E-ISSN: 2709-9423
P-ISSN: 2709-9415
JRC 2021; 2(1): 63-67

www.chemistryjournal.net
Received: 13-11-2020
Accepted: 21-12-2020

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Comparative studies of phytochemistry and proximate analysis of essential oil of leaves and flower of mistletoe plant

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Abstract
Fresh leaves and flower of the sample (mistletoe) was extracted using a soxhlet extraction method. The extracts of the leaf and flower of mistletoe plant was subjected to qualitative and quantitative analyses of phytochemicals and nutritive value of essential oils. The result of the qualitative analyses of mistletoe leaves and flower shows the presence of tannin, flavonoid and saponins; alkaloid and phenol was highly concentration while terpenoid was absent in the flower extract. Saponins were present in leaf and absent in the flower. The presence of phenol and tannin is detected in the leaf extract. The quantitative result revealed tannin 2.01 ± 0.66mg/100g, alkaloid 0.93. ± 0.33mg/100g, phenol 3.22 ± 0.70mg/100mg, flavonoid 2.66 ± 0.17mg/100g, saponin 2.35 ± 0.17/100g. The quantitative result revealed tannin 0.84 ± 0.09mg/100g, alkaloid 4.47 ± 0.14mg/100g, phenol 8.46 ± 0.24mg/100mg, flavonoid 1.71 ± 0.08mg/100g. The result reveals that the leaves and flower have potential to promote good health and reduce diseases risk.

Keywords: Comparative, phytochemistry, proximate, essential, plant

Introduction
Mistletoe plant is a hemi-parasitic plant that grows on trees such as cocoa, mango, guava, kolanut trees is known scientifically for its nutritive content such as carbohydrate, protein, fat, fiber, energy value, and ash. Mistletoe leaves have been known for its use in the treatment of some ailments including hypertension, epilepsy, infertility, arthritis, cancer and diabetes or used as a diuretic agent (Simeon *et al.*, 2013) [15]. Essential oils are concentrated hydrophobic liquids containing volatile aroma compounds found in some plants. Mistletoe leaves also contains essential oil which could be a good anti-inflammatory substance since it contain n-hexadecanoic acid that have been reported to exhibit anti-inflammatory properties. (Odunayo & Ibrahim 2016) [9]. Ogunmefun *et al.* (2013) [10] reported that in Nigeria, the Hausa and Fulani tribes in Nigeria use mistletoe in the treatment of cancers and inflammation. The aim of this research is to compare the quantified phytochemicals and proximate composition of the essential oil content of mistletoe leaves and flowers.

Materials and Method
Collection and Preparation
The mistletoe samples used were collected from Cross River University of Technology Calabar, Cross River State, Staff Quarters from a cocoa tree. The plant was identified in the Department of Biological Sciences of Cross River University of Technology Calabar. The identified plant materials were separated into flower and leaf and some fresh ones obtained were used for oil extraction and moisture content while the rest were dried in an electron oven for 3 hours. After drying, the samples were crushed to powdered form using mortar and pestle and were respectively stored in bottle till require for analyses.
15g was weighed into the soxhlet extractor attached to a round bottom flask containing 250ml of methanol and a pitch of anti-bumping granules, clamped to a retort stand attached to a heating mantle connected to a power source. The soxhlet extractor is connected to a condenser with an inlet that allows the flow of water into the system and outlet that allows water flow out of the system. The inlet and outlet were connected to a water source with rubber tubing. The water flowing into the condenser aids in cooling the systems and prevents the escape of the vapour from the system as the methanol is being heated.

The vapour dropped back as a result of the cooling effect of the inlet and outlet in the condenser into the round bottom flask through the reflux arm of the soxhlet extractor that was fitted with glass wool. The extraction was completed when the extract passing through the reflux arm of the soxhlet extractor into the round bottom flask was clear. The methanol was distilled and the plant extract was left in the round bottom flask. The extracted sample was used for further analyses to determine the phytochemical content.

Oil Extraction Procedures

Fresh leaves and flowers of the sample (mistletoe) were fed into a round bottom flask and placed in a soxhlet extraction and 100ml of distilled water was poured into it. The soxhlet apparatus was setup with condenser connected to the tap inlet and outlet, few chips of antibumbing was placed in the round bottom flask containing the sample with distilled water. The flask was heated on the hot plate for 10hours. The flask was removed when the clear extract was observed and the extract was turn into a separating funnel and few drops of n-hexane was added and shaken for five minutes and kept for some time. Since the oil is lighter than the water, the oil was observed as the upper layer while the water was the bottom layer. The tap of the separating funnel was opened and the water layer was separated from the oil.

