Phytochemical and Proximate Composition of Cucumber (*Cucumis Sativus*) Seed Oil

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***Abstract:* The phytochemical and proximate properties of cucumber (*Cucumber Sativus*) seed oil had been evaluated and characterized for its suitability in pharmaceutical, foods, and skin-care product formulations. The seed-oil was isolated using acetone and ethanol with an automated soxhlet extractor, while, the triplicate values of the oil yield were calculated, according to AOAC standard. The moisture content of 7.21 %; ash content of 5.48 %; crude fibre of 27.8 %; crude protein of 4.50 %; crude fat is 2.143 %, carbohydrate content of 52.86 %, and other physiochemical properties of the oil were determined. Some good health support inorganic elements identified include, Potassium (6.830 ± 0.0040); Sodium (7.2287 ± 0.0058); Iron (6.2213 ± 0.0043) and Arsenic(3.1113 ± 0.0003). Major phytochemical compounds identified in both ethanol and acetone extracts are saponins, terpenoids, phytosterols and flavonoids. The Viscosity of 7.066, Specific gravity of 0,882 g/cm^3, Bulk density of 0.393 g/cm^3 and Tapped density 3 0.680 g/cm^3, are good properties of edible oil, if further purified. The Phytochemicals and other inorganic elements identified revealed that cucumber seed oil can serve as raw materials for pharmaceutical and cosmetic formulations.**

***Keywords:* Cucumber seed, Phytochemicals, Proximate properties, Solvent- extraction.**

I. INTRODUCTION

Cucumber (*Cucumis Satitivus* L.), which belongs to the family of *Curcurbitaceae*, is widely cultivated as vegetable in all parts of the world, according to Paris et al, (2011) and Vivek et al, (2017). Cucumber is the fourth most important vegetable crop after tomato, cabbage and onion, Tatligou,(1993) and Uzuazokaro et al, (2018). The plant has large leaves that form canopy over the fruits. Cucumber fruit grows from its flowers and contains dozens of seeds that may be used to cultivate future generation of cucumber plants. Cucumber fruit, which is roughly cylindrical, elongated with tapered end is about 96 % of water content, and is always eaten fresh, when unripe, because, ripe ones are usually bitter and sour. The fruits could also be used in dishes, like deserts, pastries, syrups, sauces, smoothies, etc. People tend to discard the seeds as wastes, after enjoying the juicy fruits. Cucumber fruits could be beneficial for hydrated health cases, due to its high water content. The water extract from *C. sativus* fruits had been reported as good additive in soap and detergent formulation, Monice et al, (2014). They also reported that some industrial products contain *(Cucumis Sativus*) fruit water extract, which could be used in baby skin's lotion formulations, around eye area or mucous membranes to remove black-shades, in cosmetic spray products such as face, neck, body, hand sprays, and could possibly be inhaled.

Studies had shown that, most ingredients derived from cucumber fruits can function well as skin-conditional agents.

More researches had been carried out on the juicy cucumber fruits than on the seeds, which people discard indiscriminately, because, there is not yet an economical value for it. Researches had shown that, extracts from cucumber juice had some medicinal applications, but, yet from the seed oil. However, the calorie and nutritional values of cucumber fruits had been reported to be low, although, it is a primary source of vitamins and minerals in human diets (Mandey *et al.,* 2019). Furthermore, Grubben and Denton, (2004) and Wang *et al.*, (2007), in their studies observed that, *C. sativus* juice contains some essential vitamins and antioxidants which are effective to human healthy life. Kumar et al, (2010) discovered also that cucumber seed oil contains tocopherols and tocotrienole, which are organic fat-soluble compounds that are referred to as vitamin E. The high content of potassium (50-80mg/100) of cucumber seed makes it highly useful for both low and high blood pressures (Kashif *et al.*, 2008).

Reports had shown that Cucumber seed oil contains polyunsaturated fatty acids which alleviate cardiovascular diseases, some cancers and arthritis (Ursin, 2003). The seeds contain oleic acids which is a diuretic (Wright et al., 2007) which reduces blood pressure (Teres *et al.*, 2008) and also prevents cancer (Waterman and Lockwood, 2007). *C. sativus* oil decreases cholesterol and hence can reduce cardiovascular diseases (Achu et al., 2008).

