International Journal of Advanced Research in Chemical Science (IJARCS)
Volume 5, Issue 7, 2019, PP 7-11
ISSN No. (Online) 2349-0403
DOI: http://dx.doi.org/10.20431/2349-0403.0507002
www.arcjournals.org

Phytochemical Analysis of Methanolic Extract of Mistletoe Leaf

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Abstract: *The study was carried out for analysis of qualitative and quantitative analysis of phytochemicals and nutritive value of essential oils in mistletoe leaves harvested from Cross River University of Technology Calabar staff quarters. The result of the quantitative analysis shows that tannins 2.01± 0.66mg/100g, alkaloid 0.93 ± 0.33mg/100g, phenol 3.22 ± 0.70mg/100g, flavonoid 2.66 ± 0.17mg/100mg, saponin 2.35 ± 0.17mg/100g. While saponin was only present in the leaves.*

1. INTRODUCTION

Mistletoe plant is a hemi-parasitic plant that grows on trees such as cocoa, mango, guava, kola nut trees and many more is known scientifically for its nutritive content such as carbohydrate, protein, fat, fiber, energy value, and ash. This nutritive content contributes remarkably in animals and human health. 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). The aim of this research was to determine the phytochemical constituents

2. MATERIALS AND METHODS

2.1. Collection and Preparation

The mistletoe samples used were collected from Cross River University of Technology Staff Quarters from a cocoa tree. The plant material was identified by the department of biological sciences of Cross River University of Technology (CRUTECH). The leaves were air dried under shade for two weeks. After drying, the samples were crushed to powder form using mortar and pestle. The sample in powdered form was stored in bottle till require for analysis.

2.2. Extraction of Sample for Phytochemical Analysis

15 g of the sample 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 essence of adding the anti-bumping granules is to aid direct the vapour of the methanol and avoid cracking or breaking of the round bottom flask containing the methanol due to heat. The soxhlet extractor is connected 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 form 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

2.3. Qualitative Analysis of Phytochemicals

Qualitative analysis of the crude extracts was carried out as described previously (Brain and Turner, 1975; Sofowora, 1993; Edeoga *et al.;* 2005; Trease and Evan, 2000; Harbone 1973; Osuagwu*et al* 2007) to identify the presence of secondary metabolites. The detail procedures involved in the phytochemical screening are as described by Ushie and Adamu (2010)

2.4. Test for Alkaloids

1.oml of the methanolic sample extract was measured into a test tube, 5.0ml of 2% HCl was of the filtrate was treated by adding 5 drops of Wagner's reagent and shake. A reddish brown colouration added and placed on a steam bath for 10mins. It was filtered with the aid of whatman filter paper. 1.0ml was observed, indicating the presence of alkaloids.

2.5. Test for Saponins

1.0ml of the methanolic sample extract was boiled with 5.0ml of distilled water in test tube for 5minutes in water bath. It was decanted while still hot. The filtrate was used for the following test;

2.6. Frothing Test

1.0ml of the filtrate was diluted with 4.0ml of distilled water and shaken vigorously for stable froth on standing. The stable froth was observed for 2minutes indicating the presence of saponins.

2.7. Test for Flavonoids

1.0ml of the methanolic sample extract was measured into a test tube, 1.0ml of 10% lead acetate was added and shaken for 30seconds and kept to stand. Formation of yellow precipitate was taken as a positive result for flavonoid.

2.8. Test for Tannins

1ml of methanolic sample extract was measured into a test tube and 1ml of 5% bromine water was added and shaken. The formation of greenish to red precipitate was recorded as evidence for the presence of tannin.

2.9. Test for Terpenoid

5ml of the methanolic sample extract was measured into a test tube, 2ml of chloroform was added, and 2ml of concentrated H_2SO_4 was added carefully by the side of the test tube to form a layer. No reddish brown colouration at the interface was formed, indicating absence of Terpenoid.

2.10. Test for Phenol

1ml of the methanolic sample extract each was measure into a test tube, 1ml of 10% ferric chloride was added and shaken. The formation of a greenish brown colouration was taken as evidence for the phenolic.

2.11. Quantitative Analysis of Phytochemical

Quantitative determination of the detected secondary metabolites was carried out to know their percentages in the *S. macrophylla*leaves by the methods described by Pavia*et al*., 2006, Iqbal, *et al.* (2011), Mudasir, (2012) , Sathya (2013) and Ushie*et al.*, 2018 with modification.

2.12. Quantitative Analysis of Alkaloids

0.5g of the leaves sample was dissolved in 96% ethanol, 20% tetraoxosulphate (vi) acid (1:1) 1ml of the filtrate was added to 5ml of 60% tetraoxosulphate (vi) acid and allowed to stand for 5mins. Then 5ml of 0.5% formaldehyde was added and allowed to stand for 3hrs. The reading was taken at absorbance of 565nm.

