Original Research Article

Quantitative Phytochemical andProximate analysis of *Mangifera indica* possible ability to induce sedation and remedy insomnia.

ABSTRACT

Aims: The study was aimed at the Phytochemical and Proximate analysis of the flower, leaf, stem bark and seed of *Mangifera indica* for its ability to induce sedation.
Place and Duration of Study: The fresh flower, leaf, stem bark and seed of *Mangifera indica* (Mango) were collected using secateurs and taken to the Department of Biological Sciences, Nigerian Defense Academy, Kaduna for specific identification and authentication by an expert.
Methodology: The phytochemical analysis was carried out following the procedures described by Harbourne, (1998) while the methods of the Association of Official Analytical Chemists (AOAC, 2000) were used for the determination of moisture and ash content, crude fiber, lipids, proteins and carbohydrates and Nitrogen was determined using the micro Kjeldahl method described by (Gadelha et al., 2009).
Results:A very significant percentage of protein, Flavonoid and terpenoids were detected in the plant samples especially *Mangifera indica* flower.
Conclusion: A high presence of Flavonoid, Terpenoid and Protein in*Mangifera indica*plant parts is a sign of the possibility that the plant sample could contain a reasonable percentage of Apeginin, Myrcene and Tryptophan which are known active components to induce sedation and can feature in the future as a good source of sleep aid against insomnia.

Keywords: Phytochemicals, Proximate, Sedatives, Mangifera indica Plant parts.

1. INTRODUCTION

In recent years the nervous (mainly stress and anxiety) and sleep disorders increased considerably, becoming prevalent diseases affecting a high percentage of the world population, some of which are sleep disorders are sleep apnea, leg cramps, narcolepsy, jet legs, restless legs syndrome, sleepwalking, insomnia [6]. Often times sleep related problems such as the difficulty to initiate and sustain sleep are treated with long-term use of Benzodiazepine sedatives such as triazolam, lorazepam, temazepam, flurazepam, and quazepam and non-benzodiazepine sedatives such as zolpidem, eszopiclone, and zaleplonthat can help induce sleep [7]. These drugs show limited benefits with obvious side-effects, such as impaired cognitive function, low memory and general daytime performance (Nilsson *et al.,* 2011) while its long-term administration can result in tolerance and dependence, severe side effects such as weight gain, anticholinergic side effects and diabetes [7]. Evidence-based options for treatment of sleep related disorders are limited. Scholarly articles have shown that pharmacological therapy is currently the most commonly used treatment for mood disorders. Although many drugs appear to have an important role in such cases there have been many complaints that the drugs are not effective for all patients and incur diverse adverse effects, as well as tolerance (if used for a long time). Therefore, it is desirable to seek fast acting, better-tolerated, more effective and fewer side effects antidepressants to help the vast majority of the world population known to be prone to sleep problems such as students, individuals with other health conditions and military veterans that have been exposed to different sleep patterns and psychological stress.

Over the years, various parts of *Mangifera indica* have been known to possess antioxidant, hypolipidemic, anti-inflammatory, analgesia mediated by opioid receptor affinity, sympathomimetic, endocrine, antimicrobial, antiparasitic, sedative, hypotensive, properties, soothing cure for rheumatism and toothache [2]. The previous use of these plants as sedatives in the treatment of insomnia[16], [20] and other related illnesses is a convincing factor that the plant might have the ability to induce sleep (sedation). Numerous phytochemical and proximate content of *Mangifera indica* reviewed by[8] in other locations do not just clarify its edibility but its therapeutic effects as well with little or no risk of addiction due long usage as observed in conventional drugs. The availability, accessibility and affordability of the plant is an important factor that will be of great advantage in their use to induce sleep thereby being a good source of combating insomnia.

2. MATERIAL AND METHODS

2.1 PLANT SAMPLE PREPARATION

The collected and identified flower, seed, leaf and stem of *M. indica* were carefully washed using tap water to remove the dusts and then dried in an oven at 28°C for 8hrs. The dried samples were then milled to fine powder separately using mortar and pestle. The powdered samples were transferred to a plastic sealed cans and kept at room temperature before the extraction process.

2.2 QUALITATIVE PHYTOCHEMICAL ANALYSIS

Basic phytochemical screening was carried out using simple chemical tests to detect the presence of secondary plant constituents such as alkaloids, tannins, flavonoids, saponins, and phenols in the sample. The methods used were those outlined by [12] except otherwise stated.

