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Table 1: Phytochemicals Qualitative Analysis

Phytochemicals	Unit	Leaf	Bark
Tannin	mg/100 g	+	+
Oxalate	mg/100 g	+	+
Cyanide	mg/100 g	+	+
Phytate	mg/100 g	+	+

Tablen2: Phytochemicals Quantitative Analysis

Phytochemicals	Unit	Leaf	Bark
Alkanoids	mg/100 g	125.22	22.5
Tannins	mg/100 g	2.967	0.754
Phenols	mg/100 g	5.83	3.041
Saponins	mg/100 g	0.070	0.023
Flavonoids	mg/100 g	24.22	9.203

Samples Preparations

40 g weight of each of the powdered sample was extracted with 50 ml of 80 % methanol. The mixture was heated for 30 minutes and the resulting liquid was filtered using filter paper (Whatman No 3, Whatman Ltd., England). Extraction was repeated five times and the filtrates were combined in one vessel. The solvent was removed on a water bath at 40 oC. The resulting dried mass was then powdered, packed into a glass vial and stored in a desiccator over silica gel until use.

Phytochemical screening (Qualitative Analysis)

Chemical tests were carried out on the methanolic extracts for the qualitative determination of phytochemical constituents following AOAC standard. Below is a brief description of the methods used.

Alkaloids: The chloroform extracts were evaporated to dryness and the residues were heated with 2% HCl solution on a boiling water bath. The extracts were cooled, filtered and then treated with the Mayer's reagent. The sample was then observed for the presence of yellow precipitation.

Flavonoids: 1.5 ml of a 50% aqueous methanol was added to 4 ml of plant extracts. The solution was warmed and magnesium turning was added. 5 drops of concentrated HCl was added to the solution and observed for red coloration.

Tannins: To 0.5 ml of extract solution, 1 ml of distilled water and 1 to 2 drops of ferric chloride solution was added to it, and observed for blue or green black coloration.

Saponins: 2 ml of distilled water was added to 2 ml of the test solution and shaken very well till frothing was observed.

Phenols: Ethyl alcohol was added to 2 ml of the test solution and few drops of ferric chloride solution and observed for coloration.

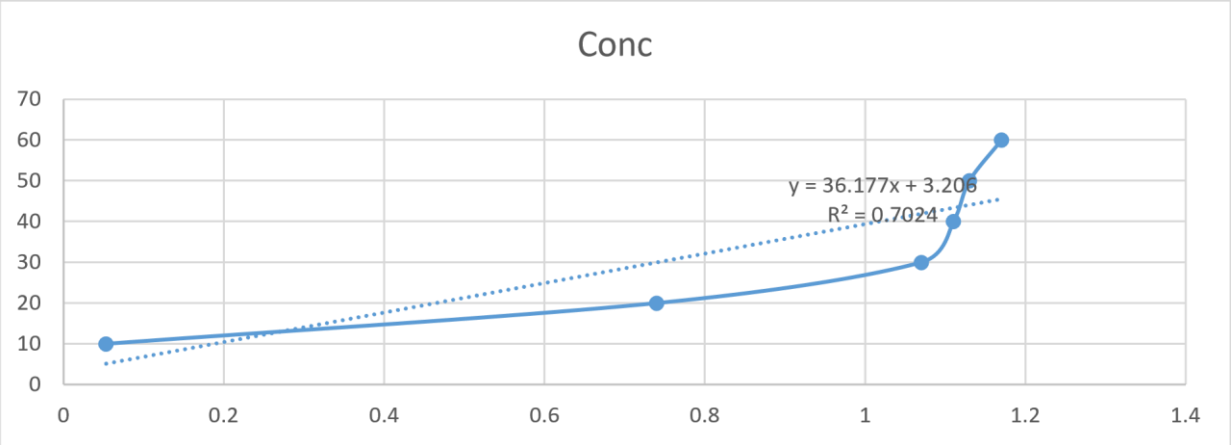
Quantitative Analysis

Determination of Alkaloids

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in Ethanol. The beaker was covered and allowed to stand for 4 h. It was then filtered and the extract concentrated on a water-bath to one-quarter (1/4) of the sample volume. Concentrated ammonium hydroxide (NH_4OH) was added dropwise to the extract until precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide (2 M) and then filtered. The residue if available is the alkaloid which is then dried and weighed.

