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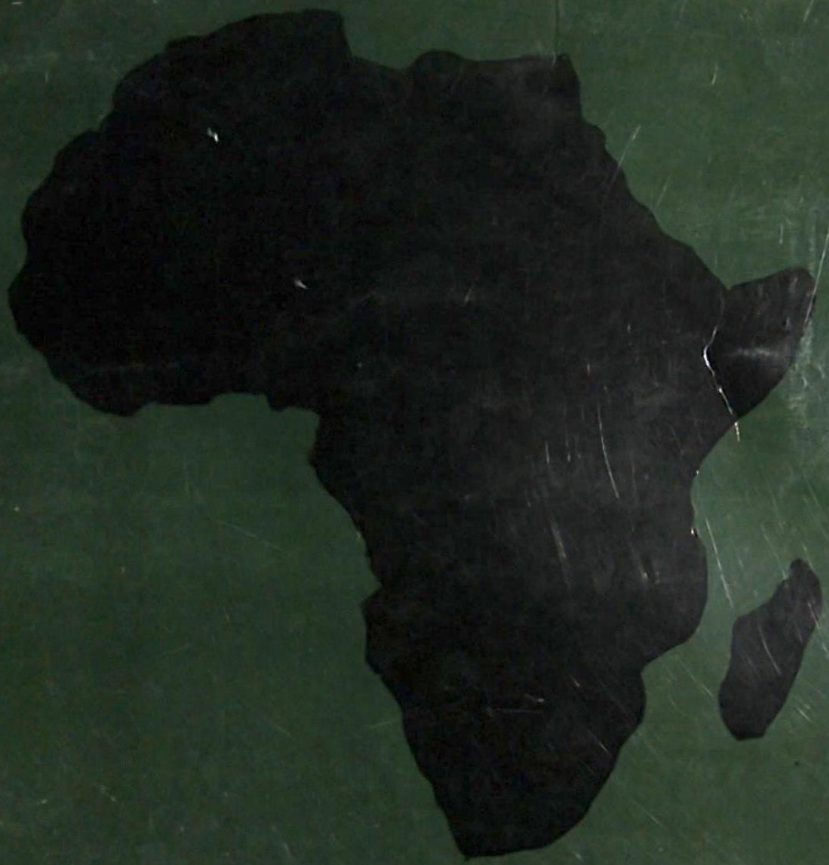

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Antibacterial and Antifungal activities of *Quassia undulata* and *Quassia amara* extracts *in vitro*

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Summary

Extracts of *Quassia undulata* and *Quassia amara* (Simaroubaceae) were screened for *in vitro* antibacterial and antifungal properties respectively. A total of eight extracts, comprising hexane and methanol extracts of the leaves and stems of each of the two plants were investigated. At a concentration of 5 mg/ml all eight extracts exhibited marked antibacterial and antifungal activities in most cases higher than the standard reference drugs included in the study. The extracts inhibited the growth of *Escherichia coli*, *Streptococcus faecalis*, *Stapylococcus aureus* and *Aspergillus niger*, even when the standard reference drugs utilized in the study did not. *Quassia amara* leaf methanol extract singularly exhibited the highest activities in both assays, which included the use of six clinical strains of bacteria and five fungi. The agar cup (10 mm diameter) diffussion and broth dilution techniques were used in both assays, utilising eleven human pathogenic microorganisms. Ampicillin and tioconazole were also included in the assay as reference compounds, while methanol was used as control. Diameter of zones of inhibition ranged between 11.0-29.0 mm for the tested extracts/drugs. All the extracts have shown impressive activities against the commonly encountered microorganisms and have thus confirmed the folklore uses of the plants in the African ethnomedicine.

Keywords: *Quassia undulata*, *Quassia amara*, extracts, antibacterial, antifungal activities.

Resumé

Les extraits du *quassia undulata* et *quassia amara*(Simaroubacee) étaient investigués in vitro pour des propriétés antibactérienne et antifungale respectivement. Un total de 8 extraits compris les extraits l'hexane et le métanol des feuilles et racines de chacune des 2 plantes étaient examinés. A la concentration de 5m/ml, les 8 extraits montraient des activités antibactérienne et antifungale remarkable et dans la plupart des cas plus elevées que les médicaments standard de reférence utilises dans cette étude. Les extraits arretaient le developpement de *l'escherichia coli*, *streptocoque fecale*, *staphylocoque aureus* et *l'aspergillum niger* meme titre que les médicaments standard de reference. L'emploi d'extrait au metanol des feuilles du *Quassia amara* singuliérenment montrait des activités plus elevees dans les 2 analyses qui inclu l'utilisation des 6 sous-type clinique des bactéries et cinq fungi. Les techniques de diffusion du verre d'agar (10mm de diamétre) et la diffusion de Broth étaient utilisées dans ces analyses avec les microorgaanismes pathogénes a l'homme. L'amplicilline et Tioconazole étaient aussi inclu dans l'analyse comme produit de reférence alorsque le métanol était utilisé comme le controle. Le diamétre des zones d'inhibition variait entre 110-290mm pour les extraits testés. Tous les extraits ont montré des activités impressive contre les microorganismes commun et ont ainsi confirmés l'utilisation des variétés de plantes en éthnomédicine Africaine.

