OPEN ACCESS Freely available online

Natural Products Chemistry & Research

ISSN: 2329-6836

Research Article

Standardization and Antimalarial Evaluation of Crude Methanol and Endophytic Fungiextracts of Azadirachtaindica Leaves

Nwankwo Ogechukwulucy[1*], Okoye FBC[2], Onyegbule FA[2]

[1]*Department of Pharmacognosy& Traditional Medicine, Faculty of Pharmaceutical Sciences, NnamdiAzikiwe University, Awka-Nigeria*

[2]*Department of Pharmaceutical & Medicinal Chemistry, Faculty of Pharmaceutical Sciences, NnamdiAzikiwe University, Awka-Nigeria*

ABSTRACT

Background & objectives: Endophytes are capable of synthesizing bioactive compounds responsible for plants defense against pathogens, thus the evaluation of Azadirachtaindica antimalarial potential.

Methods: Extraction,phytochemical and physicochemical evaluation of the powdered leaves of Azadirachtaindicawas done using standard methods. Isolation of the endophytic fungi was done using malt extract agar (5 days). Fungal fermentation was done (21 days) and ethyl acetate extracted. Acute toxicity tests were performed on the crude and endophytic fungal extracts. Antimalarial curative study was done for both extracts, with artemeter-lumefantrine as positive control to evaluate the percentage curative effect (PCE). HPLC analysis was based on the retention time and UV absorption spectra.

Results: Phytochemical and physicochemical analyses revealed the presence of alkaloids, saponins and tannins and low moisture content (7.5 %). Seven endophytic fungi were isolated. Fungal fermentation yielded an appreciable amount ofendophytic fungi. The oral acute toxicity study of the crude extract in mice revealed lethality reaction at a dose of 4472.14 mg/kg while that of the seven endophytic fungi extracts were above 5000 mg/kg. The crude extract had its highest PCE (83.3 0%) at 125 mg/kg. AMRc and AMRyendophytic extracts gave the highest PCE at 125 mg/kg (95 and 95.72 %). Artemeter-lumefantrine showed PCE of 98.62 % at 0.9 mg/kg. HPLC analysis of the endopyhtic extractsidentifiedmethyl– 2- (4-hydrophenyl) acetate, neurolenin B, aniquinazoline, palitantin, genistein, procynanidin and pipernonaline. Conclusion: The findings of this study demonstrated the antimalarial potentials of Azadirachtaindica which may be exploited in the management of malarial infections.

Keywords: Endophyte, microscopy, artemeter-lumefantrine, percentage curative effect.

INTRODUCTION

The need for new and useful compounds to provide relief in all aspects of the human health condition is ever growing. Drug resistance in bacteria, the appearance of life threatening viruses, the recurrent problems of diseases in persons with organ transplant, and the tremendous increase in the incidence of fungal infections, emergence of resistant strains of malaria parasites in the world's population all underscore our inadequacy to cope with all these medical problems.1Natural products are chemical compounds derived from living organisms. The most prominent producers of natural products can be found within different groups of organisms including plants, animals, marine and microorganisms. The success of several medicinal drugs from microbial origin such as antibiotic penicillin from Penicillin sp., the immunosuppressant cyclosporine from Tolypocladiuminflatum, etc,has shifted focus of drug discovery from plants to microorganisms. Fungi are

Correspondence to: Nwankwo Ogechukwulucy, Department of Pharmacognosy& Traditional Medicine, Faculty of Pharmaceutical Sciences, NnamdiAzikiwe University, Awka-Nigeria, Tel: +2347030529221; E-mail: ol.nwankwo@unizik.edu.ng

Received: July 31, 2020; **Accepted:** August 31, 2021; **Published:** September 11, 2021

Citation: Ogechukwulucy N, Okoye FBC, Onyegbule FA (2021) Standardization and Antimalarial Evaluation of Crude Methanol and Endophytic Fungiextracts of Azadirachtaindica Leaves. Nat Prod Chem Res. 09:p412.

Copyright: © 2021 Ogechukwulucy N. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

among the most important groups of eukaryotic organisms that are well known for producing many novel metabolites which are directly used as drugs or function as lead structures for synthetic modifications.2Endophytes are believed to carry out a resistance mechanism to overcome pathogenic invasion by producing secondary metabolites.3 So far, studies reported a large number of antimicrobial compounds isolated from endophytes, belonging to several structural classes like alkaloids, peptides, steroids, terpenoids, phenols, quinines and flavonoids.4 This study evaluated the antimalarial activity of methanol extract and endophytic fungi extract isolated from Azadirachtaindica(Figure 1).