2.2.1. Test for Alkaloids

A known quantity of the extract (0.1 mg) was added to 6ml of dilute hydrochloric acid and boiled, allowed to cool and filtered. The filtrate was then divided into three portions and subjected to the following tests. To the first portion, 2 drops of Dragendorff's reagent was added - the formation of a red precipitate indicated the presence of alkaloids. To the second portion, 2 drops of Meyer's reagent was added - creamy white precipitate indicated the presence of alkaloids. To the third portion, 2 drops of Wagner's reagent was added - a reddish-brown precipitate indicated the presence of alkaloids.

2.2.2 Test for Terpenoid

The extract (1 ml) was added to 10 ml of deionised water and then treated with 3 drops of ferric chloride. A greenish-brown precipitate it displayed indicated the presence of tannins.

2.2.3 Test for Flavonoids

A known quantity of the extract (0.2mg) was boiled in ethyl acetate (10 ml) for 3 minutes, filtered and cooled. Then the filtrate (4 ml) was shaken with 1ml of dilute ammonia solution. An intense yellow coloration indicated the presence of flavonoids.

2.2.4 Test for Saponins

A 5.0ml aliquot of the extract was diluted with 20ml of deionized water, shaken vigorously and observed. Persistent foaming indicated the presence of saponins.

2.2.5 Test for Phenols

The test sample 0.1 g was added to 10 ml of distilled water. The solution was heated in a boiling water bath for 3 min and filtered. A 2 ml aliquot of the filtrate was placed in 3 test tubes. The filtrate in one of the

test tubes was diluted with distilled water in the ratio 1:4. A blue/greenish color indicated the presence of phenols.

2.2.6 Test for Tannins

A0.5g of powdered sample of each plant was boiled in 20ml of distilled water in a test tube and filtered 0.1% $FeCl_3$ is added to the filtered samples and observed for brownish green or a blue-black coloration which shows the presence of tannins.

2.2.7 Test for Glycosides

One (1) ml of concentrated H_2SO_4 is prepared in test tube 5 ml of aqueous extract from each plant sample is mixed with 2ml of glacial CH_3CO_2H containing 1 drop of $FeCl_3$. The above mixture is carefully added to 1ml of concentrated H_2SO_4 so that the concentrated H_2SO_4 is underneath the mixture. If cardiac glycoside is present in the sample, a brown ring will appear indicating the presence of the cardiac glycoside constituent.

2.2.8 Test for Steroid

To 1ml of the plant extract, 2 ml of chloroform and 1 ml of Sulphuric acid (H_2SO_4) were added. Formation of reddish-brown ring at interface indicates the presence of steroids.

2.3 QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF PLANT SAMPLES

Phytochemical analysis of the organic extract was carried out according to the general method of [12].

2.3.1Determination of total Alkaloids

1 g of each plant sample was macerated with 20 ml of ethanol and 20% sulfuric acid (H_2SO_4) (1:1 v/v). 1 ml of the filtrate was added to 5 ml of 60% H_2SO_4. After 5 min, 5 ml of 0.5% formaldehyde in 60% H_2SO_4 were mixed with the mixture and allowed to stand for 3 hrs. The absorbance was then read at 565 nm using a spectrophotometer.

2.3.2 Determination of total Terpenoids

The plant sample (1 g) was macerated with 50 ml of ethanol and filtered. To the filtrate, 2.5 ml of 5% aqueous phosphomolybdic acid solution was added and 2.5 ml of concentrated H_2SO_4 and mixed. The mixture was left to stand for 30 min and then made 89/up to 12.5 ml with ethanol. The absorbance was taken at 700 nm.

2.3.3 Determination of total Flavonoids

The plant sample (1 g) was macerated with 20 ml of ethyl acetate for 5 min and filtered. To the filtrate, 5 ml of diluted ammonia was added and shaken for 5 min. The upper layer was collected and the absorbance read at 490 nm.

2.3.4 Determination of total Saponins

The plant sample (1g) was macerated with 10 ml of petroleum ether and decanted into a beaker. Another 10 ml of the petroleum ether was added into the beaker and the filtrate evaporated into dryness. The residue was then dissolved in 6 ml of ethanol. The solution (2 ml) was put into a test tube with 2 ml of chromogen solution added into it and left to stand for 30 min. The absorbance was read at 550 nm.

2.3.5 Determination of total Phenols

The plant sample (1 g) was macerated with 20 ml of 80% ethanol and filtered. The filtrate (5 ml) was then added to 0.5 ml of folin ciocalteu's reagent and allowed to stand for 30 min. Then 2 ml of 20% sodium carbonate was added and absorbance measured at 650 nm.

2.3.6 Determination of total Tannins

About 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 pipetted out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min (Van-Burden and Robinson, 1981).

