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Phytochemical analysis and antibacterial activity of broad leave plants' mistletoe (*Viscum album*) for its medicinal applications

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Abstract – Mistletoe (*Viscum album*) from four broad leaves plants: Kolanut (*Cola nitida*), Orange (*Citrus*), Cocoa (*Theobroma cacao*) and Guava (*Psidiumguajava*) were obtained from farms in Osun state between the months of March and April, 2017 and prepared into easily extractable form (tea). The Phytochemical constituents of the extracted solutions were determined using the standard methods of analysis. The results show that the mistletoe extracts contain alkaloids (2.31± 0.01 %), flavonoids (5.47 ± 0.32 mg/g), tannins (3.01 ± 0.21 mg/g), total phenols (428 ± 12.31 mg/g), glycosidic cyanide (0.35 ± 0.06 mg/g) and saponins (1.28 ± 0.11 mg/g). The antibacterial activities of the samples extract on *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonia*. The results indicated that the antibacterial activities of the extract are concentration dependent with the minimum inhibitory concentration ranging from 15- 65 mg/ml. The statistical analysis of the data showed that no significant differences occurred in most values (P> 0.05). The results established the effectiveness of mistletoe plants in folk treatment of various aliment and therefore reinforced the potential of the mistletoe tea for its medicinal usage.

Keywords:*Mistletoe (Viscum album), Phytochemical, analysis, antibacterial, activity.*

1.0 INTRODUCTION

Mistletoe (*Viscum album*) belongs to the *Loranthaceae*family. It is a semi -parasitic plant which grows the branchesand trunks of other trees rather than on the soil.The seeds of mistletoe unlike most seeds of other plants require sunlight for it germination (Anselm, 2008). The leaves of mistletoe also produce chlorophyll even inthe darkness unlike other plants leaves that turn yellowish when denied of light hence, mistletoe is sometime referred to as evergreen plant (Daij and Mumper, 2010).

The plant has been used for various medicinal purposes from ancient times.It has been reported to possess a number of therapeutic properties which make it applicable in folkmedicine for the treatment and management of a wide range of diseases such as diabetes mellitus, blood pressure,cramps,stroke, stomach problems and others (Essam*etal*., 2010). The presence of various phytochemicalslike glycosides, alkaloids, viscotoxins,phenylpropannoids, tannins, lignnins and sugars has been reported in the mistletoe collected from differenthost plants (Atmani*et al*., 2009). Generally, phytochemicalsare bioactive and non-nutrient plants compounds with physiological action on human body (Okwu, 2004). They are natural occurring compounds which have both stimulatoryand inhibitory properties.*Viscm album* plant living on different host treesis endowed with different antioxidant activity and theantioxidant capacity of the extract could differ accordingto the harvesting time of the plant as well as nature of the host tree (Njoku and Akumefula, 2007).

Mistletoe grown on guava (*Psidiumguajava*) was reported to be effective in treating cancer because its highconcentration of lectins; a type of proteins that was reported to destroy cancerous tumors and cells. *V. album*from kolanut (*Cola nitida*) was reported to be potent for the treatment of hypertension, nervousnessand insomnia, while those from cocoa (*Theobroma cacao*) wasnoted in the treatment of diabetes (Anselm, 2008). The aim of this work was to determine the differences in the phytochemicalconstituents and antibacterial activity of *Viscum album*Phytochemicals fromdifferent host plants in order to justify their efficacy of their medicinal applications.

2.0 MATERIALS AND METHODS

Fresh leaves and twigs of mistletoe (*Viscum album*) from four different host trees werecollected from different farms inOsun state between the month of March and April, 2017. The host tree plants areKolanut (*Cola nitida*), Orange (*Citrus*), Cocoa (*Theobroma cacao*) and Guava (*Psidiumguajava*). The samples were washedand air dried separately on the laboratory benches for two weeks the dried leaves were separately grinded into fine powder with mortar and pestle and kept in sterile container separately with appropriate labels for further analysis.

