Fungitoxicity spectra of crude extracts of three Ghanaian medicinal plants

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SUMMARY

Steam distillates from three Ghanaian medicinal plants, *Ocimum gratissimum* (L.), *Xylopia aethiopica* (Dunal) Rich and *Cymbopogon citratus* (D.C.) Stapf, were tested for fungitoxicity against 20 fungal species. A fungitoxicity spectrum (FS) was calculated for each extract based on per cent inhibition of fungal growth. High FS values of 4.8 and 3.5 (maximum assignable value = 5) were, respectively, associated with extracts of *O. gratissimum* and *C. citratus*. A low value of 1.4 was recorded for *X. aethiopica*. Whereas extract of *O. gratissimum* exhibited complete fungicidal activity towards 14 of the 20 fungi, that of *C. citratus* was fungicidal to only three. The extract of *X. aethiopica* was fungistatic but not fungicidal.

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RÉSUMÉ

AWUAH, R.T.: *Spectre fongitoxicité des extraits bruts de trois plantes médicinales ghanéenes.* Les produits de distillation à vapeur de trois plantes médicinales ghanéenes, *Ocimum gratissimum* (L.), *Xylopia aethiopica* (Dunal) Rich et *Cymbopogon citratus* (D.C.) Stapf, étaient analysées pour fongitoxicité contre 20 espèces de fongus. Un spectre de fongitoxicité (FS) était calculé pour chaque extrait basé sur le pourcentage d'inhibition de la croissance fongique. Les hautes valeurs FS de 4.8 et 3.5 (valeur maximum assignable = 5) étaient respectivement associées avec les extraits de *O. gratissimum* et *C. citratus*. Une valeur basse de 1.4 était enregistrée pour *X. aethiopica*. Alors que l'extrait de *O. gratissimum* exposait une activité fongicide complète envers 14 du 20 fongus, celui de *C. citratus* était fongicide seulement à trois. L' extrait de *X. aethiopica* était fongistatique mais pas fongicide.

Introduction

Extracts of certain West African plants have been shown to possess anti-microbial effects against human bacterial pathogens (Gyane, 1976; Boakye-Yiadom, Fiagbe & Ayimo, 1977; Benjamin & Lamikanra, 1981; Akinde, 1986). Studies involving fungi are, however, few. Awuah (1989), for example, found extracts of *Oximum gratissimum*, *Xylopia aethiopica* and *Cymbopogon citratus* to be toxic to *Ustilago maydis* (D.C.) Cda., *Ustilaginoidea virens* (Cke.) Tak., *Curvularia lunata* (Wakk.) Boed. and a *Rhizopus* species. The biologically active constituents which make these plants fungitoxic have been reported to be citral for *C. citratus* (Purseglove, 1975), eugenol and thymol (Tripathi, Benerji & Sharma, 1986; Sainsbury & Sofowora, 1971) for *O. gratissimum* and xylopic acid/other diterpenes for *X. aethiopica* (Boakye-Yiadom, Fiagbe & Ayim, 1977). Because only four fungi were used by Awuah (1989), it is now necessary to assay extracts of these plants against a wider range of fungi.

The paper presents results of tests conducted with 20 fungi to establish the spectra of fungitoxicity of the three plant extracts so that the most potent could be identified for possible development as a broad-spectrum fungitoxicant. A scheme for quantifying and objectively comparing fungitoxicity spectra of plant extracts is also presented and discussed.

