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Qualitative and Quantitave Evaluation of the Phytochemicals in Dry, Wet and Oil Extracts of the Leaf of Morinda lucida

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Abstract
This study was carried out to assess the quality and quantity of phytochemicals present in the dry, wet and oil of the leaves of *Morinda lucida* using standard methods. The phytochemicals detected were flavonoids, cardiac glycoside, tannins, saponin, terpenoids and steroids. The quantitative analyses showed that oil extract had the highest total phenolics content (TPC: 2.11 mg GEA/g), total flavonoids content (TFC: 10.21 mg QE/g), total saponins content (TSC: 14.95 mg DE/100g) and Total tannins content (TTC: 1.09 mg GAE/g) followed by dry leaf extract (TPC: 1.34 mg GEA/g; TFC: 5.18 mg QE/g; TSC: 10.24 mg DE/100g; and TTC: 0.55 mg GAE/g) and the least was recorded for the wet leaf extract (TPC: 0.45 mg GEA/g; TFC: 2.61 mg QE/g; TSC: 7.19 mg DE/100g; and TTC: 0.37 mg GAE/g). The overall results showed that the oil extract of a *Morinda lucida* leaf is rich in phytochemicals than dry and wet leaf extracts and any analyses should be done using the oil.
Keywords: Phytochemicals, leaf, *Morinda lucida,* dry, wet, oil

1. Introduction

Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmacological. The medicinal power of these plants lies in phytochemical constituents that cause definite pharmacological actions on the human body (Akinmoladun *et al.*, 2007). Phytochemical, natural compound occur in plants such as medicinal plants, vegetables and fruits that work with nutrients and fibers to act against diseases or more specifically to protect against diseases.

Morinda lucida known as Oruwo in the South-Western part of Nigeria is a medium sized tree with a crooked hole and rather short twisted branches. It belongs to the family Rubiaceae. It has a rough bark, grey in colour, flaking off in irregular patches. Its leaves are about 7 to 15 cm long by 3.5 to 7.5 cm broad, and flowers are white with a narrow glabrous corolla-tube about 2.5 cm. Stem bark, roots and leaves infusion is used as an antimalarial, antidiabetic, jaundice and dysentery treatment (Burkill, 1997). M. lucida extracts have been reported to have antioxidant and reducing activities (Ogunlana *et al.*, 2008), and anti-microbial activity (Ogundare and Onifade, 2009; Adomi, 2006, 2008). This research work aims at identifying the phytochemicals present in *Morinda lucida* and ascertain the quantities of certain chemical constituents present in oil, dry and wet extracts of the leaves of *Morinda lucida* plant which may account for their use in traditional medicine.

2. Material and Methods

Chemicals

Ethanol, distill water, aqueous HCl, methanol, chloroform, concentrated sulphuric acid, Ammonia solution, picric acid, Hexane.

2.1 Sample Collection

The leaves of *Morinda lucida* (oruwo) were collected from the local suppliers in Akure, Ondo state (Nigeria). The leaves were used for the purpose of phytochemical analysis. The plants collected were identified botanically in School of Agriculture, Federal University of Technology, akure. Fresh and tender leaves of selected plants were used for phytochemical analysis.

2.2 Preparation of plant extract

The leaves of *Morinda lucida* (oruwo) were removed from the plant and then washed under running tap water to remove dust.

Fresh leaves extract

The leaves were crushed, 5 % HCl was added and the concentrated aliquot extracts obtained were used for phytochemical analysis.

Dry leaves extract

The leaves were air dried for few days and crushed into powder and stored in air-tight stopped glassware for use. The plant powder was taken in a test tube and distilled water was added to it such that plant powder soaked in it and shaken well. The solution was then filtered with the help of filter paper and filtered extract samples were taken and used for further phytochemical analysis.

2.3 Extration of oil from *Morinda lucida* (oruwo) leaf

10 g portion of the milled sample was soaked in 100ml of n hexane and reflux at 60°C 3 times. The evaporation of the n hexane was performed using a rotary evaporator on a water bath for 1 h leaving the oil. The oil were concentrated and stored in air tight bottles until used.

2.4 Qualitative phytochemical analysis of *Morinda lucida* (oruwo) leaves and Oil

Chemical tests were done on the wet and dry leaves and oil obtained from *Morinda lucida* (oruwo) using standard procedures to identify the constituents as described by Sofowora (1993).

