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PHYTOCHEMICAL INVESTIGATION, PROXIMATE AND TRACE ELEMENTS ANALYSIS OF THE LEAVES OF MANGO (*MANGIFERA INDICA*) GROWN AT TANNING AREA

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ABSTRACT

This study compared the phytochemicals, composition, and trace elements of Mangifera indica leaves with a control sample. Seven phytochemicals were found in the methanolic extract of Mangifera indica leaves, while eight were found in the control. Tannery effluent is an environmental pollutant that can be harmful to ecosystems. Alkaloids, saponins, tannins, phenols, and quinone were present, while oxalate, steroids and reducing sugar were absent for both Mangifera indica leaves methanolic extract and control sample. Glycoside and phlobatanins were present in Mangifera indica leaves methanolic extract while absent in the control sample. Anthraquinone, resin and flavonoid were absent in Mangifera indica leaves methanolic extract but present in the control sample. The percentage composition for proximate analysis of the Mangifera indica leaves for moisture content, ash content and crude fats were 7.3%, 6.4% and 6.48%, respectively. The control percentage composition for moisture content was 8.0%, ash content was 6.3%, and crude fats were 9.32%. The percentage composition of alkaloid, saponin and lipids in the Mangifera indica leaves was 10.01 %, 9.66% and 3.66%, respectively. The control percentage composition of phytochemicals was alkaloid, 11.1%., saponin, 9.88% and crude lipids, 4.22%. Copper detected in the Mangifera indica leaves was 0.04384 mg/l while 0.00525 mg/l for control. Lead was found in Mangifera indica leaves at 0.0011 mg/l but not in the control sample. Cadmium was not detected in either sample. Further investigation is needed on other parts of the plant.

KEYWORDS

Mangifera indica, investigation, phytochemicals, proximate, NILEST

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INTRODUCTION

Mangifera indica has been the focus of intense research in the search for various biomolecules from different parts of plants, such as stems, leaves, fruits and seed kernels (Jhameer *et al.*, 2018).

The leaves exhibit exceptional biological, medicinal and metabolic properties. Otherwise, they are considered as a waste material generated mainly through the pruning of mango plants; in reality, they are almost significant resource containing a wide variety of bioactive compounds (phenolic and essential oils), crude protein, dietary fibre, minerals, and vitamins (Kumar *et al.*, 2021). The various bioactive compounds present in *Mangifera indica* leaves include phenolic compounds, flavonoids, benzophenes, sesquiterpenes, saponins, xanthones, tannins, terpenoids, and alkaloids. *Mangifera indica* leaves help treat common diseases like diarrhoea and chronic ailments like diabetes and fatty liver disease. The leaves possess antiproliferative solid activity against pancreatic, breast, human colon carcinoma, and other types of hepatoprotective action. However, when these plants grow at contaminated sites, their chemical composition becomes affected (Saeed *et al.*, 2003).

The Tannery industry is a significant cause of pollution of the environment. Due to untreated effluent discharge, it has a solid potential to cause soil and water pollution, plants, vegetables, and terrestrial and atmospheric systems. Tanning is the chemical process that converts animal hides and skin into stable and imputrescible products called leather. The transformation of hides into leather is usually done using tanning agents, generating highly turbid, coloured and foul-smelling effluent. During the tanning process, there are three types of wastes: air pollutants, water pollutants, and solid pollutants, discharge discharged during various steps, namely, beam house operations, tan yard operations, post-tanning operations and finishing operations (Geremew & Tekalign, 2017). When exposed to tannery effluents and heavy metals, vegetables grown at contaminated sites could take up and accumulate metals at toxic concentrations. Wastewater from tanning industries has a fast interaction with the environment. Hence, there is a need for a phytochemical investigation and proximate and trace elements analysis of the mango (Mangifera indica) grown at the tanning area.

MATERIALS AND METHODS

Sample Collection and Preparation

The *Mangifera indica* leaf samples were collected at the Nigerian Institute of Leather and Science Technology (NILEST) Samaru Zaria Kaduna state, Nigeria, at a tanning area of 5 m away. *Mangifera indica* leaves used as control were collected 2 km from the study area. It was identified

and authenticated in the Herbarium section, Department of Botany, Ahmadu Bello University, Zaria, with the voucher number ABU01944.