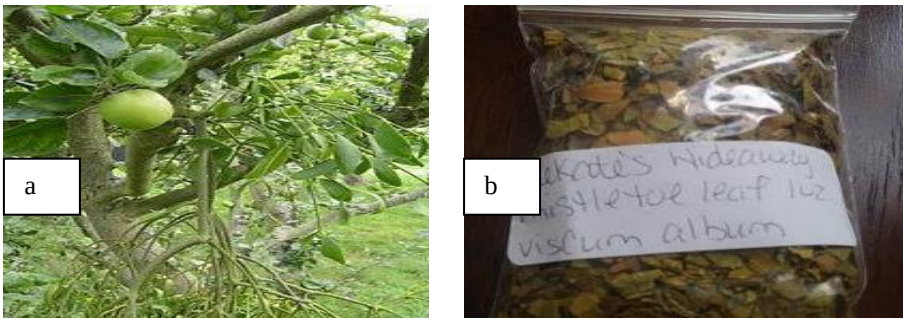

Figure 1: (a) Mistletoe on host orange plant (b) Dried mistletoe leaves (tea)

2.1 Phytochemical analysis.

Flavonoids

Flavonoid was determined bycolorimetric method as described by(Akimutini, 2006). One gramme of the powdered samplewas each weighed differently labeled beakers and 25ml of 95% ethanol was added to each and left for 24hours. The solutions were filtered separately using *Whatman* filter paper. 0.5ml of the filtrate was taken from each and 1.5ml of 95% ethanol was added, 0.1ml of 10% aluminium chloride was also added to each of the solution and the mixture was well mixed to obtain homogenous solution. 0.1ml of potassium ethanoate was thereafter added to the mixture followed byaddition of distilled water. The mixture was incubated at room temperature for 30 minutes before its absorbance was measuresat 415 nm with a visible spectrophotometer (Ejikeme*et al*., 2014).

Alkaloid

The alkaloid content of the extract was determined by gravimetric method using the procedure described by Onwuka, (2005). 5g of each powdered samples separately measured into conical flasks and 50mlof 10% acetic acid in ethanol was added to each flask. The mixture was mixed and allowed to stand for 4 hoursbefore filtration. Each filtrate was evaporated to one-quarter of the filtrate volume ona hot plate. Then 1% concentrated NH_3 was added to each of the concentrated filtrates drop-wise to precipitate the alkaloids. Each of theprecipitate was filtered with a weighed *Whatman* filterpaper (w_1) and each of the precipitate in the filter paper was dried in an electric oven at 60 ^{0}C for 30 minutes, cooled in the dessicator before and weighed (w_2). The alkaloids content of the sample was by weight difference and expressed in %.

$$\% \text{ alkaloids} = \frac{w2-w1}{Sample\ weight}$$

w_1 = Weight of empty filter paper, w_2 = Weight of filter + filtrate

Tannin

The tannin content was determinedby spectrometric the method as described by Akimutini, (2006) with title modification. 2g of each of thepowdered sample was measured and added to 10ml of distilled water in conical flasks separately. The solution was left to stand for 30 minutes before 2.5ml from each of the supernatant was pipeted into a 5ml volumetric flask and 1ml of *Folin-Denis* reagent was added to eachfollowed by addition of 2.5ml of saturated Na_2CO_3.Eachof the solution thereaftermade up to 50ml in avolumetric flask and left for 90 minutesbefore the absorbance was measured at 250nm in aspectrophotometer.

Cyanide

Cyanide constituent of the samples extract was determined using the method of Wang and Filled as described by (Onwuka, 2005). 5g of each sample was weighed and added to 50ml of distilled water in corked 250ml conical flasksseparately and allowed to stand for 24 hoursbefore filtration. The filtrates were poured into five different corked test tubes and then 4ml of alkaline was added. Each tube was incubated in a water bath at 37^0C for 5minutes before the absorbance of each was taken at 490nm.

Phenolic compounds

Phenolic compound analysis was determined by spectrophotometric method. 0.03g of the plant samples was weighed into five (5) different test tubes and 10ml of50% aqueous ethanol was and left mixtures were for 2hours. It was filtered using *Whatman* filterpapers into five (5) different 50 ml volumetric flask. 2.5ml of *Folin-Denis* reagent was added to each of the filtrate and was allowed to stand for 30 minutes. 50ml ofsaturated Na_2CO_3 was thereafter added to each tube, mixed thoroughly and allowed to stand for 20 minutesbefore the absorbance of each was taken at 760nm.

