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Phytochemical screening of African mistletoes Tapinanthus globiferus (A.Rich) Tieghem (loranthaceae) on some host species in Birnin-Kebbi, Nigeria

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ABSTRACT

African mistletoe (Tapinanthus globiferus (A.Rich) Tieghem) is a hemi –plant parasite belonging to the family Loranthaceae. It grows as a partial parasite on the branches of many trees and has various ethnomedicinal uses. Qualitative screening of extracts obtained from 10 different host species in (Fadama Area) Birnin-Kebbi Local Government, Kebbi State revealed the presence of biologically active compounds. Quantitatively. Presence or distribution of phytochemical substances in T. globiferus appeared to be partly dependent on the host plant species. The current study is aimed to make aware about the presence of certain bioactive compounds in plants which attribute them medicinal values & can provide alternative pathway for the replacement of synthetic drugs. Effort should be directed to the phytochemical screening and pharmacological potentials of T.globiferus in order to unlock the full medicinal potentials of the species.

Introduction

Mistletoe, which consists of about 1400 species around the world, belongs to the kingdom Plantae, subkingdom Tracheobionta, super-division Spermatophyte, division Magnoliophyta, class Magnoliopsida, subclass Rosidae, order Santales [1]. Recent phylogenetic studies confirm that mistletoes belong to five distinct families: Misodendronaceae, Eremolepidaceae, Santalaceae, Viscaceae and Loranthaceae. The largest family of this mistletoe is Loranthaceae which has 75 genera and over 900 species. Among them, six major genera are found in Nigeria, namely: *Tapinanthus*, *Agelanthus*, *Loranthus*, *Globimetula*, *Phragmanthera* and *Englerina*. *Tapinanthus* is far more widespread in the Nigeria savanna [1]. Mistletoe, in Yoruba speaking area in Nigeria, it is called 'afomo', in Igbo 'apari' while in Hausa it is called 'kauci' [2]. Mistletoes are evergreen, perennial plant species that form a dark green to yellowish-green, drooping bush that can reach 0.6-0.9m long on the branch of a host tree. It has thickly crowded, forking branches and round, jointed stems, the opposite, oval, lance-shaped with leathery leaves of about 5cm long. African mistletoes (Tapinanthus) occur in Guinea, Mali, Sierra Leone, Liberia, Ivory Coast, Burkina Faso, Ghana, Togo, Benin and Nigeria. Other forms of mistletoes are American (*Phoradendron flavescens*) and the European (*Viscum album*) types [3]. The seeds of Tapinanthus species are dispersed mainly through the agency of birds, bat, insect and animals at a low rate some of these animals peel the berry epicarp before swallowing. The mesocarp contains a viscous sticky fluid, which helps the seed to adhere on host branch. The seed germinates and carryout a peg like root called haustorium, which initially serves for anchorage and later as absorptive organ.

This haustorium penetrates into the vascular system of the host tree and absorbs water, inorganic ions, sugar, amino acid and other essential nutrients from the tree's xylem and phloem. The vegetative shoots are given off and later flowers and fruits are produced. The mistletoes thus form a bushy outgrowth on the host [3].

Mistletoes causes abnormal growth and decrease in productivity due to reduction in the overall photosynthetic area of the host plants. They hinder the tree from giving off branches at the positions of attachment and deprive it of food that should be available for its other activities. The host plant is thus weakened and may eventually be killed. Tree's that are heavily infected by mistletoe hardly possessed enough nutrients even for basic maintenance of growth and as such express signs of senescence and death [4]

Most genera of African mistletoes belong to the family Loranthaceae [1] In West Africa, mistletoes are found on many indigenous trees and a number of tree crops of economic importance, including citrus plants like orange (Citrus sp.), guava (Psidium guajava) Vitellaria paradoxa (Sheer butter), Anacardium occidentale (cashew) Mangeifera indica (mango) Annona squamosal (sugar apple) Azadirachta indica (Neem) and Acacia nilotica etc. Mistletoe is especially interesting botanically because it is a partial parasite (a "hemiparasite") [1]. As a parasitic plant, it grows on the branches of trunk of trees and actually sends out haustoria that penetrate into the tree and take up nutrients. Mistletoe is also capable of growing on its own; like other plants as it can produce its own food by photosynthesis [5].

Many of these parasitic plants (mistletoes) can simultaneously parasitize many host species. Since different host species may supply a parasite with different resources, a mixture of host species may be superior to a single host

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alone. Boussium et al. (2004) reported as cited in Dlama, Oluwagbemileke [1] that mistletoe (*T. globiferus*) parasitized 126 species, and believed that it is less specific compared to other mistletoe species. Despite the large host range of the majority of parasitic plants.

