibadan where voucher specimens is deposited in the Herbarium (Table 1).

Preparation of extracts

The powdered (100 g) material of each sample was extracted with 500 ml methanol for 48 hr (3 X) by cold maceration, filtered and the filtrate evaporated to dryness to obtained crude extract of *P. nitida* (PNS), *J. podagrica* fruit (JPF), *P. americana root* (PAR), *P. americana* seed (PAS) and chloroform fraction of *P. americana* seed (PASC). The dried extracts and fractions were each screened for antiplasmodial and antimicrobial activities. The fractions PN1, PN2, PN3, PN4 and PN5 are hexane: ethylacetate 50 %, ethylacetate 100 %, hexane: ethylacetate 80 %, methanol 100 % and alkaloidal mixture respectively, were obtained from the vacuum liquid chromatography (VLC) with normal silica and the solvents.

Antimicrobial testing

In vitro antimicrobial activity against a panel of microorganisms, including fungi: *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258), *C. neoformans* (ATCC 90113) and *Aspergillus fumigatus* (ATCC 204305); and bacteria: *Staphylococcus aureus* (ATCC 29213), *methicillin-resistant S. aureus* (MRSa) (ATCC 33591), *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853) and *Mycobacterium intracellulare* (ATCC 23068), was determined using modified versions of the CLSI/NCCLS methods (NCCL, 2000; NCCL, 2002). *M. intracellulare* and *A. fumigatus* was tested using an Alamar Blue method (Franzblau et al., 1998). All organisms were obtained from the American Type Culture Collection (Manassas, VA). Samples, dissolved in DMSO, were serially diluted in saline and transferred in duplicate to 96 well micro plates. Susceptibility testing was performed for all organ cate to 96-well flat bottom micro plates. Microbial inocula were prepared by correcting the OD630 of microbe suspensions in incubation broth to afford final target inocula. Controls [fungi: amphotericin B; bacteria: ciprofloxacin (ICN Biomedicals, OH)] were included in each assay. All plates were read at 530 or 544(ex)/590(em) nm (*M. intracellulare* and *A. fumigatus*) prior to and after incubation. Percent growth was plotted versus test concentration to afford the IC_{50} using XLFit (Alameda, CA).

Antimalarial/Parasite LDH Assay

The *in vitro* antimalarial assay procedure utilized was an adaptation of the parasite lactate dehydrogenase (pLDH) assay developed by Makler et al., 1993.The assay was performed in a 96-well microplate and included two *P. falciparum* clones [Sierra Leone D6 (chloroquine-sensitive) and Indochina W2 (chloroquine- resistant)]. In primary screening the crude plant extracts were tested, in duplicate, at a single concentration of 15.9 íg/mL only on the chloroquine-sensitive (D6) strain of *P. falciparum*. The extract showing >50 % growth inhibition of the parasite was subjected to screening. For bioassay-guided fractionation, the column fractions were also tested only at single concentration. The pure compounds were subjected to additional testing for determination of IC_{50} values. The standard antimalarial agents chloroquine and artemisinin were used as positive controls, with DMSO (0.25 %) as the negative (vehicle) control. The selectivity indices (SI) were determined by measuring the cytotoxicity of samples on mammalian cells (VERO; monkey kidney fibroblast). All experiments were carried out in duplicate.

Results

Air-dried parts of the plants tested were pulverized, extracted and the percentage yield calculated as shown in Table 1. The percentage yield of 11.25, 3.45, 40.68 and 12.85 were obtained for *P. nitida*, PNS1, PNS5, *P. americana* root and PAS, respectively.

The antibacterial and antifungal activities of *P. americana* and *Jatropha podagrica* against a panel of organisms are shown in Tables 2 and 3. The results showed that the plant extracts were effective against both bacterial and fungal organisms. The plant extracts showed different antimicrobial activity (IC_{50} values) to the test organisms. The methanol extract of *Persea americana* seed (PAS) showed activity against all the bacterial and fungal isolates. A very good activity was observed against *C. neoformans* at IC_{50} value of 8 µg/mL. The chloroform fraction (PASC) also exhibited high inhibitory activity against *C. neoformans* (Tables 2 and 3).

The antimalarial activity of *Picralima nitida* total extract and fractions against two strains of *Plasmodium falciparum* demonstrated high inhibitory activity against the two species (Table 4).

Discussion

The current study was carried out on the extracts and fractions obtained from 3 plants used in Nigerian ethnomedicine for malaria and bacterial infections. The vacuum liquid chromatography of the *P. nitida* was done in line with standard operating procedures in pre-fractionation technique to separate the non-polar (n-hexane), intermediate (hexane: ethylacetate), and polar (ethylacetate: methanol) compounds. We have evaluated these extracts and fractions for activities against a panel of pathogenic fungi, bacteria, and the protozoa to explore the beneficial effects of these herbs.

Traditional herbal practitioners in Nigeria have achieved success with the use of *P. nitida* as remedy against malaria. Here, we report the antiplasmodial activity of the crude extracts and VLC fractions on two strains of *Plasmodium falciparum* (*P. falciparum* D6 and *P. falciparum* W2). Primary antimalarial evaluation of the methanol extract of *P. nitida* showed 91% growth inhibition in *P. falciparum* D6 clone at a concentration of 15867 µg/ml. Further evaluation of the methanol extract of *P. nitida* (Table 2) revealed moderate dose-dependent activity with IC_{50} values of 6.33 mg/ml and 6.00 mg/ml against D6 and W2 clones with a selectivity index ranging from >7.5 and >7.9, respectively. The other fractions of PNS

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showed activity against the two strains of *Plasmodium falciparum*. PNS1 gave a strong activity at IC_{50} of 3.50 and 2.82 mg/ml for D6 and W2 respectively, at a concentration of 4.70 mg/ml, and a selectivity index of >1.4 and >1.7.

