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PHYTOCHEMICAL ASSAY OF SOME MEMBERS OF THE FAMILY LORANTHACEAE FROM LOCAL AREA OF GWALE, KANO NIGERIA

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Abstract

An investigation of the phytochemical constituent of some species of the Loranthaceae family from Gwale local area was carried out in the Department of Biological Sciences, Bayero University Kano to study the taxonomic relationship existing among some members of the Loranthaceae Family which included Tapinanthus dodoneifolius, Tapinanthus begwensis and Tapinanthus buttingii between the period of January 2008 and December 2012. The phytochemical screening of the leaf extract of members of the Loranthaceae revealed the presence of Tannins, steroids, glycosides, saponins and reducing sugars. In all the members studied, Resins were absent in T. dodoneifolius but were present in the other members studied. Flavonoids were absent in T. Begwensis but present in T. buttingii and T. dodoneifolius. Alkaloids were absent in all the species studied. The results of Thin Layer Chromatography showed that R_f value of spots in the members of the Loranthaceae family ranged from 0.18 to 0.96. There were similarities at R_f values of 0.68, 0.90 and 0.96 while differences were observed at 0.18, 0.30, 0.4 and 0.43 respectively. The result could be used in the separation of members into the genus, Tapinanthus. The result of HPLC phytochemical profiling in the members of the Loranthaceae family studied showed more similarities than differences among members. The members of the Loranthaceae family studied showed similarities and differences at 360nm the similarities were found at 44, 46, 49 and 54 minutes. There were differences with respect to Tapinanthus dodoneifolius at 15, 41, 35 and 37 minutes. The difference at the 15 and 41 minutes were confirmed to be catechol and Benzoic Acid respectively. The potentials of members of

Loranthaceae Family to be used as raw materials in the production of drugs in orthodox medicine could be explored.

Keywords: Taxonomy, Loranthaceae, Phytochemical, Chromatography, Catechol, Benzoic acid.

INTRODUCTION

The Loranthaceae has 73 genera and over 900 species. Loranthaceae is the largest family of mistletoes. The *Loranths* are always resolved as monophyletic and with strong support from both nuclear and chloroplast genes (Vidal-Russell and Nickrent, 2007). The family occurs mainly in tropical areas worldwide, although they are also found in temperate habitats (Barlow, 1983) hypothesized that by the late cretaceous, Loranthaceae had a wide distribution in Gondwana, moreover following the breakup of the supercontinent, major radiations occurred in Malaysia, Australia, and South America. Different genomic basal stocks were proposed. Afro-Indian, Indian-Indonesian, Australian-papuasian and South American. Barlow (1990) proposed that these four evolutionary lines were isolated by the fragmentation of the supercontinent.

The genus *Tapinanthus* is far more widespread in the Nigerian Savanna (Omolaja and Gamaye, 1988). The taxa infest many wild and domesticated trees and shrub, species of ethnobotanical and economic value, causing various degree of structural and economic damage (Bako *et al.,*2001). Mistletoes are very important in curative medicine. They are known to be highly potent in curing circulatory problem and also as anticancer agents (Kafaru, 1994) mistletoe extracts are widely used in complementary and alternative cancer therapy in Europe. The extracts possess cytotoxic as well as immunostimulatory effect (Delinassios, 2007).

In Nigeria several herbal preparations form leaves and twigs of mistletoes e.g. *Tapinanthus begwensis* (Engl. and K. Krause) danser have become popular for the treatment of variety of diseases such as diabetes and hypertension which have been reported to be on the increase in the country (Olapade, 1995).

The type of host tree seems to largely influence the chemical compounds (especially alkaloids) found in the respective mistletoes, and mineral content in mistletoe has been found to be much higher than that in the host especially relative to the infected branch. These variations indicate that the same species occurring on different hosts in the same locality might have differences in their metabolites. This variation in metabolites had been observed by earlier workers (Deeni and Sadiq, 2002). Where *A.dodoneifoluis* on eleven different hosts were screened for metabolites. The differences noted in the chemical constituents of this parasite present on different hosts might justify why the hemi-parasites are highly variable morphologically (Wahab *et al.,* 2010).

Kafaru (1993) described the mistletoe plant as an "all purpose herb" because of its rich folkloric uses. Kingsley (2010) reported the presence of flavonoids in the methanolic extracts of leaves of Tapinanthus begwensis. The flavonoids have been shown to be hepatoprotective (Seevola *et al.*,1984). Chemical analysis of different extracts from *T. dodoneifolius* yielded components as triterpenes, sterols, carotenoids, saponosides, anthracenosides, anthocyanosides and tannins (Oeudraogo *et al.*,2005).

