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Pharmcognostic Standardization of Capsicum Chinese's Nsukka Drilus Leaf, Fruit and Seed Solanaceae

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Abstract: Capsicum chinense Nsukka drilus is an erect herb or sub-shrub belonging to the family of Solanaceae. It is found in South East of Nigeria. It is commonly cultivated in Nsukka local government area of Enugu state. The plant is used as food additives, ingredient in local traditional dishes and treatment of various diseases such as inflammation, cancer, neuralgia and rheumatism. In this study, the Pharmcognostic profiles of the leaf and fruit were carried out to determine its macroscopic, microscopic and phyto-constituents. The macroscopic character showed simple alternate, dark green and yellowish colours. The microscopic analysis of the leaf and fruit revealed: calcium oxalate, epidermal cells, stomata, trichome and fibres. The result of analytical standard for fruit showed 13.75, 0.37, 8.45, 4.95, 0.40 and 1.60% a for total ash, acid insoluble ash, sulphated ash, alcohol soluble extractive, water soluble extractive and moisture content and 18.8, 0.41, 5 .55, 9.89, 1.5 and 2.15% for total ash, acid insoluble ash, sulphated ash, alcohol soluble extractive, water soluble extractive and moisture content for the leaf. The result of phytochemical analysis revealed: carbohydrates, saponins, tannins, acidic compound and proteins were found in the leaves. Alkaloids, flavonoids, saponins, tannins, steroids and terpenoids were found in the fruit. From the result, it can be observed that alkaloids were absent in the leaf.

Key words: Capsicum Chinense Nsukka Drilus · Standardization · Phytochemical Analysis

INTRODUCTION

Plants and animals have been recognised as sources of natural bioactive compounds [1]. Over the years, they have been utilised in treatment of various diseases in traditional medicine in the form of concoctions, decoctions, infusions, tinctures, etc. Their proper uses are highly dependent on correct identification, collection and preparation. Following the increased patronage of herbal products in our markets, some unscrupulous herbal dealers have resorted to adulteration of crude drugs to maximise profit. This, therefore, calls for a tentative African Pharmacopeia, that has the monographs of medicinal plant to help in fishing-out these adulterated crude drugs and products.

Capsicum chinense nsukka drilus is a variety of pepper commonly cultivated in various parts of Nsukka in Nsukka Local Government area of Enugu State of Nigeria. It is so peculiar to this region such that attempts to cultivate them outside this area have not yielded any good result [2]. Hence, it is called Nsukka-yellow pepper. It is cultivated and consumed locally as food additive and ingredient in local traditional medicine. Its sparkling yellow colouration and characteristic hotness coupled to its sweetness have endeared it to housewives and hoteliers who preferentially use it in preparation of local dishes, especially stew and pepper soup dishes.

Capsicum chinense nsukka drilus is from Solanaceae family and genus Capsicum [3]. The Solanaceae family, which is known as nightshade or potato family, is created for so many phytochemical of diverse medicinal properties [4]. Some medicinally important genera of this family are Solanum, Atropa, Capsicum, Datura, Withania, Hyoscymus and Nicotiana [5]. The Capsicum species is said to have originated from Central and South America [6]. According to Dagnoko *et al*. [7] there are 30 species of peppers of which, only five are domesticated. These five species include *Capsicum annuum L., Capsicum frutescens L., Capsicum chinense Jacq, Capsicum baccatum L.* and *Capsicum pubescens*

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Ruiz and Pav. However, Denniss Ashilenje [8] reported that there were 40 species of pepper and that only five of these were eaten by humans. These five species are the same as those mentioned by Dibulo *et al*. [9]. Suffice it to say that the number of species of pepper is yet to be ascertained or resolved. What we can say for sure is that these five species mentioned above are widely domesticated, distributed and consumed.

Standardisation of crude drug is a code of conduct that ensures the correct substance in correct amount for desired therapeutic effect (Safety, quality and efficacy) is ensured at every point in time, Ekhuemelo and Olatunji [10]. It is a means of differentiating authentic crude drugs from adulterants. Standardisation expression is used to describe all measures, which are taken during manufacturing process and quality control leading to a reproducible clinical application. Standardisation of herbal medicine is not an easy task as numerous factors influence the bio production and reproducibility of therapeutic agents. Factors such as environmental conditions (Temperature, humidity, rainfall, sunshine, etc.), time of collection, method of preparation, preservation, etc., are very critical in the quality of crude drug. Methods of standardisation and evaluation of herbal medicines can be grouped into three [11].

