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Research paper

Quantification of bioactive constituents of mistletoe leaves (tapinanthus globiferus a. rich) from four different host plants in the EWU community

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Abstract

Background: Pharmacological activities of the medicinal plant are determined by the chemical substances it contains, these substances are also affected by factors of which geographical and all that surround such plant plays a vital role. Mistletoe is a plant that depends solely on its host. This study evaluated the different phytoconstituents concentrations in Mistletoe (*Tapinanthus globiferus*) leaves growing on four different host plants (Avocado pear (*Persea americana*), Orange (*Citrus sinensis*), Guava (*Psidium guajava*), and Kolanut (*Cola nitida*)) in the Ewu community of Edo State, Nigeria.
Methods: Qualitative and quantitative phytochemical analyses were carried out using standard methods.
Results: The qualitative test revealed the presence of alkaloids, Tannin, Phenolic compounds, Flavonoid, Saponin, while the quantification estimation showed these phytochemicals in varying concentrations. Guava had the highest concentration of Tannin (323.4±0.07), Saponin (138.6 ± 0.02) while Orange has the highest concentration of Flavonoids (632.61 ± 0.12) followed by Guava (407.88 ± 0.05), the highest concentration of Phenolics was found in Avocado pear (519.9 ± 0.29) followed by Guava (273.5 ± 0.34). The highest Alkaloids were found in Orange (2.20 ± 0.06) followed by Avocado pear respectively.
Conclusion: From this present study, it can be concluded that Mistletoe from different host plants has a different concentration of phytochemicals and is thus expected to have different bioactivities.

Keywords: *Quantitative Phytochemical Screening; Tapinanthus Globiferus; Persea Americana; Citrus Sinensis; Psidiumguajava; Cola Nitida.*

1. Introduction

Sustainable operation of traditional medicinal factories coffers is important, not only because of their value as an implicit source of new medicines but due to reliance on traditional medicinal companies for health. The vast majority (70-80%) of people in Africa consult traditional medical practitioners for healthcare (Cunningham, 1993). Traditional medicine producing companies have been considered to be veritably successful in addressing several infections and habitual conditions with the advantage of being completely natural (Nigeria Natural Medicine Development Agency (NNMDA), 2009). Several plants have been evaluated for efficacy (Owolabi *et al.* 2019a, Owolabi *et al*. 2019b) and phytoconstituents responsible for some of the medicinal plants' efficacy identified (Owolabi *et al.* 2021).
Mistletoe, with the common name (s); raspberry lime, all-heal, devil's fuge, Iscador, etc is a general term for woody shoot spongers in several plant families especially Loranthaceae and Viscaceae (Burkill, 1985, Parker & Riches, 1993, Polhill & Wiens, 1998) and utmost class of African mistletoes belong to the family Loranthaceae (Polhill & Wiens, 1998). Seven classes of the Loranthaceae – Helixanthera, Berhautia, Englerina, Globimetula, Agelanthus, Tapinanthus, and Phragmanthera – with about five dozens or further species are recognized in West Africa (Burkill, 1985) and the group term mistletoe is used for all these species. In West Africa, mistletoes are planted on numerous tree crops of profitable significance including the shea adulation tree (Vitellaria paradoxa Gaertn.f.), the neem tree (*Azadirachta indica* L.), citrus species, especially sweet orange (*Citrus sinensis* L.), and grape (*Citrus paradise* L.), cocoa (Theobroma cacao L.) and rubber (Hevea brasiliensis Muell Arg) (Bright & Okusanya, 1998; Overfield *et al.* 1998; Gill & Onyibe, 1990; Begho *et al.* 2007). Often, the host trees that have lots of mistletoes suffer from them as the triumph of mistletoes leads to poor growth and productivity and eventual death of the same host, especially during unfavorable rainfall conditions and if the host tree is simply a shrub or a small tree. Either, the host plant is considered as important as the mistletoe since the distinction is made between hosts and not between mistletoes. This current study aimed at quantifying the phytoconstituents of several mistletoes growing of different hosts.

2. Material and methods

2.1 Chemicals

All chemicals and reagents used were of analytical grade and the water used was glass distilled.

2.2 Preparation of plant material

Fresh young leaves of *Tapinanthus globiferus* (Mistletoe) were harvested from Kolanut (*Cola nitida*), Orange (*Citrus sinesis*), Guava (*Psidium guajava*), and Avocado pear (*Persea americana*) trees; identification and authentication were done at Paxherbal Clinic and Research Laboratories Herbarium. The leaves were rinsed, drained, air-dried for 24 hrs and later oven-dried at 50°C for 24hrs before being blended into a fine powder and kept in an airtight container for analysis.