Figure 1: Picture of Azadirachtaindicaplant showing the seed, leaf and stem.

MATERIALS AND METHODS

Collection of Plant Materials

Leaves of Azadirachtaindica were collected from their trees growing in natural environment in the Faculty of Pharmaceutical SciencesAgulu, NnamdiAzikiwe University on the 2nd of December, 2015, with no visible symptoms of disease. The plants were identified, examined and authenticated in the Department of Pharmacognosy and Traditional Medicine, NnamdiAzikiwe University, Awka.

Experimental Animals

Swiss Albino mice (25-30 g) were employed for the study. All the animals were obtained from the animal house of the Department of Pharmacology and Toxicology, NnamdiAzikiwe University, Agulu. The animals were housed in standard laboratory condition of 12 h light, room temperature, 40-60 % relative humidity and fed with rodent feed (Guinea Feeds Nigeria Ltd). They were allowed free access to food and water.

Parasite

The parasite, Plasmodium bergheiused for this study was obtained from the Department of Pharmaceutics, University of Nigeria Nsukka. The strain was maintained in the laboratory by serial blood passage from mouse to mouse.

Experimental Procedures

Phytochemical Analysis Crude Plant Leaves

The qualitative and quantitative analyses of the extracts were carried out using standard methods.5

Methanol Extract Preparation

One hundred grams (100 g) of the dried, pulverized plant leaves was weighed using a weighing balance (Camry EK350 Model, China) and cold macerated in 500 ml of methanol for 72 h with intermittent shaking. The resulting solutions were filtered with Whatman filter paper. The filtrate was concentrated in vacuo using rotary evaporator (RE300 Model, United Kingdom) at 4 0C.

Isolation of Endophytic Fungi

The plant leaves were rinsed thoroughly with sterile water to remove dust and debris in the laboratory before being disinfected by immersion in 70 % ethanol for 3 min and 2 % sodium hypochlorite for 2 min. Then, they were rinsed with sterile distilled water and blotted dry on sterile blotting paper in a lamina flow cabinet. Sterile scalpel was used to cut the leaf blade and mid-rib approximately 1 cm in length. About 5-6 segments were placed aseptically on malt agar media which was prepared with 250 mg chloramphenicol per liter of the media to suppress bacterial growth. The cut end of the leaf blade and midrib were made to be in contact with the media. The plates were then incubated at 25-28 0C for five days but monitored every day. Most of the fungal growth was initiated within 5 days of inoculation. The hyphae growing from the plant materials were sub-cultured repeatedly until a pure culture was obtained.

Fungal Fermentation and Extraction Of Metabolites

Fungal fermentation was done using solid state fermentation. A 100 g of unpolished rice was sterilized with 200 ml of water at 121 0C for 30 min. After cooling, blocks of actively growing pure fungal isolates were transferred onto the rice media under aseptic conditions, plugged with cotton wool and foil and left on fermentation shelf for 21 days.On the 21st day, the fermentation was stopped by adding 500ml of ethyl acetate until the rice media was completely covered. A sterile aluminum rod was used to break apart the mycelia growth on the rice media and to loosen the media. The flasks were allowed to stand for 2 days with intermittent agitation. Filtration was done using a sterile cheese cloth to remove the mycelia, the rice media and finally through Whatman filter paper. The filtrates being the extracts were concentrated using rotary evaporator at the speed of 7 rpm at 50oC.The filtrates were used for the biological assay.

Acute Toxicity Study

Acute toxicity tests were performed using modified method of OECD- 423 guidelines.6A total of 13 mice were employed and done in two phases:

Phase I: In this phase the animals were grouped into three groups of three mice per group-

Group 1 – 10 mg/kg of the extract par oral; Group 2- 100 mg/kg; Group 3- 1000 mg/kg

The animals were constantly monitored for 2 h, intermittently for the next 6 h and after 24 h for behavioral changes and mortality. From the result of the 1st phase, the 2nd phase was carried out.

Phase 2: In this phase 4 mice were used and grouped into 4 groups of one mouse per group. The animals received treatment as follows:

Group 1- 2000 mg/kg; Group 2-3000 mg/kg; Group 3- 4000 mg/kg; Group 4- 5000 mg/kg

The animals were monitored as in phase 1 for behavioral changes and mortality,

The LD50 was calculated by the formula:

$LD50 = \sqrt{D0 \times D100}$

D0 =Highest dose that gave no mortality; and D100 = Lowest dose that produced mortality.