Pepper Plant: Pepper plant is an erect herb or sub-shrub, which grows up to 2.5m tall, much-branched and cultivated as an annual but in home gardens sometimes as a short-term perennial. It also has a strong taproot which is supported with numerous lateral roots. While the stem is irregularly angular to subterrneous, up to 1 cm in diameter, green to brown-green, it is often softly hairy with purplish spots near nodes. Leaves are simple, alternate, very variable and almost glabrous with pale to dark green. Capsicum species have a solitary flower that starts at the axial of the first branching node with subsequent flowers forming at each additional node. The flower is complete, bisexual, hypogynous and usually pentamerous. The most actively growing organs of a pepper plant after flowering is the fruit. The fruit is ordinarily seeded, but parthenocarpic forms exist. The seed set affects development and subsequent growth of the fruit. On the average, there is a direct linear relationship between the number of seeds per fruit and final fruit size [12]. Typically cultivated fruit reaches the mature green stage in 35-50 days after the flower is pollinated. The fruits are characterized as non-climacteric in ripening [13]. Pepper tolerates a wide range of soil and climatic conditions. According to Kelly and Boyhan [14] pepper grows best in deep medium textured sandy loam or loamy, fertile, well-drained soils that are friable. If not irrigated, an annual rainfall of at least 600 mm is required for optimum growth and reproduction. Water-logging causes poor fruit setting, diseases and fruit rotting. A pH range of 6.5-7.5 is optimum for its production. Kodama *et al*. [15] reported that low soil pH and magnesium deficiency can result in stunted plants growth. Low pH can also contribute to toxicity from aluminium and severe flooding or drought which is injurious to plant growth.

Fig. 1: *Capsicum chinenes nsukka drilus* plant

Fig. 2: Longitutidinal section of Capsicum chinense nsukka drilus fruit

Significance of the Study: Capsainoids, which are phenolic compounds, have been created for the characteristic hotness (Pungency) of peppers of solanaceae family. This hotness makes it good anti-inflammatory agent. Together with carotenoids, the content of phenolic compounds in capsicum species has been correlated with its pharmacological activities, especially antioxidant activity [16]. The studies carried by Mai Hazekawa *et al*. [17] and Marcelis *et al*. [18] have shown the uniqueness of *Capsicum chinense nsukka drilus* in pungency and carotenoid content. They maintained that the pungency level is higher in it than found in atarugu, Miango and tatase. This makes its standardisation is necessary for proper utilisation in disease management.

Aim of this Study: Owing to the diversity of *Capsicum chinense nsukka drilus* and widespread uses in ethno-medicine, the aim of this study is to investigate its

phytochemical constituents and standardise it for easy identification, usage and possible inclusion in the herbal pharmacopeia.

MATERIALS AND METHODS

Collection and Identification: The leaf and fruit of *Capsicum chinense nsukka drilus*, were obtained from the local farmer in Ozalla Nsukka, Nsukka Local Government Area of Enugu State, Nigeria. These were identified by Mr. A.O. Ozioko, a taxonomist with Bio-resources and Development Centre Programme (BDCP), Nsukka.

Preparation: The fresh fruits and leaves of *Capsicum chinense nsukka drilus* were collected, dried under shade for fourteen days and pulverised. Fresh fruits and leaves samples were used for macroscopic study and for transverse sectioning, while the powdered fruits and leaves were used for microscopic study, analytical standardisation and preliminary phytochemical analysis.

Phytochemical Analysis: Phytochemical tests were performed on the powered seeds, rind and leave samples in order to detect the presence or absence of major secondary plant metabolites of Pharmcognostic importance which include, alkaloids, steroids, tannins, saponins, resins, flavonoids, oils, etc., following standard procedure [19].

Test for Carbohydrates: A 0.1g of the powered material was boiled with 2 ml of water and filtered. To the filtrate, a few drops of naphthol solution in ethanol (Molisch's reagents were added). Concentrated sulphuric acid was then gently poured down the side of the test tube to form a layer. A purple interfacial ring indicates the presence of carbohydrates.

Test for Reducing Sugar: A 0.1g of the power was shaken vigorously with 5ml of distilled water and filtered. The filtrate was used in the following test:

Fehling's Test: A 1ml portion of the filtrate was added equal volumes of Fehling's solution A and B and boiled on water bath for few minutes. A brick red precipitate indicates the presence of reducing sugar.

Benedict's Test: A 1ml portion of the filtrate was added to 2 ml of Benedict's reagent. The mixture was shaken and heated on water bath for 5 minutes. A rusty brown precipitate indicates the presence of reducing sugar.