The ethnomedicinal uses of mistletoes had, for a very long time, been in the hands of very few herbal practitioners who claimed a general use to counter sorcery and magical powers, to treat mental conditions, sterility, and health problems associated with urino-genital system, rheumatism and pain. These hemi-parasitic plants, mistletoes of the Loranthaceae and Viscaceae, are widely used in various cultures in almost every continent to treat various ailments including hypertension, cancer, and diabetes, or used as a diuretic agent [6]. For example, the tea made from Loranthaceae spp. is believed to cure bone fracture and body pain [7]. Remedies for tumour (Tanachaa) in South-western Ethiopia were reported to be prepared by crushing fresh leaves of *T. globiferus* (A.Rich.) Tiegh. and mixed with cold water to be administered orally [8, 9]. Also in the Ebolowa region of Cameroon, one handful of the fresh leaves of *T. globiferus* is usually mixed with one handful of the root bark of Boswella odorata, the ingredients are macerated in 5L of local beer and one glassful is taken twice a day for two weeks to cure syphilis [9]. In Saudi Arabia, fresh stems of *T. globiferus* (local name, Hadhal) are given orally to all types of livestock for the treatment of fever and removal of placenta after parturition [10].

This study was aimed at determining the both qualitative and quantitative phytochemical compositions T. globiferus sourced from ten different host species Lawsonia inermis, Acacia nilotica, Psidium guajava, Ceiba pentandra, Balanites aegyptiaca, Citrus sinensis, Annona squamosal, Faidherbia albida, Albizia zygia Faidherbia albida and Azadirachta indica so as to confirm its phytochemical variations.

Materials and Methods

Study area

The study was conducted in Birnin-Kebbi Local government area of Kebbi State, Nigeria. Birnin-Kebbi lies approximately at an altitude of 200 meters and latitude 12^{o}N and longitude 4^{o}E in the Sudan savanna vegetation zone in the north western Nigeria. The area has a semi-arid climate that is characterized by long dry (October-May) and short wet (June-September) seasons with a mean annual rainfall of 665mm average over the period 1980-1998. This is far exceeded by the potential evapotranspiration of 1770mm KARDA, (1998) as cited in [11].

The minimum and maximum mean temperature of Birnin-Kebbi ranged between 20^{oc} and 35^{oc}, respectively [12].The condition produce a rather fragile agro ecosystem and agricultural production is hampered by such hazards as drought, wind and water erosion and high soil temperature [13]. Kebbi State occupies a total land area of 36,800 square kilometres. It shares boundaries with Sokoto State on the North-Eastern axis, Zamfara State on the Eastern part, Niger State on the Southern part and Republic of Niger on the Western part. According to NPC (2006) Kebbi State has an estimated population of 3,662,103 people. The state has four major tribes, which include: Hausa, Fulani, Dakarkari and Gungawa, Others minor tribes include Zabarmawa, Dandawa, Kambari, Yorubas and Igbos [14]. Inhabitants are predominantly farmers even though the condition is fragile agro ecosystem and agricultural production is hampered by such hazards as drought, wind and water erosion and high soil temperature [15]. Vegetation of the area is scattered major tree species, farm produce and some animal species[15].

Sample Collection and authentication

Fresh leaves of Tapinanthus were collected in August 2009 from ten different hosts' plant species in (fadama Area) of Birnin-kebbi local government and labeled 1-10 respectively. The leaves were packed separately in clean sterilized polythene bags and brought to the Department of Biological Sciences (Botany unit) herbarium, Usmanu Danfodiyo University, Sokoto, for identification. Identification was further confirmed by Jemilat A. I. at the herbarium of National Institute for Pharmaceutical Research and Development (NIPRD) Abuja, Nigeria. Voucher specimens were deposited in the two herbaria as recommended by [16].

Preparation of Sample

The fresh leaves of the samples were oven dried, thermostatically controlled at 40^{o}C for 48 hours. The dried leaves were grinded into powder. One gram each of the powdered samples was extracted separately in 100ml of distilled water for 24 hours. The filtrate was obtained using muslin cloth, and kept the stock solution. The stock solution was later subjected to phytochemical analysis. The methods of [17-19] were employed.

Results and discussion

Table 1. Woody species infested by T. globiferus in Birnin-Kebbi.