2.3 Qualitative phytochemical screening

The powdered plant samples (100 g) of (*T. Globiferus* growing on, Kolanut, Orange, Guava, and Avocado pear trees) were separately macerated with 200 mL of double distilled water with stirring for 45 minutes and tested for the presence of bioactive compounds by using the following standard methods (Harborne JB (1973), Trease GE & Evans WC (2002) with slight modifications as follows;

2.3.1. Test for alkaloid

Filtrate (3mL) + 3mL of 1% HCL + steam (30 minutes) + cooling + centrifuging at 2000-3000rpm for 10 minutes

a) 1mL of supernatant + 1mL of Drangedroff reagent
b) 1mL of supernatant + 1mL of Mayer's reagent
c) 1mL of supernatant + 1mL of Wayner's reagent

2.3.1.1 Results for confirmation

Dangedroff reagent	-	Orange ppt
Mayer's reagent	-	Creamy ppt
Wagner's reagent	-	Reddish brown ppt

2.3.2. Test for cardiac glycoside

2mL of filtrate + 2mL of glacial acetic acid + 1mL of 0.1% $feCl_3$ + 1mL of Conc. H_2SO_4 acid. Green-blue colouration indicates the presence of Cardiac glycoside.

2.3.3. For flavonoid

2mL of the filtrate + 2mL dilute ammonia, add 1mL of Conc. H_2SO_4 acid. Yellow coloration reveals the presence of flavonoid, which disappears upon standing.

2.3.4. Test for phlobatannins

2mL of filtrate + 2mL of 1% HCL acid + steam for 30 minutes. A red deposit at the base of the test tube shows the presence of phlorotannins.

2.3.5. Test for reducing sugar

2mL of filtrate + 2mL (Fehling solution A & B) + steam for 30 minutes. Red coloration reveals the presence of reducing sugar.

2.3.6. Test for saponin

0.5mL of the filtrate + 5mL of distilled water and shake vigorously. Persistent fronting means saponin is present.

2.3.7. Test for starch/polysaccharide

2mL of filtrate + 6 drops of iodine solution. Blue-black coloration reveals the presence of starch.

2.3.8. Test for steroid

0.5mL of sample + 0.5mL of acetic acid anhydride + cool in ice + 0.5mL chloroform + 1mL Conc. H_2SO_4 acid is added carefully using a pipette. A reddish-brown ring at the interphase of the two liquids reveals the presence of steroids.

2.3.9. Test for tannins

2mL of filtrate + 2mL of 0.1% $FeCl_3$ solution. A blue-black coloration indicates the presence of hydrolyzable tannin and brownish-green color indicates the presence of condensed tannin.

2.3.10. Test for terpenoid

2mL of filtrate + 6 drops of Brady's reagent. A yellowish-orange coloration reveals the presence of Terpenoid.

2.4. Quantitative phytochemical analysis

The phytochemicals present in the water extracts of leaves of T. Globiferus of Kolanut, Orange, Guava, and Avocado pear trees were determined and quantified by standard procedures.

2.4.1. Quantification of alkaloid

Quantification of alkaloid content was determined using the Gravimetric method as described by Onwuka, (2005). Using this method, 5 g of each powdered sample was weighed separately into 4 beakers, 50 mL of 10% acetic acid in ethanol was added. The mixture was shaken and allowed to stand for 4 hours before filtering each using Whatman filter paper No. 1. The four different filtrates were concentrated to ¼ of their original volume each. 1% concentrated ammonia was added to each of the concentrates dropwise to precipitate the alkaloid. Each of the precipitates was filtered using a pre-weighed over-dried Whatman filter paper No. 1 and recorded as W_1. Each of the precipitates on the filter paper was dried on an electric oven until a constant weight was obtained and noted as W_2. By weight difference, the weight of alkaloid was determined and expressed in percentage (%) as shown below:

$$\% \text{ alkaloids} = \frac{W_2 - W_1}{W_3} \quad X100$$

Where W_1 = weight of empty filter paper
W_2 = weight of filter paper + precipitate
W_3 = weight of the sample.

2.4.2. Quantification of flavonoids

The flavonoids content was determined by the Aluminium trichloride method using quercetin as a reference compound (Kumaran and Karunakaran, 2006). About 100 µL of the extracts in methanol (10 mg/mL) was mixed with 100 µL of 20% $AlCl_3$ in methanol and a drop of acetic acid (100%), and then diluted with methanol to 5 mL. The absorption at 415 nm was read after 40 min. Blank samples were prepared similarly except without extract. A standard calibration curve was prepared from Quercetin solution (0.5 mg/mL) with different concentration (0.02 – 0.1 mg/mL) was measured under the same conditions. All determinations were carried out in triplicates. The amount of flavonoids in plant extracts in QE was extrapolated from the standard Quercetin graph.

