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Phytochemical Screening of *Dialium Indum* Leaf extract (Velvet Tarmarind)

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Abstract

The present research was carried out to evaluate the phytochemical constituents of the methanol extracts of *Dialium indum* leaf, the percentage yield was calculated for the methanol extract comparatively to the chloroform extracts, it was revealed that methanol as an extracting solvent gave a higher yield of *Dialium indum* extract than chloroform. A total of fifteen and fourteen Phytochemical was determined qualitatively and quantitatively respectively. From the results of the phytochemical screening it were observed that saponins had a higher concentration of 9.240%, the concentration of alkaloids (1.120%), steroids (0.146%), terpenoids (2.345%), flavonoids (0.323%), phenolics (0.142%), tannins (0.581%), cardiac glycosides (1.009%) and cyanogenic glycosides (0.370%) were all determined. The presence of these phytochemicals might be the reason *Dialium indum* leaf is used as a therapeutic remedy for the treatment of diseases caused by *Bacillus typhi, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella typhi, Staph albus, Staphylococcus aureus, Streptococcus muteus, Aspergillus flavus, Aspergillus niger* and *Candida albican*. The results indicated that the leaf contained Protein (6.887%), Carbohydrate (9.593%), and Oil (47.760%).The results also showed the scientific bases for the use of *Dialium indum* in herbal medicine and as a source of nutrients for animals. Finally, the study recommends the isolation and purification of these metabolites so as to harness the maximum therapeutic and nutritive potentials**.**

Keywords: *Dialium indum*, qualitative and quantitative phytochemical analysis, Nutritive value, percentage yield.

1. Introduction

Compounds which exert various physiological effects of therapeutic value are collectively known as drugs [1]. Herbal medicine sometimes referred to as herbalism or Botanical medicine is the use of herbs for their therapeutic or medicinal value [2].

Out of hundreds of plants species that are recognized as having medicinal values, four out of every five are collected from the wild forest while most of them are from the floras of developing countries [2]. Nearly 50 percent of drugs used in medicine are of plant origin [3].

It has been shown that different parts of the tree (*Dialium indum*) have been used in folkloric medicine for the treatment of different diseases such as cancer, headache and pains (bark), fever, prenatal pains and oedema (leaves) and diarrhea (fruits) [4].

Evaluation of the analgesic activity of the methanolic stem Bark extract of *Dialium Guineensse* (wild) specie of the *Dialium genus*; Showed that the stem bark have analgesic properties [5]. The stem bark and the leaves of *Dialium indum* have been employed in the treatment of guinea worm infections [6].

Therefore, this research is aimed at giving in-depth screening into the qualitative and quantitative phytochemical constituents of *Dialium indum* leaf; so as to determine and quantify the class of secondary metabolites as well as the essential nutrient present in the leaf of *D. indum* methanol extracts that made it a potent antimicrobial agent in herbal medicine as claimed by the residents of Amike-Aba.

2. Materials and Methods

2.1 Plant Collection, Identification and Preparation

The leaves of *Dialium indum* used in this study were collected from Nnodo Amike-Aba Abakaliki Ebonyi state. It was identified and authenticated as *Dialium indum* by Prof. S.S.C Onyekwelu of the Department of Applied Biology Ebonyi State University. The Fresh leaves samples were dried under sunlight; pulverized and stored in a Glass jar for subsequent analysis.

2.2 Extraction and Fractionation into different classes [3]

500g of the pulverized leaves were soaked in 2000ml and 500ml of methanol/water mixture in a ratio of 4:1 for about 72hour. The mixture was filtered and the filtrate heated on a water bath to one-tenth (1/10) of the volume at temperature of about 40˚C. The filtrate was then acidified with 2ml of 2M H_2SO_4 and then extracted with chloroform. The mixtures were separated using separating funnel. The chloroform extract was heated to dryness and re-dissolved with chloroform giving the chloroform extract [3]. The fresh leaves were also qualitatively analyzed simultaneously with the methanol extracts while the quantitative analysis was carried out on the fresh dried leaf only.