2.3.7 Determination of total Glycoside

Eight (8)ml of plant extract was transferred to a 100ml volumetric flask and 60ml of H_20 and 8ml of 12.5% lead acetate were added, mixed and filtered. 50ml of the filtrate was transferred into another 100ml flask and 8ml of 47% Na_2HPO_4 were added to precipitate excess Pb^{2+} ion. This was mixed and completed to volume with water. The mixture was filtered twice through same filter paper to remove excess lead phosphate. 10ml of purified filtrate was transferred into clean Erlyn-Meyer flask and treated with 10ml Baljet reagent. A blank titration was carried out using 10ml distilled water and 10ml Baljet reagent. A blank titration was carried out using 10ml distilled water and 10ml Baljet reagent. This was allowed to stand for one hour for complete color development. The color intensity was measured colorimetrically at 495nm.

Calculation;

$$\% \text{ of total glycosides} = \frac{A \times 100}{77} g\%$$

$$where\ A = Absorbance$$

2.3.8 Determination of total Steroid

One (1) ml of test extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70±20 C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank.

2.4 PROXIMATE ANALYSIS OF PLANT SAMPLES

The methods of the Association of Official Analytical Chemists [3] was used for the determination of moisture and ash content, crude fiber, lipids, proteins and carbohydrates while Nitrogen was determined using the micro Kjeldahl method described by (Gadelha *et al.,* 2009).

2.4.1 Moisture content.

Sample was thoroughly mixed and 2g was weighed into a silica dish which has been previously ignited and weighed. Sample was allowed to oven (Genlab-210 UK) dry for 24hours at 100^0c to constant weight [3].

Calculation;

$$\%moisture = \frac{100 \times (wt.of\ dish\ + sample\ before\ drying) - wt.of dish\ + sample\ after\ drying}{wt.of sample\ taken}$$

2.4.2 Ash content

Samples was heated in a muffle furnace at 500^oC, until white/gray ash color was obtained. Weight of ash was noted down directly [3].

2.4.3 Crude fiber

This was measured by treating the samples with 1.25% sulfuric acid (H_2SO_4) 1.25% sodium hydroxide (NaOH) and 1% nitric acid (HNO_3), then filtered and washed with hot water after each step. The residue obtained was oven dried at 130^oC and ashed at 550^oC in the furnace. The loss in weight on ignition was expressed as content of crude fiber [3].

2.4.4 Lipids

This was extracted from the sample with petroleum ether (60-80^oC) in a Soxhlet apparatus for about 6-8hours. The residual solvent was evaporated in a pre-weighed beaker and an increase in weight of the beaker gave the total lipid of the sample [3]

2.4.5 Crude Protein content

Nitrogen in sample was converted to ammonium-nitrogen by digestion with sulphuric acid using catalyst. The ammonia liberated when this digestion was reacted with sodium hydroxide was removed by steam distillation and collected with 4% boric acid-indicator mixture. This is then titrated with HCl to give the estimated nitrogen using micro Kjeldahl (Markham 230 Foss USA). This was used to obtain the protein content of the samples by multiplying the evaluated nitrogen by 6.25 [3].

Calculation: $\%\ crude\ protein\ = \%nitrogen \times 6.25$

2.4.6 Carbohydrate

The value of carbohydrate was calculated by;

100 – (Percentage of ash + percentage of total lipid + percentage of protein + percentage of crude fiber)[3].

2.5 STATISTICAL ANALYSIS.

Data was collected, recorded and analyzed for interpretation using Microsoft Excel and Statistical Package for the Social Sciences (SPSS). All data were expressed as mean± SD. Probability of $P > 0.05$ were considered significant. Results were presented in tables, graphs, figures and pictures which formed the basis for discussion, conclusion//'n and recommendation.

3. RESULTS AND DISCUSSION

Table 1 Qualitative phytochemistry of the flower, leaf, stem and seed of *Mangifera indica* (mango).

Phytochemicals	M/flower	M/leaf	M/stem	M/seed
Tan.	+	+	+	+
Phb.	-	-	-	+
Sap.	+	+	+	-
Flav.	+	+	+	+
Terp.	-	-	-	-
Gly.	-	-	-	+
Phen.	+	+	+	+
Ster.	-	-	-	-
Alk.	+	+	+	+

Keys:
M=Mango
Tan.=Tanins
Phb.=Phlobotanins
Sap.=Saponins
Flav.=Flavonoids
Quin.=Quinone
Terp.=Terpenoids
Gly.=Glycosides
Phen.=Phenols
Ster.=Steroids
Alk.=Alkaloids
- = Absent
+ = Present

