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Mycelial growth inhibition of basidiomyceteous fungi by extracts of *Anacardium occidentale* Linn, *Bridelia ferruginea* Benth and *Ficus sur* Forsskal

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ABSTRACT:
Aqueous extracts of the leaves, barks and the roots of Anacardium occidentale, Bridelia ferruginea and Ficus sur were bioassayed at full concentrations for their antifungal potentials by determining their inhibitory effects on the mycelial growth of Rigidiporus lignosus and Coriolopsis occidentalis. Statistically significant differences at $P < 0.05$ between plants and plant parts were studied as well as the resistance of the test fungi to treatment with different plant part extracts. The results indicated that all the plants and their parts contain water soluble active ingredients with antifungal properties which proved to be good inhibitors of the growth of R. lignosus and C. occidentalis. The crude extracts from all the parts of Ficus sur performed best of all extracts as it totally inhibited the mycelial growth of R. lignosus and C. occidentalis.

INTRODUCTION

The use of different parts of plants as natural repellent to protect crops in different parts of the world has been reported by many workers (Qasem, 1996; Enikuomehin and Kehinde, 1999; Adetogun *et al*, 2003; Adetogun and Atayese, 2006). These plant parts have been reported to contain various secondary metabolites that possess antimicrobial properties.

Anacardium occidentale Linn. (commonly called Caju of Brazilian origin) is the most abundant member of the Anacardiacae family. Probably as a result of the Portuguese influence, it is now grown in many tropical countries (Tyman and Lam, 1978). Many parts of *A. occidentale* plants such as seed shell, barks, leaf and root contain a biologically active compound called anacardic acid, though at different percentage composition, that is potent to many micro organisms (Kubo and Murol, 1993, Kubo *et al*, 1993 Adetogun, 1998, Adetogun and Adegeye, 2002)

Cashew syrup is a good remedy for coughs and colds. Cashew apple juice is said to be effective for the treatment of syphilis. Root infusion is an excellent purgative. Old cashew liquor in small doses cures stomach-ache. The oil obtained from the shell by maceration in spirit is applied to cure cracks on the sole of the feet, common in villagers. Cashew apple is anti-scorbutic, astringent and diuretic, and is used for cholera and kidney troubles. Bark is astringent, counterirritant, rubefacient, vesicant, and used for ulcer. Cashew nut shell oil is anti-hypertensive and purgative; it is used for blood sugar problems, kidney troubles, cholera, cracks on soles of feet, hookworms, corns and warts. (Olajide *et al* 2004). The kernel is a demulcent, an emollient and is used for diarrhea. Buds and young leaves are used for skin diseases. The resinous juice of seeds is used for mental derangement, heart palpitation, rheumatism; it was used to cure the loss of memory that was a sequel to smallpox.

According to Salim et al (2002) *Bridelia. ferruginea* Benth occurs in savannah and open Coastal Plains, sometimes on rocky soil. A common shrub or small, straggly tree up to 45ft. high and, young stems thorny and rather zigzag, bark grey and scaly, slash crimson, branchlets rusty-pubescent, leaves up to 4in, broadly elliptic, margins undulate, lower surface rusty-pubescent, with 6-8 prominent nerves and parallel tertiary nerves, secondary forest, in

swamp forest, along forest edges, in riverine woodland and in gallery forest. It does well in a wide variety of climates. It is naturally distributed from the Sudan in the north to the Eastern Cape in South Africa. It can withstand light frost but is not drought resistant.

A bark decoction is taken as a remedy for stomach-ache and tapeworm. The bark is also boiled to make a soup for treating diarrhea in children, or is mixed with milk and drunk as a tonic. A decoction of roots is drunk to cure aching joints. The leaf sap is used as an application to sore eyes and, in a decoction with a number of other plants, for the treatment of conjunctivitis. The root is used as a remedy for severe epigastric pain and is applied to the scalp to relieve headache. A decoction of the root is drunk as a purgative, an anthelmintic or an antidote for poison, as it causes vomiting or diarrhea that gets rid of the poison. An infusion made from the root is taken orally for coughs. The powdered bark is applied to burns to speed healing. The plant is said to contain saponin. Edible fruits are sometimes used as fish bait.

Ficus sur is widely distributed in upland forest, opebn grassland, riverine and rocky areas and sometimes in savannah. It occurs naturally from the Democratic Republic of Congo and Tanzania in the north to the Eastern Cape in South Africa. Trees are relatively drought resistant.