2.3 Phytochemical screening of plant extract

2.3.1 Qualitative phytochemical screening of the crude *Dialium indum* leaves

The phytochemical analysis of the crude samples was performed using the method recommended by Harbone [3]. They were screened for the following:

a) Determination of acidic component

0.2g of each of the pulverized plant sample was placed in different clean test tubes and sufficient distilled water was added. This was warmed in a hot water bath at 40°C and cooled. Two pieces of indicator litmus paper were dipped into each filtrate and the colour change was observed.

b) Determination of flavonoids

0.2g of each sample was heated with 10ml of ethyl acetate in boiling water for 3 minutes. The mixture was filtered and the filtrate was used for the following test

Aluminum Chloride Test:

2ml of each filtrate was stirred with I ml of 1% aluminum chloride solution and observed for colour Change in the ethyl acetate layer. A yellow colour in the aluminum chloride solution showed the presence of flavonoids.

d) Determination of saponins

20ml of distilled water was added to 0.2g of the crude dug powder (stems and leaves) each in a 100ml beaker and boiled gently on a hot water at a temperature of 40^{o}C for 20 minutes. The mixture was filtered hot into 100ml beaker and allowed to cool. Then the filtrate was used for the following tests:

Frothing Test: 1ml of each filtrate was diluted with 4ml of distilled water and vigorously stirred in a test tube. Persistent (foaming) frothing showed presence of saponin.

Fehling test: 5ml of Fehling's solution was measured with syringe and added into 5ml of each filtrate and heated. Reddish precipitate confirmed the presence of saponin. This was further heated with 2drops of sulphuric acid and brick red precipitate confirmed the presence of saponin.

e) Determination of reducing sugar

0.2g of pulverized crude sample each was stirred vigorously with 5ml of distilled water and filtered. The filtrate was used for the following test:

Fehling Test: 1ml, portion of filtrate was added in equal volume of Fehling's solution I and solution II and boiled on water bath at 40^{o}C for few minutes. Presence of white precipitate indicated the presence of reducing sugar.

f) Determination of Carbohydrate

0.2g of pulverized crude sample each was stirred vigorously with water and filtered. Few drops of iodine was added to the aqueous filtrate followed by vigorous stirring, then 1ml of conc. sulphuric acid was carefully added to form a layer below the aqueous solution. A slight blue-black coloration with iodine and a brown ring at the interface on the addition of conc. sulphuric acid indicated carbohydrate.

Two drops of Molish reagent was added to the filtrate, a purple colouration indicated the presence of carbohydrate.

g) Determination of Tannins

Pulverized crude sample (0.2g) each was boiled with 5ml of 45% ethanol for 2 minutes. The mixture was filtered and used for the following tests:

Ferric Chloride Test: 1ml of each filtrate was added to 1ml of 5% iron (iii) chloride. Presence of a brownish green precipitate indicated presence of tannin.

Potassium Hydroxide Test: 1ml of each filtrate was added to 1ml of 10% KOH solution, presence of brownish green precipitate showed tannin.

h) Determination of resins

Pulverized crude sample (0.2g) each was extracted with 15ml of 90% ethanol. The alcoholic extract was poured into 20ml of distilled water in a test tube. The presence of a pale yellow and a brown precipitate indicated the presence of resin for the leave and stem respectively.

i) Determination of Steroids

10ml of 96% ethanol was added to 2g of the powered crude sample each in a round bottom flask. This was refluxed for few minutes and then filtered. The filtrate was concentrated to 2.5 ml on a boiling water bath followed by the addition of 5ml of distilled water.

The mixture was allowed to stand for 1hour. Presence of waxy matter indicated the presence of steroids.