2.4.3. Quantification of phenolic compounds

Folin–Ciocalteu procedure given by Yu *et al*. (2002) was used to estimate the total phenolic contents in the extracts of the plants. Following this method, 0.1 mL of extracts were diluted to 1 ml with distilled water. To this solution, 0.5 mL of Folin–Ciocalteu reagent (2N, 1:1) and 1.5 mL of 20% sodium carbonate solution were added. The mixture was incubated for 2 h at room temperature. The volume was raised to 10 mL with distilled water and the absorbance of the blue-colored mixture was measured at 765 nm (SURGISPEC SM-23D Spectrophotometer, England). The amount of total phenol was calculated as mg TAN/g from the calibration curve of Tannic acid standard solution

2.4.4. Quantification of tannin

The tannin content was quantified using the spectrophotometric method as described by Akinmutini, (2006). 2g each of the powdered samples was weighed into four different conical flasks, 10ml of deionized water was added separately and the solution was left to stand for 30mins. 0.04ml from each supernatant was taken into three different test tubes, 0.46ml of deionized water, 0.25ml of Folin-Ciocateus reagent, and 1.25ml of 20% $NaCO_3$, was added to each test tube. The test tubes were incubated in dark for 10mins and the absorbance was measured at 725nm.

2.4.5. Quantification of saponin

The samples were ground and 20 g of each were put into a conical flask and 100 cm3 of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue was re-extracted with another 200 mL 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90°C. The concentrate was transferred into a 250 mL separatory funnel and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded.
The purification process was repeated. 60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to constant weight; the saponin content was calculated (Obadoni & Ochuko, 2001).

3. Results and discussion

The filtrates of the aqueous extracts of the samples were subjected to phytochemical screening to check for the presence of the phytochemicals of interest in them and the results presented in the table below indicate the presence of the phytoconstituents of interest.

Table 1: Results of the Qualitative Phytochemical Screening of the Samples

Parameter	Guava	Orange	Pear	Kola
Alkaloid	+	+	+	+
Flavonoids	++	+++	++	+++
Saponin	++	+++	+	+++
Tannns	+	+++	+	++

Key:
+ = Mildly present
++ = Moderately present
+++ = Abundantly present

The quantitative estimation of the phytoconstituents studied showed that the leaves of the Mistletoe obtained from four different host trees (Avocado Pear, Orange, Guava, and Kolanut) is present in varied concentration. Mistletoe obtained from the Guava tree had the highest concentration of Tannin (323.4 ± 0.07) and the ones from Avocado pear contained the lowest concentration (84.28 ± 0.17); in phenolic compounds determination, Mistletoe from Avocado pear had the highest concentration (519.9 ± 0.29) while that of Kolanut has the least in phenolic compound concentration (33.7 ± 0.06), Mistletoe from Orange tree contains the highest concentration of flavonoid (632.61 ± 0.12) followed by Guava in flavonoid concentration, while Kolanut Mistletoe contains the lowest concentration (223.64 ± 0.07), Mistletoe gotten from Guava tree contain the highest concentration of saponin (138.6 ± 0.02) followed by pear tree and Mistletoe from Orange had the least concentration (66.37 ± 0.08). Finally, Mistletoe obtained from the orange tree contained the highest concentration of alkaloid (2.20 ± 0.06) followed by Avocado pear Mistletoe (1.20 ± 0.03) and Mistletoe obtained from Kolanut had the least concentration. Therapeutic effects of any medicinal plant are believed to occur from one or a combination of phytoconstituents such plant contains (Owolabi, 2021).

Table 2: Results of the Quantitative Phytochemical Analysis of T. Globiferus Leaves from Four Different Host Trees

Plant sample	Tannins (mg/100g)	Phenolic (mg/100g)	Flavonoid (mg/100g)	Saponin (mg/100g)	Alkaloid (%)
Mistletoe from Pear	84.28 ± 0.17	519.9±0.29	326.7±0.35	101.37±0.03	1.20±0.03
Mistletoe from Orange	85.26 ± 0.11	56.78±0.08	632.61±0.12	66.37±0.08	2.20±0.06
Mistletoe from Guava	323.4 ± 0.07	273.5±0.34	407.88±0.05	138.6±0.02	0.08±0.19
Mistletoe from Kolanut	136.22±0.04	33.7±0.06	223.64±0.07	75.24±0.08	0.06±0.08

Data represent means ± S.E, n= 3

4. Conclusion

The concentration of the tested phytoconstituents varied with different host trees. This work thereby suggests that the mistletoe (Tapinanthus globiferus) possess different biological activities. However, the use of Guava Mistletoe might give better health benefits since it contains the highest concentration of Flavonoids which have been researched to have antioxidant activities.

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