Materials and methods

Two hundred grams of chopped fresh leaves of *C. citratus* and *O. gratissimum* and 200 g of crushed

dried fruits of *X. aethiopica* were separately steam-distilled with 600 ml tap water. The volume of distillate during a 15-min period, after the first trickle, was collected in an oven-sterilized 500 ml Erlenmeyer flask, allowed to cool and tested for fungitoxicity as follows: Mycelial plugs (7 mm diameter) were removed from the margin of an actively growing culture of each test fungus on potato-dextrose agar (PDA) plate and flooded (3 min) in a sterile Petri dish with the extract. The treated mycelial plugs were subsequently transferred, top down, onto plates of PDA containing chloramphenicol (250 ppm) to suppress bacterial growth. Four replicate transfers were made and incubated on a laboratory bench at 25 - 30 °C. The diameters of the fungal colonies which developed were measured within 24-74 h and expressed as cm/24 h. A parallel set up in which mycelial plugs were treated with sterile distilled water served as control. Per cent inhibition of a test fungus obtained with each extract was calculated. Fungitoxicity classes were established as per cent inhibition values with the following scheme: 0 = no inhibition; 1 = 0.01 - 20.00 per cent inhibition; 2 = 20.01 - 40.00 per cent inhibition; 3 = 40.01-60 per cent inhibition; 4 = 60.01 - 80 per cent inhibition and 5 = 80.01 - 100 per cent inhibition. With this scheme, a fungitoxicity spectrum (FS) was calculated for each extract as follows:

$$FS = \frac{N_o + N_1 + N_2 + N_3 + N_4 + N_5}{N_T}$$

where N_o, N_1, N_2, N_3, N_4, N_5 are, respectively, the numbers of fungi in class 0×0, 1×1, 2×2, 3×3, 4×4, 5×5 and N_T is the total number of fungi tested.

The following 20 fungi used in the test, *Alternaria citri* Ell. & Pierce, an *Aspergillus* sp., *Aspergillus niger* van. Tieghem, *Bipolaris maydis* (Nisikado) Shoemaker, *Choanephora cucurbitarum* (Berk. & Rev.) Thaxt., *Cladosporium fulvum* Cke., *Colletotrichum dematium* (Pers. ex Fr.) Grove, *Coriolopsis polyzona* (pers.) Ryv., *Curvularia lunata* (Wakk.) Boedjin, *Fusarium oxysporium* Schlect, *Gloeophyllum striatum* (Sw. & Fr.) Murr., a *Mucor* sp., *Nigrospora sphaerica* (Sacc.) Mason, a *Penicillium* sp., a *Pestalotia* sp., a *Pleurotus* sp., *Pycnosporus sanguineus* (Lk. ex Fr.) Murr., *Rhizopus stolonifer* (Ehr. ex Fr.) Lind., *Sclerotium rolfsii* Sacc. and *Trichoderma viride* Pers. ex Fr. *Pleurotus* sp., *Coriolopsis polyzona*, *Gloeophyllum striatum* and *Pycnosporus sanguineus* were obtained from the pathology section of the Forest Research Institute of Ghana (FORIG), Kumasi. The rest of the fungi were isolated from natural substrates following standard procedure (Tuite, 1969).

Results

The results are summarized in Table 1. The *Ocimum gratissimum* extract had the highest FS value of 4.8 out of a maximum assignable value of 5. With this extract, fungicidal activity was obtained in 14 of the 20 fungi tested and fungistatic effect on the remaining six test fungi was very high. The extract of *C. citratus* was intermediate in activity, being fungicidal to only three of the test fungi. The *X. aethiopica* extract had a very low FS value of 1.42. This extract was not fungicidal but merely suppressed growth in 16 of the test fungi and was ineffective against three.

Discussion

Inhibition of four phytopathogenic fungi in a previous study by extracts of *O. gratissimum* and *C. citratus* (Awuah, 1989) provided evidence of possible broad spectrum efficacy of the extracts. Significant inhibition of all 20 fungi with the extract of *O. gratissimum* and 16 fungi with that of *C. citratus* obtained in the present study confirms the broad spectrum fungitoxic properties of these two plant extracts, making the plants potential candidate sources of broad spectrum fungitoxicants. This is particularly true with *O. gratissimum*, the extract of which was fungicidal to 14 of the 20 fungi and exhibited high fungistatic activity towards six others. Both plants occur locally and are easy to propagate. Thus, should on-going *in vivo* experi-