Extracts obtained from *P. americana* and *J. podagrica* were also evaluated for their *in vitro* antimicrobial activities, and the results in Tables 3 and 4.

Table 1: Plants collection, parts tested and voucher number

Plant	Voucher specimen no.	Locality	Family	Part tested	Extraction yield
Picralima nitida Durand	FHI109572	Benin City	Apocynaceae	stem	11.25
PNS1					2.23
PNS2					1.45
PNS3					2.23
PNS4					1.08
PNS5					3.45
Persea americana Mill	FHI 107767	Ovia north east local government area, Edo State	Lauraceae	Root, seed	40.68 (R)
PASC					5.78
PARP					2.34
PAS					12.85
Jatropha podagrica Hook	FHI 93265	Owan east local government area, Edo State	Euphorbiaceae	fruit	7.02%

Table 2: Percent Inhibitions of *Persea americana* and *Jatropha podagrica* against bacteria and fungi

Test organism	% inhibition @ 50µg/mL			
	PARP	PASC	PAS	JPF
C.albicans	29	55	52	0
C. glabrata	16	49	40	8
C. krusei	37	68	58	17
A. fumigatus	13	96	12	6
C.neoformans	45	100	100	82
S. aureus	35	47	21	22
MRSA	84	97	14	10
E.coli	24	40	33	23
P. aeruginosa	6	8	2	38
M.intracellulare	0	54	0	0

Table 3: Antimicrobial activities of extract and fractions of selected plants

Test organism	IC_{50} (µg/mL) of extracts and fractions					
	PARP	PASC	PAS	JPF	Amphotericin B	Ciprofloxacin
C.albicans	>200	32.2	157.4	>200	0.27	NT
C. glabrata	150.9	30.6	116.5	>200	0.39	NT
C. krusei	`142.2	15.5	71.1	>200	0.65	NT
A. fumigatus	>200	14.7	195.1	>200	1.18	NT
C.neoformas	>200	<8	8.2	16.9	0.24	NT
S. aureus	51.3	40.3	>200	>200	NT	0.12
MRSA	8.7	35.2	>200	>200	NT	0.10
E.coli	>200	>200	>200	>200	NT	0.006
P. aeruginosa	>200	171.3	>200	58.7	NT	0.09
M.intracellulare	>200	95.7	186.1	>200	NT	0.40
NT: Not tested						

The primary assay (Table 3) showed that the two plants had antifungal activity against *Candida krusei* (68 and 58%) and *Candida albicans* (55 and 52 % for JPF). These two organizms are known to contribute to opportunistic fungal infections. The antimicrobial results indicated that pet. ether extracts of *P.americana* root, chloroform fraction of *P. americana* seed and methanol fraction of *P. americana* seed showed strong antifungal activity against *Candida krusei* (IC_{50} 142.20 mg/ml,15.51mg/ml and 71.10 mg/ml respectively). Previous reports had indicated that *P. americana* is used for treatment of skin diseases related to fungal infections, such as white lesions of vulva, vitiligo, psoriasis, tinea capitis, and lichen amyloidosis. This investigation provides a significant experimental evidence for development of PA as a potential antifungal agent. As well, the fraction chloroform

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extract of *P. americana* seed, methanol fraction of PAS and methanol fraction of JPF showed strong activity against *Cryptococcus neoformans* at 100,100 and 82 % inhibition with IC_{50} values of < 8, 8.211 and 16.93 µg/ml respectively. Only the petroleum ether of *P. americana* root, showed low activity against *C. neoformans* at IC_{50} of > 200 mg/ml. PARP and PASC showed a significant activity against methicillin-resistant *Staphylococcus*

Table 4: Activity of *P. nitida* extract and fractions against *Plasmodium falciparum*

Extract/fractions	IC_{50} (µg/mL) of extracts and fractions				
	P. falciparum D6 IC_{50}	*P. falciparum* D6 SI	*P. falciparum* W2 IC_{50}	*P. falciparum* W2 SI	VERO IC_{50}
PNS	6331.3	>7.5	6005.6	>7.9	>47600
PNS1	>35051.8	>1.4	28376.9	>1.7	>47600
PNS2	>4760	1	>47600	1	>47600
PNS 3	>4760	1	>4760	1	>4760
PNS4	16992.1	>2.8	15902.6	>3	>4760
PNS5	>4760	1	>4760	1	>4760

aureus (IC_{50} 8.695 mg/ml and 35.189 mg/ml respectively). All of the extracts were inactive against *E. coli*, PASC was the only extract active against *Mycobacterium intracellulare* 54% (IC_{50} 95.93) and *Aspergillus fumigatus* 96% (IC_{50} 14.728).

Extract of *P. americana* exhibited significant *in vitro* antimicrobial activity against of microorganisms, *C. neoformans*, methicillin-resistant *S. aureus*, *E. coli*, *M. intracellulare*, and *A. fumigatus*. The fractions of *P. nitida*, demonstrated strong *in vitro* antimalarial activities against chloroquine susceptible (D6) and resistant (W2) strains of *Plasmodium falciparum* with IC_{50}s ranging from 120 - 270 ng/mL.

The *in vitro* antibacterial assay of JPF showed complete inactivity to bacteria. A cursory electronic literature search did not reveal any reports of significant antibacterial activity for the fruit of *J. podagrica*, though the root and stems were documented to possess antibacterial activity.

Conclusion

The results indicated promising antimicrobial and antiplasmodial actions of *P. americana and P. nitida*. Further evaluation of *in vivo* antimicrobial and antiplasmodial activities in an animal model is needed

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Conflict of interest: There is no declaration of interest

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