It had been thought of that cucumber seeds could be munched while enjoying the softy fruits without any oral effect, such as, irritation, sensitivity, toxicity, etc. Therefore, it has been considered that, some chemical components, active ingredients or derivatives can be isolated from the cucumber seeds, and be used as raw materials in cosmetics, pharmaceuticals or food industries. Kumar *et al.*, (2010) in their studies, suggested that cucumber seed could consist of some major phytochemicals such as, taninns, flavonoids, phytosterols, etc.

The aim of this study is to determine the phytochemicals, heavy metals, natural products of cucumber seed oil and to characterize the seed oil as a potential bioactive compound for cosmetics, drug formulation, or as edible oil.

II. MATERIALS AND METHODS

2.1.1 Cucumber Seed oil Extraction

Matured cucumber (*C. sativus*) fruits were obtained from the Ministry of Agriculture, Awka, Nigeria. The seeds were isolated from the fruits, washed and sun-dried for 7 days. An electric grinding machine was used to crush the seed samples into powdered form. The oil extraction was carried out according to the standard of Association of Official Agriculture Chemists (AOAC, 2000), using ethanol and acetone solvents and an automated soxhlet extractor used at different boiling temperatures.

2.1.2 Determination of Proximate Chemical Composition

These analyses were carried out to determine the following;

i. Percentage moisture content of the oven-dried seeds sample was determined and calculated according to American Society for Testing and Materials, (ASTM D4442).

ii. The percentage ash content was determined according to AOAC (2000) standard and calculated using the formular (a):

$$\text{Percentage (\%) Ash content} = \frac{w_2 - w_0}{w_1 - w_o} \times 100 \text{ ----------------(a)}$$

Where: w_0 = weight of empty crucible(g) ; w_1= weight of crucible with powdered sample (g); w_2 = weight of crucible and ash sample(g).

iii. Crude fibre, crude protein, crude fat, carbohydrate, bulk density and tapped density and other mineral contents were carried out accordingto,(AOAC(2000) standard.

The percentage (%) crude fibre was calculated using the formular according to AOAC,

(2000), as shown in (b) Crude fibre= $\frac{w_1 - w_2}{w_0} \times 100$ -----(b)

Where: w_0 = weight of seed sample before drying; w_1 = weight of oven-dried sample;

w_2 = weight of seed-ash sample.

Crude Protein: This is the measurement of the amount of Protein content by determining the Percentage of analyzable Nitrogen content of the seed- oil. This was determined using Kjeldahl method and calculated as:

Percentage crude protein = % Nitrogen × 6.25 ----------(c)

Where: 6.25 is the protein conversion factor.

The mineral Nitrogen content was obtained by Kjeldahl method, as calculated in (d) below, before determining the crude protein content using formular (c)

$$\text{Note: \% Nitrogen} = \frac{(A-B) \times 1.4007}{Wt.\ of\ sample} \times \frac{100}{1} \text{ -------------- (d)}$$

Where: A is vol. (mls) of HCl × Normality of HCl

B is vol. (mls) of NaOH × Normality of NaOH

V. Crude Fat: This was determined using AOAC methods(2003) and calculated as in (e).

$$\text{\% Crude fat} = \frac{F - T}{S} \times \frac{100}{1} \text{ -------------- (f)}$$

Where: F is weight of cup + fat residue (g); T is the weight of cup (g);

S is test portion weight (g).

2.2 Physicochemical Characterization

The physico-chemical properties of the seed oil, such as, specific gravity, viscosity, free fatty acid, acid value, iodine value (wjis), peroxide value and saponification value (mlKOH/g) were determined according to the their respective standard methods as follows:

i. Specific Gravity

This was carried out at 20 ^{0}C using weighed density bottle according to MIl standard (MIL 45662 A) and calculated as

$$\text{Specific gravity} = \frac{M_2 - M_1}{M_3 - M_1} \text{ ------------(h)}$$

Where: M_1 is weight of density bottle ; M_2 is weight of density bottle + oil ; M_3 is density bottle + water; $M_2 - M_1$ is weight of oil and M_3-M_1 is weight of water.

ii. Viscosity according to (ASTM D2162) Standard

This was determined using an Ostwald viscometer at 25 ^{0}C.