Phytochemical Screening Assay

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by (Tabe, *et al.*, 2019a) [16], (Tabe, *et al.*, 2019b) [17] (Ushie *et al.*, 2013) [18] and (Ushie *et al.*, 2016) [19].

Quantification of Phytochemicals

Qantitative determination of the detected secondary metabolites was carried out to know their percentage in the essential oil of leaves and flower of mistletoe plant by the methods described by Igbal *et al.*, (2011), Mudansir (2012), Sathya (2013) [14] and Ushie *et al.*, (2018) [20].

Determination of Saponins

5.0g of dried sample (mistletoe leaf & flower) each were measured accurately with an analytical top loading balance into a thimble and was transferred into the soxhlet extractor connected to the condenser and round bottom flask of known weight, 100ml of methanol was used for the extraction for 3 hours to obtain the lipid and the pigment content from the samples. After the extraction the methanol was distilled off leaving the saponins. After the evaporation the flask and the content were reweigh. The difference between the final and the initial weight of the flask represent the weight of the saponins in the sample.

$$\text{Calculation for \% saponins} = \frac{s-t\ x\ 100\%}{w}$$

Flavonoid Determination

1.0ml of each sample methanolic extracts (mistletoe leaf and flower) was put in separate test tubes. 1ml each of 5% NaOH was added and shaken for the formation of precipitate. The mixture was decanted to a cuvette for uv-vis spectrophotometer measurement at the wave length of 540nm and result was recorded as absorbance sample.

$$\text{Calculation for \% flavonoid} = \frac{Abs\ sample \times Std\ cone \times 100\%}{Abs\ sample\ standard}$$

Terpenoid Determination

1.0ml of methanolic extract of sample (mistletoe leaf and flower) was measured into test tube with a stopper, 3.0ml of acetic anhydride was added gently to the test tube, shaken and cooled in an ice bath for 10 minutes. The colour changed after addition of two drops of concentrated H_2S0_4 to bluish colouration. 0.1mg of standard terpenes tablets was weighted and extracted with 5ml of methanol in separating funnels. 1.0ml of this standard extract was measured into a test tube and treated as the above sample and the colour was allow to develop before Ultraviolet (UV) spectrophotometer measurement at a wavelength of 520nm and the result was recorded.

$$\text{Calculation for \% Terpenoid} = \frac{Abs\ sample \times std\ con\ c \times 100\%}{Abs\ std}$$

Tannin Determination

1.0g of the grinder power of mistletoe leaves and flower at room temperature were weight into four different test tubes and dispensed in 10ml of distilled water and agitated and were left to stand for 30 minutes before centrifuged to obtain supernatant in 5 minutes. The extract obtained 2.5ml of the sample were displaced into 5ml volumetric flask with stoppers. The 5th 50ml flask was fixed with 0.1 of standard tannic acid solution was prepared.

1.0ml of Folin-Denis reagent was measured into each of the five flask containing four samples extract and one Tannin acid extract of the standard followed by 2.5ml of saturated sodium carbonate (Na_2C0_3) solution was diluted to the mark of 50ml flask with distilled water and incubated for 90 minute at room temperature. The absorbance was measured at 700nm spectrophotometer Jenway model 6405 Uv-Vis spectrophotometer, the reagent blank was measured at zero. The result of the sample and blank were recorded.

$$\text{Calculation for \% Tannin} = \frac{absobance\ of\ sample \times stabdard\ concentration \times 100\%}{Absobance\ of\ standard}$$

Phenol Determination

0.1mg of phenol was extracted with methanol in separating funnel. 1.0ml of the extract phenol standard 10% ferric measuring in Ultraviolet (UV) spectrophotometer before the wave length 560nm and result is recorded.

$$\text{Calculation for \% Phenols} = \frac{Absorbance\ sample \times Standard\ Concentration \times 100\%}{Standard}$$

Alkaloids Determination

1.0ml of the sample extract was measures into a test tube, 5.0ml of 2% HCl was added to the test tube on steam bath for 10mins and filtered with the aid of what man filter paper, 1.0ml of the filtrates was treated by adding 5 drops of Wegner's reagent and shake, a reddish brown coloration was observed to be the precipitate for alkaloid. The sample was measured with the Ultraviolet (UV) spectrophotometer with wave length of 520nm for the percentage content and comparing with standard alkaloid (table) 0.0lg with standard and the result was recorded.