Test for Alkaloids: A 20ml of 5% sulphuric acid in 50% ethanol was added to about 2g of the powered material and heated on a boiling water bath for 10 minutes, cooled and filtrate tested with the following reagents: Mayer's reagent, Dragendorff's and 1% picric acid solution. The remaining filtrate was placed in 100ml separating funnel and made alkaline with dilute ammonia solution. The aqueous alkaline solution was separated and extracted with two 5ml portions of dilute sulphuric acid. The extract was tested with a drop of Mayer's, Wagner's and Dragendorff's and picric acid reagents. Alkaloids give; cream precipitate with one drop of Mayer's reagent, brick red precipitate with one drop of Dragendorff's reagent, orange precipitate with one drop of Wagner's reagent yellow precipitate with one drop of picric acid.

Test for Glycosides: A 5ml of dilute sulphuric acid added to about 0.1g of the powder in a test tube and boiled for 15 minutes on a water bath, then cooled and neutralized with 20% potassium hydroxide solution. 10ml of a mixture of equal parts of Fehling's solution A and B was added and boiled for 5 minutes. A dense brick red precipitate indicates the presence of glycosides.

Test for Saponins: A 20 ml of water was added to 0.25g of the powdered plant material in 100 ml beaker and gently boiled on hot water bath for 2 minutes. The mixture was filtered while hot and allowed to cool. The filtrate was used for the following test:

Frothing Test: A 5ml of the filtrate was diluted with 20ml of water and shaken vigorously. A stable froth (Foam) upon standing indicates the presence of saponins.

Emulsion Test: Two drops of olive oil were added to the frothing solution and the content shaken vigorously. The formation of emulsion indicates the presence of saponins.

Haemolysis Test: 1 ml of the filtrate was added to 1 ml of a solution of blood in normal saline. Haemolysis occurring (Shown by sedimentation of the cells would indicate the presence of saponins).

Test for Tannins: A 1 g of the powdered sample was boiled with 50 ml of water, filtered and the filtrate was used for the following tests:

Ferric Chloride Test: To 3 ml of the filtrate, a few drops of ferric chloride were added. A greenish black precipitate indicates the presence of tannins.

Lead Acetate Test: A few drops of lead acetate were added to 3 ml of the filtrate, a cream precipitate appearing at the interface indicates the presence of tannins.

Test for Flavonoids: A 10 ml of ethyl acetate was added to 0.2 g of the powdered plant material and heated on water bath for 3 minutes. The mixture was cooled, filtered and filtrate used the following tests.

Ammonium Test: A 5 ml of filtrate was shaken with 1ml of dilute ammonia solution. The layers were allowed to separate and yellow colour in ammonical layer indicates the presence of flavonoids.

1% Aluminium Chloride Solution Test: A 5 ml portion of the filtrate was shaken with 1ml of 1% aluminium chloride solution. The layers were allowed to separate. A yellow colour in the aluminium chloride layer indicates the presence of flavonoids.

Test for Resins: A 0.2g of the powdered material was extracted with 15 ml of 90 % ethanol. The alcoholic extract was then poured into 20 ml of distilled water in a beaker. A precipitate occurring indicates the presence of resins.

Test for Protein: A 0.2g of the powered material was placed in a test tube and 10 ml of water added. The mixture was heated on a water bath for 15 minutes, cooled and filtered. The filtrate was used for the following tests.

Million's Test: Two drops of million's reagent was added to a little portion of the filtrate in a test tube. A white precipitate indicates the presence of proteins.

Xanthiproteic Reaction Test: A 5 ml of the filtrate was heated with few drops of concentrated nitric acid a yellow colour which changes to orange on addition of an alkaline indicates the presence of proteins.

Picric Acid Test: Few drops of picric acid were added to a little portion of the filtrate in the test tube. A yellow precipitate indicates the presence of proteins.

Test for Oil: A O.1g of the powered materials was pressed between fitter paper and the fitter paper observed. Translucency of the filter paper indicates the presence of oil.

Test for Steroids: A 9 ml of ethanol was added to 1g of the powder and refluxed for a few minutes and filtered. The filtrate was concentrated to 2.5 ml on boiling water bath and 5 ml of hot water was added. The mixture was allowed to stand for 1 hour and waxy matter filtered off. The filtrate was extracted with 2.5 ml of chloroform using separating added to 0.5 ml of the chloroform extract in a test tube to form a lower layer. A reddish brown interface shows the presence of steroids.

Test for Terpenoids: Another 0.5 ml of the chloroform extract from (K) above was evaporated to dryness on a water bath and heated with 3 ml of concentrated sulphuric acid for 10 minutes on a water bath. A grey colour indicates the presence of terpenoids.

