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Phytochemical and antifungal activities of Uvaria. chamae leaves and roots, Spondias mombin leaves and bark and Combretum racemosum leaves

OMTB Okwuosa[1], EI Chukwura[2], GO Chukwuma[1], CN Okwuosa[3], IB Enweani[1], NR Agbakoba[1], CM Chukwuma[1], PO Manafa[1] and CU Umedum[4].

Departments of Medical Laboratory Sciences and Microbiology, Nnamdi Azikiwe University, Awka Nnewi Campus, Department of Medical Laboratory Sciences, University of Nigeria, Enugu Campus and Department of Microbiology, Anambra State University, Uli, Nigeria

Abstract

The effects of crude and dilutions of aqueous, methanolic and n-hexane extracts of Uvaria chamae (roots and leaves), Spondias mombin (leaves and bark) and Combretum racemosum (Leaves), on pathogenic candida albicans and aspergillus niger was studied. the aim was to contribute to the search for a cheaper, conventional cure for both fungi. phytochemical analysis revealed varying degrees of alkaloids, glycosides, saponin, lipid and oil, tannins, flavonoids, terpenoids and acids. Agar diffusion method was used for anti fungal assay. Minimum inhibitory concentration (mic) used was 10mg/ml of extract and dilutions of the non polar solvents of 10^{-1} and 10^{-2} was used. results showed that none of the plant parts was active against aspergillus niger. combretum racemosum had no antifungal effect on tested organisms as well as the different dilutions. however crude methanolic extract of uvaria (roots and leaves), spondias (bark and leaves), and n-hexane extracts of uvaria (leaves and roots), produced anti candidal effects with diameters in this order 14.67 $\pm$ 0.72mm, 10.67 $\pm$ 0.52mm, 11.00 $\pm$ 0.47mm, 15.00 $\pm$ 0.47mm, and 14.67 $\pm$ 0.72mm respectively. Some of the plant parts especially uvaria had zones of inhibition at a confidence limit comparable with control drug which is ketoconazole and it had inhibitory effects at a diameter of 20.06 $\pm$ 0.40mm.

Keywords: *Phytochemical, antifungal, leaves, roots, bark, uvaria. chamae, spondias. mombin, combretum racemosun*

Résumé

Les effets de pétrole brut et les dilutions des extraits aqueux, méthanolique et le n-hexane de Uvaria chamae (racines et feuilles), Spondias mombin (feuilles et l'écorce) et Combretum racemosum (feuilles), sur les Candida albicans pathogène et l'Aspergillus du fleuve Niger ont été étudiés. L'objectif était de contribuer à la recherche d'un produit moins cher, un remède traditionnel pour les deux champignons. Une analyse phytochimique a montré de divers degrés d'alcaloïdes, de glycosides, de la saponine, de lipides et de l'huile, des tanins, des flavonoïdes, des terpénoïdes et des acides. La méthode de diffusion en gélose a été utilisée pour le dosage de la lutte contre des champignons. La concentration minimale inhibitrice (CMI) utilisée était de 10mg/ml de l'extrait et des dilutions des solvants non polaires de 10^{-1} et 10^{-2} ont été utilisés. Les résultats ont montré qu'aucune des parties de la plante n'était active contre l'Aspergillus Niger. Le Combretum racemosum n'a eu aucun effet antifongique sur les organismes testés ainsi que sur les différentes dilutions. Cependant l'extrait méthanolique brut de Uvaria (racines et feuilles), la Spondias (écorce et feuilles) et les extraits n-hexane de Uvaria (feuilles et racines), ont produit les effets anti Candidaux avec des diamètres de l'ordre de 14,67 + 0,72mm, 10,67 + 0,52 mm, 11,00 + 0,47mm, 0,47mm 15,00 +, et 14,67 + 0,72mm respectivement. Certaines des parties de la plante en particulier l'Uvaria avaient des zones d'inhibition à une limite de confiance comparable à celles du médicament pilote c'est-à-dire kétoconazole et il a eu des effets inhibiteurs à un diamètre de 20,06 + 0,40 mm.

Introduction

clinical microbiologists have every reason to be interested in the topic of antimicrobial plant extracts, since it is likely that those phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed for humans. It is reported that on average, two or three antibodies derived from microorganisms are launched each year [1]. Fungal infections, especially candidosis and the increasing emergence of drug resistance have induced the interest in ethnopharmacology and the past 20years has shown a rapid rate of plant species extinction, it is important that studies should be conducted to harness the potentials in these herbs [2]. Spondias mombin Uvaria

Correspondence: Dr. G.O. Chukwuma, Department of Medical Laboratory Sciencess, Nnamdi Azikiwe University, Awka Nnewi Campus, Nigeria. E-mail: georgechuma@yahoo.com

chamae, Combretum racemosun are tropical herbs that grow naturally and can be thus cultivated in tropical, savanna, grassland or lightly wooded forests of west africa. these plants can also be found in other tropical regions of the world, including asia, asia minor, south america and possibly the south-western, western and plains regions of the united states. the active medicaments from these plants include, but are not limited to glyceryl esters, saponins and several derivatives of alkaloids, glycosides,tamins, fats and lipids.