Delimitation of genera within the Loranthaceae family has long presented taxonomic difficulties. Tieghem (1894) accepted 118 genera, yet just three years later Engler demoted all of these to various sections within one genus *Loranthus* (Vidal – Russell and Nickrent, 2008). Much work remains to fully resolve interspecific and in some cases Intrageneric phylogenetic relationship among the various mistletoes. These studies would not only provide new insights into the phylogeny and taxonomy of these plants but would also generate data critical for evaluating hypothesis stemming from other disciplines such as anatomy, morphology, population biology and ecology (Mathiason *et al.*,2008).
The research work focuses on the similarities and differences of phytochemical constituents in some members of the Loranthaceae family in order to have a better understanding of classification in the family.

MATERIALS AND METHODS

Study Site

The study site for the collection of members of the Loranthaceae family was Bayero University, Kano in Gwale Local Government Area (Lat 11^{o} 58'40.39"N and Long 7^{o}55'47.13"). The site is endowed with trees on which the mistletoes parsitise on. *Tapinanthus dodoneifolius* was collected from *Parkia biglobosa*, *T. begwensis* was collected from *Cassia sabriena*,and *T. buttingii* was collected from *Azadirachta indica*.

Extraction of phytochemicals

The extraction of phytochemicals were carried out according to the protocol described by (Oyeleke and Manga, 2008).

Ethanol Extraction

Fifty grams (50g) of air – dried powder of the leaves of *Tapinanthus* spp was percolated with one liter of ethanol in separate conical flasks and allowed to stand for one week with intermittent shaking. The prepared sample were decanted, filtered and labeled E1. The extracts were repercolated twice for three weeks and labeled E2 and E3 respectively. Each extract was concentrated using a Rotary evaporator. The residue was weighed and labeled as ethanol fraction of *Tapinanthus dodoneifolius* one (EFTDI). The same procedure was used for the remaining species of *Tapinanthus*.

Hexane Fraction

Ethanol fraction of Tapinanthus dodoneEFRTDI was macerated with 450ml of n – hexane using 50ml. This was repeated nine times. The fractions was evaporated to dryness using a Rotary evaporator and the residue obtained was labeled as Hexane fraction of *Tapinanthus dodoneifolius* leaf one (HFTD1) The insoluble fraction was labeled (HFTD2). The same procedure was followed for the other species of *Tapinanthus*.

Chloroform Fraction

Hexane fraction of *Tapinanthus dodoneifolius* leaf two (HFTD2) was macerated with 850ml of chloroform using 100ml each time for six times for each sample and then followed by 50ml aliquots for five times. The fractions were put together and evaporated to dryness using Rotary evaporator. The residue obtained was labeled as chloroform fraction of *Tapinanthus dodoneifolius* leaf one (CFTD1) and the insoluble fraction was labeled chloroform fraction of *Tapinanthus dodoneifolius* two (CFTD2). The same procedure was followed for the remaining samples of *Tapinanthus*.

Butanol Fraction

CFTD2 was macerated with 200ml of n – butanol using 50ml four times for each sample. The combined extracts were evaporated to dryness using Rotary evaporator. The residue obtained was labeled as butanol fraction of *Tapinanthus dodoneifolius* one (BFTD1). The insoluble fraction was labeled as butanol fraction of *Tapinanthus dodoneifolius* two (BFTD2) . The same procedure was followed for the remaining samples of *Tapinanthus* .

Phytochemical Tests

The extracts were subjected to phytochemical tests to determine the groups of natural products present in the plant material according to the protocol of (Oyeleke and Manga, 2008).

Test for Alkaloids

Four milligrams per milliliter (4mg/ml) of the ethanol, hexane, and chloroform and n-butanol fractions were treated with three drops of the following reagents and observations noted for each reagent added; viz Meyer's reagent, Dragendoffs reagent and Haga's reagent. Production of a turbid suspension or precipitate with any of the reagents indicated a positive test for alkaloids (Oyeleke and Manga, 2008).

Test for Tannins

Four milligrams per milliliter (4mg/ml) of the fractions were stirred with distilled water and the filtrate mixed with Ferric chloride ($FeCl_3$). Blue – black, green or blue – green precipitate indicates a positive test for tannins (Oyeleke and Manga, 2008).