Calculation for % Alkaloid $= \frac{Absorbance\ \ sample\ \times standard\ \ Concentration\ \ \times 100\%}{Absorbance\ \ STancarde}$

2.13. Quantitative Analysis of Flavonoids

The determination of flavonoids on the leaves sample was done by acid hydrolysis of spectrophotometric method. 0.5g of the processed leaves sample was mixed with 5ml of dilute hydrochloric acid and boiled for 30mins. The boiled extract was allowed to cool and filter. 1ml of the

filtrate was added to 5ml of ethylacetate and 5ml of 1% ammonium. This was then scanned from 420nm and 520nm for the absorbance.

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

2.14. Quantitative Analysis of Phenols

The quantity of phenol is determined using spectrophotometric method. The leaves sample is boiled with 50ml diethyl ether or petroleum spirit. 5ml of the boiled sample is then pipette into 50ml flask and 10ml of distilled water is added. After the addition of the distilled water, 2ml of ammonium hydroxide solution and 5ml of concentrated pentanol is added to the mixture. The leaves sample was made up to mark and left for 30mins to react for colour development and measure at 505nm wavelength using a spectrophotometric method.

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

2.15. Quantitative Analysis of Tannins

The quantity of tannins isdetermined by using spectrophotometric method. 0.5g of the leaves sample is weighed into plastic bottle; 50ml of distilled water is added and stirred for 1hr. The sample is filtered into a 50ml flask and made up to mark. 5ml of the filtered sample is then pipette out into test tube and mixed with 2ml of 0.1M HCl and 0.008M $K_4Fe(CN)_6.3H_2O$. The absorbance is measured with a spectrophotometer at 395nm wavelength within 10mins.

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

2.16. Quantitative Analysis of Saponins

5.0g of dried sample was 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, 100 ml of methanol was used for the extraction for 3 hours to obtain the lipid and the pigment content from the sample first. After the extraction the methanol was distilled off leaving the Saponins after the evaporation the flask and the container was reweigh. The difference between the final and the initial weight of the flask represent the weight of the saponins in the sample.

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

2.17. Quantitative Analysis of Terpenoid

1.0ml of methanolic extract of sample 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 Uv-Vis spectrophotometer measurement at a wavelength of 520 nm andthe result was recorded.

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

3. Result and Discussion

3.1. Result

Table1. *Result of Qualitative Analysis of Leaves.*

Phytochemical	Leaves
Tannins	++
Alkaloids	+
Phenols	+++
Flavonoids	++
Terpenoid	ND
Saponins	++

Note: ND-not detected

Table2. *Result of Quantitative Analysis of Leaves*

Phytochemical	Leaves mg/100g
Tannins	2.01 ± 0.06
Alkaloids	0.99 ± 0.33
Phenols	3.22 ±0.7
Flavonoids	2.66 ± 0.17
Terpenoid	0.00 ± 0.00
Saponins	2.35 ±0.17

Note: Result is mean of triplicate of samples

3.2. Discussion

The result of the qualitative phytochemical analysis of mistletoe leaves are presented in table 1. The result revealed that all the phytochemicals tested for were detected in all the samples except terpenoid.The quantitative analysis result of mistletoe leaves is reported in table 2. It shows that mistletoe leaves contains phenols (3.22 ± 0.07mg/100g), flavonoids (2.66 ± 0.17mg/100g), saponins (2.35 ± 0.17mg/100g), tannin (2.01 ±0.06mg/100g), alkaloid (0.93 ± 0.33mg/100g) and terpenoid (nil). Mistletoe leaves was found to contain moderate concentrations tannin, flavonoid and saponins.

The mistletoe leaf are known to carry out important medicinal roles in human body as a result of the presence of flavonoids in the hexane, chloroform and ethyl acetate extracts from the stem bark. Flavonoids have inherent ability to modify the body's reaction to allergens viruses and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial, antioxidants, Salah *et al*.; 1995 Okwu 2004 (Cushnie and Lamb 2005, Salah *et al*.; 1995 Okwu 2004).The stem bark of mistletoe leafis a very useful medicinal plant because saponins were detected. There is tremendous, commercially driven promotion of saponins as dietary supplements and nutriceuticals. It can be emphasized that mistletoe leafhas a medicinal value since it contain saponins which is useful in medicine and pharmaceutical due to its foaming ability that produce frothy effects in the food industry (George 1965). The presence of terpernoids was detected in the mistletoe leaf and hence can be used in herbal medicines (Edeoga*et al.;* 2005).

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Citation: *M. Shibdawa, et.al, "Physiochemical Analysis of Local (Fulani) Yoghurt Syrup Sold in Bauchi Metropolis". International A., Journal of Advanced Research in Chemical Science (IJARCS), 5(7), pp. 7-11, DOI: http://dx.doi.org/10.20431/2349-0403.0507002*