Antimalarial Curative Study

Antimalarial curative study of endophytic extract and methanol leaf extract were carried out according to standard method.7In this model, a total of seventy-two mice were used. They were grouped into twenty-four groups of three mice per group. Blood was collected from donor mouse infected with parasite (P. berghei) by oculus puncture and was diluted with normal saline such that 0.2 ml contains approximately 1x107 infected red cells. All the animals in each group were infected with the parasite by single oral administration of 0.2 ml of the diluted blood and were left for 72 h for the infection to be established. After infection, the animals were treated as elaborated in Table 1. A thin blood film was made from the tail blood stained with Giemsa stain and was examined for parasitemia on day 4 and 8 post-treatment. The percentage curative effect was calculated using the formula:

$$\frac{Do - DA}{Do} \times \frac{100}{1}$$

Where: Do = day 0 mean parasitaemia and DA = Treated day mean parasitemia

Table 1: Antimalarial Curative Study Groups and Treatment.

S/N	Group	Treatment (mg/kg)
1	A	500 AMRe
2	A	250 AMRe
3	B	500 ALBx
4	B	250 ALBx
5	C	500 AMRc
6	C1	250 AMRc
7	C2	125 AMRc
8	C3	62.25 AMRc
9	D	500 ALBg
10	D	250 ALBg
11	E	500 AMRy
12	E1	250 AMRy
13	E2	125 AMRy
14	E3	62.25 AMRy
15	F	500 AMRd
16	F	250 AMRd
17	G	500 ALBd
18	G	250 ALBd
19	H	500 CEA
20	H1	250 CEA
21	H2	125 CEA
22	H3	62.25 CEA
23	I	ACT 0.9
24	J	10 ml/kg 5% Tween 80

High Performance Liquid Chromatography (HPLC) Analysis

Each of the dried fungal metabolite extract (2 mg) was reconstituted with 2 ml of HPLC grade methanol. The mixture was sonicated for 10mins and thereafter centrifuged at 3000 rpm for 5 min. 100 µL of each dissolved sample was transferred into HPLC vials containing 500 µL of HPLC grade methanol. HPLC analysis was carried out on the sample using a Dionex P580 HPLC system coupled to a photodiode array detector (UV3440S, DionexSoftron GmbH, Germering,Gremany). Detection was at 235, 254, 280 and 340 nm. The separation column (125 x 4 mm; length x internal diameter) was prefilled with Eurospher-10 C18 (Knauer, Germany), and a linear gradient of nano pure water adjusted to pH 2 by addition of formic acid and methanol was used as eluent.

RESULTS

Result of Isolation of Endophytic Fungi

A total of seven fungal endophytes designatedAMRc – Azadirachta midrib c; AMRY– Azadirachta midrib y; AMRd – Azadirachta midrib d; AMRe – Azadirachta midrib e; ALBg – Azadirachta leaf blade g;ALBd - Azadirachta leaf blade d; ALBx -

Azadirachta leaf blade x were isolated. They are presented in Table 2, with their respective yields indicated.

Table 2: Yield of secondary metabolites obtained from endophytic fungi.

S/N	Endophytic Fungi	Metabolite yield (mg)	% Yield
1	AMRc	495	4.95
2	AMRy	520	5.20
3	AMRe	205.9	2.06
4	AMRb	150	1.50
5	ALBg	450	4.50
6	ALBd	400	4.00
7	ALBx	370	3.70

KEYS: A= Azadirachtaindica, LB=Leaf Blade, MR= Mid-rib

The result of extraction and phytochemical screening of Azadirachtaindica leaves

The results of the extraction of Azadirachtaindica leaves, the qualitative and quantitative phytochemical screening of the crude extract of Azadirachtaindica are presented on Tables 3, 4 and 5 respectively; showing a low yield of 2.5% and reasonable saponin content of 9.5 %.

Table 3: Yield of methanol extract of Azadirachtaindicaleaves.

	Plant	Weight of plant powder (g)	Weight of crude methanol extract (g)	Percentage yield (% w/w)
1	Azadirachtaindica	100	2.5	2.5

Table 4: Qualitative phytochemical constituents of methanol extract of Azadirachtaindica.

Phytochemicals	Observation
Tannins	+
Saponins	+
Flavonoids	+
Steroids	-
Alkaloids	+
Cardiac glycosides	-
Carbohydrates	-
Proteins	-
Starch	-
Triterpenoids	-

Key: (-) absent; (+) present

Table 5: Quantitative phytochemicalconstituents ofAzadirachtaindica leaves.