The waxy matter was filtered off and 2ml of the filtrate was poured into test lube and 1ml of sulphuric acid (H_2SO_4) was added into it in a slant form. Brownish ring colouration formed at the interface indicated the presence of steroid.

j) Determination of Terpenoids

2ml of the filtrate got from the steroid test after the waxy matter has been filtered off was heated to dryness on a water bath. 3ml of glacial acetic acid was added to it and heated to almost dryness followed by 1ml of sulphuric acid and Subsequent heating. A grey colouration indicated the presence of terpenoids.

k) Determination of alkaloids

20ml of sulphuric acid and 50ml ethanol were added to 2g of each powdered sample, heated on a boiling water bath at 40^{o}C for 10minutes cooled and filtered. The filtrate was placed in 100ml separating funnel, made alkaline with dilute ammonia solution. The aqueous alkaline solution was separated and extracted with 100ml of 5% sulphuric acid, The extract was tested with few drops of Mayer's reagent (potassium mercuric iodide solution), Wagner's reagent (iodine in potassium iodide solution) and 1% picric acid solution.

The alkaloids present gave a milky precipitate with one drop of Mayer's reagent, reddish brown precipitate with one drop of Wagner's reagent, light reddish precipitate with one drop of picric acid reagent.

l) Determination of Oil

0.2g of pulverized sample was extracted with 5ml of 90% ethanol and filtered. Three drop of 5% ferric chloride was added to the filtrate.

A light green colour observed showed the presence of oil for the leave extract whereas a brown colour indicated the presence of oil for the stem extract.

m) Determination of cardiac glycosides

0.2g of sample was extracted using 90% ethanol, and then filtered, to the filtrate was added 5ml of distilled water, followed by glacial acetic acid containing one drop of ferric chloride solution. This was under laid with conc. Sulphuric acid.

A brown ring at the interface indicated the presence of a deoxy sugar characteristic of cardenolides and a violet ring may appear below the brown ring whereas in the acetic acid layer a greenish ring may form just above the brown ring, confirming the presence of cardiac glycosides.

n) Determination of cyanogenic glycosides

Powered sample (10g) was weighed into a conical flask and covered with sufficient amount of water. A piece of sodium picrate paper was suspended above the flask and all was placed on water bath for 1 hour**.** A colour change from yellow to white showed the presence of cyanogenic glycosides

o) Determination of protein

5ml of water was added to 0.2g of the pulverized crude sample and left to stand for 3 hours and was filtered. To 2ml portion of each filtrate was added 0.1ml of million's reagent, stirred and kept for observation. The occurrence of yellowish brown precipitate showed the presence of protein for the leaves of *Dialium indum*

p) Determination of anthraquinone

The crude sample (0.2g) was extracted using 30ml of 90% ethanol. 20ml extract was added 10ml sulphuric acid and filtered. The filtrate was shaken with chloroform. The chloroform layer was pipette into another test tube and dilute ammonia was added. The aqueous ammonia layer showed yellow colour, indicating the presence of anthraquinone.

2.3.2 Quantitative Estimation of the Phytochemical Constituents of the Leaves of *D. indum*

a) Estimation of Alkaloid content

The determination of the concentration of alkaloid in the leaves of the plants was carried out using the alkaline precipitation gravimetric method described by Harbone [7].

5 g of the powdered sample was soaked in 20 ml of 10% ethanolic acetic acid. The mixture was stood for four (4) hours at room temperature. Thereafter, the mixture was filtered through Whatman (No 42) filter paper. The filtrate was concentrated by evaporation over a steam bath to 1/4 of its original volume. To precipitate the alkaloid, concentrated ammonia solution was added in drops to the extract until it was in excess. The resulting alkaloid precipitate was recovered by filtration using previously weighed filter paper. After filtration, the precipitate was washed with 9% ammonia solution and dried in the oven at 60^{o}C for 30 minutes, cooled in desiccators and reweighed. The process was repeated two more times and the average was taken. The weight of alkaloid was determined by the differences and expressed as a percentage of weight of sample analyzed as shown in equation 1

% Alkaloid = (W2-W1) x 100 (1)

Where: -W1 = weight of filter paper

W2 = weight of filter paper + alkaloid precipitate

b) Estimation of Flavonoid content

The flavonoid content of the leaves of the plant was determined by the gravimetric method as was described by [7].

5g of the powdered sample was placed into a conical flask and 50ml of water and 2ml hydrochloric acid (HCl) solution was added. The solution was allowed to boil for 30 minutes. The boiled mixture was allowed to cool before it was filtered through Whatman filter paper (No 42).

