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Proximate Analysis and Phytochemeical Screening on Root of *Cnidoscolus carumbium*

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ABSTRACT

The root of *Cnidoscolus carumbium* was soaked in water for 48 hours and filtered. The residue was oven dried at 800C for 4hrs. Further extraction process was done on the residue by soxhlet extraction method using different organic solvent mixtures of 500cm3 of hexane, acetone and methanol in the ratio 3:1:1 mixture. Proximate analysis for roots revealed Moisture content (19.1%), Fiber (17.7%), Crude protein (2.2%), Ash (15.5%), crude fat (1.0%), and carbohydrate (44.5%), indicating high nutritional value. The result for the extraction shows the presence of phytochemicals such as saponin, alkaloid, steroid flavonoid, phenol, tannin, cardiac glycosides, terpenoid and phlobatanin due to the solvent mixture possessing wide polarity thereby enabling the extraction of all the phytochemicals.

Keywords: Proximate Analysis, Phytochemeical Screening, Root, Cnidoscolus carumbium

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Introduction

Traditional medicinal plants are a therapeutic resource used by the population of the continent specifically for health care which may also serve as starting materials for drugs. Sofowora (1993). People of all continents have long applied poultice and imbibed infusions of indigenous plant dating back to prehistory for health purposes. Cowan (1999). Plants provide an alternative strategy in the search for new drugs. There is a rich abundance of plants reputed in traditional medicine to possess protective and therapeutic properties. It is likely that plants will continue to be a valuable source of new molecules which may, after possible chemical manipulation, provide new and improved drugs. Shah (2006). It comprises of therapeutic practices in existence for hundreds of years before the development of modern scientific medicine and is still in use today without any documented evidence of adverse effects.

Cnidoscolus carumbium is said natively to be of medicinal and nutritional value (containing some nutritional element) also in abating anemia (i.e. enhancing blood quality) in the human body. Also its stems and roots extracts are said to have anti-malaria and anti-microbial properties. The study of *Cnidoscolus carumbium* could bring forth advancement in the quest for actual remedies for health defects and encourage its use. This research is focused on confirming the phytochemical, proximate composition and efficiency of this plant. The study of this plant could bring forth advancement in the quest for actual remedies for primary and secondary health defects and boost it dietary patronage.

The aim and objective is o carry out the phytochemical screening of the plant extracts in different solvent system.

i. To evaluate the proximate compositions of the roots and stems of *Cnidoscoluscarumbium*.

ii. To determine the suitable solvent for the extraction of the different phytochemicals.

Materials and Methods

Sampling and Extraction

The roots of *Cnidoscolum carumbium* were obtained in the month of February 2016 at the Cross River University of Technology Staff Quarters, Calabar. Cold extraction was used for the initial extraction. The stem of *Cnidoscolum carumbium* was soaked in water for 48 hours and filtered. The residue was oven dried at 80^0c for like 4hrs. Further extraction process was done on the residue by soxhlet extraction method using different organic solvent mixtures of 500cm^3 of hexane, acetone and methanol in the ratio 3:1:1 mixture.

Proximate analysis of *Cnidoscoluscarumbium*

Moiture Content (AOAC, 2005)

The moisture content of the root were measured or determined by weighing 4.0g of the fresh root and stem (separately) into three (3) crucibles and labeled a, b, c respectively and placed in an air circulating oven at 80^0C for 12hrs. The crucible and its content were cooled in a desiccator containing magnesium sulphate as drying agent. The process was repeated until constant weights were obtained.

Determination of Ash Content in Root and Stem

5.0g of the root were weighed into crucibles of known weight with a lid and ignited in a muffle furnace for 2hrs at 55^0C. At the end of this process, the crucibles and lids were cooled in a desiccator containing magnesium sulphate as drying agent.

Determination of crude fat from root (AOAC 2005)

The fat in the root was determined by using 5.0g of the grinded sample by soxhlet extractor with ethyl acetate as extracting solvent. The sample was weighed into the thimble and

150ml of ethyl acetate was measured into 250 round flask, which was held on the hot plate for 8 hours and the fat was on the hot plate for 8 hours during the fat extraction process. The amount of lipid extracted was obtained as the difference between the weight of the flask before and after the extraction. Finally, the ether content in the root and stem was evaporation in the water bath to obtain the fat content in the root and stem.

Determination of crude protein (AOAC 1995)

This procedure measures the protein content of the samples.