Test for Acidic Compounds: A 0.1 g of the powdered material was placed in a clean dry test tube and sufficient water was added. This was warned in hot water bath and then cooled. A piece of water wetted litmus paper was dipped into the filtrate and colour change on the litmus paper observed.

Structural Standard/Qualitative and Quantitative Analysis: Structural standard parameters include: the macroscopic and microscopic characteristics. The macroscopic characteristics comprise organoleptic features (Taste, odour and colour) and morphological characters (Physical appearance-texture, fracture etc.) of the crude drug.

The microscopic characters include tissue and cellular compounds. In microscopy we have quantitative and qualitative microscopy.

Macroscopic Analysis: The fruits were examined visually. The macroscopic characters of the leaf which include the type of the margin, petiole, venation, base and so on were observed and noted. Also macroscopic features such as size, shape, surface characters, fracture and texture of the fruits and leaf were observed. Finally, the organoleptic properties like colour, odour and taste of both the leaf and fruits were observed and noted.

Microscopic Examination of Powdered Materials: Little quantity of the powdered crude drug was placed on a slide and two drops of chloral hydrate were added to moisten the powered drug. It was covered with cover slip and passed across the flame of Bunsen burner repeatedly until bubbles occur. Then it was allowed to cool. Two drops of glycerine were added for clarity of structure and the slide was viewed under microscope to reveal microscopic characters which were observed and noted.

Microscopically Examination of Transverse Sections: The staining method was described by Onwubuya *et al.* [20]. Sledge microtone was used for sectioning of the specimen. The sections were transferred into staining jar and stained in safranin for 5 minutes. The safranin was drained off and sections were washed about three times with distilled water. Then 97% alcohol was used to wash the sections for two times each. The sections were counter stained in 1% past green for 5 minutes and washed with absolute alcohol for about three to four times.

After that, the sections were transferred into a staining jar containing 50/50 alcohol/xylene and washed until they became clear. Finally, the pure xylene was used to clear the sections and the Canada balsam mountant was used to mount the sections on the slide.

Chemo Microscopic Examination of the Powdered Sample

Test for Cellulose

Method: A little quantity of the powdered drug was placed on a slide and two drops of iodinated zinc chloride solution (20g of Zinc chloride in 8.5ml of water + 1g potassium iodide and 0.5g of iodine in 20 ml of water) was added to the slide. A cover slip was used to cover the slide and then viewed under a light microscope to observe the individual colour changes.

Test for Secretory Cells and Ducts

Methods: A little quantity of the powdered drug was mounted on a slide with two drop of Sudan III solution (Prepared with equal parts of glycerine and alcohol). The slide was covered with a cover slip, viewed under a light microscope and colour was noted.

Test for Fibres

Method: A little quantity of the powdered drug was mounted with saturated aqueous solution of picric acid and allowed to stand for 5 minutes. The slide was irrigated with water and examined under a light microscope and colour noted.

Test for Calcium Oxalate Crystals

Methods: A little quantity of the powdered drug was mounted with 80% H_2SO_4 and examined under a light microscope and the colour was noted.

Test for Tannins: Method A little quantity of the powdered drug was mounted with ferric chloride solution and examined under a light microscope and the colour was noted

Quantitative Phytochemical Analysis

Tannins Estimation: Five hundred gram of each of the powdered samples were weighed and put into 100 ml of plastic bottle. 50 ml of distilled water was added and shaken for 1 hr in a mechanical shaker. This was filtered into a 50 ml conical flask and made up to the mark. Then 5 ml of the filtrate was pipetted out into a tube and missed with 3 ml of 0.1N FeCl3 in 0.1N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 530nm wavelength, within 10minutes. A blank sample was measured at the same wavelength [21]. Note: the blank sample was used to bring the spectrophotometer to zero for direct measurement of the sample extract. Absorbance percentage weight of Tannins.

$$= \frac{absorbance\ of\ sample}{absorbance\ of\ standard} X \text{ concentration of standard}$$

where, absorbance standard = 0.740 and
concentration of standard = 50 mg/dl

Total Saponins Estimation: The saponins content of the sample was determined by double extraction gravimetric method [22]. 100 g of the powdered sample was mixed with 100ml of 20% aqueous ethanol solution in a flask. The mixture was heated with periodic agitation in water bath for 90 minutes at 55°C; it was then filtered through Whitman's filter paper (No 42). The residue was extracted with 50 ml of 20% ethanol and both extracts were poured together and the combined extract was reduced to 40 ml at 90°C and transferred to a separating funnel where 40 ml of diethyl ether was added and shaken vigorously. Separation was by partitioning during which it done repeatedly and discarded and the aqueous layer reserved. Re-extraction by partitioning was done repeatedly until