10ml of ethyl acetate extract which contained flavonoid was recovered, while the aqueous layer was discarded. A pre weighed Whatman filter paper was used to filter second (ethyl-acetate layer), the residue was then placed in an oven to dry at 60^{o}C. It was cooled in desiccators and weighed. The quantity of flavonoid was determined using equation 2.0

% Flavonoid = W2-W1 x 100 (2)

Where:-

W1= Weight of empty filter paper

W2= Weight of filter paper + Flavonoid extract

c) Determination of Phenolics content

The concentration of phenols in the leaves of the leaves of the plants was determined using the folin-ciocalteau colorimetric method described by Pearson [8].

0.2 g of the powdered sample was added into a test tube and 10ml of methanol was added to it and shaken thoroughly the mixture was left and to stand for 15 minutes before being filtered using Whatman (No 42) filter paper. 1 ml of the extract was placed in a text-tube and 1ml folin-ciocalteau reagent in 5ml of distilled water was added and color was allowed to develop for about 1 to 2 hours at room temperature. The absorbance of the developed colour was measured at 760 nm wave. The process was repeated two more times and an averaged taken. The phenol content was calculated as in equation 3

% Phenol =100 /w x AU /AS x C/100x VF/ VA x D (3)

Where,

w= weight of sample analyzed

AU= Absorbance of test sample

AS= Absorbance of standard solution

C= concentration of standard in mg/ml

VF= total filtrate volume

VA= Volume of filtrate analyzed

D= Dilution factor.

Determination of Saponin content

The Saponin content of the sample was determined by double extraction gravimetric method [7].

5 g of the powered sample was mixed with 50 ml of 20% aqueous ethanol solution in a flask. The mixture was heated with periodic agitation in water bath for 90 minutes at 55^{o}C; it was then filtered through what man filter paper (No42). The residue was extracted with 50 ml of 20% ethanol and both extracts were poured together and the combined extract was reduced to about 40 ml at 90^{o}C and transferred to a separating funnel where 40 ml of diethyl ether was added with vigorous shaking. Separation was by partition during which the ether layer was discarded and the aqueous layer reserved. Re extraction by partitioning was done repeatedly until the aqueous layer become clear in color. The saponins were extracted, with 60 ml of normal butanol. The combined extracts were washed with 5% aqueous sodium chloride (NaCl) solution and evaporated to dryness in a pre-weighed evaporation dish. It was dried at60^{o} C in the oven and reweighed after cooling in a desiccators. The process was repeated two more times to get an average. Saponin content was determined by difference and calculated as a percentage of the original sample using equation 4

% Saponin = W2 –W1 x 100 (4)

Where: -W1 = weight of evaporating dish

W2 =weight of evaporating dish + sample

d) Determination of Steroid content

The steroid content of the leaves of the plants was determined using the method described by Harborne [7].

5g of the powdered sample was hydrolysed by boiling in 50ml hydrochloric acid solution for about 30minutes. It was filtered using Whatman filter paper (No 42) the filtrate was transferred to a separating funnel. Equal volume of ethyl acetate was added to it, mixed well and allowed to separate into two layers. The ethyl acetate layer (extract) recovered, while the aqueous layer was discarded. The extract was dried at 100^{o}C for 5minutes in a steam bath. It was then heated with concentrated amyl alcohol to extract the steroid. The mixture became turbid and a pre weighed Whatman filter paper (No 42) was used to filter the mixture properly. The dry extract was then cooled in a desiccator and reweighed. The process was repeated two mere times and an average was obtained.

The concentration of steroid was determined and expressed as a percentage as shown in equation 5

% Steroid = W2 –W1 x 100

(5)

Where:- W1= weight of filter paper.

W2 = weight of filter paper + steroid

e) Determination of Tannin Content

The tannin content of the leaves of the plants was determined using the Folin Dennis spectrophotometric method described by Pearson (1976).