The bark is important in local medicine, and it is used in treating colds, sore throat, dysentery, wounds, constipation, nosebleed and to stimulate lactation. Latex is used for wound fever, while an infusion of the root and fibre is taken orally to help prevent abortion. Powdered root is taken in porridge to stop nosebleed; the milky latex is dropped into the eye to treat cataracts. The sticky juice from pounded roots is used to trap small animals like hares and birds

In this study, the antimicrobial potentials of the leaf, bark and root of *Anacardium occidentale, Bridelia ferruginea* and *Ficus sur* against *R. lignosus* and *C. occidentalis* which were known to be common wood rot fungi in Nigeria were undertaken *in vitro* as natural control agents against these pathogens.

MATERIAL AND METHODS

Preparation of extracts.

Fresh leaves, barks and roots of *Anacardium occidentale, Bridelia ferruginea* and *Ficus sur* were collected from the Arboretum of the Department of Forestry and Wildlife Management, University of Agriculture, Abeokuta, Nigeria. 20 g of fresh samples of each specimen were first washed under running tap water, rinsed with distilled water; the leaves were chopped while the barks and the roots were milled with hammer mill. The protocol of Awuah (1989) was used to obtain the extracts. 10 g of chopped fresh leaves, milled barks and roots of *Anacardium occidentale, Bridelia ferruginea,* and *Ficus sur* were separately infused with 150ml distilled water in 500ml Erlenmeyer flasks. The flasks with the contents were heated on Gallenkamp steam bath at 600C for 2 h. The crude extracts obtained were filtered through two layers of cheese cloth. The extracts obtained were considered as full concentrations. The following experiments were carried out.

Experiment 1

The aqueous leaf extracts of *Anacardium occidentale, Bridelia ferruginea,* and *Ficus sur* were examined *in vitro* for activity against pure cultures of *R. lignosus* and *Coriolopsis occidentalis* obtained from the Pathology laboratory of Forestry Research Institute of Nigeria (FRIN), Nigeria. Fifteen mililitres of each of the extracts were separately added to 85ml of molten lactic acid modified potato dextrose agar (PDA) and thoroughly mixed together on a magnetic stirrer. Twenty mililitres of the resultant solutions were separately dispensed into sterile Petri dishes (11cm diameter). A disc (4mm diameter) of the test fungi, taken from 7 day old culture, was placed in the sterile Petri dishes containing the extracts and the lactic acid modified potato dextrose agar. Lactic acid modified potato dextrose agar devoid of the extract was inoculated with the pure culture of the test fungi served as the control. There were four replicates of each treatment. The Petri dishes were incubated at $25^0C \pm 2^0C$ in the dark for 21 days. Mycelial radial growths of the fungal colonies were measured at 3 days interval for 21 days.

Experiment 2

The aqueous bark extracts of *A. occidentale, B. ferruginea* and *F. sur* were examined in vitro for

activity against pure cultures of *Fomes lignosus* and *Coriolopsis occidentalis* as described in Experiment 1. Inoculated dishes were incubated and data on mycelial radial growth of the fungal colonies were taken as in earlier experiment.

Experiment 3

The aqueous root extracts of *A. occidentale*, *B. ferruginea* and *F. sur* were examined in vitro for activity against pure cultures of *R. lignosus* and *C. occidentalis* as described earlier. Inoculated dishes were incubated and data on mycelial radial growth of the fungal colonies were measured as previously described.

Statistical Analysis

Treatments in all experiments were laid out in a completely randomised block design with four replicates. Data collected were subjected to analysis of variance procedure to calculate the significance of various treatments and the standard errors of differences.

RESULTS

The aqueous extracts from the different parts of the plant species used in the study reduced radial growth of the test fungi compared with the control treatment. The inhibitory effects of these plant parts extracts varied.

Experiment 1

Toxicity of the fresh leaf extracts of *A. occidentale*, *B. ferruginea* and *F. sur* was exhibited on pure cultures of *R. lignosus* and *C. occidentalis* after 72 hours of incubation. At 21 days, *Ficus sur* extract exhibited the highest toxicity against the test organisms and totally reduced the fungal colony radially (Table1).

Experiment 2

Aqueous extracts of the barks of *A. occidentale*, *B. ferruginea* and *F. sur* reduced the colony growth of the test organisms. The extract of *F. sur* showed a significant reduction in the radial growth of the test fungi than the extract of *A. occidentale*. The extract of *B. ferruginea* also showed a promising anti-fungal potential (Table2)

Experiment 3

The fungitoxicity of *A. occidentale* extract decreased significantly with the time of incubation. After 72 hours of incubation the radial growth inhibition of *R. lignosus* and *C. occidentalis* was 44.5% and 67.6% respectively. However, at day 21, the radial growth inhibition of *R. lignosus* and *Coriolopsis occidentalis* by *Anacardium occidentale* was 9.3% and 15% respectively (Table3).

