Phytotaxonomy and phytochemicals of Eight species of the Family Moraceae in Benue State,Nigeria.

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Abstract: Phytochemical screening was carried out on eight species of Moraceae family in Benue State, Nigeria, using both aqueous and alcohol extracts. Results showed that, tannins and sterols were contained in all the 8 plant species (100%), followed by phlobatannins and saponnins which were found in 7 species(87.5%). Cardiac glycosides and reducing sugars were contained in only 1 species(12.5%). Neither alkaloids nor carotenoids were found in any of the species. There was no association between phytochemical composition by species and the type of extract used,when Chi square analysis was used at p≤ 0.05. Cluster analysis was carried out using Euclidean test to place the identified plants into a number of different groups such that similar plants were placed in the same group on the basis of phytochemical constituents. The cluster analysis did not reveal much distinction between the eight species under study. There was no much distinction between the Ficus species themselves and between the Ficus species and the *Artocarpus heterophyllus* which has been shown to belong to another tribe by other workers. This indicates that the distribution of phytochemical constituents may vary irregularly between the Ficus species and they on their own can not necessarily be used to discriminate between the species.

Keywords: *Artocarpus heterophyllus*, Cluster analysis, Euclidean test, *Ficus spp* , Moraceae, phytochemicals, Taxonomic relationship,

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1. INTRODUCTION

Phytotaxonomy or chemical plant taxonomy is one of the most significant tools used by modern taxonomist to identify, differentiate, classify, and position closely related taxa systematically. It is an approach in taxonomy in which chemical nature of plants are used in developing classification or in solving taxonomic problems. In this sense, phytochemistry of plants is defined as the scientific investigation of the potentialities of chemical characters for the study of problems of plant taxonomy and plant physiology (Ankanna *et al*., 2012). The system of chemotaxonomic classifications relies on the chemical similarity of taxa; it is based on the existence of relationship between constituents and among the plants. It gives the close relationship between chemical constituents of plants and their taxonomic status. Chemotaxonomy establishes relationship between the position of the plant and exact understanding of biological evaluation and natural relationship. Depending upon chemical evidence, plants are classified accurately as containing alkaloids, flavonoids, carotenoids, polysaccharides, terpenoids etc. (Anukul, 2011).

Anukul (2011) also divided the chemical characters into three categories: directly visible characters such as starch grains, raphides, silica, gypsum; chemical test characters such as phenols, betalains, oil fats, waxes, alkaloids; and Proteins. The natural characters of plant products are also divided into two groups on the basis of molecular weight: low molecular weight compounds i.e, molecular weight of 1000 or less called micromolecules such as amino acids, alkaloids, fatty acids, terpenoids, flavonoids; and the high molecular weight compounds with molecular weight of more than 1000 called macromolecules such as proteins, DNA, RNA, and complex polysaccharide.

Based on taxonomic and chemical knowledge, chemotaxonomy is classified into descriptive, dynamic, and serotaxonomy. Descriptive taxonomy deals with the classification of plants and secondary metabolite and other products like sugar and amino acids. It is also concerned with evolutionary change, chemical convergence and divergence in the plants.

Dynamic taxonomy is based on biosynthetic pathway while serotaxonomy or semantics is based on pathway of specific proteins and amino acids sequencing in proteins. Serotaxonomy is further classified as: DNA-primary semantics; RNA-secondary semantics; and proteins-tertiary semantics.

Plant parts used for extracting crude extract for chemical analysis are roots,

leaves,or bark. **S**ome authors use methods such as paper, thin layer, gas or high pressure liquid chromatography (Philip *et al.*, 2003) for separating a crude plant extract. One or more types of spectroscopy are used (Geoffrey *et al.*, 2006., Baranska *et al.*, 2005) to elucidate the structure of chemical compounds.

Phytochemical studies of theplants are largely carried out for pharmaceutical use and / or antimicrobial activities, while little is done on the use of chemical components for the sake of taxonomy of these plants.The aim of this study is to investigate and establish the phytochemical characters of members of Family Moraceae in Benue State, Nigeria.