iii. pH Determination

The pH value of the sample oil was determined after standardizing the electrode with a buffer solution. The value was recorded accordingly.

iv. Fatty Acid Value

The free fatty acid value was determined according to CODEX STAN, 2- 1999) standard and calculated using the formular below:

$$\text{fatty acid vaue} = \frac{\mathbf{56.1 \times V \times N}}{\mathbf{w}} \text{ --------------(i)}$$

Where: V=volume of standard potassium hydroxide; N=Normality of potassium hydroxide;

and w= weight in gram of the oil sample.

v. Iodine Value

This was tested according to ASTM D5768-02(2018) and calculated using the formular

$$Iodine\ value = \frac{(b-a) \times 1.269}{wt.\ of\ sample} \text{-----------(j)}$$

Where: (titration volume = a ml); the blank was carried out at the same time commencing with 10 ml of carbon tetrachioride (titration – b ml).

vi. Peroxide value

The peroxide value was determined using Japanese Association of Oil Chemists: Standard methods of oil analysis in Japan, (1972).

vii. Saponification value (ml KOH/g)

This was carried out according to ISO(International Standard Organization, 2020) for fats and oils and calculated as:

$$Saponification = \frac{(b-a) \times 28.05}{wt.\ of\ Sample} \text{----------------(k)}$$

Viii. Percentage yield determination (Wernerova and Hudlicky, 2010).

The percentage yield of the oil was determined using the formlar:

$$Percentage\ oil\ yield = \frac{Weight\ of\ extracted\ seed\ oil}{Weight\ of\ sun-dried\ sample} \times 100 \text{-------(l)}$$

ix. Refractive Index

Abbe refractometer was set with a light compensator (water at 20^{o}C) and the oil sample was smeared on the lower prism of the instrument and closed. The reading was taken according to standard.

x. Acid Value

25 ml diethyl ether was mixed with 25 ml ethanol and 1 ml of plenolphthalein carefully neutralized with 0.1M NaOH. 3g of th oil was dissolved in the above material and titrated with aqueous 0.2 M NaOH shaking constantly until pink colour which persisted for 15 seconds was obtained.

$$Acid\ value = \frac{Titre\ value\ (Cm\ 3) \times 5.61}{Weight\ of\ the\ smaple} \text{------------------}(m)$$

2.3 Phytochemical Screening

The phytochemical screening of the extracted seed oil was carried out to evaluate the plant nutrients or phyto-components present in the sample. Some of the parameters tested for include:

i. Presence of Alkaloid using (Wagner test)

This was carried out using few drops of Wagner's reagent (A solution of iodine and potassium iodide). Alkaloids give a reddish brown precipitate with the reagent.

ii. Test for Flavonoids (Shinoda Test)

A piece of magnesium ribbon was added in Conc. HCl and mixed with 2 mls of the oil sample. Formation of a pink colour after few minutes indicates presence of flavonoids.

iii. Presence of Terpenes (Salkowski Test)

2 mls of chloroform and 3 mls of Con. H_2SO_4 were each added to the oil extract. Formation of a yellow ring at the interface of the two liquids which turns reddish brown colour later, shows presence of triterpenoids or terpenoids.

iv. Protein Contents Using Biurret Test

1 % NaOH (Sodium hydroxide) solution was added to the oil sample, followed by few drops of aqueous 1% Copper (II) Sulphate solution. The appearance a purple solution indicates the presence of protein.

v. Determination of Phenol using 10 % $FeCI_3$

10 % of Ferric solution was prepared and diluted with 0.1 M of NaOH solution, drop by drop until a permanent brown colour appeared. This solution was filtered and the filtrate used for the phenol test. I ml of the ferric solution was added into 5 ml of each sample extracts. Appearance of fresh bluish-black color indicates the presence of polyphenols.

vi. Presence of Carbohydrate by Fehling Test:

5 mls of Fehling solution A and B were mixed; 2 drops of the oil were added and boiled. Brick red precipitate of cuprous oxide shows presence of carbohydrate.

vii. Test for Glycosides using Concentrate H2SO4.

