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Research Paper

Comparative antifungal and toxicological effects of the extract of mistletoes growing on two different host plants in Akure North, Nigeria

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Abstract. Antifungal property of methanolic-extract (60%) of mistletoe (*Viscum album)* leaves was screened on *Aspergillus niger, Fusarium oxysporium, Penicillium oxalium* and *Microsporum canis*. The combined extract doses from the two host plants showed a better antifungal efficacy [Cocoa + Cola (1:3)] when compared with the standard antifungal agent used than the extract doses from individual host plant. The results of acute oral toxicity recorded the LD_{50} as 5.754 ± 452 mg kg^{-1} (oral dose) as it was interpolated from the probit vs. log-dose curve. The toxicological assay was carried out on albino rats orogastrically fed with the *V. album* extract for 14 days using paracetamol (2 mg/kg body weight) as positive control. The alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine-aminotransferase (ALT) activities of rat liver and serum were investigated and compared with the control, the activities of these liver enzyme biomarkers showed no significant difference ($P < 0.05$) for *V. album* growing on cocoa (1000 to 5000 mg/kg) but there was a corresponding significant increase in the serum enzymes ($P < 0.05$) for *V. album* growing on cola at 4000 and 5000 mg/kg doses. The results indicated that the methanolic extract of *V. album* leaves growing on cola (≥ 4000 mg/kg) has brought about induction of synthesis of the liver enzymes studied which is an important biochemical symptom of cytolysis.

Keywords: *V. album,* methanolic-extract, antifungal, toxicological assay, biomarkers, cytolysis.

INTRODUCTION

The search for plants with antimicrobial activity has gained importance consequent to the alarming increase in antibiotic resistant microorganisms (Onifade, 2010). As a result, numerous Nigerian plants have now been investigated. Their natural products have been isolated and assayed for biological activities. Therefore, the use of medicinal plants have always been part of human culture, as some plants possess therapeutic properties, which can be, and have been utilized in the treatment of human and other animal diseases (Cowan, 1999). Herbs have for centuries been used to treat and manage various ailments (Khosh and Khosh, 2001). In fact, herbal medicine still remains the first line of medication amongst a vast majority of Africans (Barbara and Theiss, 1992).

Although, remarkable success have been achieved in the detection, treatment and management of diseases using conventional medicine, many patients still resort to the orthodox medicine since many of which are reputed to offer a complete cure with less serious side effects (personal comm.). Most of these plants have been ingested indiscriminately without minding their toxicological safety. The mistletoe leaf extract is said to possess antidiabetic (Obatomi et al., 1994), immunomodulatory (Solar et al., 1998), bacteriostatic (Fulder, 1998), antimicrobial (Yusuf et al., 2013) and therapeutic values for many other ailments. We quite observed that most of the reports about mistletoe therapy in hypertension and some other ailments are related to

the English species of mistletoe. Little literature is however available about the toxicological properties of Nigerian species of mistletoe, although, many of our traditional healers have widely claimed success with mistletoe therapy.

The growing interest in herbal medicine demands information on the toxicity risk assessment on the various plant preparations used in the management of diseases. Therefore, the present study was set out to provide information on the antifungal properties, and toxicological effect of *V. album* leaf extracts on albino rats.

MATERIALS AND METHODS

Plant materials

Fresh leaves of mistletoes (*V. album*) were collected from the parent plant growing on cocoa and cola host trees at Ogbese, Akure North of Nigeria. The leaves were identified and confirmed at the Crop Soil Department of the Federal University of Technology Akure. The leave extracts was prepared adopting the method used by Yusuf et al. (2013).

Antifungal testing

Pure cultures of pathogenic strains of *Aspergillus niger, Fusarium oxysporium, Penicillium oxalium* and *Microsporum canis* were obtained from the stock cultures of the University Teaching Hospital, Ibadan, Oyo State, Nigeria. The identities of the organisms were confirmed using standard methods of the morphological and biochemical characteristics of each organism at the Microbiology Department Federal University of Technology, Akure, Nigeria.

The agar pour plate diffusion technique was used. A 0.2 ml of 18 h old peptone broth culture of the test organisms was added to 20 ml sterile molten TSA (Tryptone Soya Agar) at 45°C. This was homogenized and poured into sterilized petri dishes in triplicates and allowed to set. Wells of 4 mm diameter were cut and labeled accordingly. The wells were then filled with 0.2 ml of 30 mg/ml extract concentration. The center well was filled with sterile distilled water as control. The extracts were allowed to diffuse into the medium for 1 h, after which the plates were incubated at 25°C for 36 h.