Saponin

Saponin quantitative determination was carried out using the method reported by Obadoni and Ochuko, (2002) and Ejikeme*et al*., (2014).100 cm^3 of 20% aqueous ethanol was added to 5 grams of each powder sample in a 250 cm^3 conical flask. The mixture was heated over a hot water bath for 4 hours with continuous stirring at a temperature of 55°C. The residue of the mixture was re-extracted with another 100 cm^3 of 20% aqueous ethanol after filtration and heated for 4 hours at a constant temperature of 55°C with constant stirring. The combined extract was evaporated to 40 cm^3 over water bath at 90°C. 20 cm^3 of diethyl ether was added to the concentrate in a 250 cm^3 separator funnel and vigorously agitated from which the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice and 60 cm^3 of n-butanol was added and extracted twice with 10 cm^3 of 5% sodium chloride. After discarding the sodium chloride layer, the remaining solution was heated in a water bath for 30 minutes, after which the solution was transferred into a crucible and was dried in an oven to a constant weight. The saponin content was calculated as a percentage:

$$\% \text{ Saponin} = \frac{\text{Weight of saponin}}{\textit{Weight of sample}}$$

2.2 Antimicrobial activity

The minimum inhibitory concentration (MIC) was determined by agar dilution method, as reported byNwankwo and Egbuonu, 2011. One two-foldserial dilution of the extracts (2.5 mg/ml) were prepared in sterile distilled water and poured into separate sterilized Petri dishes. This concentration was selected based on the preliminary sensitivity tests on themicroorganisms previouslyreported (Cleidson*et al*., 2007; Esimone*et al*.,2003). 28 g of nutrient agar powder wasweighed and dispersed in 1 litre of distilled sterile water.

The mixture was allowed to stand for 10 minutes before swirling to mix properly, sterilized by autoclavingfor about 15 minutes at 121ºC and cooled to 40ºC. 20 ml of molten nutrient agar was poured into the Petri dishes, swirled slowly and then allowed to set and dry. Each set of agar plate was streaked with the brothculture of bacteria (*E. coli,S. aureus, P. aeruginosa and K. pneumonia*). The agar plates

without extract (the negative control) werealso streaked with the micro organisms. The agar plates were then incubated at 37°C for 24 h (for the bacteria). The inhibition zonediameter, the measure of activity, was consequently determined by plotting the square of the inhibition zone diameter (IZD^2) against the log concentration of theextract and the MIC calculated from the intercept on the log concentration axis.

2.3 Statistics

The values are mean ± SD of triplicatedeterminations. The data were analysed by ANOVA followed by Turkey's HSD test for significant differences using SPSS 16.0 computer software.

3.0 Results and Discussion

The result of the phytochemical analysis presented inTable 1 revealed the presence of medicinally active compounds in mistletoe samples (*Viscum album*). The quantitative estimation of the phytochemical constituents of the plant extract showed that the of the plant (*Viscum. album*) obtained from four different host trees: kolanut (*Colanitida*), Guava(*Psidum. guajava*)orange (*Citrus spp*.) and Cocoa (*Thebroma cacao*) is relativelyrich inalkaloid, flavonoid, saponin, tannin, glycosidic cyanide and phenolic compound which varied in concentration with host plant. Mistletoe obtained from cacao contained the highest concentration of tannin (3.43 mg/g ± 0.15). Orange mistletoe contained highest concentration of flavonoids (6.89 mg/g) which was in line with the value reported by (Taiga, 2013). Flavonoids have been reported to have biological activities that are beneficial in the prevention and management of many ailments (Li and Lin, 2010). Therefore the presence of flavonoids in relatively high concentration in *Viscum album* (mistletoe) extract from different host plants has established their potentials anduses for medicinal purposes. Flavonoids were reported to inhibit platelets aggregation due to the possible efficient detoxification and antioxidant activities (Obasi*et al*., 2011).

Mistletoe from guavahas the highest concentration of phenolic compounds. Alkaloid concentrations values were higher than those reported for *Viscum album* mistletoe extracts from the same plants under study except the orange value which was in agreement with the value previously reported.Sponinn and glycosides values were in agreement with the previous studies (Taiga, 2013).Aqueous leaf extracts of many plants containing tannins, terpenoids, flavonoids, and alkaloids have been implicated for the various pharmacological activities of the plant including antibacterial and antidiabetic properties (Osadebe and Ukwueze, 2004; Osadebe*et al*., 2004; Murali*et al*., 2011).