Introduction

Quassia undulata (Guill & Perr) D. Dietr and *Quassia amara* L. are plants of the family Simaruobaceae and indigenous to the tropics. Both plants grow predominantly in Southern Nigeria. *Quassia undulata*, synonymous with *Hannoa klaineana* [1] is a tall tree with bore up to 0.9-1.2m in diameter. It may attain a height of 37.5m and dominate upper storey canopy with a large crown. *Q. amara* also known as Bitterwood does not grow so flambouyantly. In most places were they are found they hardly grow above a height of 6.0m. Both plants are used ethnomedically in different parts of Africa for the treatment of fever and to relieve colic. Decoction of wood and root is used for hypertension [2-4]. In Central Africa and other parts of the World, infusions from *Quassia* plants have been used as inhalant febrifuge, antirheumatic, treatment of dysmenorrhoea, gonococcal infections, bronchial pneumonia and for gastro-intestinal infections [2,5]. *Q. amara* has particularly been used in treating syphillis [6] and has demonstrated *in vivo* antifertility properties in male rats [7].

Compounds isolated from *Quassia* plants have exhibited a battery of biological activities. The plant family is characterised by the presence of quassinoids, ß-carboline alkaloids and cathin-6-one alkaloids. These compounds have been studied for use as chemotherapeutic agents world-wide. Quassinoids have been found to be useful as antimalarial, antitumour, antileukemic, antifertility, antiviral, antimicrobial insect anti-feedant anti inflammatory and antimicrobial agents [8-13]. In addition to quassinoids, ß-carboline alkaloids isolated from the plant family Simaroubaceae and other sources have been found to have, plant growth regulators, anti herpes simplex virus type 2, antileukemic, anti larval , antimicrobial and antitumour properties [14-16]. Cathin-6-ones also found in this plant family, have been found to inhibit cultures of resistant strains of human malaria parasite, *Plasmodium falciparum* [17]. Squalene triterpenoids from *Quassia sp* have also been found to possess anti cancer properties [18,19].

In continuation of our studies of Nigerian Simaroubaceae plants and a systematic search for biologically active natural compounds and derivatives based on folkloric uses [15, 20, 21], we present the antibacterial and antifungal studies of eight extracts from *Quassia undulata* and *Q. amara* including investigation of parts of both plants which have hitherto not been studied.

Materials and methods

Plant collection and authentication

Leaves (1.05 kg) and stems (1.31kg) of *Quassia undulata* were collected from a single tree in Abadina in the campus of University of Ibadan (UI). While the leaves (0.40 kg) and stems (0.92 kg) of *Q.amara* were collected from the Botanical garden within the campus of the same university in September 1997. Voucher

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specimen were authenticated in the Herbaria of Botany Department, UI and the Forestry Research Insitute of Nigeria (FRIN). Ibadan. *Q. undulata* was identified under FHI 40875 and *Q. amara* under FHI 055879 at FRIN.

Extraction of plant materials

The air dried plant materials were each ground with a Hammer mill. The four plant samples were consecutively extracted with redistilled hexane and methanol by percolation at room temprature for 72 hours respectively. After removal of solvents, yields of of the eight extracts so obtained were determined and stored in the refrigerator until needed.

***In-vitro* antimicrobial Assay**

Micro-organisms

The micro-organisms used in this study were obtained from the laboratory stock of the Department of Pharmaceutical Microbiology, UI, Ibadan. They were made up of cultures of 6 human pathogenic bacteria of which 3 were Gram positive and 3, Gram negative. For the antifungal studies, 5 fungi were used, consisting of 3 yeasts and 2 molds.

Media

Nutrient broth no. 2, pH 7.4; nutrient agar, pH 7.4; sabourand dextrose agar (SDA); tryptone soya agar; all products of Oxoid Laboratories, U.K. were used in the studies. Methanol (Merck) was also included in the assay to solubilize the extracts/drugs and as negative control.

Antimicrobial agents

The following chemotherapeutic agents were included in the assay as reference compounds : ampicillin, 2.5μg/ml (Lab Oftalmiso, Spain), tioconazole cream, 1mg/ml (Pfizer Inc. New York).