Mangifera indica leaves for sample and control were washed thoroughly with distilled water to remove dust, soil particles, and other impurities. The leaves were air-dried for two weeks under shade to prevent ultraviolet rays from inactivating the chemical constituent (Das *et al*., 2010; Ncube *et al*., 2008) and later pulverized (ground into powder form) using a laboratory mill.

The leaves were subjected to exhaustive extraction using a soxhlet extractor. Then, 100g of it was weighed and mixed with 99% methanol, which was used in the extraction process. The crude extracts were packed in plastic bottles with proper labelling for phytochemical proximate composition and mineral determination.

Qualitative Determination of Phytochemicals

The extracts were analyzed for alkaloids, resins, tannins, saponins, flavonoids, glycosides, phenols, anthraquinones, cardiac glycosides, steroids, phlobatanins, oxalate and reducing sugars.

Test of alkaloid

The methanolic extract of the sample and control of each of 0.50 cm^3 were evaporated to dryness, and 2 % hydrochloric acid was added to the residue heated in a boiling water bath. After cooling, the mixture was filtered and treated with a few drops of Mayer's reagent. The samples were then observed for turbidity or yellow precipitation (Kumar *et al.*, 2014).

Test for glycoside

To 0.5cm^3 of the extract solution in glacial acetic acid, a few drops of ferric chloride and concentrated sulphuric acid were added. A reddish-brown colouration at the junction of two layers and a bluish-green colour in the upper layer indicated the presence of glycoside (Siddiqui & Ali, 1997).

Test for terpenoid and steroid

The plant extracts of 4.00 g were treated with 0.50 cm^3 of chloroform. A concentrated solution of sulphuric acid was added slowly to the extracts until a red-violet colour was observed for terpenoids and a bluish colour for steroids (Siddiqui & Ali, 1997)

Test for flavonoid

The plant extracts of the 4.00 cm^3 were treated with 1.50 cm^3 of 50 % methanol solution, which was warmed and metal magnesium added. 5-6 drops of concentrated hydrochloride acid were

added to the solutions to the solutions. The formation of red colour indicated the presence of flavonoids and orange colour, flavones (Kumar *et al.*, 2014).

Test of reducing sugar

To 0.50 cm^3 of the extracts each, 1.00 cm^3 of distilled water and 5-8 drops of fehlings solution were added, heated and observed for brick red precipitate (Siddiqui & Ali, 1997; Harbone, 2009).

Test for tannins

To 0.50 cm^3 of the extracts, 1.00 cm^3 of water and 1-2 drops of ferric chloride were added. Blue was observed for gallic tannins, and green and black were observed for catholic tannins.

Test for saponins

The solutions were each diluted with 20.00 cm^3 of distilled water and agitated in a graduated cylinder for 15 minutes. The formation of a 1.00 cm layer of foam indicated the presence of saponins. The frothing was mixed with three drops of olive oil and shaken vigorously. The presence of saponin resulted in the formation of an emulsion (Kumur *et al*., 2014).

Test for resins

To 5.00 cm^3 of the extracts, a solution of copper acetate was added; the resulting solution was then shaken vigorously and allowed to separate. A green-coloured solution indicates the presence of resin.

Test for anthraquinones

To 0.20 g of the extracts, 4.00 cm^3 of benzene was added and mixed vigorously. The mixtures were then filtered, and 2.00 cm^3 of 10 % ammonia solution was added to the filtrate.

The mixtures were shaken, and the presence of pink, red, or violet in the ammoniacal (lower) phase indicates the presence of free anthraquinones (Harbone, 2009).

Test for phenols

To 0.20 g of the extracts, ferric chloride was added. A green or dirty green precipitate indicates the presence of a phenolic compound (Kumar *et al*., 2014).

Test for phlorotannins

Distilled water was added to 0.50 g of the extracts and filtered. The filtrate was then boiled with a 2 % hydrochloric acid solution. The formation of red precipitate shows the presence of phlobatanins (Harbone, 2009).

Quantitative Determination of Phytochemicals

Determination of total alkaloids

The sample powdered extracts were weighed, and 5.00 g of each was transferred into a 250.00 cm^3beaker. Then 2000.00 cm^3 of 10 % acetic acid in ethanol was added, covered and allowed to stand for 4 hrs. It was filtered, and the extracts were concentrated to one-quarter of the original volume in a water bath. Concentrated ammonium hydroxide was added dropwise to the extracts until the precipitate was collected, washed with diluted ammonium hydroxide, and filtered. The alkaloid content residue was calculated, as presented in Equation 1 (Harbone, 2009).