Test for alkaloids

For the purpose of phytochemical analysis of wet and dry leaves and oil obtained from *Morinda lucida* (oruwo), 5 ml of the extract was added to 5mL of aqueous HCl (1%) in a steam bath. The solution was filtered and the filtrate was treated with a few drops of Dragendorff's reagent. Turbidity or precipitate showed the presence of alkaloids (Trease and Evans, 1978).

Test for saponins

1 ml of the extract was shaken with 5mL of water in a test tube and warmed. Frothing indicated the presence of saponins (Trease and Evans, 1978).

Test for steroids

2ml of acetic anhydride was added to 0.5ml of each plant extract with 2ml H_2SO_4. The colour change from violet to blue or green indicates the presence of steroids (Edeoga, 2005).

Test for tannins

5 ml of the extract was stirred with 10mL of distilled water. The mixture was filtered and the filtrate treated with ferric chloride. A blue-green – black-green precipitate indicated the presence of tannins (Trease and Evans, 1978).

Test for cardiac glycosides

Keller – Killiani test

5 mL of the extracts was treated with 2mL of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1mL of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for flavonoids

For the confirmation of flavonoid in the wet and dry and oil obtained from the leaves of sour-sop, 0.5 g of each extract were added in a test tube and 10 ml of distill water, 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of 1 ml concentrated H_2S0_4. Indication of yellow color shows the presence of flavonoid in each extract (Sofowora, 1993).

Test for terpenoids

An amount of 0.8 g of the dry, wet and oil extracts were taken in test tubes, then poured 10 ml of methanol, shake well and filtered to obtain 5 ml extracts of each sample. Then 2 ml of chloroform were mixed with each extract of the sample and 3 ml of sulphuric acid were added to the samples extract. Formation of reddish brown color indicates the presence of terpenoids in the samples (Edeoga, 2005).

Test for phlobatannins

Plant wet, dry and oil extracts were separately mixed with distill water in test tubes, then shaken well, and filtered to take plant extract. Then to each extract, 1% aqueous hydrochloric acid was added and each sample was then boiled with the help of Hot plate stirrer. Formation of red colored precipitate confirmed a positive result.

2.5 Quantitative phytochemical analysis

The phytochemicals which are present in the wet crushed leaves and aqeous extracts of dry powdered leaves and the oil extracts of sour-sop were determined and quantified by standard procedures.

Determination of total phenolics

100 mg of the extract of the sample was weighed accurately and dissolved in 100 ml of distilled water. 1.5 ml of this solution was transferred to a test tube, then 1 ml 2N of the Folin-Ciocalteu reagent and 2 ml 20% of Na_2CO_3 solution was added and ultimately the volume was made up to 8 ml with distilled water followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid (Hageman *et al.,* 2000).

Determination of total flavonoids

The total flavonoid content of the extract was determined using a slightly modified method reported by Meda *et al.* (2005). 0.5mL of the extract samples were mixed with 0.5mL methanol, 50µl of 10% $AlCl_3$, 50µl of 1mol L^{-1} potassium acetate and 1.4mL water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of each reaction mixture was subsequently measured at 415 nm. The total flavonoid was calculated

using quercetin as standard by making use of a seven point standard curve (0-100 µg/mL). The total flavonoids content of samples was determined in triplicates and the result was expressed as mg quercetin equivalent per gram of the sample.

Determination of total tannins

500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipette out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferro-cyanide. The absorbance was measured at 120 nm within 10 min (Van-Burden and Robinson, 1981).

Determination of total saponins

Estimated of total saponins content was determined by the method described by Makkar *et al.* based on vanillin-sulphuric acid colorimetric reaction with some modifications (Makkar *et al.,* 2007). About 50 µL of the extract was added with 250 µL of vanillin reagent (800 mg of vanillin in 10 mL of 99.5 % ethanol) was added. Then 2.5 mL of 72% sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60 ^{o}C for 10 min. After 10 min, it was cooled in ice cold water and the absorbance was read at 544 nm. The values were expressed as diosgenin equivalents (mg DE/g extract) derived from a standard curve.