Antimicrobial activity determination

The agar cup diffusion and agar broth tube dilution procedures [22] were utilised in the studies. Each organism was passed through a series of culturing and subculturing to obtain a standard suspension for the test. An overnight broth culture of 1-2 X 10^7 CFU of each bacterium was used to seed sterile molten agar medium maintained at 45°C. Sterile tryptone soya agar plate was also seeded with the fungi. The seeded plates were allowed to solidify after which 5 wells were bored in each plate (9cm in diameter) using a 10mm aseptic cork borer.

Three concentrations of each extract (20 mg/ml, 10 mg/ml and 5mg/ml) were made in methanol. With the aid of a sterile Pasteur pipette, each well was filled with 80 μl of the extract. The plates were incubated at 37 °C for 24 h (bacteria) and at 25°C for 48 h for the fungi. Thereafter, diameters (mm) of zones of inhibition were determined. When seeded with bacteria, each plate had wells filled with methanol as well as ampicillin and while seeded with fungi, tioconazole was filled in one of wells in the plate.

Results of the antimicrobial assays were expressed as means and standard errors. The significance of difference between means of control and treated groups was determined by the Student's "t " test within 95% confidence limits $P < 0.05$.

Result

Percentage yield of extracts

The denotation of the eight extracts employed in this investigation and their respective yields (%) are presented in Table 1. The methanol extracts had higher yields than the hexane extracts

Table 1. Percentage Yield of Extracts of *Quassia undulata* and *Q. amara*

Plant	Part	Extract	Yield (%)
Q. undulata	leaf	hexane - QULH	2.40
Q. amara	leaf	hexane - QALH	2.50
Q. undulata	stem	hexane - QUSH	0.65
Q. amara	stem	hexane - QASH	0.24
Q. undulata	leaf	methanol - QULM	2.90
Q. amara	leaf	methanol - QALM	10.6
Q. undulata	stem	methanol - QUSM	4.30
Q. amara	stem	methanol - QASM	1.90

Antimicrobial activity

The antibacterial properties of the extracts are presented in Table 2 and the antifungal activities are presented in Table 3. The results presented in the two Tables are at a concentration of 5 mg/ml. The results of activities at 20 mg/ml and 10 mg/ml are not presented because at those concentrations, all extracts displayed good activities and the extracts inhibited the growth of all microorganisms much more than the standard drugs without any differentiation.

Table 2: Antibacterial activities of *Q. undulata* and *Q. amara* extracts

Extract/Drug[a]	Diameter of zone of Inhibition of Bacteria (mm) ± SEM					
	Gram +ve				Gram -ve	
	B. cereus	*B. subtilis*	*S. aureus*	*S. faecalis*	*P. aeruginosa*	E. coli
QULH	17.70 ± 0.6	13.3 ± 0.8	14.3 ± 1.7	12.0 ± 0.5	16.7 ± 2.0	10.7 ± 0.6
QALH	13.0 ± 0.7	12.7 ± 1.4	10.0 ± 1.1	14.0 ± 0.5	13.0 ± 1.0	13.0 ± 0.5
QUSH	18.5 ± 0.8	13.7 ± 1.2	13.0 ± 1.5	12.3 ± 0.2	16.7 ± 0.6	11.0 ± 1.0
QASH	15.0 ± 0.5	13.5 ± 1.8	17.7 ± 1.4	14.7 ± 0.1	10.7 ± 0.6	15.3 ± 0.3
QULM	25.6 ± 0.2	13.3 ± 1.8	14.6 ± 0.8	16.0 ± 0.0	16.3 ± 0.8	12.7 ± 2.4
QALM	29.0 ± 0.5	22.0 ± 1.5	28.7 ± 0.6	25.3 ± 0.2	18.4 ± 1.4	25.3 ± 2.4
QUSM	11.0 ± 1.0	14.0 ± 1.0	14.6 ± 0.6	13.6 ± 0.6	12.3 ± 1.3	13.3 ± 0.6
QASM	14.3 ± 1.2	13.0 ± 2.0	14.3 ± 0.2	15.0 ± 1.0	18.0 ± 1.4	12.3 ± 1.2
AMP	19.0 ± 0.7	23.7 ± 0.9	10.0 ± 0.4	10.0 ± 0.0	12.5 ± 0.3	10.0 ± 0.2
MeOH	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0

$P < 0.05$

a Extract concentration = 5 mg/ml; AMP = ampicillin (2.5 mg/ml, reference drug), MeOH = methanol (control and solvent)