Test for Steroids and Terpenoids

Four milligrams per milliliter (4mg/ml) of the fractions were dissolved in their respective solvents followed by addition of few drops of acetic anhydride and thereafter the addition of concentrated sulphuric acid. Blue – black, green or mixture of these colours indicates the presence of steroids while red, pink or violet colour indicates the presence of terpenoids (Oyeleke and Manga, 2008).

Test for Flavonoids

To each of fractions a piece of magnesium ribbon was added followed by concentrated Hydrochloric acid drop wise to four milligrams per milliliter (4mg/ml) . Colours ranging from orange to red indicate flavones; red **t**o crimson indicates flavonoles while magenta indicates flavonoids (Oyeleke and Manga, 2008).

Test for Saponins

To 0.2g of the fractions four milligrams of boiled distilled water was added. The solution was filtered, allowed to cool and the filtrate vigorously shaken. Honey comb frothes higher or equal to the aqueous layer was recorded a positive test for saponins (Oyeleke and Manga, 2008).

Thin Layer Chromatography

This was carried out in order to separate and identify the components of the various fractions. The method of (Connell, 1990) was used for the thin layer chromatography. The surface of the plate had been readly coated with a thin layer of silica (ready coated). A small glass chromatographic tank contained the solvent i.e. hexane and ethylacetate in the ratio 9:1. The plant extract was dissolved in methanol and using very small capillary tubes the TLC plate was spotted. This was left to dry and was placed in a vertical position inside the tank, covered with a lid and run until after the solvent front was reached, the chromatogram was removed, the solvent front was recorded and air dried. The chromatogram was then sprayed with anisaldehyde and methanol mixed with concentrated sulphuric acid as the detecting reagent.

The chromatogram was then put in an oven at 120^{o}C for 10 minutes in order for the spots to develop. The different R_f values for the spots were then recorded.

HPLC Profiling

The HPLC analysis was achieved by the use of a Waters HPLC system which was equipped with a Waters HPLC 600 pumping system Waters 2487 Dual absorbance π detector and Waters integrated software. A Jones chromatographic C18 reverse phase column with a particle size of 5µm, and 250X 4.6mm in column dimention was used for separation process at 35^{o}C (column temperature) The mobile gradient solvent systems used were 1ml formic acid in a liter of Milli-Q water (A);1ml of formic acid in a liter of methanol (HPLC grade) B.A flow rate of 0.8ml /min with an injection volume of 10ml was used. Also another elution of two solvents was used (acetonitrile) A and (0.1% phosphoric acid in water) B. The method

was in accordance with that reported by Samee and Vororat (2007). The gradient program was started at 8% of A for the first 35 minutes and then increased to 22% for the next 10 minutes before bringing it down to 8%.Detection wavelengths used for the measurements were 254nm and 360nm.

RESULTS

Phytochemical Profiling in the Loranthaceae

The phytochemical profiling of members of the Loranthaceae family showed the presence of various phytochemicals such as tannins in the ethanol , n-butanol , and water fraction of *T. buttingii*. In *T. dodoneifolius* there was the presence of tannins in the ethanol and water fraction. The presence of tannins was also recorded in the ethanol and water extract of *T. begwensis*. The presence of steroids and glycosides was detected in the all the fractions of the three species studied i.e *T. buttingii*, *T begwensis* and *T. dodoneifolius*. Resins were found to be present in hexane and chloroform fractions of *T. buttingii,* and hexane and n-butanol fractions of *T. dodoneifolius*, while they were absent in hexane and n-butanol fractions of *T. begwensis*. Alkaloids were found to be absent in all the species of *Tapinanthus* studied. Saponins were found to be present in all the fractions of *T. buttingii* studied, but were found to be absent in all fractions of *T. dodoneifolius* except in the water extract .Saponins were also found to be present in all the fractions of *T. begwensis* except in the water extract.. Reducing sugars were found to be present in ethanol fraction of *T. buttingii* and in its n-butanol and water fractions. Reducing sugars were also found to be present in the ethanol fraction of *T. dodoneifolius* and in the n-butanol fraction and water extract. They were also found in the n-butanol and water fraction of *T. begwensis.* Flavonoids were found to be present in the hexane and n- butanol fractions of *T. buttingii* and also in the chloroform and n-butanol fractions of *T. dodoneifolius* and were found to be absent in *T. dodoneifolius*. The results are shown in Table 1.

