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Full Length Research Paper

A preliminary comparative phytochemistry of metabolites of orange (*Citrus sinensis*) and guava (*Psidium guajava*) mistletoes and their host plants

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In this project research, organic solvents were used to extract samples of guava and orange mistletoes and their hosts. The preliminary phytochemical studies revealed presence of saponins, tannins, and anthraquinones both in the host plants and in the parasites. Phlobatanins were found in guava parasite and its host only, while cardiac glycosides with steroidal nucleus were found only in orange parasite and its host. Also lipids extracts were resolved into monoacylglycerol, diacylglycerol, triacylglycerol and free fatty acids. The sugar Thin Layer Chromatography revealed the presence of a ketohexose sugar which was confirmed by selliwanoff's test. It appears that the aromatic smell evident in the orange parasite and its host is due to the cardiac glycosides with steroidal nucleus in their extracts. The aromaticity apparently acquired from the orange host by its parasite is probably a contributory factor in the medicinal value of the parasite.

Key words: Thin layer chromatography, lipids, sugars, parasites.

INTRODUCTION

Ethnopharmacognosy in conjunction with phytochemistry provide one of the greatest areas of biotechnology that pave the way for drug discovery from natural products. It is along this, that investigations have been on the increase in order to discover more beneficial plants, the exact active chemical constituents present in them and their possible roles as therapeutics in modern medicine.

Mistletoe (*Viscum album*) is a flowering plant which belongs to one of the three families: Loranthaceae, Viscaceae or Eremolepidacaea (Calder, 1983; Le'Strange, 1977). In traditional taxonomy all mistletoes were placed in the Loranthaceae, a name conjugated from Latin 'lorum' a thong or straps and the Greek anthos' a flower (Jaeger, 1944). It is a hemi-parasitic plant that grows on deciduous trees preferring those with soft bark like old apple trees, guava, cocoa, citrus and other trees. Mistletoe, called 'Afomo' in Yoruba, 'Kauchi' in Hausa and 'Apari' in Igbo, is ethno medicinally used by various ethnicities in Nigeria as a remedy for several human and animal ailments such as diarrhea, dysentery, wound, cancer, hemorrhage, convulsion (Adesina and Ojewole, 1983; Adesina, 1979), gynaecology problems and cardiovascular diseases (Adodo, 2004). It has been found to have anti-microbial properties against certain multiple-drug-resistant bacterial and fungal isolates of farm animals (Deeni and Sadiq, 2002). Sweet orange (*Citrus sinensis*) of the family of Rutaceae is commonly used for treating capillary fragility, hemorrhages, colds, constipation, and slow digestion (Paul, 2002). Guava (*Psidium guajava*) of the family of Myrtaceae is locally used for stomach disorder (Saidani et al., 2004). The

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reputed efficacies of these plants have been experienced and passed from one generation to the other. Apparently lack of scientific proof of efficacies claimed by traditional medicinal practitioners in Nigeria called for this study. This study is aimed at comparing the phytochemical constituents of orange and guava mistletoes with their host plants to justify their different medicinal usages and their parasitic behaviors.

MATERIALS AND METHODS

Sample collection and preparation

The stems and leaves of mistletoes parasites from orange and guava plants and their hosts' stem and leaves were collected from a citrus garden in Federal University of Technology, Akure, Nigeria. They were biologically authenticated by Mr. Aranilewa, Botany Department of Federal University of Technology, Akure, Nigeria. They were cleaned and air-dried at room temperature. The dried materials were ground into powdered and kept in air tight bottles for further analysis.

Extraction of plant

Three hundred gram of each powdered samples were soaked in a solvent mixture of methanol: water (4:1) for 24 h.

The extracts were filtered and the solvent evaporated to give a concentrated extract. Lipids samples were extracted with Folch mixture (chloroform/methanol/water in ratio 4:2:1), 5 g of each sample was extracted with 30 ml isopropanol for sugar analysis (Harborne, 2005).

Phytochemical screening

The extracts from the parasites and hosts' leaves and stem were screened for some secondary metabolites namely, saponins, tannins, alkaloids, anthraquinones, phlobatanins and cardiac glycosides according to the methods described by Trease and Evans(1985), Sofowora (1993). Each of the tests was qualitatively expressed as negative (–) or positive (+).

Qualitative screening

Alkaloid detection was carried out by extracting 1 g powdered sample with 5 ml methanol and 5 ml of 2N HCl; and then treating the filtrate with Meyer's and Wagner's reagents. The samples were scored positive on the basis of turbidity or precipitation. The presence of tannins was confirmed by boiling 0.5 g powdered sample in 20 ml distilled water, followed by addition of 3 drops of 5% $FeCl_3$ to the filtrate. Development of brownish-green or blue-black colouration was taken as positive for the presence of tannins. Saponins content was determined by boiling 1 g powdered sample in 10 ml distilled water for 15 min and after cooling, the extract was shaken vigorously to record froth formation. Cardiac glycosides were identified by extracting 2 g sample in 10 ml methanol. 5 ml of this methanolic extract was treated with 2 ml glacial acetic acid containing 1 drop of 5% $FeCl_3$ solution. This solution was carefully transferred to surface of 1 ml conc. H_2SO_4. The formation of reddish brown ring at the junction of two liquids was indicative of cardenolides/cardiac glycosides.