3. Results

3.1 Qualitative phytochemical analysis

These preliminary study showed that the aqueous extracts of dry, wet and oil of the leaves of *Morinda lucida* (oruwo) contained saponins, steroids, flavonoids, tannins, terpenoids and cardiac glycosides. However, steroids were present only in the oil extract. Flavonoids, saponins, terpenoids, tannins and cardiac glycosides were present in all the samples. Alkaloids and phylobatanins were absent in all the tested extracts (Table 1).

3.2 Quantitative phytochemical analysis

Total phenolics content

Total phenolics content (TPC) of oil, dry and wet leaves extracts of sour-sop plant showed that the oil extract had the highest TPC (2.11 mg GEA/g) followed by dry leaf extract (1.34 mg GEA/g) and the wet leaf had the least TPC value (0.45 mg GEA/g) (Table 2).

Total flavonoid content

The total flavonoids content was highest in oil extract (10.21 mg QE/g) followed by dry leaf extract (5.18 mg QE/g) and the wet leaf had the least content (2.61 mg QE/g) (Table 2).

Estimation of tannins content

The tannins content of the extracts was determined and found to be 1.09 mg GAE/g in oil extract, 0.55 mg GAE/g in dry leaf extract and 0.37 mg GAE/g in wet extract (Table 2).

Estimation of saponins content

The total saponins content of the samples were found to be highest in oil extract (14.95 mg DE/100g) followed by dry leaf extract (10.24 mg DE/100g) and the least value was seen in wet extract (7.19 mg DE/100g) (Table 2).

4. Discussion

Secondary metabolite studies of the dry, wet and oil leaves extracts of *Morinda lucida* medicinal plants have shown that the presence of saponins, terpenoids, tannins, steroids, flavonoids, cardiac glycosides, and phenol which are of great importance in the field of drug research. These classes' steroids, saponin, tannins, flavonoids are known to have activity against pathogens and therefore aid the antimicrobial activities of medicinal plants (Ghosh *et al*., 2010). This is similar to the reports of Adomi and Umukoro (2010) who reported presence of saponins, cardeolides and alkaloids but absence of tannins. Differences such as this may be attributed to, the chronological age of the plant, percentage humidity of the harvested material, situation and time of harvest, and whether the method of extraction was a possible source of variation for the chemical composition, toxicity and bioactivity of the extracts (Felix, 1982).

Quantitative tests showed the abundance of flavonoids, terpenoids, and cardiac glycosides in the oil extracts, followed by dry leaf extract while the wet extract had the least value. Steroids were found in plenty only in the oil extract while saponins were in abundance in all the extracts.

5. Conclusion

This research showed that oil extract from *Morinda lucida* leaves are rich in phytochemicals such as flavonoids, phenolics, tannins, saponins, steroids and cardiac glycosides at higher concentration than dry and wet leaves extracts and making use of the oil extract of the leaves should be strongly recommended for good health

Table 1: Phytochemical analysis of Oil, Dry and Wet leaves of *Morinda lucida* (oruwo)

Compounds	*Morinda lucida* Oil	*Morinda lucida* Dry leaf	*Morinda lucida* Wet leaf
Alkaloids	--	--	--
Saponins	++	++	++
Steroids	++	--	--
Flavonoids	+++	+ +	+
Tannins	+	+	+
Terpenoids	++	+	+
Cardiac glycosides	++	+	+
Phylobatanins	--	--	--

+++ = highly present, ++ = moderately present, + = present, -- = absent

Table 2: Quantitative analysis of phytochemicals (mg/g) of Oil, dry and wet leaves of *Morinda lucida* (oruwo)

Compounds	*Morinda lucida* Oil	*Morinda lucida* Dry leaf	*Morinda lucida* Wet leaf
[1]Flavonoids	10.21 ± 2.12^{b}	5.18 ± 0.21^{c}	2.61 ± 1.55^{a}
[2]Phenolics	2.11 ± 0.42^{a}	1.34 ± 0.33^{b}	0.45 ± 0.25^{b}
[3]Tannins	1.09 ± 0.37^{a}	0.55 ± 0.32^{a}	0.37 ± 0.06^{c}
[4]Saponins	14.95 ± 2.08^{b}	10.24 ± 2.19^{a}	7.19 ± 1.11^{b}

Values were performed in triplicates and represented as mean±SD. [1]; mg QE/g extract, [2]; mg GAE/g extract, [3]; mg GAE/g, [4]; mg DE/g extract. Mean values followed by different superscript in a column are significantly different ($P<0.05$)

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