Table 1: Phytochemical Constituents of Members of the Loranthaceae family Studied

S/N	Fractions	Tannins	Steroids Glycosides	Resins	Alkaloids	Saponins	Reducing sugars	Flavonoides
1.	EF (BU)	+	+	-	-	+	+	-
2.	Hef (BU)	-	+	+	-	+	-	+
3	Cf (BU)	-	+	+	-	+	-	-
4.	nBF (BU)	+	+	-	-	+	+	+
5	H_2O (BU)	+	+	-	-	+	+	-
6	EF (DO)	+	+	-	-	-	+	-
7	Hef (DO)	-	+	-	-	-	-	-
8	Cf (DO)	-	+	-	-	-	-	+
9	nBF (DO)	-	+	-	-	-	+	+
10	H_2O (DO)	+	+	-	-	+	+	-
11	Ef (Be)	+	+	-	-	+	-	-
12	Hef (be)	-	+	+	-	+	-	-
13	Cf Be)	-	+	-	-	+	-	-

14	nBf (Be)	-	+	+	-	+	+	-
15	H_2O (Be)	+	+	-	-	-	+	-

Key:(1) EF – Ethanol fraction (2) Hef – Hexane Fraction (3) CF – Chloroform Fraction (4) nBF – n – Butanol Fraction BU – *Tapinanthus butingii* DO – *Tapinanthus dodoneifolius* Be – *Tapinanthus begwensis;*

\+ Phytochemical present

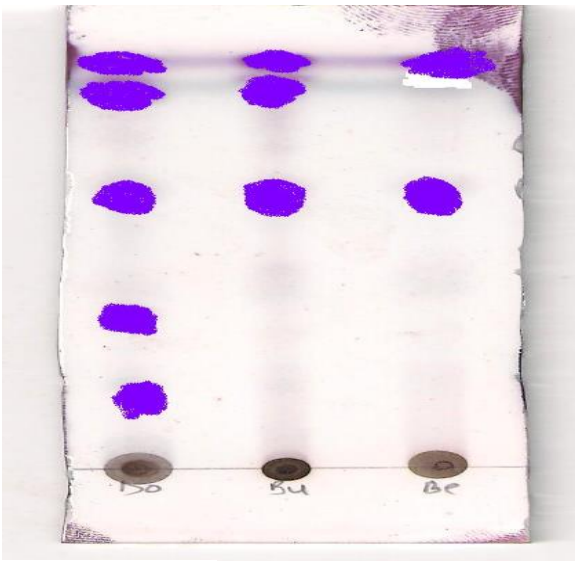
_ Phytochemical absent

phytochemical Profiling of *Tapinanthus* Species using Thin Layer Chromatography

The thin layer chromatography of members of the Loranthaceae family showed similarities at 0.68, 0.90 and 0.96. Differences with respect to *Tapinanthus dodoneifolius* were found at 0.18 and 0.30 which was absent in *Tapinanthus buttingii* and *Tapinanthus begwensis.* Table 2 (Fig. 1.)

Table 2: thin layer chromatography of ethanol extracts of *Tapinanthus* species

Plant sample	No. of Spots	R_f
1. *Tapinanthus dodoneifolius*	(5)	0.18
		0.30
		0.68
		0.78
		0.90
		0.96
2. *Tapinanthus buttingii*	(4)	0.4
		0.68
		0.90
		0.96
3. *Tapinanthus begwensis*	(4)	0.43
		0.68
		0.90
		0.96



Tapinanthus dodoneifolius *Tapinanthus butingii* *Tapinanthus begwensis*

Plate 1: Thin layer Chromatography of members of Loranthaceae showing spots similarities at R_f value 0.68, 0.90 and 0.96. Differences with respect to *T. dodoneifolius* was at R_f value 0.18 and 0.30 which was absent in *T. begwensis* and *T. buttingii*.

Phytochemical profiling of the Lorantheceae family using HPLC

The result of HPLC profiling in members of the *tapinanthus* genus showed similarity in the retention time of 46,49 and 56 minute(figure 2,3, and 4) presence of catechol at 15 minutes and benzoic acid at 41 minutes in *Tapinanthus dodoneifolius* as observed.