Thin Layer Chromatography

Thin Layer Chromatography (TLC) separation of lipids and sugars were done uni-dimensionally, samples were run along with authentic standards in ascending mode using pre-coated silica gel 60 F_{264} plates (Wagner, 2004). Different screening systems were used to obtain better resolution of the components. Spots movements were noted and the various Rf (relative solute to solvent distance) values were calculated.

Acid hydrolysis of plant: Samples for sugar analysis were hydrolysed by boiling the extracted samples in 1 molar HCI at 100°C, for 30 min.

RESULTS AND DISCUSSION

Phytochemical screening revealed that saponins, tannins, anthraquinones, cardiac glycosides (Cardenolides) were present in both parasite and hosts stems and leaves. Alkaloids were not present in all the samples. Phlobatanin was found only in the guava parasite and its host as shown in Tables 1 and 2. The parasites and their hosts have the same metabolites in their leaves and stem. Cardiac glycosides with steroidal nucleus observed in the orange parasite may be responsible for the high aromatic smell observed in citrus. The aromaticity is evident in the orange parasite.

Thin Layer Chromatography of neutral lipids revealed the presence of monoacylglycerol, diacylglycerol, triacylglycerol and free fatty acids in both parasites and their hosts as shown in Table 3. The monoacylglycerol and diacylglycerol may be useful in triacylglycerol synthesis. Lipids are stored as triacylglycerol in plants (Christie, 1982).

The result of Thin Layer Chromatography of sugar analysis in four different solvents revealed a common occurrence of a sugar moiety that co-chromatographed with fructose as shown in Table 4.

The selliwanoff's test carried out also confirmed the presence of a ketohexose sugar. Mistletoes are always located at the apex of the host plant. This location and the nutrient deprival from the host plant may be responsible for the evergreen characteristics of the parasite; since the parasite share the same metabolite types with the host, the parasite can thus be used medicinally in place of the host in dry season. Mistletoe of orange has been locally prescribed for various gynecological problems such as amenorrhea, anovulation, uterine heamorrrhage and fibroids (Adodo, 2004). The cardiac glycosides with steroidal nucleus particularly found in the orange mistletoes may give credence to the plant usage for these ailments.

Steroidal compounds are of interest in pharmacy due to their relationship with compounds such as sex hormones (Okwu, 2001). The steroidal structure could serve as potent starting material in synthesis of these hormones (Harpers, 1975). It appears that the aromaticity apparently acquired from the orange host by its parasites is probably a contributory factor to the medicinal value

Table 1. Phytochemical analysis of orange parasite and its host (stems and leaves).

Metabolites	Orange host	Orange parasite
Alkaloids	–	–
Saponin	+	+
Tannin	+	+
Phlobatanin	–	–
Anthraquinone	+	+
Cardiac glycosides		
Cardenolides	+	+
Steroidal nucleus	+	+
Deoxy sugar	+	+

Key: + = Present, - = Absent.

Table 2. Phytochemical analysis of guava parasite and its host (stems and leaves).

Metabolites	Guava host	Guava parasite
Alkaloids	–	–
Saponin	+	+
Tannin	+	+
Phlobatanin	+	+
Anthraquinone	+	+
Cardiac glycosides		
Cardenolides	+	+
Steroidal nucleus	–	–
Deoxy sugar	+	+

Key: + = Present, - = Absent.

Table 3. Rf values of neutral lipids.

Samples	Number of spot	Components	Rf	Tentative identity
Guava parasite	8	1	0.07	Monocylglycerol
		2	0.13	Diacylglycerol
		3	0.19	
		4	0.35	
		5	0.46	Free fatty acid
		6	0.59	
		7	0.70	Triacylglycerol
		8	0.82	
Orange parasite	5	1	0.07	Monocylglycerol
		2	0.13	Diacylglycerol
		3	0.24	
		4	0.46	Free fatty acid
		5	0.69	Triacylglycerol
Standards	3	1	0.07	Monoacylglycerol
		2	0.13	Diacylglycerol
		3	0.46	Free fatty acid

Table 3. Continued.

Cholesterol	1	1	0.20	
Stearic acid	1	1	0.46	Free fatty acid
Glycerol tripalmitate	1	1	0.70	Triacylglycerol

Solvent: Hexane / Ether / Acetic acid (80:20 .Iv/v).

Table 4. Rf values of sugar TLC. Staining: Aniline hydrogen phthalate.

Samples	EPW (11:5:4v/v)	BAW (4:1:5v/v)	BEW (4:1:2. 2v/v)	BAEW (9:6:5:1v/v)
Guava parasite	0.30	0.52	0.62	0.42
Orange parasite	0.30	0.52	0.62	0.42
Standards				
Fructose	o.31	0.52	0.62	0.42
Glucose	0.58	0.45	0.52	0.48
Sucrose	0.16	0.36	0.47	0.44
Mannose	0.21	0.47	0.40	0.40

Solvents: EPW = Ethyl acetate / Pyridine / Water (11:5:4 v/v), BAW = Butanol / Acetic acid / Water (4:1: 5 v/v), BEW = Butanol / Ethanol / Water (4:1:2. 2v/v), BAEW = Butanol / Acetic acid / Ether / Water (9:6:5:1v/v).

of the parasite.

Conclusion

This work has been able to establish that the hosts of mistletoe contain the same metabolites as the parasites but further work is needed to compare the concentrations of these metabolites. Also, the aromatic smell observed in the orange mistletoe may be due to the steroids contained in the cardiac glycosides of the citrus mistletoe. It appears that the aromaticity apparently acquired from the orange host by the parasite is probably a contributory factor to its medicinal values.

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