2 MATERIALS AND METHODS

2.1 Plant Specimen Collection for Phytochemical Studies

Fresh samples of some organs: leaves, stem bark, and roots of the plant species selected for the study were collected from each plant in June, 2013. These were taken to the Chemistry laboratory Benue State University, Makurdi, for phytochemical analyses. The samples were air dried at room temperature and each separately pulverized into powder by pounding using wooden mortar and pestle. After pounding, each sample was sieved using fine mesh. This gave a very fine powder of each sample which were used in the extraction of the phytochemicals using different solvents.

2.2 Extraction using Ethanol (Alcohol Extraction)

Five hundred grams (500g) each of the powdered samples of leaves, stem barks, and roots from each of the species of the Family Moraceae were separately extracted using 70% ethanol.The powder from each of the samples was macerated in 2 litres of ethanol in beakers which were covered with aluminium foil to avoid evaporation of the alcohol. The set up was allowed to stand for 48 hours with occasional shaking to obtain maximum extraction after which the alcohol extract was filtered using ash free whatman filter paper. The extract was concentrated on a water bath at a temperature of 40^{o}C and made to dry into powdered form.

2.3 Extraction using Water (Aqueous extract).

Five hundred grams (500g) each of the powdered samples of the leaves, stem barks, and roots from each of the species of the Family Moraceae were used for extraction using water. Each of the powdered plant samples as in above was soaked in 1000ml of distilled water in a beaker and boiled for 15 minutes using a heating mantle. This was allowed to stand for 24 hours and then the supernatant liquid was filtered off using ash free whatman filter paper. The extract was also concentrated on a water bath at a temperature of 40^{o}C and made to dry into powdered form.

2.4 Phytochemical screening

Phytochemical screening for major constituents was undertaken using standard qualitative methods adopted from Fadeyi *et al.* (1989), Odebiyi and Sofowora (1990), and Harborne, (1992), Abulude *et al*. (2001& 2004) ,and Abulude (2007). Tannins, saponins, phlobatannins, terpenoids, flavanoids, glycoside, anthraquinones, carotenoids, reducing sugars, alkaloids, and sterols tests were conducted using both the alcohol and the aqueous extracts.

2.5 Cluster analysis involving phytochemical constituents and as the set of measured variable

Cluster analysis was carried out using Release 7.22 DE) to classify the identified plants into a number of different groups such that similar plants were placed in the same group on the basis of phytochemical constituents

3. RESULTS AND DISCUSSION

Table 1 illustrates the phytochemical composition of members of the Family Moraceae in Benue State, Nigeria and Table 2 shows the total number of species of moraceae containing different phytochemicals in the leaves, stem bark, and roots using aqueous extract and alcohol extract. Both tannins and sterols were contained in all the 8 plant species of Moraceae, whereas saponnins and phlobatannins were contained in only 7 of them. None of the plant species contained either alkaloids or carotinoids. Using only aqueous extract, the highest number of

plant species (7) contain tannins in their leaves followed by six species containing tannins in their stem bark. When only alcohol extract was used, all the eight plant species under study showed presence of sterol in both leaves, stem bark, and roots followed by seven species containing saponnins in both leaves, stem bark, and roots. Tannins, however, were contained in seven species only in the stem bark and roots (6 species in the leaves). All the eight plant species under study showed presence of neither alkaloids nor carotenoids using both aqueous extract and alcohol extract**.** There was no association between phytochemical composition by species and the type of extract used, at p= 0.05, when chi – square (X^2) analysis was conducted. Also, there was no association between phytochemical composition by species and plant parts. Out of the 12 phytochemicals tested for; both *Ficus exasperata, Ficus ingens* and *Ficus sur* contained 6 phytochemicals each. *Ficus exasperata* was found to contain saponnins, tannins, Flavonoids, cardiac glycosides, steroids and phlobatannins but no alkaloids, carotenoids, combined anthraquinones, free anthraquinones, reducing sugars and terpenoids. The findings partially agree with the findings of Lawal *et al*. (2012) whose investigations on the phytochemical screening of the root bark extract of *Ficus exasperata*, indicated the presence of saponins, cardiac glycosides and anthraquinones in faint quantity, while tannins and alkaloids were absent. This could be due to soil conditions or to seasonal or climatic factors. However, previous studies on the phytochemical analysis of Ficus exasperata by Adebayo *et al*. (2009) showed that the root contained tannins, flavonoids, saponnins, phlobatanins, glycosides and steroids.