Calculation for % Alkaloid =

$$\frac{Absorbance\ sample \times standard\ Concentration \times 100\%}{Absorbance\ STancarde}$$

Proximate Analysis of Essential Oil of Leaves and Flower of Mistletoe Plant

The fresh leaves and flower of mistletoe were separated and some were used for moisture content while the others were dried and grinded into powder and stored in a container for other analysis. The method adopted for the study was the gravimetric method of the Association of Official Analytical Chemist (A.O.A.C, 2005) [1]. The moisture, crude fibre, crude protein, ash, crude fat and carbohydrate of the samples were determined using standard methods of the Association of Official Analytical Chemists (AOAC, 2000) [1]. All determinations were done in triplicates. The proximate values were reported in percentage. Determination of moisture content was done by weighing the sample in crucible and drying in oven at 105 0C, until a constant weight was obtained, determination of ash content was done by ashing at 550 0C for about 3 hours. The kjeldah method was used to determine the protein content by multiplication of the nitrogen value with a conversion factor of 6.25. The crude fibre content of the samples was determined by digestion method and the crude fat was done by Soxhlet extraction method. Total Carbohydrate content was estimated based on the net difference between the other nutrients and the total percentage composition (100%).

Results and Discussions

Results

Table 1: Phytochemical screening of leaf and flower extracts of mistletoe plant

Phytochemicals	Leaves	Flowers
Tannins	++	+
Alkaloids	+	++++
Phenols	+++	+++
Flavonoids	++	+
Terpenoid	ND	ND
Saponins	++	ND

Note: ND-not detected**,** Heavily present: +++; slightly present: ++; present: +

Table 2: Mean Quantification of Phytochemicals of leaves and flower oil extracts of Mistletoe Plant (mg/100g)

Phytochemicals	Leaves mg/100g	Flowers mg/100g
Tannins	$2.0^{1} \pm 0.06$	0.84 ± 0.09
Alkaloids	0.99 ± 0.33	4.47 ± 0.14
Phenols	3.22 ± 0.7	3.46 ± 0.24
Flavonoids	2.66 ± 0.17	1.71 ± 0.08
Terpenoid	0.00 ± 0.00	0.00 ± 0.00
Saponins	2.35 ± 0.17	0.00 ± 0.00

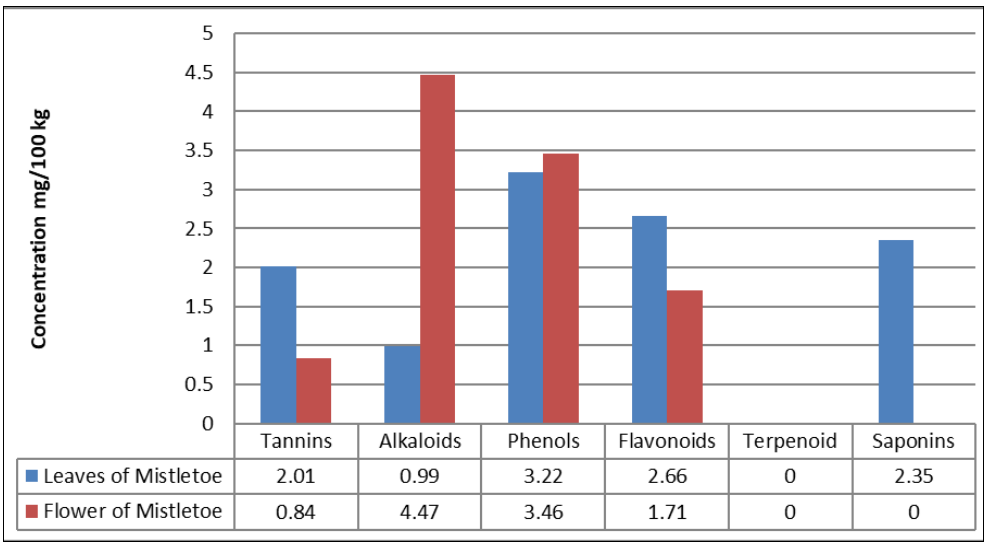

Fig 1: Mean quantification of phytochemicals in mistletoe leaf & flower

Table 3: Proximate analysis of leaves and flowers

Parameter s	Leaves %	Flowers %
Moisture content	29.04 ± 0.24	32.70 ± 0.24
Fiber content	10.05 ± 0.08	8.26 ± 0.14
Fat content	5.84 ± 0.08	5.00 ± 0.05
Protein content	2.09 ± 0.34	0.37 ± 0.17
Ash content	11.49 ± 0.08	10.19 ± 0.15
Carbohydrate	41.27 ± 0.00	43.35 ± 0.01