Plants species	Common name	Local name (Hausa)	Family
Psidium guajava	Guava	Guiba	Myrtaceae
Annona squamosa	Custard apple	Gwandan daji	Annonaceae
Citrus sinensis	Sweet orange	Lemu	Rutaceae
Ceiba pentandra	Silk Cottontree	Abdugar rimi	Bombacacea e
Albizia zygia	West African albizia	Madobiyar rafi	Mimosaceae
Azadirachta indica	Neem tree	Dogon yaro	Miliaceae
Acacia nilotica	Egyptian mimosa	Bagaruwa	Mimosaceae
Lawsonia inermis	Henna	Lalle	Lythraceae
Balanites aegyptiaca	Desert date	Aduwa	Balanitaceae
Faidherbia albida	Winter thorn	Gawo	Mimosaceae

Table 1; Woody species infested by T. globiferus in Birnin-Kebbi, collectively 10 species of plants that are infested by T. globiferus are documented in this study. From Table 1, all the 10 plants species were from different families and genera with representation of one species each with only Mimocaceae having 3 members the remaining are: Myrtaceae, Annonaceae Rubiaceae Bombacaceae, Miliaceae, Lythraceae and Balanitaceae.

The result of the preliminary phytochemical analysis (Table 2) revealed the presence of Alkaloids, flavonoids, volatile oil and tannins in all the samples, while Saponins in four samples numbering 2, 5, 8 and 10 respectively. Glycosides was also recorded in eight sample, however, cardiac and saponin glycosides were also tested with saponin glycosides having the highest records as it was observed to be absent only on sample number 5, while anthraquinones was not recorded in all the samples.

Table 2. Qualitative Phytochemical Screening of Ten Samples of *T. globiferus* infesting ten tree species in Birnin- Kebbi.

	Tree species									
Phytochemical	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Alkaloids	++	+++	++	++	+++	+++	++	+++	+++	++
Tannin's	++	++	+	+	+	+	++	++	++	++
Saponins	-	+	-	-	++	-	-	+	-	++
Flavonoids	++	+++	++	+++	++	+++	++	++	++	+++
Glycosides	-	+++	++	+	-	++	+++	+++	+++	++
Cardiac glycosides	-	+	+	-	-	+	++	++	++	+
Saponin glycosides	++	+++	+++	+++	-	++	+++	+++	+++	+++
Volatile Oil	+++	++	++	+++	+++	+++	+++	+++	+++	+++
Steroids	-	++	-	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-	-	-	-
Balsams	+	++	-	-	-	-	+	+++	+++	+

Key: +++ = Present in large amount, ++ = Present in moderate amount, + = Present in trace amount, - = absent, 1 – 10 = Trees species as indicated in Table 1.

Balsam as the last components was recorded to be present in sample 1, 2, 7, 8, 9 and 10, respectively.

Quantitative Phytochemical Analysis

The result of the mean analysis for the quantitative phytochemical constituents in the leaves of T. globiferus infesting ten tree species in Birnin-Kebbi (Table 3) revealed that for alkaloids concentration Lawsonia inermis has differed from the rest of the species tested, while Citrus pentandra and Balanites aegyptiaca were similar and least in alkaloid concentration in Acacia nilotica. In Saponin, Psidium guajava has the highest concentration with Faidherbia albida, Annona squamosa, Citrus sinensis and Acacia nilotica being similar but differed with the rest of species. Concentration with Albizia zygia differed from both Faidherbia. albida and Annona squamosa, Citrus sinensis and Acacia nilotica but were similar to Ceiba pentandra. In Flavonoids Citrus sinensis and Faidherbia alibida were high but low in Annona squamosa and Azadirachta indica and trace in Acacia nilotica, Glycosides recorded the lowest values of concentration among the all species tested.

The results obtained from the qualitative phytochemical screening of ten samples of *T. globiferus* showed the presence of biologically active compounds in the extracts. These include alkaloids, tannins, flavonoids and volatile oil, as shown in Table 2. The presence of components in species is an indication that it may have some medicinal potential [20]. This is probably due to the fact that each of the components identified has of one therapeutic usage or another.

The presence of these compounds in *T. globiferus* infesting other woody species was earlier observed by Ndukwe et al. (2001). [4, 5, 21], also reported presence of these compounds in T. dodoneifolius.

Presence of alkaloids and tannins in the plant extracts are supported by the findings of [22, 23].

Steroids were scantly present as it was recorded only in one out of the ten samples of T. globiferus infesting A. squamosa. The absence of Anthraquinones in all the samples are worth noting taxonomically and medicinally as earlier observed by [4]. Moreover, other components tested include saponins, glycosides, cardiac glycosides, saponin glycosides and balsms. Saponin was recorded in four out of the ten samples. While glycoside was noted in eight out of the ten samples, but absent in Tapinanthus globiferus infesting Psidium guajava, Albizia zygia. Saponin and cardiac glycosides seem to be present in abundance with saponin glycosides recording the highest number as it was seen in nine samples. Balsams was also recorded in six out of the ten samples and absent in samples infesting Citrus sinensis, Ceiba pentandra, Albizza zygia and Azadirachta indica. Saponin has detergent properties and also serve as lytic agent and exhibits anti-inflammatory properties [24]. Generally, glycosides are non-volatile and lack fragrance. But cleaving the glycosidic bond yields the glycan, volatile and fragrant. Glycosides serve as defense mechanisms against insects and herbivores [25]. Presence of some of these active compounds were earlier observed by Aliero, Aliero [26] and Abukakar, Ukwuani [27] on Scadoxus multiflorus and Tamarindus indica respectivily .