Materials and method

Spondias. mombin treebark and leaves, uvaria. Chamae roots and leaves and *Combretum racemosum* leaves were obtained from Oraukwu in Idemili Local Government of Anambra State, and proper identification made at Department of Botany, University of Nigeria, Nsukka.

Phytochemical screening of the crude extracts

phytochemical screening of the plant material was done to ascertain the presence of bioactive components in different plant parts. The phytochemical screening was done according to the method described by [3] and [4].

Preparation of plant extracts

the stem bark of spondias. mombin was dried under shade and pulversied. the same was done for the roots of uvaria. chamae. The leaves of the three plants were also air dried, ground into powder and the resulting powder was then extracted using water (aqueous), n-hexane and methanol in soxhlet extractor. It was done at 4-6hrs for the leaves and 6-8 hrs for the roots and bark according to the methods decsribed by [3] and [5].

The crude extracts were prepared to contain a concentration of 10mg/ml for the different plant parts. The organic solvents were also prepared as 1:100 (10-2) and 1:10 (10-1) dilutions, so that for each organic (non polar) extract, there is a crude (stock of 100mg) and 2 dilutions (10-1 and 10-2).

Preparation of fungal isolates

Stock cultures of the fungal isolates of candida. albicans and aspergillus. niger were subcultured unto sabouraud dextrose agar plate, incubated at 25°c for 24hrs.

Susceptibility tests

Agar gel diffusion method (punch hole method) was used for susceptibility test according to methods described by [6].

Table 1: Phytochemical analysis of leaf, root and bark extracts of herbs

Plants	Extracts	Alkaloids	Glycosides	Saponins	Acidity	Lipids/ oil	Tanins	Flavonoids	Terpenoids
Uvaria. chamae	Leaf extracts	+++	+	+	_	_	++	+++	+++
	Root extracts	++	++	+	_	_	+++	+++	+++
Spondias. mombin	Leaf extracts	++	+	++	++	++	+++	++	+++
	Bark extracts	+++	_	+	_	_	++	+++	++
Combretum. racemosum	Leaf extracts	+	++	+++	_	_	_	+	++

Key
+ *present*
++ *moderate*
+++ *abundant*
- *absent*

Results

Table 2: Antifungal activity of the aqueous extracts of various plants and plant parts extracts on candida. albicans and Aspergillus. niger isolates

Extracts Aqueous extracts (leaves, roots and bark)	Test organisms/ diameter of zones of inhibition (mean+_sem) in millimeters (mm) Candida. albicans	Aspergillus. niger
SMLc (400ug)	7	7
UCLc (400ug)	7	7
UCRc (400ug)	7	7
SMBc (400ug)	7	7
CRLc (400ug)	7	7
Ketoconazole 200mg: (+control)	34	30
Distilled water (-ve control)	7	7

Table 3: Antifungal activity of Spondias. mombin extracts

Extracts	Dilutions	Candida. albicans	Aspergillus. niger
	SMLc (400ug)	11.00 ± 0.47	7
	10-1 (40ug)	7	7
Methanolic and	10-2 (4ug)	7	7
N- hexane	snlc (400ug)	7	7
extracts of	10-1 (40ug)	7	7
Spondias mombin	10-2 (4ug)	7	7
leaves and	ketoconazole (200mg)	34	30
their dilutions	distilled water (-ve control)	7	7
	SMBc (400ug)	11.00±-0.47	7
Methanolic	10-1 (40ug)	7	7
extracts of	10-2 (4ug)	7	7
bark of Spondias.	ketoconazole (200mg)	34	30
mombin	distilled water (-ve control)	7	7

Tests revealed the presence of resins, saponins, tamins, glycosides, flavonoids, alkaloids, fats and lipids

Discussion

All the plants used contain abundant quantities of tannins, flavonoids, terpenoids and alkaloids. combretum though contained no tannins was not acidic or contained any lipid, however, it contained the largest quantity of saponin than others.