2 g of the powered sample was mixed with 50 ml of distilled water and shaken for 30 minutes in a shaker. The mixture was filtered and the filtrate used for the experiment. 5 ml of the filtrate was measured into 50ml volumetric flask and diluted with 3 ml of distilled water.

Similarly, 5 ml of standard tanuric acid solution and 5 ml of distilled water was added separately. 1ml of Folin- Dennis reagent was added to each of the flask followed by 2.5 ml of saturated sodium carbonate solution. The content of each flask was made up to mark and incubated for 90 minutes at room temperature. The absorbance of the developed colour was measured at 760nm wave length with the reagent blank at zero. The process was repeated two more times to get an average. The tannin content was calculated as given in equation 6

% tannin = 100/W x AY /AS x C/100 x VF/VA x D (6)

Where: - W= weight of sample analysed
AY=Absorbance of the standard solution
C= Concentration of standard in mg /ml.
VA= volume of filtrate analysed
D= Dilution factor where applicable
AS = Absorbance of test sample
VF = Total filtrate volume

f) Estimation of Cardiac glycoside Contents

The Cardiac Glycosides content of the leaves of the plants was determined using the method described by El Olemy *et al*[9].

1g of the fine powder was soaked in 10ml of 70% alcohol for 2 hours and then filtered. The extract obtained was then purified using lead acetate and Na_2HPO_4 solution before the addition of freshly prepared Buljet's reagent (containing 95ml aqueous picric acid + 5ml of 10% aqueous NaOH). The difference between the intensity of colours of the experimental and the blank (Distilled water + Buljet's reagent) samples gave the absorbance which was proportional to the concentration of the glycosides.

$$\text{Concentration (\%)} = \frac{\text{Absorbance} \times 100 \times g\%}{17} \quad (7)$$

g) Determination of terpenoid Content

The terpenoid content of the leaves of the plants was determined using the method described by Harborne [7].

50g of the pulverized sample was extracted with solvent combination of methanol and water (4:1) at room temperature for 24 hours, the solution was filtered using whatman No. 1 filter paper. The filtrate was then evaporated to 1/10 volume at 40^{o}C, the evaporated filtrate was acidified with 2m sulphuric acid (pH 0.89) followed by chloroform extraction (three time the volume), stirred and allowed to stand in a separating funnel. Out of the two layers formed, the non aqueous layer was taken and evaporated to dryness. The dried extract contains the terpenoid components. Weight of dried extract was estimated and it is equivalent to the terpenoid content

h) Determination of protein

Estimation of protein was done using the method described by Lowry *et al*[10].

5g of the dried sample was weighed and ground well with a pestle and mortar in 100ml of the buffer.

The dried powdered samples was extracted by stirring with 50ml of 50% methanol (1:5 w/v) at 25^{o}C for 24 hours and centrifuged at 7000rpm for 10 minutes. 0.2ml of extracts was pipette out and the volume was made to 1.0ml with distilled water. 5.0ml of alkaline copper reagent was added to all the tubes and allowed it to stand for 10minutes. Then 0.5ml of Folin's-Ciocalteau reagent was added and incubated in the dark for 30 minutes. The intensity of the colour developed was read at 600nm.

i) Determination of acidic component

Acidic components were calculated using the method described by Horn and Mehanan [11].

To 0.2ml sample add 5.8ml of CHM (Chloroform-Heptane-Methanol) mixture and 200mg of activated salicylic acid was added, mixed well and centrifuged. The supernatant was transferred to another tube. Standard were also made up to 6.0ml with CHM mixture, blank contained 6.0 ml of CHM mixture. To all these tubes, 2.0ml of copper nitrate-TEA (triethanolamine) solution was added and mixed on a mechanical shaker for 20 min, they were then centrifuged to give two separate phases, 2ml of the upper phase was transferred to another tube, 1.0 ml of the colour reagent was then added and shaken well, the colour developed and was read at 430 nm against a reagent blank. Free fatty acids are expressed as mg/g in tissues.

j) Estimation of carbohydrate

The total carbohydrate content was estimated by the method of Hedge and Hofreiter [12].