Table 1: Fungitoxic effect of leaf extracts of *A. occidentale*, *B. ferruginea* and *F. sur* on mycelial growth of *R. lignosus* and *C. occidentalis* over 21 days incubation periods.

Plant species	Plant part	Mycelial Inhibition (%)							
		R. lignosus				*C. occidentalis*			
		Days of Incubation							
		3	7	14	21	3	7	14	21
Anacardium occidentale	Leaf	24.2	16.3	14.8	12.3	55.9	55.0	45.0	33.0
Bridelia ferruginea	Leaf	89.1	84.3	80.7	80.3	84.4	75.5	55.5	38.5
Ficus sur	Leaf	94.8	95.5	96.3	96.3	91.2	89.0	84.3	80.0
Control		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SED (df=11)				10.496				5.993	

Table 2: Fungitoxic effect of bark extracts of *A. occidentale*, *B. ferruginea* and *F. sur* on mycelial growth of *R. lignosus* and *C. occidentalis* over 21 days incubation periods.

Plant species	Plant part	Mycelial Inhibition (%)							
		R. lignosus				*C. occidentalis*			
		Days of Incubation							
		3	7	14	21	3	7	14	21
Anacardium occidentale	Bark	42.4	27.0	25.0	21.8	58.2	39.5	35.5	23.0
Bridelia ferruginea	Bark	46.7	8.3	7.5	7.5	90.6	83.3	72.3	63.8
Ficus sur	Bark	88.8	90.0	93.8	95.0	96.5	94.3	91.0	88.0
Control		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SED (df=11)		10.387				7.370			

Table 3: Fungitoxic effect of root extracts of *A. occidentale*, *B. ferruginea* and *F. sur* on mycelial growth of *R.lignosus* and *C. occidentalis* over 21 days incubation periods.

Plant species	Plant part	Mycelial Inhibition (%)							
		R. lignosus				*C. occidentalis*			
		Days of Incubation							
		3	7	14	21	3	7	14	21
Anacardium occidentale	Root	44.5	23.0	20.5	9.3	67.6	43.0	31.8	15.0
Bridelia ferruginea	Root	54.5	31.3	31.5	28.0	92.6	85.8	73.3	76.5
Ficus sur	Root	98.8	85.3	88.5	88.5	96.5	94.5	91.5	89.5
Control		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
SED (df=11)		9.159				7.858			

DISCUSSION

The fungitoxic effects of aqueous leaf, bark and root extracts of *A. occidentale*, *B. ferruginea* and *F. sur* indicate the importance of organic plant materials as a possible alternative to the use of synthetic compounds as preservatives against wood rotting basidiomycetes. Crude extracts of the three plants and their parts inhibited the growth of *R. lignosus* and *C. occidentalis*. The results obtained is in consonance with the works of many workers who have reported the antifungal activities of different plant species and their parts and have stressed the importance of these plants as a possible source of natural repellent to most seed-borne mycoflora and basidiomyceteous fungi that incite serious decay in wood (Al-Abed *et al*, 1993, Singh,1994, Qasem and Abu-Blam,1995, Qasem-1996, Adetogun and Adegeye,2003, Adetogun *et al*, 2005; Adetogun *et al*, 2007.).In this study, the extracts of the leaves of *B. ferruginea* and *F. sur* significantly inhibited the radial growth of *R lignosus* while the same extract from the leaf of *F. sur* totally inhibited the growth of *C. occidentalis* .However ,the extracts from the various parts of *F. sur* were the most toxic to *R lignosus* and *C. occidentalis* when compared with the extracts of *A. occidentale* and *B. ferruginea* The results therefore show that extracts of different plants and their parts are substantially varied in their antifungal potentials. These differences in the antifungal potency may be connected with the difference in their chemical composition which serves as inhibition

ingredients to these micro-organisms.

The degradation of the bark and root extracts of *Anacardium occidentale* with incubation period may be due to the transformation of the active ingredients to non-toxic form and this is in consonance with the work of Al-Abed *et al*, (1993) where they reported that the fate of chemical inhibitors in plants may be due to the transformation of these materials to non-toxic forms, or the loss of some volatile inhibitors during the relatively long incubation period and the possibility of non-solubility of some inhibitors of the plants extract in water hence, the relative solubility of chemical inhibitors of different plants species in water may be a reason behind the differences obtained in the antifungal activity of different plant extracts (Qasem and Abu – Blan, 1995)

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