Table 3: Antifungal activities of *Q. undulata* and *Q. amara* extracts

Extract/drug[a]	Diameter of zones of inhibition of Fungi (mm) ± SEM				
	Yeasts			Molds	
	Penicillium sp.	*O. fusisporum*	*C. albicans*	*A. flavus*	*A. niger*
QULH	18.7 ± 1.3	11.0 ± 1.0	15.6 ± 2.0	11.0 ± 0.5	16.0 ± 0.1
QALH	26.7 ± 1.4	14.3 ± 2.0	15.3 ± 2.1	10.3 ± 0.4	16.3 ± 0.6
QUSH	18.3 ± 1.2	12.0 ± 1.5	13.3 ± 1.2	11.5 ± 2.0	15.6 ± 1.8
QASH	15.3 ± 0.6	21.7 ± 1.6	15.0 ± 1.0	19.0 ± 1.0	12.6 ± 0.3
QULM	16.6 ± 0.6	14.3 ± 0.6	11.3 ± 0.6	15.6 ± 1.1	10.7 ± 0.6
QALM	19.0 ± 0.4	24.3 ± 0.5	22.0 ± 2.0	21.0 ± 0.5	18.7 ± 1.9
QUSM	17.7 ± 1.4	14.3 ± 0.8	15.6 ± 0.8	20.0 ± 2.5	15.7 ± 1.1
QASM	20.3 ± 1.6	16.0 ± 0.7	17.0 ± 2.0	13.0 ± 2.5	11.0 ± 1.0
TIO	16.8 ± 1.7	18.3 ± 1.0	15.0 ± 0.7	15.5 ± 0.8	10.0 ± 1.4
MeOH	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0

$P < 0.05$

a Extract concentration = 5 mg/ml; TIO = tioconazole (reference drug, 1 mg/ml) MeOH = methanol (control and solvent)

The Gram positive bacteria were more sensitive to the extracts than the Gram negative as shown in Table 2. Also evident from Table 3 is the fact that the yeasts were more susceptible to the extracts than the molds.

Discussion

The results of this investigation indicate that extracts of *Q. undulata* and *Q. amara* have exhibited outstanding antibacterial and antifungal activities. It is interesting to note that at a concentration of 5 mg/ml, *Q. amara* leaf methanol extract had antibacterial activities higher than ampicillin, the reference drug, for all the bacteria utilised in the study. Except for *Bacillus subtilis*, the extract exhibited a stronger antimicrobial activity to the bacteria than the ampicillin. *Streptococcus faecalis* and *E. coli*, two pathogenic and commonly encountered bacteria were found to susceptible to all the extracts used in the study while the standard reference drug was not. The extracts also inhibited the growth of *Pseudomonas aeruginosa* used in the the study to a higher extent. All the extracts exhibited various degrees of activity with *Q. amara* leaf hexane extract showing the least activty. *B cereus* was more sensitive than the other bacteria used in the study.

In the antifungal assay shown in Table 3, the extracts (5 mg/ml) of the two plants once more demonstrated high antifungal activities against all the fungi utilised in the study. In the assay, *Q. amara* leaf methanol extract exhibited the highest antifungal activity with all the microorganisms used. With each fungus, it had a wider diameter of zone of inhibition than the standard reference drug, tioconazole (1mg/ml). The *Penicillium sp* used was more sensitive to the extracts than all the other fungi.(15-27 mm). *Candida albicans* which is a difficult yeast infection to treat, was also inhibited by almost all the extracts much more than tioconazole. The least sensitive of the fungi was *Aspergillus niger*. Generally, all the extracts displayed considerable antifungal activity, even when the micro-organisms were resistant to tioconazole, as shown in Table 3. Methanol was used as vehicle, and did not display any activity against the reference compounds or the micro-organisms. It is well known that intensive use of antibiotics is often accompanied by appearance of resistant strains [23], so there is a high need for continous search for new antibiotics. It is even more imperative to seek them from natural sources rather than from chemical agents as this reduces the risk of resistance. Plants continue to be a rich source of such new therapeutic agents in tropical Africa which has a predominantly evergreen forest. *Q undulata* and *Q. amara* have demonstrated from this study that they are likely candidates for identification and isolation of such drugs.

It is imperative to note the susceptibility of cultures of *E coli, S faecalis, S aureus* and *A. niger* to all the studied extracts when the reference drugs did not show any antimicrobial activity. Also worthy of note is the outstanding activity of *Q. amara* leaf methanol extract to all the micro-organisms used in the study. Though many studies have been done on *Q. amara* stem wood, this is the first report on Q. *amara leaf* [7, 16, 24-28]. It is also important to note that there is no report in literature concerning the antimicrobial properties of leaves of *Q. amara* and *Q. undulata* or components thereof.

Conclusion

Considering the antibacterial and antifungal activities that were exhibited by all the extracts of *Q. undulata* and *Q. amara* investigated, and in addition to the popularity of these plant drugs in the treatment of gonococcal infection, bronchial pneumonia, gastro intestinal among other microbial infections in the African ethnomedicine, the two plants could be sources of development of antimicrobial agents.

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