Table 2 Phytochemical composition of *Mangifera indica* (Mango) flower, leaf, stem and seed (% Mean ± S.D)

Phytochemicals	M/Flower	M/leaf	M/stem	M/seed

Tan.	5.24±0.3	0.3533 ± 0.02082[a]	1.9367 ± 0.02082[a]	1.1467 ± 0.03055[a]
Phb.	0.0000 ± 0.000	0.0000 ± 0.000[b]	0.0000 ± 0.000[b]	1.1400 ± 0.02000[a]
Sap.	1.15 ± 0.02	2.1400 ± 0.0200[a]	6.9400 ± 0.03000[a]	0.0000 ± 0.000[b]
Flav.	18.35±0.01	13.5333±0.2510 [a]	7.1400±0.03001 [a]	1.8733 ± 0.23629[a]
Quin.	0.0000 ± 0.000	0.0000 ± 0.000[b]	0.0000 ± 0.000[b]	0.7733 ± 0.06807[a]
Terp.	3.18 ± 0.09	0.0000 ± 0.000[b]	0.0000 ± 0.000[b]	0.0000 ± 0.000[b]
Gly.	5.33 ± 0.4	0.0000 ± 0.000[b]	0.0000 ± 0.000[b]	0.3133 ± 0.07572[a]
Phen.	11.69±0.9	0.1467±0.0378 [a]	1.0967±0.08963[a]	0.7267±0.59119 [a]
Ster.	3.28±0.01	0.0000 ± 0.000[b]	0.0000 ± 0.000[b]	0.0000 ± 0.000[b]
Alk.	0.2±0.1	1.1700±0.0002 [a]	6.3567±0.05033[a]	2.4567±0.03055[a]

Values indicated by superscript 'a' are significantly different down the group from the M/leaf group using one way anova (dunnett's test) p (<0.05)

Keys:

M=Mango
Tan.=Tanins
Phb.=Phlobotanins
Sap.=Saponins
Flav.=Flavonoids
Quin.= Quinone
Terp.=Terpenoids
Gly.=Glycosides
Phen.=Phenols
Ster.=Steroids
Alk.=Alkaloids
- = Absent

+ = Present

Table 3Proximate Composition of *Mangifera indica* (Mango) flower, leaf, stem bark and seed (%)

Proximate	**M/Flower**	**M/leaf**	**M/stem**	**M/seed**
Moist content	61.56±0.96	1.14±0.92[a]	1.30±0.22 [a]	1.67±0.34 [a]
Ash content	7.8±4.86	6.48±0.02 [a]	9.24±0.67 [a]	3.11±0.12 [a]
Crude protein	2.22±0.70	11.03±0.11 [a]	3.94±0.14 [a]	4.82±0.98 [a]
Crude Fibre	11.50±0.12	3.55±1.56 [a]	13.42±0.69 [a]	4.32±1.34 [a]
Crude lipid	5.21±0.17	2.85±0.24 [a]	1.63±0.34	5.01±0.57 [a]
Carbohydrate	11.72±1.41	74.95±0.55 [a]	70.47±0.56 [a]	81.07±±0.79 [a]
Nitrogen free Extract	23.22±1.33			

Values indicated by superscript 'a' are significantly different down the group from the M/leaf group using one way anova (dunnett's test) p (<0.05)

Keys:

M/leaf=Mango leaf
M/stem=Mango stem
M/seed=Mango seed

*Mangifera indica*have been counted as plants with great medicinal and phytochemical properties such as antioxidant, hypolipidemic, anti-inflammatory, analgesia mediated by opioid receptor affinity, sympathomimetic, endocrine, antimicrobial, antiparasitic, sedative, hypotensive, properties, soothing cure

for rheumatism and toothache[2]. This study revealed that there is significant difference in the quality of secondary metabolite present in each part of the sample studied. Mango flower contains highest amount of flavonoids (18.35±0.02) more than other parts of the plant studied as shown in Table 2. This high Flavonoid content in Mangifera indica flower as against all other plant parts used is in agreement with the assertion by [9] that phytochemical compounds such as flavonoids are more expressed and easily recognized as flower pigments. Preclinical studies carried out by [4] and that of [15] revealed that the flavonoid contains apigenin which produces sedative effects throughmodulation of y-aminobutryic acid (GABA) receptors. Apigenin's muscle relaxant properties, as well as its well-known anti-anxiety and sedative effects, were recently established in animal experimental models in a recent study by[4] and [15] which is accomplished through the involvement of the dopaminergic system in apigenin's possible antidepressant effect, also known to lower the activity of monoamine oxidase, which in turn raises the level of serotonin in the brain and that is linked to remission of depressive symptoms[15]. Chances are, *Mangifera indica* high flavonoid content could make it a good source of apigenin and consequently induce sedation.