TABLE 1

Effect of Extracts of Three Ghanaian Medicinal Plants on Radical Growth of 20 Fungal Species

	Extract of O. gratissimum			*Extract of* X. aethiopica			*Extract of* C. citratus		
	Mean diameter of colony (cm) from mycelial discs immersed in			*Mean diameter of colony (cm) from mycelial discs immersed in*			*Mean diameter of colony (cm) from mycelial discs immersed in*		
Test fungus	*Distilled water*	*Plant extract*	*Per cent change*	*Distilled water*	*Plant extract*	*Per cent change*	*Distilled water*	*Plant extract*	*Per cent change*
Alternaria citri	1.01	0	100*	1.05	0.96	8.57	0.84	0.15	82.14*
Aspergillus sp.	0.78	0	100*	0.60	0.44	26.67	0.39	0.03	92.31*
A. niger	1.03	0	100*	0.44	0.44	0	1.08	0	100*
B. maydis	1.63	0.83	49.08*	1.39	1.23	11.51	1.80	0	100*
C. cucurbitarum	1.50	0	100*	1.28	0.93	27.34*	1.45	0	100*
C. fulvum	0.42	0	100*	-	-	-	0.37	0.29	20.95*
C. dematium	0.85	0.09	89.41*	0.76	0.63	17.11*	0.65	0.54	16.92
C. polyzona	0.40	0	100*	0.53	0.51	3.77	0.39	0.01	97.44*
C. lunata	1.25	0.56	55.4*	1.18	1.07	9.32*	1.19	0.23	96.25
F. oxysporum	0.66	0.05	92.42*	0.61	0.58	4.92	0.74	0.17	77.02*
G. striatum	0.45	0	100*	0.39	0.30	23.08	0.30	0.28	6.67
Mucor sp.	4.03	0	100*	2.43	2.28	6.17*	3.37	0.68	81.90*
N. sphaerica	0.17	0	100*	0.67	0.56	16.42*	0.58	0.38	34.48*
Penicillium sp.	0.24	0	100*	0.20	0.05	75.00*	0.16	0.12	25.00
Pestalotia sp.	1.11	0	100*	0.55	0.24	56.36*	1.15	0.65	43.48*
Pleurotus sp.	0.67	0	100*	0.79	0.83	-5.06	0.60	0.09	51.25
P. saguineus	0.59	0.06	89.83*	0.53	0.68	-28.30*	0.29	0.36	-24.13
R. stolonifer	3.60	0.20	94.44*	3.48	2.76	20.69*	2.56	0.16	93.75*
S. rolfsii	1.44	0	100*	1.47	0.93	36.73	1.31	0.20	85.11*
T. viride	2.11	0	100*	2.78	1.95	29.86	2.39	1.85	22.59*
FS		4.8			1.42			3.5	

*An asterisk indicates a significant per cent change in colony diameter ($P = 0.05$).

ments aimed at control of specific diseases with extract from the two plants prove successful, large-scale cultivation and exploitation of the plants for fungitoxicants should not be difficult.

While the anti-microbial property of *Cymbopogon citratus* is attributed to the aldehyde citral (Gyane, 1976), that of *O. gratissimum* has not been determined with certainty. Tripathi *et al.* (1986) reported the major and most biologically active principle of *O. gratissimum* to be eugenol but this was contradicted by Sainsbury & Sofowora (1971) who reported thymol to be the major component of *O. gratissimum* occurring in Nigeria. They did not detect eugenol in the plant, neither did they allude to potential fungitoxicity of thymol which El-Said *et al.* (1969) had earlier speculated to be the biologically active principle in the plant. The subject, therefore, needs further study. A gas chromatographic analysis could readily reveal the components of *O. gratissimum* extract.