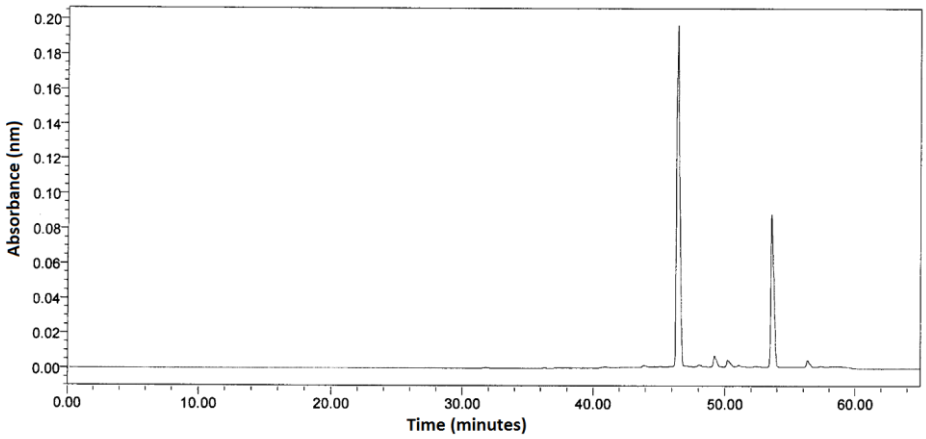


Figure 1: HPLC chromatogram of *Tapinanthus begwensis* at 360nm showing retention time peaks at 46, 49, 50 and 56 minutes.

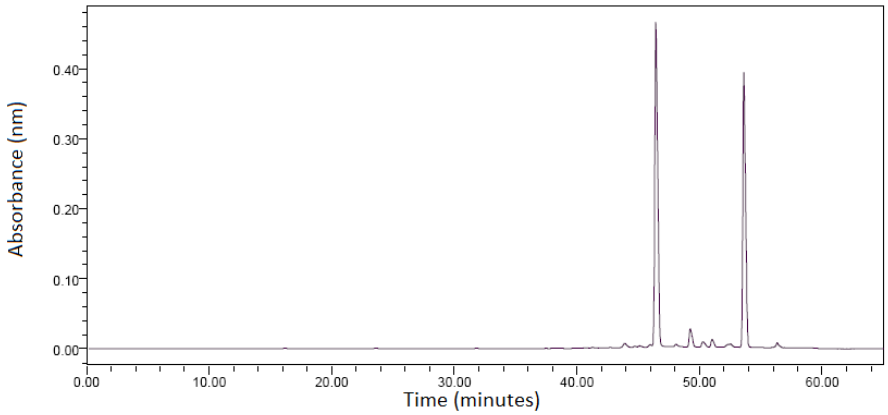


Figure 2: HPLC chromatogram of *Tapinanthus buttingii* at 360nm showing retention time peaks at 44,46, 49, 53 and 56 minutes.

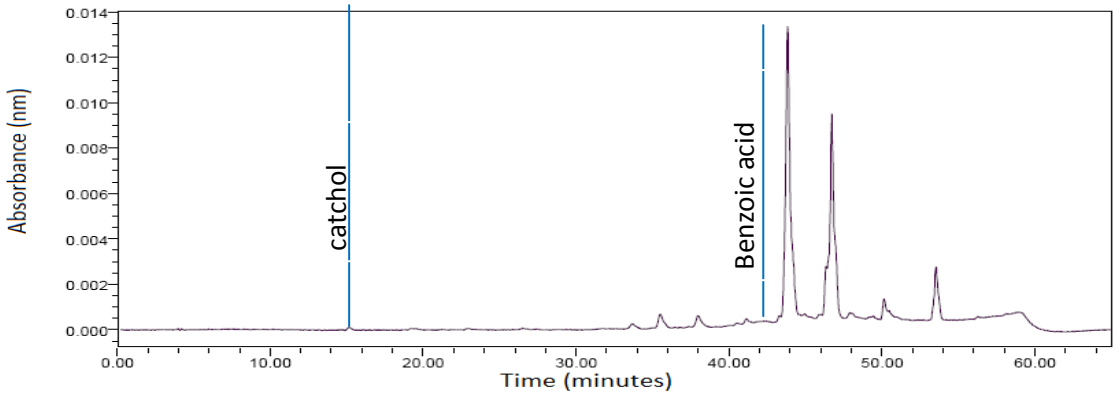


Figure 3: HPLC chromatogram of *Tapinanthus dodoneifolius* at 360nm showing the presence of catechol at 15 minutes retention time and benzoic acid at 41 minutes retention time.

DISCUSSION

The presence of various phytochemicals in members of the *Tapinanthus* genus was obtained from this study such as tannins, steroids, glycosides, resins, saponins, reducing sugars and flavonoids as reported by Ekhaise *et al.*(2010).