Alkaloids, one of the most varied families of secondary metabolites in terms of structure and biosynthesis processes due to its variety of medicinal properties such as the Morphine which is a powerful opioid used to alleviate pain, although its addictive properties restrict its effectiveness, they are also good sources of sedatives and analgesic. These Alkaloids were however found to be significantly high in *Mangifera indica* stem bark (6.35±0.050) as recorded in Table 2 and that is lower than the percentage obtained in a similar study conducted in the eastern part of Nigeria. "Weather as well as period of harvest could have a lasting effect on the percentage and expression of phytochemicals" as stated by[19]. According to [10], alkaloids found in the stem-barks and leaves of *Mangifera indica* promotes its medicinal relevance especially for malaria and fever treatment in Nigeria. Some plants are known to have higher levels of phenols, also known as polar phenols, which form a complex phenol-protein complex which include tyrosine, a common amino acid present in most proteins, epinephrine (adrenaline), a stimulating hormone produced by the adrenal medulla and serotonin, a neurotransmitter in the brain. Polyphenols are capable of not only protecting the human body against oxidative chemical species (OCS), but also of reaching the target organs and tissues. Table 2 shows *Mangifera indica* stem bark has (1.0967±0.0896 of phenols), The result of phenol obtained in this study was found to be higher when compared to a similar study of *Mangifera indica* by [10] which found the stem bark and leaf to contain (0.75% and 0.09% phenol). These changes could be due to the fact that the plants used for both studies were obtained from locations with different weather and climatic temperature.

The result of the proximate content (crude protein, lipids, carbohydrates, crude fiber, ash and moisture) of the plants sampled in this study indicates that *Mangifera indica*is a good sources of carbohydrates and protein which seem to be significantly higher than that obtained from another analysis conducted by [13] and [5] which could be pointing to the fact that climatic factors, period of harvest and method of processing can cause variation on the nutritional composition of plants. It is evidently clear that (*Mangifera indica*) contained a significant amount of carbohydrates as seen in Table 3 where *Arachis hypogaea* seed had 28.56±0.44% carbohydrate (the least percentage obtained in this study) which differs from the percentage obtained from other parts of the samples analyzed, it was still found to be higher than the level obtained from other studies by [13] and [5]. Mangifera indica seed contained (81.07±±0.79) which explains its high utilization as animal feed and this percentage is higher than some of the commercial fruits that are known to be good source of carbohydrate such as Apple-13.4%, Banana-27.2%and Fresh dates-33.8% as registered by the Food Agriculture Organization. Crude protein acts as an enzyme catalyst, mediates cell responses, and regulates cell development and differentiation [14] Crude protein content observed for *Mangifera indica* (Table 3) in this study is appreciable since [1] considered food plants with 12% and above in protein content as a rich source of protein. [21] also reported that Tryptophan which is an essential amino acid naturally found in plant to be an innocuous health food used for the treatment of premenstrual syndrome, stress, depression, behavioral disorders and insomnia, is a product of protein although it could be quite toxic for pregnant women in high percentage [21]. Crude fiber is the insoluble residue of a feed after multiple boils with dilute acid and alkali. It's a fraction of a food's total carbohydrate that can withstand acid and alkali treatment. These ingredients have no nutritional value, but they offer the bulk required for appropriate peristaltic activity in the intestine[11]. Table 3. shows the percentage fiber *Mangifera indica* stem bark (13.42%) and that is

comparatively higher than all other values obtained in other studies consulted which could be tied to different methods of processing the samples for the analysis.

4. CONCLUSION

Although only a few of the antinutritional content of these plants have been analyzed, the phytochemical properties however, were critically assessed for the potential usage of the said plants as medications or possible drugs to manage insomnia in future through their tendency to cause sedation. Sedatives come in a variety of forms, but most of them function by increasing the activity of the neurotransmitter gamma-aminobutyric acid (GABA), which is a type of brain molecule that communicates between brain cells. Despite the fact that each sedative has its different mechanism of action, they all increase GABA activity, which has a very calming effect [17]. The proximate content examined in this study through proximate analysis has indicated an appreciable percentage of carbohydrates and proteins alongside other nutritional contents. Tryptophan which is known to be an amino acid with muscle relaxant properties, sedatives and antianxiety effects is a category of protein which could possibly be present in the samples analyzed. The presence of proteins and phytochemicals such as flavonoids, alkaloids, terpenoids with tryptophan, apigenin, and myrcene as active ingredients in the plant samples is a step in the right direction to discovering an alternative sleep aid.

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