To 5 mls oil sample, 2 ml of glacial acetic acid, one drop of 5 % Iron Chloride and 1 ml of Conc. H2SO4 were added. Presence of Glycosides is marked by the appearance of brown ring colouration in interphase.

viii. Starch was tested using drops of iodine solution (Harborne, 2005).

ix. Phytosterols (Liberman test, 2014)

Presence of phytosterols was determined using Solkowski method and Libermann Burchurd's test.

X. Amino acid (Ninhydrin Test)

The amino acid contents were determined by hydrolyzing the seed protein with 6M HCl for three hours. The amino acid was extracted and analyzed using HPLC (High –Performance Liquid Chromatographic methods).

III. RESULTS AND DISUSSION

3.1 Results of proximate analysis on the cucumber seed oil

The result on the moisture content (7.211%) of the cucumber seed as shown on Table 1.0, suggested that, solvent extraction method is suitable for the seed-oil isolation and ensures a longer shelf life or storage period, and easier for transportation, according to, Mahmoud, et al,(2014). He suggested also, that oil seeds with moisture content between 4.78 to 10 % could be used as edible oil, good in milling, has longer bench or storage life, and are easier to extract (Olayanju, 2006). The value is also good for metabolism activities in enzymes or co-enzymes. He reported that the moisture content of oil-seeds is an inevitable property that can affect harvesting and post-harvesting processes.

The result for the crude fibre, which is the cellulose and lignin content of the seed, is 27.80 % as shown in Table 1.0. This is a good dietary fibre value for most oil seeds, according to Olayinka, et al (2016). Weickert and Pfeiffer (2008) and Aina et al., (2012) had reported that dietary fibre can reduce the chances of gastro-intestinal problems, such as, constipation and diarrhoea, by increasing the weight and wetness of stool. (Yohanna, (2013) reported that C. sativus helps to overcome hypotonic which aids constipation. Our findings are therefore in line with their observation, with regard to the health benefits of cucumber seeds.

The percentage yield for cucumber seed oil was 48.7 %, as shown in Table 1.0, and is within the range of most seed oils, such as, moringa seed (40.60%), cashew seed (49.34 %), sesame seed (47.90 %) and is considered good for commercial purposes, according to AOAC, (1990). The crude protein which is the amount of mineral Nitrogen content of the seed is 4.40 %, showing a good source of protein when converted. The value of ash (5.48 %) as in Table 1.0, shows that cucumber seed does not contain much carbon compounds, thus, has good nutritional value and suitable for elemental analysis.

The results from the proximate analysis of the seed sample and oil were evidence that it could be good for human consumption and formulation of livestock feed meals. Carbohydrate content of the seed sample is considerably normal, therefore, could provide energy and strength when taken.

Some mineral elements are displayed in Table 1.0. The Iron content of 6.2213±0.0043 ppm was observed, which suggests that cucumber seed is a good source of iron. Iron is a vital property for haemoglobin formation and is also needed to transport oxygen in the body, (medicalnewstoday, 2/02/2021). The value for copper is 4.8183±0.0006 ppm and in conformity to other studies, where copper had been verified as a very necessary metal in anaerobic cytochrome oxidase metabolic activities of some enzymes. Researches had shown that cucumber seed oil is essential for adequate growth, lung elasticity, cardiovascular integrity, neuroendocrine function, iron metabolism, e.t.c, Georgatsou, (1997). Most plant nutrients, like carbohydrate, fat, proteins found in the seed are very essential for some physiological functions in human body system. The composition of other heavy metals, like lead, and Arsenic are discovered in a very minute quantity, while cadmium and cobalt are completely absent.

Table 1.0: Proximate Composition and Chemical Analysis of *C. Sativus*.

Constituents	Measured Value
Percentage yield (%) Moisture content (%)	48.7 7.211
Ash content (%)	5.48
Crude fibre (%)	27.81
Crude protein (%)	4.50
Crude fat (%)	2.14
Carbohydrate (%)	52.86
Bulk density (g/cm^3)	0.393
Tapped density (g/cm^3)	0.680
Inorganic components	Value (ppm)
Potassium	6.8309±0.0040
Sodium	7.2287±0.0058
Cadmium	ND
Cobalt	ND
Arsenic	3.1113±0.0003
Iron	6.2213±0.0043
Copper	4.8183±0.0006
Lead	0.0168±0.0007