Ficus ingens was found to contain saponnins, tannins, combined anthraquinones, free anthraquinones, steroids and phlobatannins but no flavonoids, alkaloids, cardiac glycosides, carotenoids, reducing sugars and terpenoids. This results is not in conformity with the findings of Aiyelero *et al.* (2009), who revealed the presence of alkaloids, flavonoids, glycosides, saponins and tannins in *Ficus ingens.* This could also be due to soil conditions or to seasonal or climatic factors *Ficus sur* was found to contain saponnins, tannins, combined anthraquinones, free anthraquinones, steroids and phlobatannins but no flavonoids, alkaloids, cardiac glycosides, carotenoids, reducing sugars and terpenoids. This finding is in line with the findings of Solomon-Wisdom *et al.*(2011), who detected saponnins, steroids and tannins but no alkaloids, triterpenoids and glycosides.

Both *Ficus polita, Ficus trichopoda* and *Artocarpus heterophyllus* were found to contain 5 phytochemicals each. *Ficus polita* was found to contain saponnins, tannins, phlobatannins, steroids and terpenoids but no flavonoids, cardiac glycosides, alkaloids, carotenoids, reducing sugars, combined anthraquinones and free anthraquinones. *Ficus trichopoda* like *Ficus polita,* was found to contain saponnins, tannins, phlobatannins, steroids and terpenoids but no flavonoids, cardiac glycosides, alkaloids, carotenoids, reducing sugars, combined anthraquinones and free anthraquinones. This finding partially agree with the findings of Balogun *et al*. (2011), who indicated the presence of both reducing sugar, alkaloids, saponnins, tannins and free amino acids/amines. *Artocarpus heterophyllus* on the other hand, was found to contain tannins, flavonoids, reducing sugars, steroids and terpenoids but no saponnins, cardiac glycosides, carotenoids, phlobatannins, combined anthraquinones and free anthraquinones.

Kumbhani *et al.* (2011) in their pharmacognostic and phytochemical evaluation of leaves of *Artocarpus heterophyllus* showed the presence of phenolics, sterols, triterpenoids and carbohydrates and tannins but no alkaloids, flavonoids and cardiarc glycosides. Gupta *et al.* (2011), in another study found that secondary metabolites including alkaloids, saponins, flavanoids and phenolics were present in the jackfruit seeds.

Both *Ficus platyphylla* and *Ficus thonningii* were found to contain 4 phytochemicals each. *Ficus platyphylla* was found to contain saponnins, tannins, phlobatannins and steroids but no terpenoids, flavonoids,

cardiac glycosides, alkaloids, carotenoids, reducing sugars, combined anthraquinones and free anthraquinones. Mudi *et al*.(2011) in a study tested for the presence of secondary metabolite in *Ficus platyphylla* which showed the presence of alkaloid, reducing sugar, saponins, tannins, resins and flavonoids in ethanol soluble fraction. Another study by Chinenye *et al*. (2011), revealed that the extract contains saponnins, tannins, flavonoids volatile oils, glycosides and steroids but no alkaloids and cardiac glycosides. *Ficus thonningii* was found to contain saponnins, tannins, phlobatannins and steroids but no terpenoids, flavonoids, cardiac glycosides, alkaloids, carotenoids, reducing sugars, combined-anthraquinones and free-anthraquinones.

When only aqueous extract was used, the highest number of 7 species were found to contain tannins in their leaves followed by 6 species containing tannins in their stem-barks. Only one species was found to contain both combined-anthraquinones and reducing sugars in both leaves, stem-barks and roots.
When only alcohol extract was used for both leaves, stem-barks and roots, all the 8 species of Moraceae showed the presence of steroids in both leaves, stem-barks and roots followed by 7 species containing saponnins in both leaves, stem-barks and roots. Tannins, however, were contained in 7 species only in the stem-barks and roots (6 species in their leaves). No species was found containing alkaloids or carotenoids.

Compounds of considerable taxonomic value may be found in any part of a plant, but it is reasonable to assume that the most important ones occur in phylogenetically old, conservative, little specialized organs. All plants are subject to variation and different specimens of the same species sometimes differ considerably. Certain compounds may be missing in some of them or occur in such small amounts that they escape observation. This can be due to soil conditions or to seasonal or climatic factors and one should therefore, always examimine several specimens of the same species, if possible specimens grown under different conditions (Erdtman, 2006). According to him, tissues such as the heartwood of trees, usually show a more constant chemical composition than living organs, since they are much less subjected to the influence of environmental factors.