Each sample (100g) was weighed into a boiling tube, hydrolysed by keeping it in a boiling water bath for three hours with 5.0 ml of 2.5 M HCl and cooled to room temperature. This was neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 100 ml and centrifuged, the supernatant was collected. 0.2 to 1.0 ml was used for analysis. The standards were prepared by taking 0.2-1.0 ml of the working standards. 1.0 ml of water served as a blank. The volumes were made up to 1.0 ml in all the tubes with distilled water, and 4.0 ml of anthrone reagent was added. This was heated for eight minutes in a boiling water bath, cooled rapidly and the green to dark green colour was read at 630 nm.

k) Determination of Cyanogenic glycosides

This was done according to the method described by A.O.A.C [5].

1g of the samples was soaked for 4 hours in distilled water. The suspension was steam-distilled into a dilute NaOH solution. The distillate was then treated with dilute KI and titrated against $AgNO_3$ to a faint and permanent turbidity. The Hydrocyanate was calculated taking 1ml of 0.02 $AgNO_3$ as equivalent to 1.08mg HCN.

l) Determination of oil

The oil content was estimated using the method described by AOAC [14], and modified by Ani *et al*[15].

Oil content was calculated by solvent extraction method using Soxhlet apparatus with hexane as solvent. 2.0 g of powdered sample was weighed into a filter paper and wrapped; the filter paper was placed inside the inner part of the Soxhlet extractor. The apparatus was then fitted to a round bottom flask, which contained 200ml of hexane solvent. It was then attached to a reflux condenser. The set-up was clamped and heated in a water bath such that

extraction is considered completed when the extracting solution becomes clear. The solvent was distilled off in the distillation set. The oil was then poured into a bottle and left for 5 days for the remaining solvent to evaporate. The oil was then weighed and the percentage oil content determined using the following expression:

$$\text{Oil content (\%)} = \frac{\text{weight of oil} \times 100}{\text{Weight of dried sample}} \quad (8.0)$$

m) Determination of reducing sugar

The reducing sugar was determined using the method described by Harbone[3].

Procedure: 100ml glucose stock solution of 10mg/ml concentration was prepared. Glucose standards ranging from 0.2, 0.3, 0.4, 0.5, 0.6 and 1.0 mg ml^{-1} (total sample volume 3ml) was prepared. 2ml of Dintrosalicylic Acid (DNS) reagent was added to 3ml of glucose standard solutions in a test tube. 3ml glucose solution of unknown concentration was taken and 2ml DNS reagent was added to it. The test tubes were kept in boiling water bath for 5 minutes and were left to cool. The absorbance was measured at 540 nm against reagent blank. A graph of glucose concentration on x-axes and absorbance at y-axes. This was the standard calibration curve. The value of glucose concentration in unknown samples was determined using the standard calibration curve.

3. Results and Discussions

Table 1: Results of Qualitative analysis of *D. indum* Fresh Leaves and Crude Methanol Extracts

Test	Fresh Leaves	Crude Methanol *D. indum* Leaves Extract
Acid Component	-	-
Carbohydrate	+	+
Oil	++	+++
Protein	+	+
Resin	-	-
Saponin	++	+++
Reducing Sugar	+	+
Tannins	+	++
Alkaloids	+	+
Flavonoid	+	++
Terpenoid	++	++
Cardiac Glycosides	-	+
Cyanogenic Glycosides	-	+
Steriods	+	+
Anthraquinone	++	++

NOTE: - Absent.
++ Present in high concentration
+ Present in low concentration.
+++ Present in very high concentration

Table 2: Results of quantitative phytochemical screening of *D. indum* leaves extracts

S/N	Phytochemical (%)	Mean±SEM
1	Alkaloid	1.120±0.000
2	Steroid	0.146±0.000
3	Saponin	9.240±0.015
4	Terpenoid	2.345±0.000
5	Flavonoid	0.323±0.000
6	Phenolics	0.142±0.000
7	Tannin	0.581±0.003
8	Protein	6.887±0.000
9	Carbohydrate	9.593±0.000
10	Reducing Sugar	2.440±0.009
11	Cyanogenic Glycosides	1.009±0.000
12	Cardiac Glycosides	0.370±0.000
13	Acidic Components	1.890±0.010
14	Oil	47.760±0.000