Constituent	Percentage yield (% w/w)
Alkaloid	5.00
Tannin	1.17
Saponin	9.50
Flavonoid	0.13

The Results HPLC Screening Of the Endophytic Fungal Extract From Azadirachtaindica

The results HPLC screening of the endophytic fungal extract from Azadirachtaindica are presented on Table 6 and Figure 2-8; showing the identified phyto-constituents of the endophytic fungal extractof Azadirachtaindica leaves. The reported pharmacological activities are presented on Table 7.

Figure 2: HPLC chromatogram of ALBd of endophytic extract of Azadirachtaindicashowing A - citreodrimene (Rt= 26.27 min), B - carbonarones (RT=30.40 min), C - Plasminogen activator inhibitor type 1 (Rt= 31.77 min), D- Plasminogen activator inhibitor type 1 (Rt= 32.56 min).

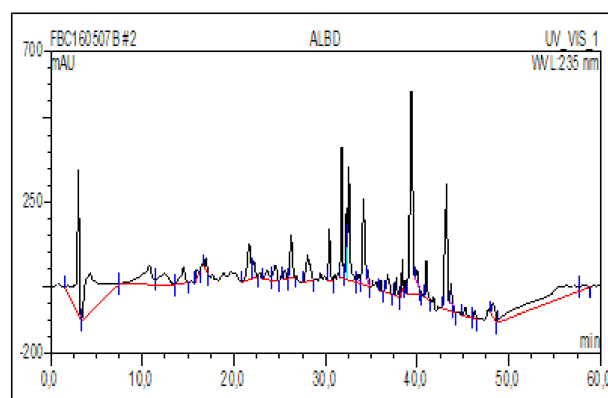

Figure 3: HPLC chromatogram of ALBg of endophytic extract of Azadirachtaindicashowing E - ethyl 2- (4- hydroxyphenyl) acetate (Rt = 15.12 min), F-Ruspolinon (Rt=20.81 min), G - PAI-1 (Rt=31.76 min), H –Citreodrimene (Rt= 32.75 min).

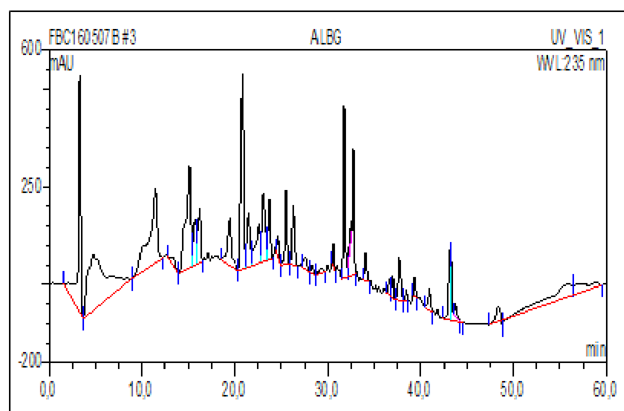

Figure 4: HPLC chromatogram of ALBx of endophytic extract of Azadirachtaindicashowing I - Ethyl 2 – (4 – hydroxyphenyl) acetate (Rt=14.26 min and 14.75 min).J - amin-chlor-phe-Essigsr. (Rt= 28.48 min), K- neurolenin (Rt=30.51 min).

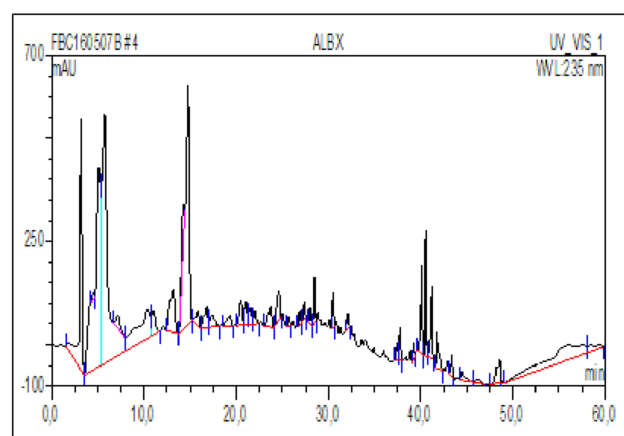

Figure 5: HPLC chromatogram of AMRb of endophytic extract of Azadirachtaindicashowing L - halenadinon (Rt= 20.42 min), M- dipiperamide (Rt= 22.08 min), N-pestaliopyrone (Rt= 23.05 min) , O- aniquinazoline (Rt=23.44 min), P- aureonitol (Rt= 38.32 min).