Determination of the toxicity of *V. album* extract on albino rat

Seven groups of six rats each were used to determine the toxic effect of the extract on healthy rats. The rats in group 1 were fed with 1000 mg *V. album* extract, group 2 with 2000 mg, group 3 with 3000 mg, group 4 with 4000 mg, group 5 with 5000 mg /kg/ body weight, group 6 served as the negative control, that is, were maintained only on the basal diet and sterile water for the period of 14 days treatment and group seven were given 2 g/kg b.w of paracetamol to serve as the positive control since paracetamol was reported to induced hepatotoxicity in rats (Setty et al., 2007). The treatment groups were orogastrically fed with the *V. album* extract (Laurence et al., 2005) alongside the basal diet but no water. The animals were observed for respiratory, GIT, symptoms, behavioural patterns and mortality. The LD_{50} was determined by graphical method (Miller and Tainter, 1944). Blood samples were collected into EDTA bottles for hematological assay. The hematological parameters were determined using the methods of Aning et al. (1998).

Biochemical markers assay

The biochemical marker enzymes assayed for were, Aspartate aminotrasferase (AST), Alakine aminotransferase (ALT), and Alkaline-phosphatase (ALP). Reflotron (Boehringer Marnherm Company, Germany) was used for the analysis of some major serum biochemical markers that could reveal the effect of the extracts on the internal organs of rats. The general procedure involves pipetting standardized amount of sample that was then applied on the test zone of the appropriate test strip. The strips were inserted into the test chamber and the flap closed. The result was then displayed after some seconds on the frame computer monitor. Test was carried out at 25°C.

Biochemical principle involved in the analyses

AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenyl-drazine. For ALT, alpha - oxoglutarate and L-alanine are converted into L – glutamate and pyruvate in the presence of ALT. This was measured by monitoring the concentration of pyravate formed with 2, 4-dinitrophenyl-hydrazine. The absorbance of the sample was read against the sample blank after 5 min at 546 nm. The activities of AST and ALT were obtained from the standard tables. The reagents contained phosphate buffer 100 mmol/pH 7.4, L-aspartate 100 mmol/L alpha - oxoglutarate 2 mmol/L and phenolphthalein which at alkaline pH values, turn into pink colour that can be photometrically determined.

Statistical analysis

Data were presented as means of 5 replicates ± standard deviation (SD); the multiple comparisons of the mean

Table 1. Antifungal assay of the *V. album* leave extracts.

Organisms	C_1 (30 mg/ml)	C_2 (30 mg/ml)	C_3 (30 mg/ml)	C_4 (30 mg/ml)	C_5 (30 mg/ml)	Fulcin (30 mg/ml)
Aspergillus niger	10.66 ± 1.45^{cd}	12.00 ± 1.15^{d}	7.00 ± 0.58^{ab}	9.17 ± 0.17^{bc}	6.50 ± 0.29^{a}	17.33 ± 0.33^{e}
Fusarium oxysporium	1.67 ± 0.88^{bc}	3.33 ± 0.33^{d}	0.00 ± 0.00^{a}	2.00 ± 0.00^{c}	1.00 ± 0.58^{b}	3.33 ± 0.33^{d}
Penicillium oxalicum	3.00 ± 0.58^{c}	6.00 ± 0.00^{d}	0.00 ± 0.00^{a}	2.00 ± 0.00^{b}	0.00 ± 0.00^{a}	6.67 ± 0.33^{d}
Microsporum canis	1.67 ± 0.33^{b}	6.33 ± 0.33^{d}	0.00 ± 0.00^{a}	4.66 ± 0.37^{cd}	4.00 ± 0.50^{c}	11.33 ± 0.67^{e}

Figures within the same row with the same superscript are not significantly different at probability ($P < 0.005$) level. C_1 = Cocoa + Cola (1:1), C_2 = Cocoa + Cola (1:3), C_3 = Cocoa + Cola (3:1), C_4 = Cola, C_5 = Cocoa. (Zones of inhibition in mm ± SD)

Table 2. Effect of the administration of *V.album* from cocoa on the biochemical indices of albino rat serum.