Method:

5.0g of the grinded fresh root was weighed 350 ml standard. Kjeldah flask containing 1 table of kjeldah catalyst, some anti-bombing chips and 30 ml of conc. H_2SO_4 was introduce into the flask. The flask was taken into the digestion rack and heated gently for 1 hour.

The flask was then subjected to vigorous heating for 8 hours until a clear blush colour was obtained. After the digestion, the flask was cooled in tap water and quantitatively transferred into 100ml standard volumetric flask and make up to mark with distilled water. 10ml portion of the digested sample was measured into a semi micro kjidahinackham distillation apparatus and treated with 30ml of 40% HCl solution. 10ml of boric acid plus 2 drops of double indicator.

The tap of the condenser receive was unmeshed in the boric acid and the distillation contained until about three times the original volume was obtained.

Determination of Fibre content in *Cnidoscolum Carumbium* Roots

The procedure for estimation of this fibre was carried out in different stages.

a. Acid digest: 5.0 of sample was weighed into 250ml beaker containing 2% H_2SO_4solution and mixed properly then heated for 30 minutes with constant stirring.

   After the boiling, the sample was filtered and washed with distilled water to remove acid content until the result was acid.

b. Base digestion: the residue was further treated with 50ml of 2% NaOH solution and heated as the above for 30 minutes with constant stirring. The residue was made base free by filtration and rinsed with distilled water

c. The residue left was treated with methanol and filtered.

   The residue left was dried in a crucible of known weight at 100^0, this followed by recognition in furnace at 500^0 c

   The weight of ash left in both root was noted, recorded and calculated.

Determination of total carbohydrate (AOAC 2005)

The calculation is conveniently done by the differential method.

% CHO = 100 – (% moisture % ash + fat + % protein + % fibre)

Determination of Phytochemical

Determination Terpeniods AOAC (1995)

Method: 5ml of the extracted sample was mixed carefully with 20.0ml of chloroform and 2.0ml concentrated H_2SO_4 by the down side the test tube to have a layer. A reddish brown colouration formed on the interface, shows the presence of terpeniods. This procedure was applied for both root and stem samples.

Determination for cardiac glycoside AOAC (2000)

Keller – killiani test

2.0ml of the extract was added with 1.oml of lead acetate, shaken and filtered. The filtrate was extracted in equal volume of chloroform. The residue left was dissolved in 3ml of 4% ferric chloride dissolved in glacial acetic acid and left to stand for 1 minute. 1.0ml of concentrated H_2SO_4 was added down side of the test tube. At the end failure of blue coloration to occur indicates the absence of cardiac glycoside in the samples.

Determination of steroid AOAC (2000)

Method: **Salkwoski test**

1.0ml of the extract was dissolved in 2.0ml of chloroform in test tube before 1.0ml of concentrated H_2SO_4 was carefully added at the side of the test tube. Presence of reddish brown coloration indicates the presence of steroid nucleus in the sample.

Determination of Phlobatannin AOAC (2000)

Method

1.0ml of the extracted samples was boiled with 4ml of 1% aqueous hydrochloric acid (HCl) for 5 minutes. The presence of deposition of red perceptible indicates the presence of phlobatannin

Determination of flavonoid

1.0ml of the extract plus 1.0ml of 10% lead acetate solution was added to the lest lube plus 2ml of 15% NaOH solution and shake the presence of precipitate shows the presence of flavonoid.

Determination of saponin

1.0ml of extract from the samples wereboiled with 5.0ml of distilled H_2O (water) in a test tube in the water bath for 5 min then decanted while still hot. When shaken, the presence of stable froth indicates the presence of saponin.

Determination of Tannins

This method used was described by freeman N.T & whiteman (1982)

Method 1.0ml of the sample extract was added to equal volume of bromine water and shaken the formation of greenish to red precipitate is taken as evidence for the presence of condensed tannins.

Determination of Alkaloid

1.0ml of the extract was mixed with 5ml of 2% HCl, and placed on a steam bath then filtered. 1ml of the filtrate was treated with 0.5ml of Wagner's reagent. The formation of reddish brown and cream coloration precipitate respectively was an indication of the presence of alkaloid.