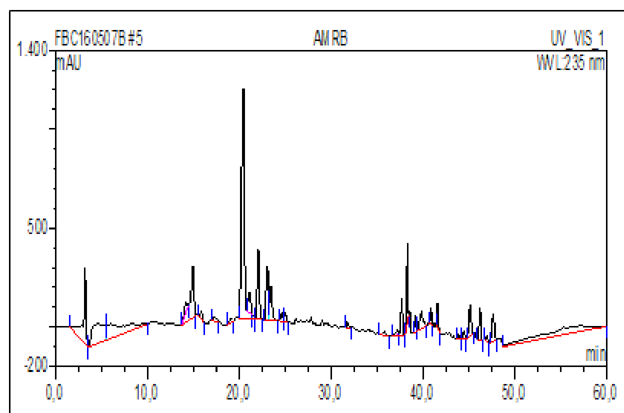

Figure 6: HPLC chromatogram of AMRc of endophytic extract of Azadirachtaindicashowing Q- Methyl -2- (4-hydrophenyl) acetate (Rt=14.86 min), R- Dicaffeoylchina (Rt= 22.18 min), S- callyaerin (Rt=26.13 min), T- antibiotic 3192C (Rt= 31.00 min), U – Dragmacidonamine (Rt= 34.34 min), V – Rubrofusarin (Rt= 35.50 min).

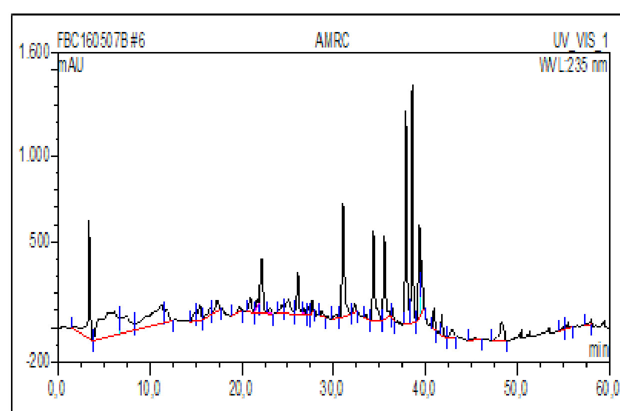

Figure 7: HPLC chromatogram of AMRe of endophytic extract of Azadirachtaindicashowing W- Monocerin (Rt= 22.26 min), X- Dehydropipernonaline (Rt= 25.25 min), Y- (E) – 4 – [(2 – Methylpropyl) amino] – 4 – oxo – 2 – butenoic acid (Rt= 27.81 min), Z- Aerophobin (Rt= 29.46 min), A1- Monocerin (Rt= 34.51 min).

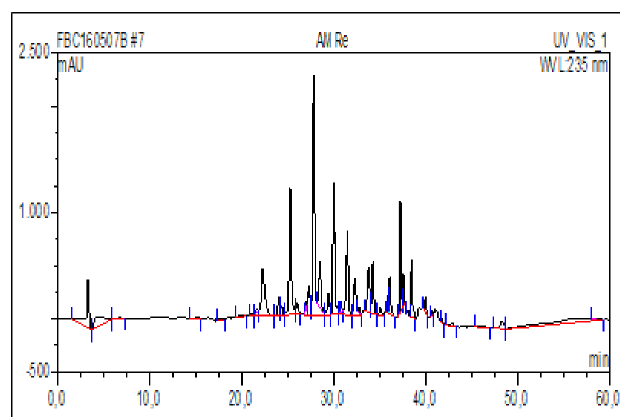

Figure 8: HPLC chromatogram of AMRy of endophytic extract of Azadirachtaindica showing A2- Bastadin 4 (Rt= 20.48 min), A3- A new aflavinine derivative (Rt= 21.92 min), A4- Pestaliopyrone (Rt= 23.16 min), A5- Genistein (Rt= 24.76 min), A6- Aniquinazoline (Rt= 27.39 min), A7- tetrahydroaltersolanol (Rt= 28.11 min), A8- Aniquinazoline (Rt= 29.69 min).

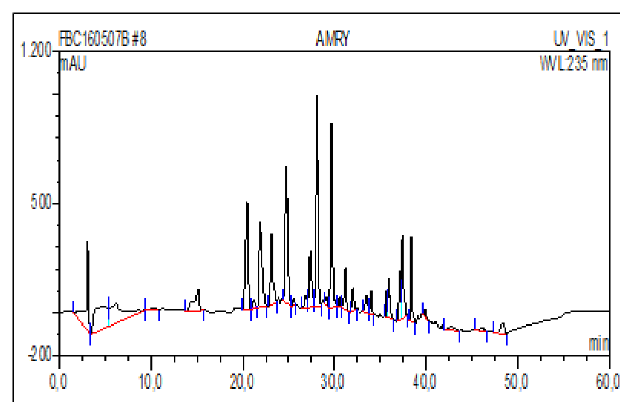

Table 6: HPLC result of the endophytic fungal extract from Azadirachtaindica.