Treatment (mg/kg/ b.wt)	AST (U/L)	ALP (U/L)	ALT (U/L)
0.00	63.00 ± 0.707^{a}	54.80 ± 0.860^{a}	16.60 ± 0.510^{a}
1000.00	62.80 ± 1.113^{a}	53.60 ± 1.077^{a}	17.00 ± 0.949^{a}
2000.00	62.80 ± 1.068^{a}	54.20 ± 1.463^{a}	17.00 ± 0.837^{a}
3000.00	63.40 ± 1.167^{a}	53.60 ± 0.927^{a}	16.60 ± 0.678^{a}
4000.00	63.20 ± 1.157^{a}	55.00 ± 1.140^{a}	17.00 ± 1.049^{a}
5000.00	63.00 ± 1.304^{a}	54.40 ± 1.364^{a}	17.20 ± 0.860^{a}
2 g/kg (paracetamol)	112.00 ± 0.949^{b}	99.60 ± 2.73^{b}	34.20 ± 1.428^{b}

AST = Aspartate aminotransferase; ALP = Alkaline-phosphatase; ALT = Alanine-aminotransferase.

values were carried out using one-way ANOVA. The statistical significance was considered at $p < 0.05$ and all analysis were performed using SPSS version 16 software.

RESULTS AND DISCUSSION

Table 1 shows the antifungal assay of the *V. album* leaf extracts on *Aspergillus niger, Fusarium oxysporium, Penicillium oxalium* and *Microsporum canis*. Tables 2 and 3 show the effect of the administration of *V. album* on the biochemical indices of the albino rat serum.

The result of the antifungal assay showed that 30 mg/ml of the combined *V. album* leaf extracts growing on cocoa and cola (1:3) exhibited a higher antifungal potency than the combined extract (1:1) and (3:1) of cocoa: cola. However, in the uncombined extracts dose used, extracts from cola is more antifungal than extracts from cocoa. The extracts was more potent on *Aspergillus niger,* showing the highest inhibition zones (10.66 ± 1.45 mm) in all the treatment used. The standard antifungal agent, fulcin, exhibited the highest antifungal activity compared to all the treatments employed (Table 1).

The result obtained from the toxicological assessment revealed that *V. album* (at doses of 1000 to 5000 mg/kg/body weight of the animal) from cocoa (Table 2) showed no significant difference ($P < 0.05$) in the level of all the biomarkers as compared with the value from the control experiment. However, at doses 4000 and 5000 mg/kg/body weight of the animals, that from cola (Table 3) showed a significant increase in the level of all serum enzymes biomarkers ($P < 0.05$). ALT is the enzyme produced within the cells of the liver, recording increases in conditions where liver cells have been inflamed or undergone cell death. Elevated activities of AST and ALT in the serum of rats as shown in this study (Table 3) might be as a result of the leakages of the enzymes into the serum. It is the most sensitive marker for liver cell damage (Adedapo et al., 2004).

An increase in blood AST, ALT and ALP has been reported as an indication to liver damage and possible damage to the heart (Oyetayo and Osho, 2004). Therefore from the result, *V. album* from cocoa has no hepatotoxic effect on the liver and the heart since no increase was observed ($p < 0.05$) in the obtained values with oral dose (1000 to 5000 mg/kg). Result from this study showed that *V. album* probably have a very wide safety margin and is probably non-toxic.

The result of acute oral toxicity, LD_{50} was recorded as 5,754 ± 452 mg kg^{-1} (oral dose), with 95% confidence interval of 5.302 to 6.206 as it was interpolated from the probit vs. log-dose curve.

The results verified the folkloric utilisation of the leaves of mistletoe as an all-purpose antimicrobial agent and a promising sources of broad spectrum antimicrobial agent (Yusuf et al., 2013). The combined extracts have a better antifungal agent. Whereas the single extract showed a very mild antifungal activity. So, should not be recommended for the treatment of wounds or any other topical infections associated with pathogenic fungi, as this may result in the development of resistant strains of

Table 3. Effect of the administration of *V. album* from cola on the biochemical indices of albino rat serum.

Treatment (mg/kg/b.wt)	AST (U/L)	ALP (U/L)	ALT (U/L)
0.00	63.00 ± 0.707[a]	54.80 ± 0.860[a]	16.60 ± 0.510[a]
1000.00	62.60 ± 0.924[a]	53.40 ± 1.029[a]	16.20 ± 0.883[a]
2000.00	62.40 ± 0.812[a]	54.40 ± 1.029[a]	16.60 ± 0.812[a]
3000.00	63.00 ± 0.707[a]	55.00 ± 1.303[a]	17.00 ± 0.707[a]
4000.00	82.40 ± 0.927[b]	68.20 ± 2.596[b]	21.40 ± 0.509[b]
5000.00	95.00 ± 1.703[c]	82.20 ± 2.517[c]	25.20 ± 1.392[c]
2 g/kg (paracetamol)	112.00 ± 0.949[d]	99.60 ± 2.73[d]	34.20 ± 1.428[d]

AST = Aspartate aminotransferase; ALP = Alkaline-phosphatase; ALT =Alanine-aminotransferase.

such fungi. This findings also revealed that the extract is toxicologically safe, but indiscriminate use should be avoided.

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