The standard deviation and the standard error of the mean (SEM) was used to estimate the precision of the measurements

Table 3: Results of the percentage yield for methanol and chloroform solvents

Extract	Percentage yield (%)
D. indum Leaves methanolic	11.00
D. indum Leave chloroform	7.00

Mass of dried *D. indum* Leaves = 500g
Mass of dried *D. indum* leaves methanolic extract=55g
Mass of dried *D. indum* chloroform leaves extract= 35g

4. Discussions

The values of the mean were taken to estimate the quantitative phytochemical constituents of the leaf extract. The results as shown in Table 1 and Table 2 revealed that saponins had a higher concentration of 9.240±0.015%, the concentration of alkaloids, steroids, terpenoids, flavonoids, phenolics, tannins, cardiac glycosides and cyanogenic glycosides were estimated at 1.120±0.000%, 0.146±0.000%, 2.345±0.000%, 0.323±0.000%, 0.142±0.000%, 0.581±0.003%, 1.009±0.000% and 0.370±0.000% respectively. Saponins have been reported to possess antiprotozoa, antifungal and antibacterial properties [16]. Saponins also aid in the control of cholesterol in the body by preventing its re-absorption into the body. They are also responsible for tonic and stimulating activities observed in Chinese and Japanese medical herbs[17]. The combination of saponins and steroids are known to show curative activity against pathogens [18]. Saponins are reported to be effective in the treatment of syphilis rheumatism and certain skin diseases; treatment of abscesses and other swellings, ulcer, septic wounds, and management of inflammation [19].

Also the diuretic, antibacterial and antifungal, antiplasmodial and antimycobacterial activities, cytotoxic and antiprotozoal activity of flavonoids containing plants have been reported [16, 17,20,21]. Tannins die and tan the skin and the mucosa, easing the resolution of inflammatory processes and wound healing [22]. The antimicrobial powers of tannin are involved in healing of burns using herbs [23,24]. Flavonoids are known to have hypoglycemic activity also used in the treatment of diabetes, exhibit anti-allergic, anti-inflammatory, anti-microbial and anti-cancer properties [25,26]. Cardiac glycosides action is to increase the contractive strength of the heart and regulate its beat rhythm. Terpenoid are reported to have a wide spectrum of biological activities including bactericidal, fungicidal, antiviral, cytotoxic, analgesic, anticancer, spermicidal, Cardiovascular and anti allergic [27]. Terpenoid, flavonoids and tannins compounds are described as potent biologically active compounds found in medicinal plants parts which are precursors for clinically useful drugs [28]. The potency of medicinal plants is attributed to the action of the phytochemical constituents [29].

The concentration of Protein (6.887±0.000), Carbohydrate (9.593±0.000), Oil (47.760±0.000), this revealed that *D. indum* leaf extract can serve as a good nutrient source for livestock.

From Table 3, it was revealed that methanol had a higher yield of *D. indum* leaf extract than chloroform. The higher yield of methanol can be attributed to its polarity. Methanol can extract both hydrophilic and lipophilic compounds from plant tissues due to its amphiphilic nature. To some extent methanol is used in the extraction of non-polar components of plant parts. The chloroform yield for *D. indum* leaf was lesser than those of methanol; chloroform is a non-polar solvent and mostly used in the extraction of non-polar constituents of plant parts

5. Conclusion

The findings of this research have revealed the phytochemical content of *D. indum*. The concentration of these Phytochemicals revealed the medicinal and nutritive potentials of the plant as claimed by ethno-medicine users since these compounds are known to be biologically active on fungi and bacteria organisms.

Recommendations

Based on the results of the study, it is recommended that

1) The toxicological and clinical study should be carried out on the plant to further determine it toxicity and threshold limiting value (TLV)for human consumption
2) Further research should be done with the aim of stabilizing the phytochemical so that they can be prevented from losing their potency
3) The compounds responsible for the biological activity be isolated, purified, characterized and structures determined

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