NB: Ppm = parts per million ND = not detected

3.2 Results on the Phytochemical Compositions of Cucumber Seed Oil

There is high content of terpenoids from acetone and ethanol extracts, as shown in Table 2.0. This indicates that cucumber seed oil is rich in terpenoids, which had been studied as one of the primary metabolites that enhances the role of hormones in the body, components of electron transfer systems, protein modification, membrane fluidity determinants, anti-oxidants. These diverse roles have evolved early in the history of green plants and terpenoids had been reported to be good as an anti-inflammatory (Olorunju *et al.*, 2012), anti- viral anti- malarial inhibition of cholesterol synthesis (Njagi *et al.*, 2015) and anti- bacterial properties (Wadood *et al.*, 2013).

Phytosterols have been discovered in both acetone and ethanol extracts of cucumber seed, as shown in Table 2.0. Castro, *et al.*, (2005) stated that, phytosterols have significant hypocholesterolemic effect, and other researches had proved that phytosterols have several bioactive properties in human health. Elke and Isabelle (2007), suggested phytosterol can lower blood cholesterol effect via partial inhibition of intestinal cholesterol absorption. They suggested that daily intake (2 g) of phytosterols can lower the cholesterol absorption by 30-40%. Physterol had been suggested to have anti-atherogenic effects, stimulate immunity and anti-inflammatory activities. Plant sterols have been suspected as inhibitor against development of different types of cancers, like colorectal, breast and prostate cancers.

Milagros, et al (2011) also reported that phytosterol are known to reduce serum low-density lipoprotein cholesterol level without changing high-density lipoprotein cholesterol or triglyceride levels. They concluded that daily consumption of phytosterols-enriched foods is widely used as a therapeutic option to lower plasma cholesterol and atherosclerotic diseases. It also provides great anti-aging benefits, by slowing down of collagen production from ultraviolet exposure, thereby preventing photo-damaging of the skin. It can therefore help to generate new collagen which can make human skin glowing, firm and elastic.

Saponin, a biological active surfactant was observed in the two solvents' extracted oil samples, as stated in Table 2.0. Saponin has good emulsifying properties; they could precipitate and coagulate red blood (Okwu and Josiah, 2006). They exhibit foaming properties and cell memebrane-permeabilizing properties, (Chu *et al.,* 2002). Recently, there had been increased demand of saponin applications in various biological, medicinal, and pharmaceutical formulations. Our observation of saponin from Cucumber seed oil has added to the already existing sources of saponin as an important phytochemical, which has great commercial significance in foods, cosmetics, and pharmaceutical industries.

Flavonoids were also discovered from the seed oil, in both acetone and ethanol extracts, as shown in Table 2.0. According to Saxena, *et al.,* (2013) and Mandey et al., (2019), flavonoids have been reported to exert multiple biological property including anti-microbial, anti- oxidant, cytotoxicity, anti-inflammatory, as well as anti- tumour activity. Shashank and Abhay (2013), had reported that different types of flavonoids could be used in pharmaceutical purposes, while the cost-effective bulk production of flavonoids could be made possible with the help of microbial biotechnological approach. Many reviewers have highlighted the structural features of flavonoids, their beneficial roles in human health, and significance in plants as well as their microbial production. Determination of flavonoids as one of the phytochemicals in cucumber seed oil, had proved the seed, a good raw material for flavonoid-rich drugs, foods, cosmetics, etc.

Alkaloids were observed to be absence in both acetone and ethanol oil extracts, using Wagner and Dragenodoff tests, as shown in Table 2.0. This shows that cucumber seed oil is not associated with any negative effect of alkaloids, as in the case of caffeine. Some alkaloids are regarded as illicit drugs and poison, such as, tubocurarine, strychnos species, etc., Joanna (2019).