the aqueous layer became clear in colour. The saponins were extracted with 60 ml of normal butanol. The combined extracts were washed with 5% aqueous sodium chloride solution and evaporated to dryness in a pre-weighed evaporation dish. It was dried at 60°C in the oven and reweighed after cooling in desiccators. The process was repeated two more times to get an average. Saponins content was determined by the difference and calculated as a percentage of the original sample thus:

$$\% \ saponins \ = \frac{(W2 - W2)}{Weight \ of \ the \ sample} \times 100$$

where, w1 = weight of of evaporating dish
w2 = weight of dish + sample

Flavonoids Estimation: Flavonoid content of the leaves of the plant was determined by the gravimetric method described by Thakkar and Ray [23]. A 5g of the powdered sample was placed into a conical flask and 50ml of water and 2 ml 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°C. It was cooled in desiccators and weighed. The quantity of flavonoid was determined using the formula.

$$\% \text{ of Flavonoid} = \frac{w2 - w1}{w3} \times 100$$

where: W1 = Weight of empty filter paper W2 = weight of paper +flavonoid extract and W3 = initial weight of the samples.

Total Alkaloids Estimation: A 5g of the sample was weighed into a 350 ml beaker and 200 ml of 20% acetic acid in ethanol was added and covered to stand for 4hours. This was filtered and the extracts were concentrated using a water bath to one quarter of its original volume. Concentrated ammonium hydroxide was added in a drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration using filter paper and weighed [24, 25]. The percentage weight of alkaloid was derived using the formula stated below:

$$\% \text{ of Alkaloid} = \frac{w2 - 1}{w3} \times 100$$

where W1 = weight of filter paper, W2 = weight of residue + filter paper, W3 = weight of the sample [26]

Determination of Analytical Standards

Determination of Ash Values: The ash content of a crude drug is generally taken to be residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but may also include inorganic matter added for the purpose of adulteration. An ash determination furnishes a basis for judging the identity and cleanliness of a drug and gives and gives information relative to its adulteration with inorganic matters. The method adopted for the determination of values follow the specification given by Vishal *et al.* [27], Wallis [28] and WHO [29].

Total Ash: A total ash value represents the amount of the residual substance not volatilized on ignition at 450°C. It is used to exclude drugs which have been coated with chalk, lime or calcium sulphate to improve their appearance.

Method: A tarred nickel crucible was ignited to a constant weight at a dull red heat. Cooled and stored in a desiccators. 2 g of the powdered materials was weighed into the nickel crucible and heated gently until all the moisture had been driven off and the materials had been completely charred. The heat was increased until most of the carbon had been vaporized, after which the materials were heated to about 450°C to make the residue carbon free. The residue was cooled and weighed. The heating and cooling were continued until a constant weight was achieved.

Acid Insoluble Ash: This acid insoluble means the ash insoluble in dilute hydrochloric acid and is often of more value than total ash value. Majority of drugs contain calcium oxalate, but in variable amounts. A total ash value therefore may vary within wide limits for specimens of genuine drug. Total ash is therefore useless in the detection earthly matter(s) adherent to such a drug. Since the calcium oxalate or carbonate yielded by the incineration is soluble in hydrochloric acid, one can therefore remove all the variable constituent of the ash by means of dilute hydrochloric acid and weighing the residue which is known as acid-insoluble ash. This way,

one can obtain evidence of the presence of excessive earthly matter (s), which is likely to occur with leaves that are densely pubescent, clothed with abundant trichomes or those that tend to retain earthly matter(s) splashed on them during heavy rain fall.

Method: The total ash obtained from (a) above was transferred to a beaker containing 25ml dilute 30% hydrochloric acid, heated to a boiling on a water bath for 5 minutes and filtered with an ash-less filter paper. The beaker and crucible were made free of acid. The filter paper was dried in an oven, folded into a narrow cone, inserted weighed a started nicked crucible and heated at 150°C until it was completely washed. The residue was then heated more strongly and cooled in desiccators after which the crucible was re-weighed.

Water Soluble Ash: This is used to detect the presence of materials exhausted by water. The water soluble ash is subjected to a much greater reduction than the total ash and is therefore, used as an important indication of the presence of exhausted materials substituted for the genuine article.

Method: A nicked crucible was ignited to a constant weight at 450°C and reweighed after 2 g of the powdered materials had been put into it. The crucible with the drug was ignited at low heat, initially to burn off the carbon content. The heat was gradually increased until all the carbon was burnt off. The crucible was cooled in desiccators and reweighed and heating was continued until a constant weigh was obtained. The content of the crucible was transferred into a small beaker, 25 ml of distilled water was added and the beaker contents were boiled for 5 minutes and filtered through an ash-less filter paper. The filter paper together with the residue was dried in the oven and compressed into a small or narrow cone. This was then transferred into the crucible and heating was continued until the ash-less filter paper was eliminated, the acid weight was noted.