Result

Table1: Shows proximate % composition of Root of *Cnidoscolus carumbium*

	Moisture (%)	Crude fibre (%)	Crude protein (%)	Ash (%)	Fat (%)	Carbohydrate (%)
Root	19.1	17.7	2.2	15.5	1.0	44.5

Table 2: Phytochemical Screening of Root of *Cnidoscolus carumbium* extracted using soxhlet extractor of solvent mixture-hexane, acetone & methanol (ratio 3:1:1).

Stem bark - Soxhlet extraction using solvent mixture-hexane, acetone & methanol (ratio 3:1:1)

S/N	Phytochemicals	Remarks (Result in symbols)
1	Alkaloids	++
2	Saponins	+++
3	Tannins	+
4	Flavonoids	+++
5	Cardiac glycosides	+
6	Steroids	++
7	Terpenoids	+
8	Phlobotannins	+
9	Phenols	

Table 3.Determination of phytochemical screening in root of *Cnidoscolus carumbium* using different solvent system

Plant Morphology	Solvents	Tannin	Saponin	Phenol	Alkaloid	Flavonoid	Cardiac glycoside	Steroid	Terpenoid	Phlobatannin
Roots	Water	++	++	-	+	++	++	+	++	++
	Methanol	+	++	+	++	+++	++	+++	++	++
	Acetone	+	+	+	+	++	+	+	+	++
	Ethyl acetate	++	+	++	+	+	+	+	+	+
	Dichloromethane	++	-	+	++	+	-	++	++	+
	Ethanol	++	+++	+	++	+	+	+	++	+
	Chloroform	+	+	+	+	+++	-	+	++	-
	Hexane	+	+	++	++	++	+	+	++	+

+++ = High concentration (HC), ++ = Moderate concentration (M C)

+ = Low concentration (LC) **- =** Below Detection limit (BDL) or Not available.

Discussion

The phytochemical components and proximate values of the plant were obtained by analysis of the plant extracts using established procedures.

Proximate analysis for roots revealed Moisture content (19.1%), Fiber (17.7%), Crude protein (2.2%), Ash (15.5%), crude fat (1.0%), and carbohydrate (44.5%), indicating high nutritional value.

The phytochemical results show that for the extraction of tannin from the root, the preferred solvent is ethyl acetate, ethanol, water or dichloromethane. Chloroform is not recommended because if its inability to extract tannin. Ethanol, methanol and water are best solvents for the extraction of saponin from root of plant sample but dichloromethane and chloroform are unsuitable. For extraction of flavonoid from root extract, methanol, chloroform, water, acetone and hexane is suitable. For extraction of alkaloid, acetone, hexane, ethanol and dichloromethane are suitable both for root extraction but water, chloroform and methanol are not very suitable. Extraction of phenol from root is suitable in ethyl acetate and hexane but not in water and for stem extract, only hexane is most preferred while water and dichloromethane is not suitable. Recommended solvent for the extraction of terpenoid from root are methanol, hexane, water, dichloromethane and chloroform is suitable but ethyl acetate and chloroform is not suitable for both root and stem extracts. For extraction of phlobatannins, water, methanol and acetone are suitable solvent for the root while chloroform is a suitable solvent for such phytochemical extraction. For cardiac glycoside, water and methanol is preferred while chloroform and dichloromethane is unsuitable for the root extracts. The result for soxhlet extraction for roots shows the presence of all the phytochemicals due to the solvent mixture possessing wide polarity, thereby enabling the dissolution of all the phytochemicals in the solvent system.

The moisture content of any food is an index of its water activity (Olutiola et al, 1991) and is used as a measure of stability and susceptibility of microbial contamination (Urah and Izuagbo, 1990), this implies that; *Cnidoscolus*

carumbium may have relatively longer shelf life than most plants herbs. Dehydration would increase the relative concentration of other food nutrients and improve the life shelf / durability of harvested *Cnidoscolus carumbium*.

Conclusion

From the general result obtained from the analysis of this plant, we can say conclusively that this plant unveils the possibilities of it being a potent source of food nutrients and medicine. It can also be concluded that since the root contains some vital phytochemicals such as saponin, alkaloid, steroid flavonoid, phenol, tannin, cardiac glycosides, terpenoid and phlobatanin. It is an essential nutrient source and has variety of biochemical functions in the body. *Cnidoscolus carumbium* also is a potent antibiotic and blood building agent. Since each community in Nigeria has its peculiar way of treating different aliments and this plant can now on a wide range be found useful for the treatment of common disease such as cancer and for food because of its nutritional values. This plant offers enormous health benefits to our immune system and antioxidant defense.

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