Table 1: Plant species of Moraceae containing different phytochemicals (using both Aqueous and Alcohol extract for both roots, stem-bark, and leaves).

Pla*nt species*	Phytochemical											
	Sap	**Ta*n***	**Fla**	**C.gl**	**Alk**	**Car**	**C.a*n***	**F.a*n***	**Phl**	**R.su**	**Ste**	**Ter**
Ficus exasperata	+	+	+	+	-	-	-	-	+	-	+	-
Ficus ingens	+	+	-	-	-	-	+	+	+	-	+	-
Ficus platyphylla	+	+	-	-	-	-	-	-	+	-	+	-
Ficus polita	+	+	-	-	-	-	-	-	+	-	+	+
Ficus sur	+	+	-	-	-	-	+	+	+	-	+	-
Ficus thonningii	+	+	-	-	-	-	-	-	+	-	+	-
Ficus trichopoda	+	+	-	-	-	-	-	-	+	-	+	+
Artocarpus heterophyllus	-	+	+	-	-	-	-	-	-	+	+	+
Total	**7**	**8**	**2**	**1**	**0**	**0**	**2**	**2**	**7**	**1**	**8**	**3**

Key
+ = present
- = absent
Sap = Saponnins
Tan = Tannins
Fla = Flavonoids

C.gl = Cardiac glycosides
Alk = Alkaloids
Car = Carotenoids
C.an = Combined anthocyannins
F.an = Free anthocyannins
Phl = Phlobatannins
R.su = Reducing sugar
Ste = Sterols
Ter = Terpenoids

Table 2: Total number of species of Moraceae containing different phytochemicals in the leaves, stem bark, and roots using Aqueous extract and Alcohol extract.

Phytochemical	Number of species for Aqueous extract				Number of species for Alcohol extract			
	Roots	Stem-bark	Leaves	Total	Roots	Stem-bark	Leaves	Total
	O (E)	O (E)	O (E)		O (E)	O (E)	O (E)	
Saponnins	4(4)	5(5)	5(5)	14	7(7)	7(7)	7(7)	21
Tannins	5(5)	6(5)	4(5)	15	6(6)	7(7)	7(7)	20
Flavonoids	2(2)	2(2)	2(1)	6	2(2)	2(2)	2(2)	6
Cardiac glycocides	1(1)	1(1)	1(1)	3	1(1)	1(1)	1(1)	3
Alkaloids	0(0)	0(0)	0(0)	0	0(0)	0(0)	0(0)	0
Carotenoids	0(0)	0(0)	0(0)	0	0(0)	0(0)	0(0)	0
Combined anthraquinons	1(1)	1(1)	1(1)	3	2(2)	2(2)	2(2)	6
Free anthraquinons	2(2)	2(2)	2(2)	6	2(2)	2(2)	2(2)	6
Phlobatannins	5(5)	5(5)	5(5)	15	6(6)	6(6)	6(6)	18
Reducing sugar	1(1)	1(1)	1(1)	3	1(1)	1(1)	1(1)	3
Sterols	5(5)	5(6)	7(6)	17	8	8	8(8)	24
Terpenoids	1(2)	1(2)	3(2)	5(5)	3	3	3(3)	9
Total	27	29	31	87	38	39	39	116

χ^2 cal = 2.84 χ^2 tab = 33.92 df =(r- 1)(c-1) =22 $p \leq 0.05$ NS (Not significant)

χ^2 cal = 0.00 χ^2 tab = 33.92 df =(r-1)(c-1) =22 $p \leq 0.05$ NS (Not significant)

Fig.1 illustrates the clustering of the 8 species using only the phytochemical constituents of the plants. The clustering shows that *Ficus platyphylla* and *Ficus polita* are more similar to each other just as Ficus trichopoda and Ficus thonningii i.e. their clades are the closest links to the bottom of the dendrogram. Another very close link to the bottom is the clade containing Ficus sur and Ficus ingens which is somewhat very far from the other clades. There is no clear cut between Artocarpus species and Ficus species as Artocarpus heterophyllus is placed alongside with the other Ficus species. These placements are also at variance with those of other classification schemes for the family