Sulphated Ash: Sulphated ash provides a more consistent ash. In this method, in this method, all oxides and carbonates are converted to sulphates at a higher temperature.

Method: A nicked crucible was ignited to a constant weight at a dull red heat in the oven. 2 g of the powdered plant materials was re-weighed. The materials was moistened with the dilute sulphuric acid and ignited at low heat initially to burn off the carbon content.

The crucible was cooled in desiccators. More dilute sulphuric acid was added and heating continued to about 80°C with occasional cooling and reweighing until a constant weight was obtained.

Determination of Extractive Yields/Values: The determination of alcohol-soluble extractive and water soluble extractive is used as a means of evaluating drugs, the constituents of which are not readily estimated by other means. In some cases, the amount of a drug soluble in a given solvent is an index of its purity. The methods used here are in conformity with the recommendation of the Wikipedia [30] and Zaki *et al*. [31].

Method: A 5 of the powered plant materials was weight accurately and placed in a 250 ml stoppered conical flask and then 100 ml of 90 % alcohol was added. The stopper was firmly replaced and the contents of the task were shaken mechanically for 6 hours and allowed to macerate for a further 18 hours, that is, for a total 24 hours and then filtered, 20 ml of the filtrate was evaporated to dryness in a 25 beaker over a water bath. The residue was dried to a constant weight at 105°C and then weighed.

Water Soluble Extractive: A 5 g of powdered plant materials was weighed accurately and placed in a 25 ml stoppered conical flask. 100 ml of distilled water was added and the stopper was replaced firmly. The contents of the flask were shaken mechanically for 6 hours and were allowed to macerate for a further 18 hours that is a total of 24 hours and then filtered. 20 ml of the filtrate was evaporated to dryness in a 25ml beaker over a water bath. The residue was dried to a constant weight at 105°C.

Determination of Moisture Content: There are many techniques available; they include:

Loss on Drying: This involves drying at a specified temperature for a given period and weighing until a constant weight is obtained.

Toluene Distillation Method: This has the advantage of eliminating the error which results due to the interference by volatile substance contained in the drug.

Kal-Fisher's Method: This involved a chemical reaction between water contained in the drug and the iodine and sulphur dioxide in the presence of pyridine methanol solution.

Only one technique which is loss on drying was used to determine the moisture content of the plant materials due to lack of equipment and high cost involved in carrying out the other techniques.

Method of Loss on Drying: A tarred evaporating dish was heated to a constant weight and stored in desiccators. 2g of the powered plant was added to the dish and kept in an oven maintained at a temperature of 105°C. It was allowed to dry until a constant weight was achieved. The difference in weight of the evaporating dish was noted.

RESULTS

Organoleptic Analysis: This study revealed the presence of characteristic sharp aroma by the fruit. However, the leave presented almost completely opposite of these feature. The Flavour of the fruits was intensely pungent, while leaves were not; they were a little beat salty.

Macroscopic Analysis: The leaves are simple, alternate, very variable and almost glabrous with pale to dark green. The margin is entire and apex aristae. The venation is network with a sheathing leaf base. The fruits are yellowish when ripe but greenish when unripe and yellowish-brown when dry. They are oblong-conically shaped, varied in size and shining when fresh. The powdered dry fruits were brown and shining.