Percentage of Alkaloid= $\frac{weight\ of\ Alkaloid}{weight\ of\ powder\ sample} \times 100$ ......………………………………… (1)

Determination of total saponins

The ground sample of 20.00 g was put into a conical flask, and 100.00 cm^3 of 20 % aqueous ethanol was added. The samples were heated over a hot water bath for 4 hours with continuous stirring at about 55^oC. The mixtures were then filtered, and the residue was re-extracted with another 200.00 cm^3 of 20 % ethanol. The combined extracts were reduced to 40.00 cm^3 in a water bath at a90. Each concentrate was then transferred to a 250.00 cm^3 separatory funnel, and 20.00 cm^3 of diethyl ether was added and shaken vigorously. The aqueous layer was recovered, while the ether layer was discarded.

The purification process was repeated, and 60.00 cm^3 of n-butanol was added. The combined n-butanol solution was heated in a water bath. After evaporation, the sample was dried in the oven to constant weight, after which the saponin content was calculated as presented in equation 2 (Obdoni & Ochuko, 2011)

Percentage of saponin = $\frac{weight\ of\ saponin}{Weight\ of\ sample} \times 100$...…………………………………………….….(2)

Determination of total lipid

The *Mangifera* leaves for sample and control of 100 g were each added into a thimble connected to a soxhlet extractor chamber with a preweighed flat bottom. Petroleum ether of 100 cm^3, enough to cause reflux, was added to the flask, and the lipid from the sample and control was extracted for 3 hours by heating on an electric hot plate at 50^oC. The extract and (petroleum ether) were distilled off, the lipid was removed by cooling the flask in a desiccator, and its value was calculated by reweighing the flask and content. The percentage of lipid content was thus calculated using (Chukwuma *et al.,* 2016).

Percentage Lipid = $\frac{weight\ of\ lipid}{weight\ of\ sample}\ x\ 100$ ……..……(3)

Digestion of sample and chemical analysis: The sample and control each of 0.5 g was digested with 10 cm^3 aqua regia (3:1 HCL: $HNO_{3)}$. After that, it was heated for 30 minutes. The samples and control were then cooled at room temperature and diluted to 50 cm^3 distilled water. Each dilution was filtered through Whatman no. 41 filter paper. The sample and control solutions for copper, lead, and cadmium were analyzed using an atomic absorption spectrophotometer (Riyadh, 2020).

Proximate Composition

Determination of moisture content

Ten millilitres of sample and control were each measured in a clean crucible using a sensitive balance. The crucible with the content was placed in an air-dry oven at 105 ^{o} C and left to stay overnight. This was transferred to the oven again and weighed after 2 hours. This was repeated until a constant weight was obtained.

Moisture content % = $\frac{(W2-W1)-(W2-W1)}{W2-W1}$ x100 ……..……..(4)

Where:

w_1 = weight of empty crucible

w_2 = weight of crucible + sample

w_3 = weight of crucible + dry sample

Determination of total ash

An empty crucible was accurately weighed, and then 10 ml of sample and control each were weighed using a sensitive balance. It was placed in a muffle furnace at 550 C for more than 3 hours until white to grey ash was obtained, then the crucible was removed from the furnace to a desiccator to cool and then weighed (AO AC, 2005).

Ash content % = $\frac{W2-W1}{W3}$ x 100 ……..……(5)

Where:

w_1 = weight of empty crucible

w_2 = weight of crucible with ash

w_3 = weight of sample

Determination of crude fat

A clean and dried thimble was weighed (w_1), and a 5 g oven-dried sample and control concentrate were added and re-weighted (w_2). A round bottom flask was filled with petroleum ether 40-60 C up to ¾ of the flask. The soxhlet extractor was fixed with a reflux condenser to adjust the heat source so that the solvent boiled gently; the sample and control were each put in the thimble and inserted into the soxhlet apparatus, and extraction under reflux was carried out with petroleum ether for 6 hours. After that, the barrel of the extractor was emptied, the condenser and the thimble were removed, taken into the event at 100^{o}C for 1 hour, and later cooled in the desiccator and weighed (AOAC, 2005),

Fat (%)= $\frac{W2-W1}{W3-W1}$ x100 .....(6)