The differences noted in the chemical constituents of this parasite present on different hosts might justify why the host is as important as the parasite in pharmacognosy and why the use of this parasite in the treatment of an ailment is usually dependent on a particular or specific host [28, 29].

Table 3. Mean Analysis for the quantitative phytochemical constituent in the leaves of *T. globiferus* infesting ten tree species in Birnin-Kebbi.

	COMPONENTS				
Host species	**Alkaloids (%)**	**Saponins (%)**	**Flavonoids (%)**	**Tannin (%)**	**Glycosides %**
Psidium guajava	$7.6\ 0^{h}$	31.10^{a}	10.50^{d}	33.90^{c}	2.31^{b}
Annona squamosa	22.00^{c}	$23.5\ 0^{b}$	4.50^{f}	40.70^{c}	1.78^{d}
Citrus sinensis	$13.0\ 0^{g}$	23.00^{b}	16.00^{a}	30.70^{f}	1.90^{c}
Ceiba pentandra	22.67^{b}	$19.9\ 0^{d}$	7.70^{c}	43.50^{b}	2.34^{b}
Albizia zygia	16.00^{f}	21.20^{cd}	6.00^{ef}	25.70^{hi}	2.38^{b}
Azadirachta indica	20.73^{d}	14.00^{e}	5.20^{f}	27.20^{h}	2.19^{c}
Acacia nilotica	$7.0\ 0^{i}$	24.20^{b}	13.30^{bc}	37.70^{d}	2.05^{c}
Lawsonia inermis	24.07^{a}	$19.5\ 0^{d}$	12.20^{cd}	32.90^{ef}	2.35^{b}
Balanites aegyptiaca	$22.6\ 0^{b}$	10.40^{f}	12.50^{c}	28.50^{fg}	2.42^{b}
Faidherbia albida	17.00^{e}	$23.1\ 0^{b}$	14.50^{ab}	47.10^{a}	2.72^{a}
L.S.D (P=0.05)	0.590	1.440	1.760	2.320	0.299

Means followed by same letters in each of the columns were statistically similar at 5% level of significance.
Means followed by different letters in each of the columns were statistically different at 5% level of significance.

The values obtain for alkaloids concentration were in agreement with the one earlier reported by [6]but higher than those reported for Tamarindus indica (4.32%) by [27]. Saponins concentration reveals that the values in all the samples of T. globiferus were comparable with findings on the same plant by [6], but still higher than those reported by [27] on Tamarindus indica (2.2%).

Flavonoids concentrations were observed to be lower than the values reported by Ndukwe et al. (2001) (6.0%). But higher than those reported by [5] and [27] in T. dodoneifolius and Tamarindus indica respectively. The concentration of tannins showed higher values than those reported by Adekunle, Oyewo [30] (2.26%). Funatogawa, Hayashi [31]. Tannins helps to regulate the growth of tissues and as well as effective in protecting kidney [32]. Glycosides concentration (1.59%) in T. globiferus was comparable to the Tamarindus indica as reported by Abukakar, Ukwuani [27].

The statistical analysis of MUSTAT package and mean comparison reveals that there is significant difference among the variables at 5% level of significance and highly significant at 1% level of significance.

Conclusions

Phytochemicals help plants defend against environmental challenges and also provide humans with protection against various diseases as well. Hence it is not surprising while they are harvested for medicinal purposes. Most similarities in chemical compositions are found between the leaves and the mistletoe in the ten plants probably because both are leaves and the mistletoe most often attach themselves to the branches where leaves grow). The results of the phytochemical screening of the chemical constituents shows alkaloids concentration in Lawsonia inermis has differed from the rest of the species tested, while Citrus pentandra and Balanites aegyptiaca were similar and least in alkaloid concentration in Acacia nilotica. In Saponin, Psidium guajava has the highest concentration with Faidherbia albida, Annona squamosa, Citrus sinensis and Acacia nilotica being similar but differed with the rest of species. Concentration with Albizia zygia differed from both Faidherbia. albida and Annona squamosa, Citrus sinensis and Acacia nilotica but were similar to Ceiba pentandra. In Flavonoids Citrus sinensis and Faidherbia alibida were high but low in Annona squamosa and Azadirachta indica.

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