The phytochemical profiling of the species *Tapinanthus buttingii*,*Tapinanthus dodoneifolius* and *Tapinanthus begwensis* showed similarities as well as differences though there were more similarities based on presence of these phytochemicals in the species. The absence of alkoloids in the members studied showed similarity among the Tapinanthus species and could be used to an extent in contributing to the nature of classification of the species . Harborne (1973) reported that the system of chemotaxonomic classification relies on the chemical similarity of the taxon. It is based on the existence of relationship between constituents and among plants Rasool *et al.* (2010).

The presence of tannins in these species has shown the importance of these species medicinally as having properties such as inhibiting the growth of yeasts fungi and bacteria (Chung *et al.*,1998). There was also the presence of steroids, glycosides in the species studied i.*e T. buttingii, T. begwensis and T. dodoneifolius*.

There was also the presence of flavonoids in *Tapinanthus buttingii* and *Tapinanthus dononeifolius*. There was similarity based on the presence of flavonoids in these two species, (Alston, 1967) reported flavonoids to be of great taxonomic value. Species reported to contain flavonoids had anti-allergic anti oxidant cyto-toxic, anti-tumur an vascular activities (Horborne and Williams 2000).

The presence of reducing sugars in all the *Tapinanthus* species studied showed similarity among them. Despite the fact that there was also similarity based on the presence of saponins, it was absent in *Tapinanthus dodoneifolius*. Ankanna *et al.,* (2012) reported that it is

important to note that phytochmicals are present in different plant species connoting taxonomic affinity these differences in their connections uniquely confers individualism on each species and thus support their being treated as taxonomic species.

The findings on the Phytochemical profiling from the present study was in accordance with that of (Wahab *et al.,* 2010) who worked on the TLC Phytochemical screening of some Nigerian Loranthaceae who reported that some samples did not show the actual orange colour expected after testing with dragendoff for the presence of alkaloids. The findings of the present study also did not show the presence of alkaloids. Wahab *et al.,*2010 reported that the chemical profile of the different samples may assist only in grouping species into the different genera but this was not adequate for the identification to the species level. The earlier findings of Deeni and Sadiq (2002) reported that the same species of *A. dodoneifolius* occuring in different hosts in the same locality might have differences in their metabolites. The differences noted in the chemical constituents of the parasite present on different hosts might justify why the hemiparasitic nature of the plants appears to make them highly variable morphologically. This probably influenced the description of many species all of which may not be taxonomically distinct.

HPLC Profile of Members of the Loranthaceae Family

The HPLC phytochemical profiling of members of the *Tapinanthus* genus showed similarities between *Tapinanthus dodoniefolius*, *Tapinanthus buttingii* and *Tapinanthus begwensis*. The similarity confirms the classification of these species into a similar genus i.e *Tapinanthus*. Their HPLC profile has showed that there is the existence of relationship between the phytochemical constituents of the species of the *Tapinanthus* genus studied. Ankanna (2012) had stated that phytochemistry besides being helpful with the identification of plants specimens, it informs of patterns of chemical variation within general and aggregate species and it may ultimately demonstrate how one pattern of plants constituents evolve from the proceeding one.

Tapinanthus dodoneifolius showed distinct profile in that it showed difference independently when compared with *Tapinanthus begwensis* and *Tapinanthus buttingii*. Its HPLC profile showed the presence of benzoic acid. The phenolic compound benzonic acid has been used as an antifungal, analgesic, antiseptic and expectorant. Benzoic acid and its salts have also been used as a food preservative (Chung, *et al.,*1998).

Catechol was also found to be present in *Tapinanthus dodoneifolius*, it is a compound which has been used as flavors and fragrances and is also used as a precursor to pesticides.
The fact that *Tapinanthus dodoneifolius* showed difference when compared to its allied species has showed that the specie has possibly undergone diversification and may ultimately change based on its phytochemical constituent. Its treatment as a seprate specie is therefore re-emphasized.

CONCLUSION

The Phytochemical profile of members of the Loranthaceae family studied was similar thereby confirming the grouping into a similar genus i.e. *Tapinanthus*. Though differences could be seen in the HPLC profiling with respect to *T. dodoneifolius*, results obtained in this research showed close relationship based on the Phytochemical screening between members of the *Tapinanthus* genus studied, although the HPLC profile showed differences with respect to *T. dodoneifolius* due to the presence of catechol and benzoic acid. The potentials of members of Loranthaceae to be used as raw materials in the production of drugs in orthodox medicine could be explored.

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