RESULTS AND DISCUSSION

Table 1: Qualitative phytochemical screening of methanolic extract of *Mangifera indica*

Chemical Constituents	Methanolic extract (Sample)	Methanolic extract (Control)
Alkaloid	+	+
Saponins	+	+
Flavonoid	-	+
Glycoside	+	-
Tannin	+	+
Phenol	+	+
Oxalate	-	-
Steroids	-	-
Quinine	+	+
Resins	-	+
Anthraqunine	-	+
Phlobatanin	+	-
Reducing sugar	-	-

Key: + = Present - = Absent

The qualitative phytochemical screening on the leaves of *Mangifera indica* leaves, and the control sample revealed some differences in the phytochemical constituents of the tested methanolic extract. Seven of the thirteen phytochemicals screened were present for *the methanolic extract of Mangifera indica leaves*, while eight were for the control samples. In Table 1, Alkaloids, saponins,

tannins, phenols, and quinone were present, and oxalate, steroids and reducing sugar were absent for *Mangifera indica leaves* and control methanolic extract. Similarly, glycoside and phlobatanins were present in *Mangifera indica leaves* while absent in the control methanolic extract. Anthraquinone, resin and flavonoid were absent in the sample methanolic extract but present in the control methanolic extract. This may be due to some chemical reactions that occur during the growth and storage of the plants (Ketaren *et al.*, 2015).

Table 2: Proximate analysis of *Mangifera indica for sample and control*

Proximate	*Mangifera indica sample* (%)	*Mangifera indica control* (%)
Moisture content	7.30	8.00
Ash content	6.40	6.30
Crude fat	6.48	9.32

In Table 2, The percentage composition for proximate analysis of the *Mangifera indica leaves* for moisture content, ash content and crude fats were 7.3 %, 6.4 % and 6.48 %, respectively. The control percentage composition for moisture, ash, and crude fats were 8.0 %, 6.3 % and 9.32 % respectively. The *Mangifera indica leaves* moisture content is slightly lower than the control sample, possibly due to the absence of rainfall in the study area (Okerulu *et al*.,2017). The ash content for a sample is slightly higher than the control, which may be due to the presence of mineral elements due to the tannery effluent (Okerulu *et al*.,2017). The sample crude fat is much lower than the control, suggesting that the sample leaves contain low quantities of lipid molecules and cannot be the primary source of biomolecules essential for body metabolism (Okerulu *et al*.,2017).

Table 3: Quantitative phytochemical screening of *Mangifera indica for sample and control*

Chemical constituent	*Mangifera indica sample* (%)	*Mangifera indica control* (%)
Alkaloid	10.01	11.10
Saponin	9.66	9.88
Lipid	3.66	4.22

In Table 3, The percentage composition of alkaloid, saponin and lipids in the sample was 10.01 %, 9.66 % and 3.66 %, respectively. These values were slightly lower than the control percentage composition for alkaloids: 11.1 %., saponin, 9.88 % and crude lipids, 4.22 %. This is an indication of the effect of the effluent on the phytochemicals.

Table 4: Heavy metals analysis of *Mangifera indica for sample and control*

Heavy metals	*Mangifera indica sample* (mg/l)	*Mangifera indica control* (mg/l)
Copper	0.04384	0.0525
Lead	0.0011	Not detected
Cadmium	Not detected	Not detected

In Table 4, Copper detected in the *Mangifera leaves* and control samples were 0.04384 mg/l and 0.00525 mg/l, respectively. The Copper detected in the control sample is slightly lower than in the sample. Lead was detected in the sample at a negligible amount but absent in the control sample. However, cadmium was not detected in either sample or control.

CONCLUSION

The present investigation aimed to study the phytochemicals and carry out proximate and trace element analysis of the mango leaves (*Mangifera indica*) grown at the NILEST tanning area. Seven were detected for the thirteen phytochemicals screened for the sample, while eight were for the control. The proximate analysis revealed lower moisture content and crude fat for the sample than the control. However, the ash content was higher for the sample than for the control. The quantitative analysis revealed fewer alkaloids, saponin and lipids in the sample than control. Heavy metals analysis for the sample and control revealed the absence of cadmium in both samples. A negligible amount of lead was present in the sample while absent in the control. Copper in the sample and control was present in a negligible amount. Based on the results of the present study, the tanning area has little effect on the mango leaves. However, there is a need to investigate other parts, such as the root, bark and fruit.

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