In previous tests, the test extract was incorporated into chloramphenicol-amended potato-dextrose agar plates before being seeded with mycelial discs of the assay fungus (Awuah, 1989). This method, with its various modifications, is commonly utilized in testing for fungitoxicity of plant extracts. Fungicidal and fungistatic effect of an

extract could then be distinguished more easily only by seeding extract-free agar plates with the inhibited mycelial discs (Garber & Houston, 1959; Kishore, Dixit & Dubey, 1989). The method used in the present study obviates the need to make a second transfer to fresh plates to ascertain the nature of activity of the plant extract. Since the mycelial discs are treated directly with the test extract, lack of mycelial growth from a disc after a period of incubation implied fungicidal nature of the extract. This method of testing adopted in this study is also less cumbersome.

A scheme for quantifying the degree of pathogenicity (Pathogenicity Index) of a plant pathogen to susceptible plants (Wheeler, 1976) was applied here, to quantify the fungitoxicity spectra of plant extracts. In that scheme, diseased plants are grouped into disease classes on the basis of disease severity (corresponding to fungitoxicity classes in the present study) and numbers ranging from 0 to usually 10 assigned to the classes. The Pathogenicity Index (corresponding to the fungitoxicity spectrum in the present study) is then a summation of the individual numbers assigned to the diseased plants divided by the total number of plants used in a pathogenicity test. Application of this scheme to studies on fungitoxicants of plants avoids the tendency to make descriptive statements on the fungitoxicity spectra of plant extracts, by providing FS values which can be objectively compared.

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REFERENCES

Akinde, B.E. (1986) Phytochemicals and microbial evaluation of the oils from the leaves of *Acalypha wilkesiana*. In *The state of medicinal plants research in Nigeria* (ed. E.A. Sofowora), pp. 262-363. Ibadan, Nigeria: University of Ibadan Press.

Awuah, R. T. (1989) Fungitoxic effects of extracts from some West African plants. *Ann. appl. Biol.* **115**, 451-453.

Benjamin, T.V. & Lamikanra, A. (1981) Investigations of *Cassia alata* a plant used in Nigeria in the treatment of skin diseases. *Quart. J. Crude Drug Res.* **19**, 93-96.

Boakye-Yiadom, K., Fiagbe, N.I.Y. & Ayim, J.S.K. (1977) Antimicrobial properties of some West African medicinal plants. IV. Antimicrobial activity of *Xylopia aethiopica* (Annonaceae). *Lloydia* **40**, 543-545.

El-Said, E., Sofowora, E.A., Malcolm, S.A. & Hofer, A. (1969) An investigation into the efficacy of *Ocimum gratissimum* as used in Nigerian native medicine. *Planta med.* **17**, 194-200.

Garber, R.H. & Houston, B.R. (1959) An inhibitor of *Verticillium albo-atrum* in cotton seeds. *Phytopathology* **49**, 449-450.

Gyane, D.O. (1976) Preservation of shea butter. *Drug Cosmet Ind.* **118**, 36-38, 138-140.

Kishore, N., Dixit, S.N. & Dubey, N.K. (1989) Fungitoxic studies with *Chenopodium ambrosioides* for control of damping-off in *Phaseolus aureus* (Moong) caused by *Rhizoctonia solani*. *Trop. Sci.* **29**, 171-176.

Purseglove, J.W. (1969) *Tropical Monocotyledons*. Vol. 1 & 2. London: Longman.

Sainsbury, M. & Sofowora, E.A. (1971) Essential oil from the leaves and inflorescence of *Ocimum gratissimum*. *Phytochemistry* **10**, 3309-3310.

Tripathi, R. D. Banerji, R. Sharma, M. L., Balasubrahmanyan, V. R. & Nigam, S. K. (1986) Toxicity of essential oil from a new strain of *Ocimum gratissimum* (*Clocimum*) against betelvine pathogenic fungi. *Hort. Abstr.* **56**, 228.

Tuite, J. (1969) *Plant pathological methods, fungi and bacteria*. Minneapolis, MN: Burges Publishing Company.

Wheeler, B. E. J. (1976) *An introduction to plant diseases*. New York: John Wiley and Sons Ltd.