Uvaria chamae leaf extracts, root extracts and the bark extracts of spondias contained no acid and lipids. moreso, the bark extract of spondias had no glycosides while the glycosides found in uvaria roots and spondias leaf were those of anthracene derivatives. The leaf of spondias mombin contained all the tested phytochemicals in varying degrees and is the only plant part studied that contained lipids/ oil and acid. The difference in variation may be due to differences in extracting capability.

These phytochemicals pocessed by these plants prove that they have antimicrobial activity. Acids like phenols contain hydroxylated compounds which have great activity against fungi, acting by enzyme inhibition by oxidized compound through reaction with sulfhydryl groups or by non specific interaction with proteins [7]. Tannins, flavonoids and terpenoids have been proved to have antifungal activities [8]. The antimicrobial properties of tannins and found them to be toxic to filamentous fungi, yeasts and fungi [8].

Terpenoids gives flavour to plants. They are highly branched terpenes which are found to be very active against fungi. Sixty percent of essential derivatives from them are inhibitory to fungi while 30% inhibited bacteria by disrupting membranes. They have a broad spectrum of antifungal activity. Alkaloids, heterocyclic nitrogen compounds have vast antibacterial activity [9]. The major biologically active plant parts are alkaloids, sequiterpenes, flavonoids, sterols, coumarines, saponins, resins, tamins, among others and all the plant parts studied contains them in varying degrees.

Table 4: Antifungal activity of methanolic and n-hexane extracts of Uvaria. chamae and Combretum. racemosum extracts on Candida albicans and Aspergillus niger isolates

Extracts	Dilutions	Candida. albicans	Aspergillus. niger
	UMLc (400ug)	10.67 ± 0.54	7
	10-1 (40ug)	7	7
Methanolic and	10-2 (4ug)	7	7
N-hexane extarcts	UNLc (400ug)	15.00 ± 0.47	7
of uvaria leaves	10-1 (40ug)	7	7
and its dilutions	10-2 (4ug)	7	7
	Ketoconazole (200mg)	34	30
	Distilled water	7	7
	UMRc (400ug)	14.67 ± 0.72	7
Methanolic and	10-1 (40ug)	7	7
N- hexane extracts	10-2 (4ug)	7	7
of roots of uvaria.	UNRc (400ug)	7	7
chamae and	10-1 (40ug)	7	7
its dilutions	10-2 (4ug)	7	7
	Ketoconazole (200mg)	34	30
	Distilled water	7	7
	CMLc (400ug)	7	7
Methanolic and	10-1 (40ug)	7	7
N- hexane extracts	10-2 (4ug)	7	7
of leaves of	CNLc (400ug)	7	7
combretum.	10-1 (40ug)	7	7
racemosum	10-2 (4ug)	7	7
	Ketoconazole (200mg)	34	30
	Distilled water	7	7

Key

7mm : No activity
HERBc : crude extract of plant part
UML : Uvaria chamae leaves(methanolic extract)
UMR :Uvaria chamae roots (methanolic extracts)
d/w : distilled water (negative control)
UNR : Uvaria chamae. roots (n-hexane extracts)
UNL : Uvaria. chamae leaves (n-hexane extracts)
SMB Sondias. mombin bark (methanolic extracts)
SML: Spondias. mombin leaves (methanolic extracts)
SNL: Spondis. mombin leaves (n- hexane extract)
CML: Combretum. racemosum leaves(methanolic extracts)
CNL: Combretum. racemosum leaves (n- hexane extracts)

Subscript (1) : 1: 10 dilution of crude drug
" (2) : 1: 100 dilution of crude drug

Methanolic extracts of spondias bark,, leaves, uvaria chamae leaves and roots showed marked antifungal activities at mic concentration of 400ug/ml (0.04mg/ml) when compared with control (ketoconazole, 200mg/ml i.e 200,000ug/ml). The clearance zone diameter was almost comparable with control drugs, considering that their concentration doses were small. Only the n-hexane extract of Uvaria chamae leaves showed a clearance diameter of (15.00 +- 0.47) at 400ug concentration (0.04mg/ml). The remaining n-hexane plant extracts were not active against either of the fungal organisms and the dilutions of the extracts were also devoid of reaction (7mm = 0). Again, n- hexane extract had the highest clearance rate (15.00 +- 0.47) at 0.04mg/ml concentration. This could be attributed to differences in molecular weight, diffusion coefficient and dissolution capability.

Conclusion

The antifungal activity of these plants, justifies their use in folk medicine in treating many skin infections. Since all the solvents were completely removed by

evaporation during extraction, it is clear that the antifungal activities are due to the bioactive ingredients in plant extracts. It is therefore necessary that more of these compounds be subjected to human and animal studies to determine their effectiveness in whole organ system including toxicity studies to check their side effects.

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