Note: Result is mean of triplicate of samples

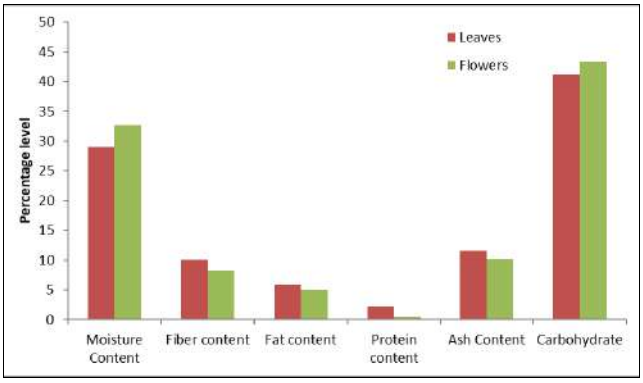

Fig 2: Proximate compositions of mistletoe leaf & flower

Discussion

The result of the qualitative analysis of mistletoe leaves and flower are presented in table 1. mistletoe leave and flower was found to contain moderate concentrations tannin, flavonoid and saponins; alkaloid had low concentration and phenol was highly concentration while Terpenoid was absent in the flower extract. Saponins were present in leaves they were absent in flowers. The quantitative analysis result of mistletoe leaves and flower is reported in table 2. It shows that mistletoe leaves contains phenols (3.22 ± 0.07 mg/100 g), flavonoids (2.66 ± 0.17 mg/100 g), saponins (2.35 ± 0.17mg/100g), Tannin (2.01 ± 0.06 mg/100g), Alkaloid (0.93 ± 0.33 mg/100 g) and Terpenoid (nil).The result of the alkaloid content is in within the range (Taiga, 2000). The high phenol profile can be exploited for antiseptic use. The flower was found to contain alkaloids (4.47 ± 0.14 mg/100 g), phenol (3.46 ± 0.24 mg/100g) flavonoid (4.47 ± 0.08mg/100g), tannin (0.84 ± 0.09) saponins and Terpenoid were nil.

Tannins, alkaloids, flavonoids, phenols and sponnins were found to be present in the leaf and flower extracts of mistletoe plant extracts. The presence of tannin in the medicinal plant suggests the ability of these plants to play key roles as antifungal antidiarrheal, antioxidant and antihemorrhoidal agent (Asquith and Butter, 1986) [2]. Alkaloids have been found to have microbiocidal effect and the major anti-diarrheal effect is probably due to their effects on small intestine and antihypertensive antifungal, anti-inflammatory, antifibrogenic effect (Ghosal *et al.*, 1996) [5]. Alkaloids in plants are used in medicine as an aesthetic agents (Herourat *et al*., 1998) [6]. The result revealed the presence of flavonoid in the extracts and hence, mistletoe plant can be use to modifies the body's reaction to allergens, virus and caranogens. It has been reported to show anti-inflammatory, antifungi, antibacterial and antimicrobial activities based on the literature (Cushnie and Lamb 2005) [3].

The presence of saponins in the seeds can be useful in treating inflammation. Some of the characteristics of saponins include formation of foams in aqueous solutions, haemolytic activity, cholesterol binding properties and bitterness (Rita *et al*., 2015) [12]. Also in nature, saponins appear to act as antibiotics that protect plants from microbes (Opara *et al.*, 2019) [11]. Phenols are present in the extracts of mistletoe plant thus can normally be involved in defense against ultraviolet radiation or aggression by pathogens, parasites and predators, as well as causative to plants colours. They are ubiquitous in all plant organs and are therefore an integral part of the human diet (Dai and and Mumper 2010) [4]. Also, phenolic compounds, can inhibit the absorption of amylase in the treatment of carbohydrate absorption, such as diabetes (Sales *et al*., 2012) [13].

The results of the proximate compositions of mistletoe leaf & flower are presented in table 3 indicates that moisture, crude fibre, fat, protein, ash, carbohydrate and energy content are 29. 4% 10.05%, 5. 84%, 2, 09% 11.49%, 41.27%, and 227. 1kcal/100g for leaves and 32.70% 8.26%, 5.00%, 0.37%, 10.19%, 43.35% and 219.7 kcal /100g for flower respectively. The leaf is rich in carbohydrate, fats, fiber, ash, with low protein content. It also has a high energy value the moisture content was high (29.04%) the high moisture content of the fresh leaves show that it is very nutritious and useful for medicinal and industrial purposes. The fat content was 5.84% for leaf and 5.00% for flower, this foods have been recommended to avoid problem of obesity (Lintas, 1992). Crude fiber contents in the leaf is (10.05%) and (8.26%) for flower. Crude fiber as reported by Ishida.*et al*., 2000) [7] helps in lowering the level of serum cholesterol, lower breast cancer risks as well as risks of coronary heart diseases.

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