Table 2.0: Qualitative Phytochemical Constituents of *Cucumis sativus seed*

Parameter	Ethanol extract	Acetone extract
Alkaloids: (Wagner test Dragenoff test)	- -	- -
Carbohydrate: (molish test	++	+
Barfoed test Benedict test	- +	- +
Glycoside	-	-
Saponins	+	+
Proteins(Biuret)	-	-
Amino acids(ninhydrin test)	-	-
Xanthoproteic test	+	+
Flavonoids	+	+
Phytosterols (liberman test)	+	+
Phenols	-	-
Terpenoids	++	
Starch(Iodine test)	-	-
Carboxylic acid	-	+

+ = slightly positive ++ = highly positive - = negative

Xanthoproteic is a noncrystallizable yellow substance derived from proteins upon treatment with nitric acid was also dictated from the oil sample. Lower intake of amino acids from animal protein had been proved to prevent risk of cardiovascular disease, via cholesterol regulation by an inhibited hepatic phospholipid metabolism according to Krajcovicova-Kudlackova and Babinska(2005). When plant protein is high in non-essential amino acids, down-regulation of insulin and up-regulation of glucagon is a logical consequence. Reports had shown that Cucumber seed-oil is rich in tocopherols and tocotrienols which are organic fat-soluble oils, that are commonly referred to as "Vitamin E" according to Murad H. and Nyc, M.A., (2016). They suggested that these fat-soluble organic compounds are the moisturizing agents of cucumber seed oil, which tend to protect against sun-rays, environmental pollution, and convert free radicals compounds that could result into skin wrinkles or aging. Cucumber seed oil is an excellent anti-ager, keeping the skin's protective barrier strong, and against both anti-bacterial and anti-inflammation

Table 3.0: Physicochemical Analysis of the seed oil

Parameter	Measured Value
Moisture content (%)	50.718
Specific gravity	0.882
Viscosity (centistoke)	7.066
Free fatty acid (%)	4.243
Acid value (%)	8.443
Iodine value (wijs)	29.013
Peroxide value (milimoles/kg)	14.425
Saponification value (mgKOH/g)	32.400

% = percentage;g/cm^3= gram per centimetre cube; Wijis = wjis unit; mgKOH/g = milligram potassium hydroxide per gram

IV. RESULTS ON THE PHYSICOCHEMICAL VALUE OF THE SEED-OIL

The moisture content as shown in Table 3 agrees with the report of Aina *et al.,* (2012) that, fresh fruits have high percentage of moisture. The increase in the moisture content value of the oil sample as compared to the value of the seed, as shown on Tables 3.0 and 1.0 respectively. This could be attributed to the water absorbtion during processing and extraction of the oil. The peroxide value was 14.425 milimoles/kg, which is ensures good shelf life. However, reports have shown that peroxide values of fresh oils could less than 10 milliequivalents/kg. Peroxide s values between 30 and 40 milliequivalents/kg will exhibit a rancid tastes Nassan, et al(2019).

The iodine value (29.013), as shown in Table 3.0 indicates that the cucumber seed oil is a non-drying oil and could be used in food production and soap making. Drying oils had been reported to have higher iodine contents(about 190) and could be used only in paint, varnish making, while, semi-drying oils has iodine value within 130. The saponification value or

Saponification number of the oil (32.40), which is the amount of base (sodium hydroxide or potassium hydroxide) reacts with any fat to form glycerol and soap molecules, shows that the oil could be good for soap making. Other physicochemical values as stated in Table 3.0 are accepted compare to the values of most seed oils, (Aremu, et al, 2015).

V. CONCLUSION

Plants' seeds are naturally composed of different kinds of phytochemicals with diverse medicinal and biological properties. The emergence and spread of most chronic diseases or viruses, such as, corona-virus, human immune deficiency virus (HIV) which causes acquired immune deficiency syndrome (AIDS), other terminal diseases like high blood pressure, kidney or heart diseases, etc, requires intensive researches to explore more plants' phytochemicals. Researchers are expected to have an insight to isolate other plants' medicinal or pharmacological derivatives that could boost human immune system and fight against some pathogens.

Cucumber seed oil had been evaluated and considered as one of those oils that may play vital roles, if well characterized and used in drug, food, or cosmetic formulations to preventing some diseases, such as inflammation, bacterial infection, fever, constipation, etc. The demand for vegetable oils had increased, and more under-utilized seeds oil, like cucumber seed oil need to be harness and introduced to consumers for desired applications.

However, it is expected that important phytochemical properties and proximate compositions identified in this sample will also be helpful in compounding ingredient for domestic delicacies, such as in baking, frying, cooking, etc, (Sarwal, 2013). Also vitamins, carbohydrates, fatty acids, etc, identified in this sseed are very essential in human diet and were identified from cucumber seed.

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