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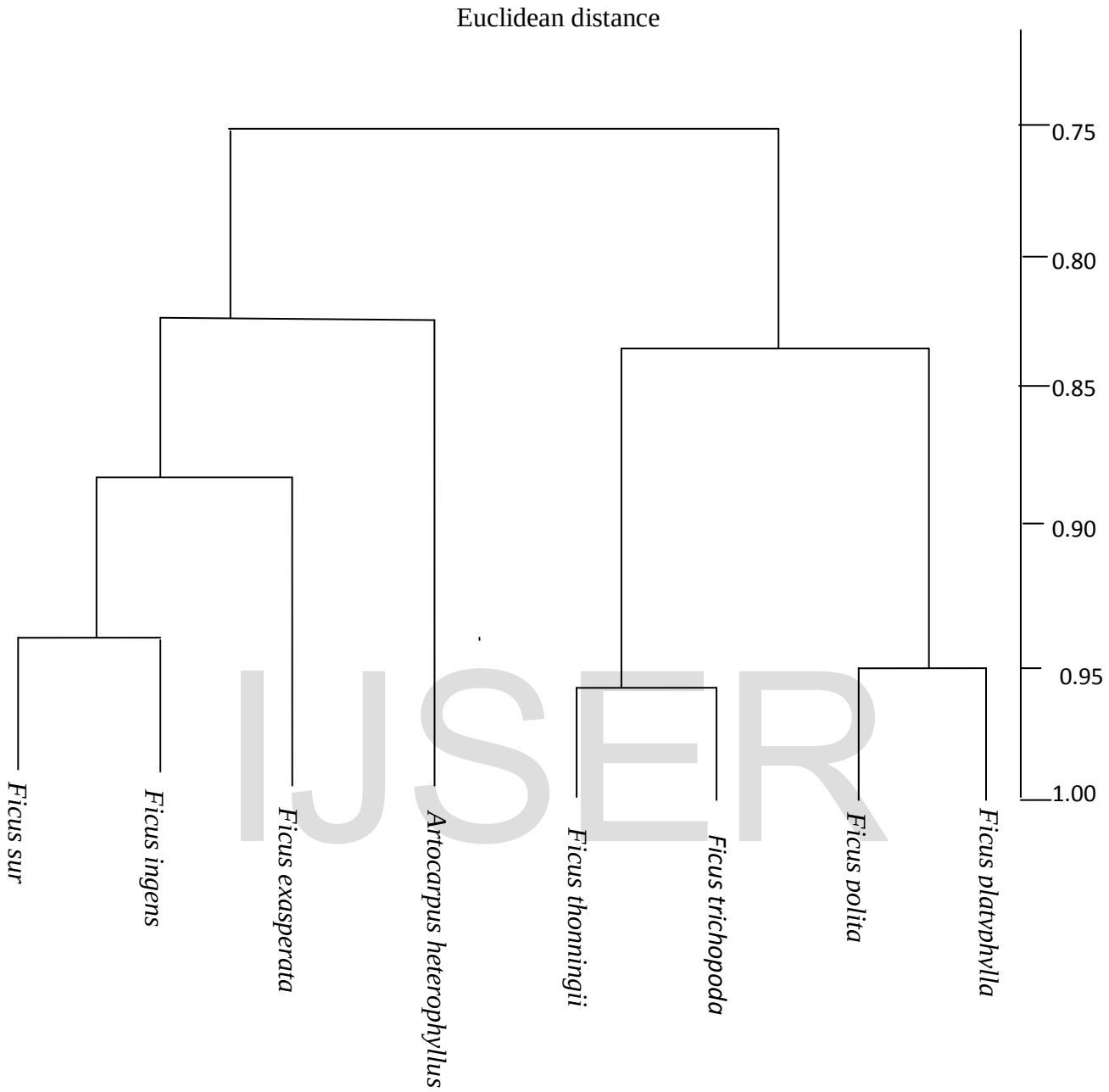

Fig.1: Taxonomic relationship between the different species of Moraceae on the basis of only phytochemical constituents as the set of measured variable

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