Determination of flavonoid contents

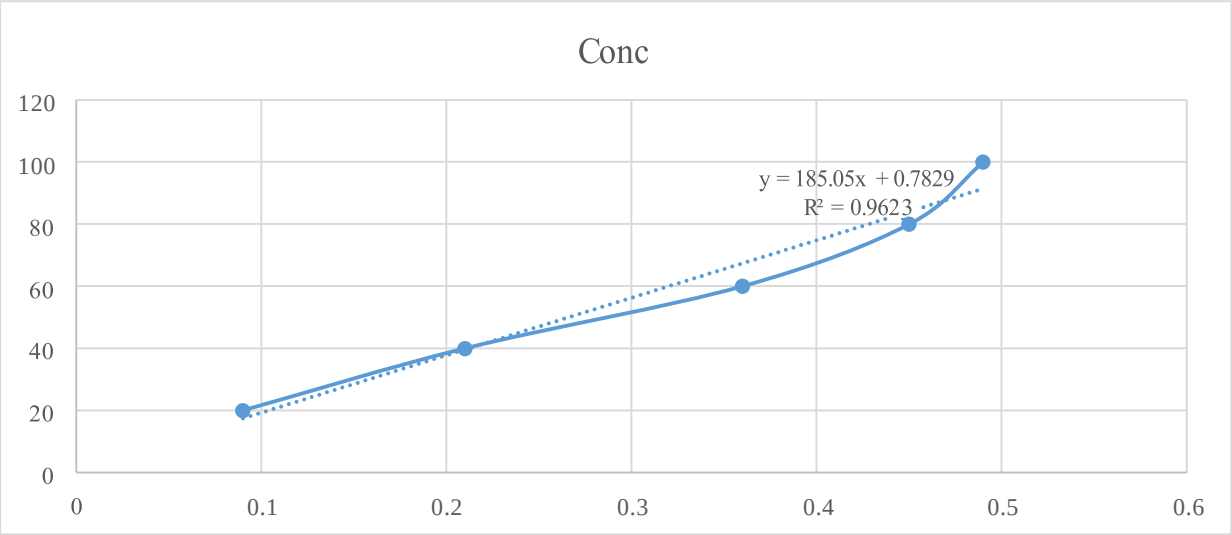
The Aluminium chloride colorimetric method was used to measure the flavonoid content of the plant extracts. 0.25ml of Extract solution of each plant extract was added to 1.25 ml of distilled water. The 5% Sodium nitrite solution (0.75ml) was then added to the mixture and was incubated for 5 minutes after which 0.15ml of 10% aluminium chloride was added. The mixture was allowed to stand for 6min at room temperature before 0.5ml of 1 M sodium hydroxide was finally added and the mixture diluted with 0.275 ml distilled water. The absorbance of the reaction mixture was measured at 510 nm with a UV/VIS spectrophotometer immediately. Quercetin was used as the standard for the calibration curve. Flavonoid contents were expressed as mg quercetin equivalent (QE)/g dry weight (D.W.).



Determination of total phenolic content

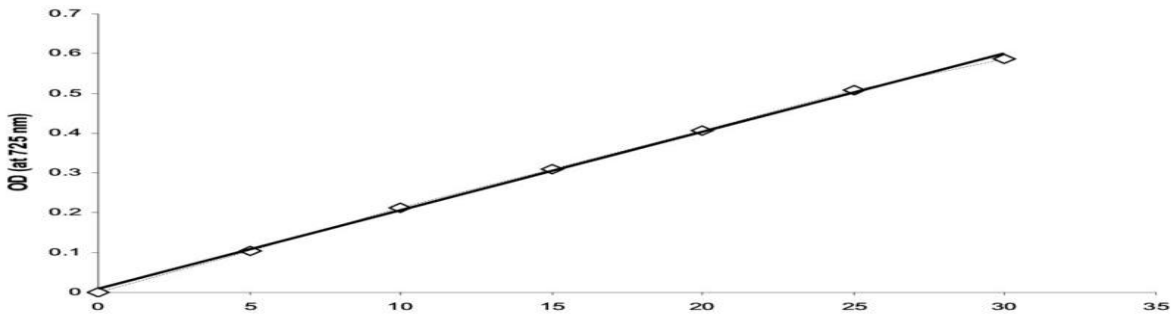
Total phenol content was estimated using Folin-Ciocalteu reagent based. To one ml of each extract

(100µg/ml) in methanol, 5ml of Folin-Ciocalteu reagent (diluted tenfold) and 4 ml (75 g/l) of Na2CO3 were added. The mixture was allowed to stand at 20°C for 30 min and the absorbance of the developed colour was recorded at 765 nm using UV-VIS spectrophotometer. 1 ml aliquots of 20, 40, 60, 80, 100µg/ml methanolic gallic acid solutions were used as standard for calibration curve. All determinations were performed in triplicate. Total phenol value was obtained from the regression equation: y = 185.05x + 0.7829 and expressed as mg/g gallic acid equivalent using the formula, C = cV/M; where C = total content of phenolic compounds in mg/g GAE, c = the concentration of gallic acid (mg/ml) established from the calibration curve, V = volume of extract (0.5ml) and m = the weight of pure plant methanolic extract (0.052g) (diluted ten times).



Tannin

2 g of the sample was extracted with 10 mL of 70% aqueous Acetone (v/v) for 24 h at room temperature. The extracts were centrifuged at 1500 rpm for 30 min and the supernatant was analyzed for tannins. In a 10 mL test tube containing 0.5 mL Folin- Denis reagent, was added 0.5 mL of the tannins extract and 1 mL of saturated sodium carbonate solution. The volume was made up to 10 mL with distilled water. After 30 min, tannin content was measured at 760 nm with the spectrophotometer against experimental blank adjusted to zero absorbance. Tannic acid was used as a standard compound.



Saponins

A total of 20 g of the extract was placed in a conical flask and 100 mL of 20% aqueous ethanol was added to it. The mixture was placed in a water bath and heated at a temperature of 55 °C for 4 h with continuous stirring. The mixture was filtered and re-extracted with 200 mL of 20% ethanol. The filtrates were combined and the volume was reduced to 40 mL using a water bath at a temperature of 90 °C. The concentrated filtrate was transferred into a 250 mL separating funnel and 20 mL of diethyl ether was added to

it. This was shaken vigorously and allowed to separate into two layers. This extraction was carried out three times and the aqueous layer was recovered. The aqueous layer was extracted three times with 60 mL of n-butanol. The n-butanol extract was washed three times with 10 mL of 5% NaCl. The washed n-butanol extract was heated in a water bath to evaporate the n-butanol. The n-butanol extract was dried in the oven at a temperature of 50 °C to a constant weight to give the saponins. Vanillin-acetic acid (0.2 mL, 5% w/v) and 0.8 mL of perchloric acid were added to 50 µL of the n-butanol extract and placed in an oven at a temperature of 70 °C for 15 min. The mixture was cooled on an ice bath for 1 min, and then 5.0 mL of glacial acetic acid was added to it. The mixture was then scanned on a UV/Vis spectrophotometer at a wavelength of 550 nm.