Table 1: Quantitative Analysis of mistletoe (*Viscum album*) from different host trees

Mistletoe samples	Tannin (mg/g)	Flavonoids (mg/g)	Total phenol (mg/g)	Alkanoids (mg/g)	Saponin (mg/g)	Glycosides (mg/g)
Kolanut	2.53± 0.33	6.22± 0.43	30.10± 1.45	2.12± 0.23	1.06± 0.11	0.34± 0.04
Guava	3.04± 0.23	4.49± 0.33	411± 9.13	2.43± 0.34	1.34± 0.15	0.28± 0.02
Orange	3.11± 0.16	5.89± 0.53	48.71± 3.33	1.68± 0.16	1.49± 0.16	0.31± 0. 04
Cocao	3.43± 0.23	5.32± 0.45	50.30± 4.16	2.54± 0.13	1.12± 0.13	0.42± 0.05

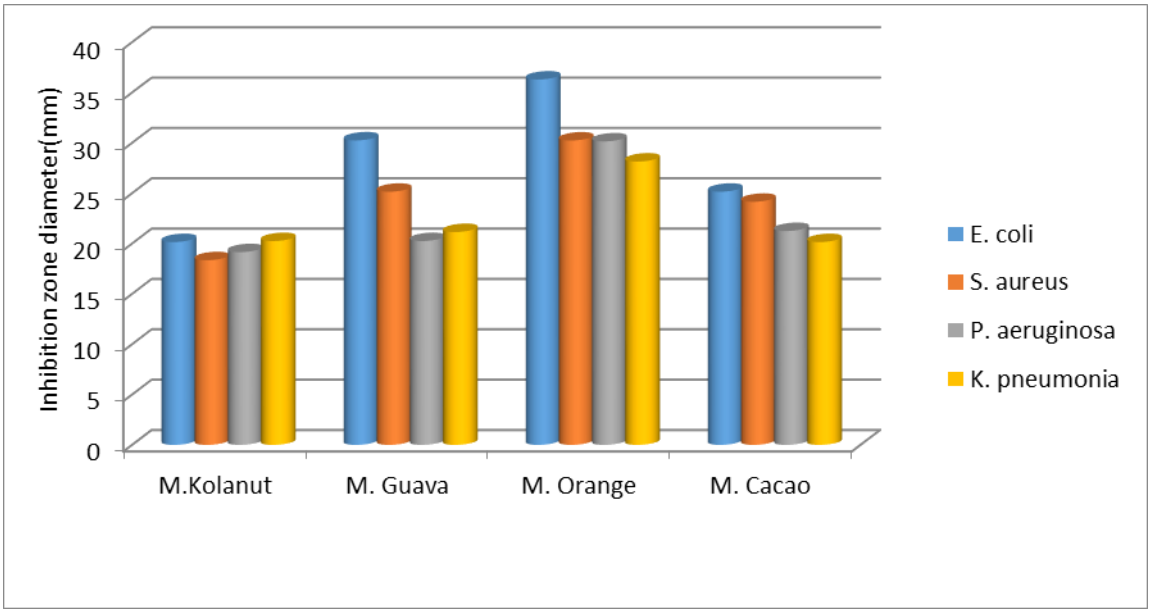

Figure 1: Antimicrobial Screening of the Extracts (IZD in mm) at 2.5 mg/ml for extracts

The different degree of anti bacterial activities on mostof the tested pathogens suggests that mistletoe (*Viscum album*) from different host plants could be of benefit in the development of potent drugs against diseases caused by these pathogens. In particular, all the extracts of Mistletoe (*V. album*) from different host plants in the present study were active against *P. aeruginosa. P.aeruginosa* is a gram negative bacterium, which was less susceptible (Gould and Booker, 2000). This is an important observation suggests the potential of harnessing (*V.album*)mistletoe in the development of drug against *P. aeruginosa*. Previuos study has showed that another mistletoe extract (*L. micranthus Linn*) hosted to *kola acuminate* plant was found to be active against *P aeruginosa.*

4.0 Conclusion

The present study has established the potential of mistletoe plants extract as medicinal remedy in the treatment and management of diseases. It is therefore recommended that comprehensive investigation of different mistletoes on different host plants be investigated.

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