Microscopic Analysis

Microscopy of Seed

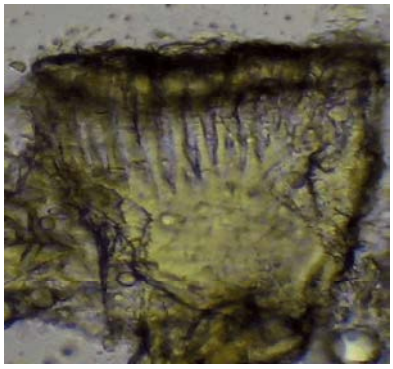

Fig. 3: Fragment of epidermal cell

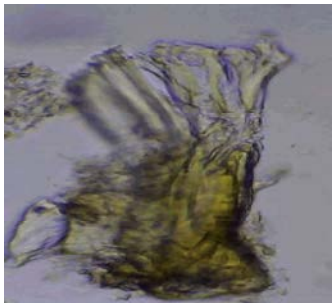

Fig. 4: Group of sclerenchymatous cells

Fig. 5: Unicellular Trichomes

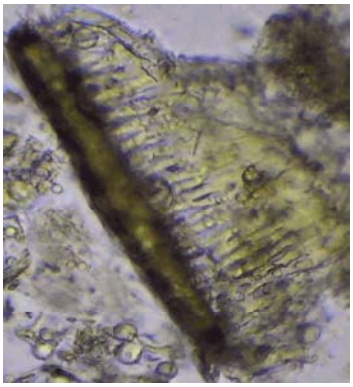

Fig. 6: Scalariform vessels

Microscopic Feature of the Fruit Rind

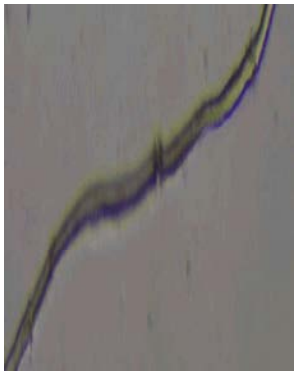

Fig. 7: Enlongated unicellular trichome

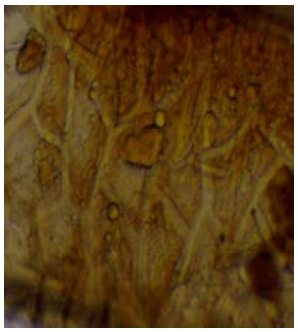

Fig. 8: Epicarp X 100

Fig. 9: Epidermal cells with stomata

Fig. 10: Isolated sclereid cell with pitted lumen

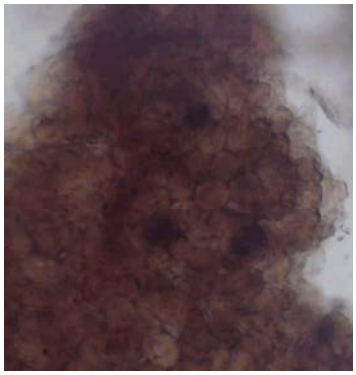

Fig. 11: Palisade cells *X100*

Microscopy of Powdered Leafs

Fig. 12: Annular xylem vessel

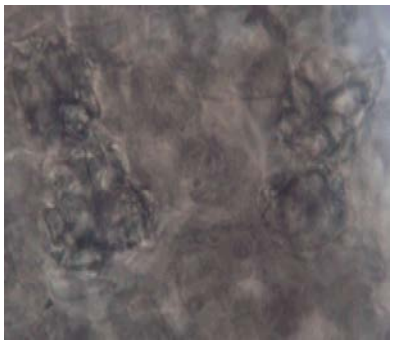

Fig. 13: Cluster of calcium oxalate

Fig. 14: Palisade cells

Fig. 15: Epidermal Cells Showing Anomocytic Stomata X100

Fig. 16: Fractured non-granular multicellular trichome

Fig. 17: Group of parenchyma cells

Fig. 18: Isolated calcium oxalate

Fig. 19: Phloem parenchyma cells X10

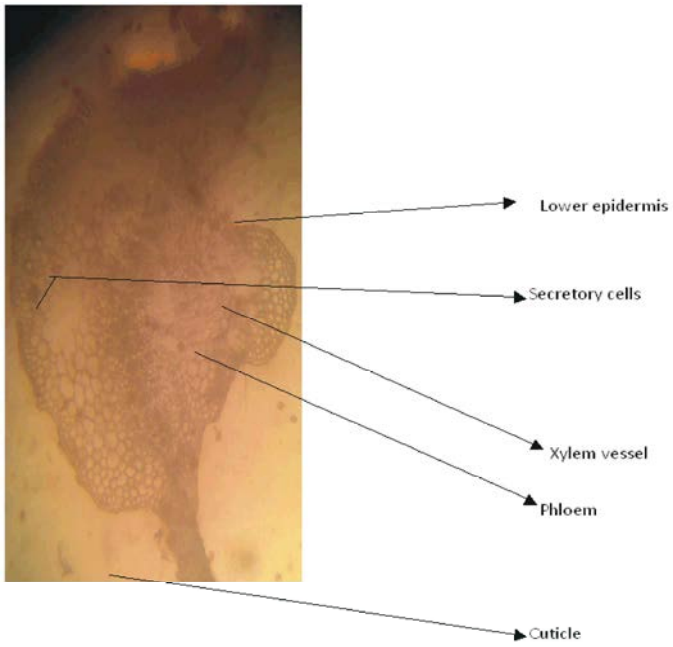

Fig. 20: Section of *Capsicum chinense nsukka drilus* leaf X100

Table 1: Results of Qualitative Phytochemical Analysis

	Inference		
Metabolite	Leaf	Rind	Seed
Carbohydrates	++	+	+
Reducing sugars	+++	++	++
Alkaloids	-	++	+++
Glycosides	-	-	-
Saponins	+	+	+
Tannins	++	+	+
Flavonoids	-	+++	+++
Resins	-	-	-
Proteins	+	+++	++
Oils	++	+++	+++
Steroids	-	+	+
Terpenoids	-	+++	++