Endophytic Fungal Extract	Comparable Peak

AMRB	Aureonitol, Halenadinon, Dipiperamide, Pestaliopyrone, Aniquinazoline, chloramphenicol.
AMRC	Dragmacidonamine, Rubrofusarin, Antibiotic 3192C, Callyaerin, Dicaffeoylchina
AMRe	Dehydropipernonaline, (E) – 4 – [(2 – Methylpropyl) amino] – 4 – oxo – 2 – butenoic acid, Aerophobin, Monocerin.
AMRy	Aniquinazoline, Bastadin 4, A new aflavinine derivative, Pestaliopyrone, Genistein, tetrahydroaltersolanol, (2)-5-4-hydrobenzylidene) – hydantoin, Questin, Palitantin,.
LBx	Amin – Chlor-Phe-Assignr, Neurolnin, Ethyl 2 – (4 – hydroxyphenyl) acetate.
ALBg	Ethyl 2 – (4 – hydroxyphenyl) acetate, PAI, Citreodimene, Ruspolinon
ALBD	PAI, Citreodimene, Carbonarone.

Table 7: Reported Biological Activities of some of the Chemical Compounds present in the Azadirachtaindica.

Chemical Compounds	Previously isolated from	Biological Activities
Dipiperamide C34H38N2O8	An alkaloid from white pepper	Nutrient28
Aureonitol	Helichrysumsp	Antiviral29
Dragmacidonamine	Dysideasp	Antibacterial30
Aniquinazoline (alkaloid)	Aspergillusnidulans	Antimalarial17, Cytotoxic17, Antibacterial31
Callyaerine	Marine sponge, Callyspongiaaerizusa	Cytotoxic14
Cynarine (Dicaffeoylchina)	Cynaracardunculus	Inhibits taste receptors
Pipernonaline		Mosquito Larvicidal18
Monocerin	Dreschleramonoceras	Insecticidal11
Dehydropipernonaline	Piper langum L.	Vasodilating activity32
Neurolenin B		Antimalarial10
Carbonarones	Aspergilluscarbonarices	Antibacterial33
Procynanidin	Quercuspetraea – heartwood	Antibacterial,9 Antioxidant9
Pavetannin		Antiviral34
Bastadin 5	Marine sponge	Antiangiogenic13
PAI – 1 (Plasminogen activator inhibitor type 1)	Wheat plant, Triticumaestivum L.	
Genistein	Genistatinctoria	Antioxidant35
Palitantin C14H22O4	Trichophytonharzianum	Antiprotozoan, 16Antifungal16
Questin C16H12O5	Eurotiumsp	Antidiabetic36
Methyl – 2- (4-hydrophenyl) acetate		Antimalarial activity12

Result of Physicochemical, Organoleptic and Microscopical Evaluations

Results of physicochemical, organoleptic and microscopical evaluations are presented on Tables 8, 9 and 10 respectively.

Table 8: Physicochemical analysis of Azadirachtaindica crude extract.

Parameter	Yield (%w/w)
Moisture content	9.0±0.68
Total Ash	11.5±0.12
Acid insoluble ash	2.0±0.03
Water soluble ash	7.5±0.05
Water soluble extractive	0.20±0.19
Alcohol soluble extractive value	4.57±0.12

Table 9: Organoleptic evaluation of Azadirachtaindicaleaf powder.

Features	Observation
Colour	Green
Odour	Non-characteristic
Taste	Slightly bitter
Texture	Coarse powder

Table 10: Microscopical examination of Azadirachtaindica leaf powder.

Features	Observation
Epidermal trichomes	Present
Calcium oxalate	Absent
Starch	Absent
Fibres	Present
Lignified vessel	Present

DISCUSSION

The plant materials were selected based on their numerous ethno-medicinal uses which are thought to be mediated by their various bioactive metabolites.Natural plant products have remained the eventual foundation for the treatment of various ailments, including malaria. The qualitative phytochemical screening of the methanol extract showed the presence of alkaloid, tannins, saponin and flavonoid. The quantified phyto-constituents in the leaves of the plant showed saponins to be the highest, followed by alkaloid, tannin and flavonoid. The quantity and quality of the phytochemical constituents could have been affected by the age, geographical location, and climatic condition of the plant. The appreciable amount of alkaloid present implies possible excellent antimalarial activity. It has been recorded that alkaloids have antimalarial activity which must have contributed to the antimalarial activity of the plant.8The presence of these chemical constituents within endophytes is an indication that they may be potential source of precursors for drug development.

The HPLC chromatogram of the endophytic extract of Azadirachtaindica leaf showed the presence of many compounds; these compounds were identified based similarity with data (UV spectra) in an inbuilt library and literature comparison. The chromatogram of the endophytic extract revealed the presence of major compounds which include dipiperamide, aureonitol, dragmacidonamine, callyaerin, pipernonaline, monocerin, dehydropipernonaline, neuroline B, carbonarones, procyanidin, pavetannin, bastadin 6, PAI-1, genistein, aniquinazoline, palitantin, questin and methyl-2- (4-hydrophenyl) acetate. Some of these preliminarily identified compounds may have contributed to the antimalarial activity of the plant, given their reported activities.9- 15 These compounds identified also confirmed some of the ethnomedicinal claims of the plant which include; antibacterial, antimalarial, antidiabetic, antioxidant, antifugal, and antioxidant activities.

Oral acute toxicity study in mice revealed no lethality or toxic reaction at any of the administered doses between (100 mg/kg and 5000 mg/kg). This implies that endophytic extract of the plant is not toxic and has a good safety profile and will be administered in very high doses orally before the toxic level can be reached. The LD50 of methanol extract of Azadirachtaindica leaf was obtained to be about 4472.14mg/kg which also shows that toxicity will be reached at very high concentration.

In the antimalarial evaluations, three endophytic extracts showed the highest antimalarial activity(ALBx, AMRc and AMRy) at 250, 125 and 125 mg/kg respectively. The HPLC analysis showed that these three extracts had compounds with very potent antimalarial activities which may have contributed to their very high percentage curative effects (PCE). These compounds include; palitantin, neurolenin B, aniquinazoline, pipernonaline and methyl-2- (4-hydrophenyl) acetate.10,12,16-18 The high alkaloid content of the crude and other undetected compounds may have contributed to its high antimalarial activity recorded. The findings of this study demonstrated the antimalarial potentials of Azadirachtaindica which may be exploited in the management of acute malaria infections. The effective dose at 50 % curative effect (ED50) was calculated from the dose response relationship curve. At 38 mg/kg AMRy and AMRcendophytic extract, 50 % reduction in parasitemia was achieved. The ED50 of the crude extract was 40 mg/kg. The least dose that achieved 50 % parasitemia reduction was obtained; hence the dose of crude extract was higher than the endophytic extract. Therefore, results from the research work have shown that the endophytic extract is more effective than the methanol leaf extract of A. indica both in dose, antimalarial effect and toxicity profile.

The estimation of the physicochemical parameters of crude drugs is important in detecting adulteration or improper handling of crude drugs. The moisture content of crude drugs determines their shelf-life. Reduced moisture content discourages bacteria, fungi or yeast growth and ensures safe storage. The general requirement of moisture content in crude drug is that, it should not be more than 14 %19 and the value obtained in this research work (9 %) is within the accepted range. The result of the moisture content of Azadirachtaindica leaf suggests that it could be stored safely for a long period of time. The ash value is the residue remaining after incineration of the plant material. This residue simply represents foreign organic matter such as metallic salts and/or silica naturally occurring in the crude drug or sticking to its or deliberately added to its, as a form of adulteration. Acid-insoluble ash is a part of total ash that is insoluble in hydrochloric acid (e.g. sand and siliceous earth) while water-soluble ash is the water soluble portion of the total ash.20The total ash value (11.5 %) of crude powder of Azadirachtaindica leaf was found to be approximately five times more than the acid insoluble ash (2 %) and water soluble ash (7.5 %). The crude powder of Azadirachtaindica leaf was higher in alcohol soluble extractive (4.57 %) than in water soluble extractive (0.20 %).

Unwanted parts of medicinal plants may sometimes, posses a character that will raise the ash value. Ash value of crude drugs varies within fairly wide limits and any fixed difference in these values indicates a change in quality.21, 22The extractive value of a medicinal plant is the amount of active constituents in a given amount of that plant when extracted with solvents. The higher alcohol extractive value shown by Azadirachta indicia leaf suggests that the constituents may be more soluble in alcohol than in water. These values could be useful for the compilation of a suitable monograph for the proper identification of this plant.