Key: + = slightly present; ++ = moderately present; +++ = highly present; = absent

Table 2: Result quantitative phytochemical analysis

Phytochemical	Value in mg/5g of powdered leaf	Value in mg/5g of powdered rind	Value in mg/5g of powdered seed
Flavonoids		29.2±0.76	27.2±0.48
Alkaloids		58.4±0.67	52.7±0.23
Tannins	9.32±4.1	6.6±1.22	6.6±1.22
Terpenoids		8.4±0.8	8.4±0.8
Saponins	6.2±1.6	6.1±2.1	5.4±2.4

Values expressed as Mean weight (mg/5g) ± SD n = 3

Table 3: Results of analytical standard

		Values %w/w	
	Standard Ash values	Fruit	Leaf
A	Total ash	13.37	18.80
B	Acid insoluble	0.37	0.41
C	Sulphated ash	4.95	5.55
D	Water soluble ash	8.45	9.89
	Extractive yields		
A	Alcoholic-soluble extractive	0.40	1.50
B	Water-soluble extractive	1.60	2.15
C	Moisture content	0.95	0.85

DISCUSSION

Identification, examination and evaluation of the leaf and fruit of *Capsicum chinense nsukka drilus* and the various features associated with them were carried out by various analysis and tests performed. Organoleptic study is very necessary in crude qualitative identification as it demonstrates the sensory profile of the drugs. The fruit of *Capsicum chinense nsukka drilus* was very pungent and sternutatory, while the leaf was not.

The microscopic analysis showed the presence of unicellular trichomes and distinct parechymatous cells as unicellular clothing of trichomes was very distinguishing features of the seeds. The rind showed similar features, except that there wer presence of stomata of anomocytic type. The prominent features of the leaf were the presence of annular vessel (Which was very conspicuous) anomocytic stomata, granular trichomes, phloem cells and cluster of calcium oxalate crystals. Preliminary phytochemical analysis revealed that the fruit of the plant possesses carbohydrates, alkaloids, tannins, flavonoids, proteins, steroids and terpenoids. It also contained oils, acidic compounds and reducing sugars. This result is consistent with the report [9] on their study of Capsicum spp. Consequently, carbohydrate, tannins, proteins, oils, acidic compounds and reducing sugars were also found in the leaf, but at a smaller quantity. However, alkaloids, flavonoids and terpenoids were not present in the leaf. Each of these phytochemicals is known for various protective and therapeutic effects. For instance, capsaicin, which is a major alkaloid found in pepper is created to exert anticancer, anti-inflammatory and anti-diabetic effects, as well as analgesic activity [12]

The result of the macroscopic examination revealed the physical appearance of the leaf and seed, which can be seen with the naked eyes and cannot be relied on solely for the identification of the plant since other plants could possess similar morphology but it gives the idea of the fruit and leaf morphology. The microscopic investigation revealed microscopic features that can be found in fruit and leaf of the plant which can therefore be used as diagnostic tools in the identification of the different morphological parts.

Quantitative phytochemical analysis of *Capsicum chinense nsukkadrilus* revealed the various amount of phytochemical substances found in it. Alkaloids had the highest yield followed closely by flavonoids in the fruit (Seed and rind) but were not in the leaf.

The ash values and extractive values as well as the moisture content, which are useful in the determination or detection of drug adulteration, were also determined. The standardisation of crude drugs, both analytical and physical standards has become necessary and important because they are used in quality control, especially to determine the quality, purity, strength and if adulterated, the nature of adulterants [22]. It has been used also to determine the percentage yield of the crude drug materials as was indicated in the work and also in solving the problem of spoilage of herbal drugs.

CONCLUSIONS

It can be seen from this study that the fruit and leaf of *Capsicum chinense nsukka drilus* possess phytoconstituents which account for its usefulness as a medicinal plant.

The study showed that standardisation is very necessary in the determination of the quality, purity and strength of crude drugs as well as the nature of the adulterants, if any. The analytical standards were determined and methods used were adequate within the limits of experimental error.

Finally, the fruit and leaf of *Capsicum chinense nsukka drilus* which is commonly called Nsukka-yellow pepper has been identified evaluated and standardised and may possibly qualify to be included in the pharmacopoeia of herbal medicine.

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