Organoleptic and macroscopic evaluations are the first steps towards establishing the identity and purity of crude drugs.23 Microscopy of powdered drugs is necessary because once the plant is dried and grinded; it loses its morphological identity and is prone to adulteration. Organoleptic evaluation is based on the study of sensory characters such as colour, odour, taste and texture.24Organoleptic evaluation of Azadirachtaindica powdered leaf revealed that it is a coarse green powder with aromatic odour and slightly bitter taste which are useful diagnostic characters. Microscopical examination of this plant showed the absence of calcium oxalate crystals and starch granules. Microscopic character observed include lignified spiral vessel which was stained pink, group of phloem fibers and covering trichome (non-glandular trichome) which was also reported by previous workers.25, 26These are diagnostic characters that could be used to differentiate Azadirachtaindicaleaf from other closely related species and varieties thus are helpful in proper identification of this plant part.

The HPLC analysis of these endophytic fungal metabolites revealed the presence of compounds belonging to alkaloids, coumarins, phenols, cyclic dipetides and other structural types, indicating that they could be useful precursors in drug development. It has been widely suggested that virtually all plants in natural ecosystems form an intimate symbiotic relationship with one or more types of endophytic fungi.27The current standard procedure for isolating fungal endophytes only accounts for non-fastidious endophytes. The obligate biotorphs are yet to be routinely cultured, thus, the true diversity of endophytes associated with these plants is underestimated.

CONCLUSION

The results from this study showed that the medicinal plant Azadirachtaindicaharbor diverse species of endophytic fungi. These endophytic fungi can be made to produce their secondary metabolites under controlled solid state fermentation. Some of these endophytic fungal extracts displayed god antiplasmodial activity against P. berghei. The crude methanol extract of the plant also showed high antimalarial activity. The very high antimalarial activity of the endophytic fungal extract could even suggest that the endophytes actually conferred that activity on the plant. Furthermore, these endophytic fungal extracts displayed strong antimalarial activity which was very close to that of Artemeterlumefantrine, thus could have high effect in treatment of acute malaria infections. The endophytic fungal extract AMRy demonstrated the strongest antimalarial activity with PCE of about 95.72 %; this shows the potential of this endophyte in the discovery of lead antimalarial agents.

RECOMMENDATION

Isolation and characterization of active constituents of the methanol leaf extract and endophytic extract to elucidate their specific mechanisms of action and further investigation of the toxicity of this plant to evaluate its effect during long term administration is recommended.

REFERENCES

1. Strobel G, Daisy B, Castillo U, Harper J. Natural products fromendophytic microorganisms. Journal of Natural Products. 2004; 67: 257-268.
2. Bode HB, Bethe B, Hofs R, Zeek A. Big effects from small changes: possible ways to explore nature's chemical diversity. Chem Bio Chem. 2002; 3: 619-627.
3. Tan RX, Zou WX. Endophytes: a rich source of functional metabolites. Nat Prod Rep. 2001; 18: 448-459.
4. Yu H, Zhang L, Li L, Sun P, Qin L. Recent developments and future prospects of antimicrobial metabolites produced by endophytes. Microbiological Res. 2010; 165: 437-449.
5. Edoga HO, Okwu DE, Mbaebie BO. Phytochemicals constituents of some Nigerian medicinal plants. Afr J Biotechnol. 2005; 4(7): 685-688.
6. Knight DJ, Peters W. The antimalarial action of N-benzyloxydihydrotriazines: The action of cycolguanil (BRL50216) against rodent malaria and studies on its mode of action. Ann Trop Med Parasitol. 1980; 74: 393–404.
7. Dua HS, Faraj LA, Said DG, Gray T, Lowe J. Human corneal anatomy redefined: a novel pre-Descemet's layer (Dua's layer). Ophthalmology. 2013; 120(9): 1778-85.
8. Souquet JM, Cheynier V, Brossaud F, Moutounet M. Polymeric proanthocyanidins from grape skins. Phytochemistry. 1996; 43, 509−512.
9. Blair S, Mesa J, Correa A, Carmona-Fonseca J, Granados H, Saez J, et al. Antimalarial activity of neurolenin B and derivatives of Eupatorium inulaefolium (Asteraceae). Pharmazie. 2002; 57: 413-415.
10. Axford LC, Simpson TJ, Willis CL. Synthesis and incorporation of the first polyketide synthase free intermediate in monocerin biosynthesis. Angewandte Chemie International Edition. 2004; 43: 727–730.