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Evaluation of Snake Tomato (*Trichosanthes Cucumerina*) Seed and Extracted Seed-oils for Relevant Applications

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Abstract

This study reports the phytochemical screening, ascorbic acid, total sugar contents of the seed flour as well as the physicochemical and selected fatty acid profile of snake gourd (*Trichosanthes cucumerina)* seed oils in three different solvents (n-hexane, methanol and ratio 1:3 mixture of n-hexane: methanol). Qualitative phytochemical screening of the seed flour showed the absence of cyanogenetic glycosides, alkaloids and saponins. The ascorbic acid and total sugar in mg/100 g of the seed flour were found to be (0.24 ± 0.02) and (0.12 ± 0.01) respectively. Higher amounts of macro and low quantities of micro elements were observed in n-hexane and methanolic oil extracts. The physicochemical properties of the oils revealed an increase in specific gravity, acid and saponification values of the n-hexane extracted oil relative to that of methanol. The determined selected fatty acids of the oils showed oleic acid to be the most abundant fatty acid with 26.51 % and 19.47 % in n- hexane and methanolic solvent extracted oils respectively while palmitoleic acid was the least abundant fatty acid in the two oil samples. FT-IR spectroscopic studies performed on the mixture of n-hexane: methanol (ratio 1:3) extracted seed oil revealed useful characteristic peak values. The outcome of this study is an indication that *T. cucumerina* seed and seed oils could be considered as alternatives in the search for replacement of synthetic additives to further enhance industrial development.

Keywords: *T. cucumerina*, oil, extract, phytochemical, physicochemical, fatty acid, FT-IR

1. Introduction

Seed oils represent one of the cheapest key materials that can be obtained from biomass and can be cheaply processed. They are also of nutritionally, industrially and pharmaceutically importance (Oderinde et al., 2008). Plants provide a large variety of compounds with different molecular formulas and various properties that are useful to human being. These compounds possess very important biological activities which attracted several works that led to advancement in their uses to be carried out. Several researchers reported that the fatty acid composition of oils from vegetable sources varies depending on plant origin, genetic factors, ripening state, specific climatic conditions and plant response to diverse environmental stresses, including pathogen attack (Altuna et al., 2018; Garcia et al., 2019; Silva et al., 2020; Gemede et al., 2020).

United Nations in June 2013 projected that the world population will reach 9.6 billion by 2050 from the current 7.4 billion. This will by implication affect the food demand of the entire world. It is therefore estimated that oil crops must increase by 133 million tonnes to reach 282 million tonnes in order to cover the demand (Ouilly et al., (2017). Oils from different sources depend mainly on their composition and no oil from a source can be useful and suitable for all purposes (Gemede, 2020). Snake tomato (*T. cucumerina*) belongs to the family of *Cucurbitaceae* and it's an annual climber plant. It is commonly called snake gourd, viper gourd, serpent vegetable, long tomato elsewhere and 'tomato elejo' in Yoruba speaking areas of the South-western, Nigeria. Snake gourd derives its name from snake- like shape of the fruit.

The plant is grown as a vegetable in homes and gardens in Africa. In Nigeria, *T. cucumerina* is used as a substitute to the regular tomato (*Lycopersicon esculentum* L.) especially during the period of the regular tomato scarcity and its attendant exorbitant prices (Salawu et al., 2014). The soft-skinned immature fruit is similar to that of luffas and calabash. The fruit pulp has been reported to be a very good source of ascorbic acid (Vitamin C) with low oxalate content (Adebooye & Oloyede, 2005). Vitamin C is a water soluble antioxidant known to be important to health and for proper functioning of human body (Davey et al., 2000; Olayiwola et al., 2012) and is able to detoxify carcinogens and may protect cell membrane and DNA from oxidative damage.

L. esculentum is less demanding as far as soil is concerned and thus ideal for grounds lacking humus. The pulp is sweet tasting and aromatic which does not get spoiled or sour as quickly as paste of *L. esculentum* (Adebooye & Oloyede, 2006). *T. cucumerina* is a monoecious annual herb with climbing vine often left trailing on fences and climbs by 2-3 branched tendrils up to 5 to 6 meters high. The stems are always slender, green, 4-angled, and hairy with unpleasant odour (Sandhya et al., 2010). The roots are somewhat tuberous and whitish while the leaves are alternate, simple with no stipules.

Several of these plant seeds are sources of bioactive ingredients that are beneficial in treatment and prevention of several diseases (Singthong & Meesit, 2017). Phytochemicals are compounds found in plants that are required for normal functioning of the body (Leonardo, 2005) and plays an active role on the amelioration of disease. Many solvents have been used to extract active materials from plants such as alcohols (ethanol or methanol), diethyl ether, chloroform, ethyl acetate, n-butanol and water (Al-Rifai et al., 2017). In India and some other parts of the world, the plant is grown principally for the immature fruit that can be cooked and served as vegetable (Ojiako & Igwe, 2008).

Most gourd specialists agreed that *Trichosanthes cucumerina* and *Trichosanthes anguina* are the same species but recommended that the later type be reserved for its wild species (ECHO, 2000; Ojiako & Igwe, 2008). Free fatty acid (FFA) content and peroxide value (PV) are some of the important quality parameters of oil and free fatty acids (FFAs) are fatty acids liberated from their ester linkage which are unattached to any glycerol present in the oil while peroxide value (PV) is a measure of primary oxidation (rancidification) of oils. Fatty acids are widely occurring in natural fats, dietary oils and are known to have antibacterial, antifungal and among others biological properties (Idris et al., 2020).

There are several methods available to analyze and characterize the properties of compounds but fourier transform infrared (FTIR) spectrophotometer is perhaps the most powerful tool for identifying the types of chemical bonds (functional groups) present in compounds (Ashokkumar & Ramaswamy, 2014). The growing prominence of nutrition related health problems called for the need to explore alternative sources of healthy foods as well as exploring processing technologies that can be used for them. The seeds of *T. cucumerina* are often discarded as waste since it is not currently being utilized for commercial purposes therefore; this research work focussed on determining some physicochemical properties of the seed flour and the extracted seed oils using three different solvents in order to provide useful information that can open up and possibly expand the scope of its usage in functional food applications.

2. Materials and Methods

2.1 Materials

2.1.1 Sample Collection and Pretreatment

Matured *T. cucumerina L* fruits were locally collected from Ondo town, Ondo state, Nigeria. The ripe matured fruits were washed in deionised distilled water and sliced opened to separate the seeds from the juicy part. The seeds were sun dried, deshelled manually, pulverized into fine particles and stored in air tight polythene bag placed over silica gel in a dessicator ready for further analysis.

2.2 Methods

The vitamin C and phytochemical screening of the seed flour for cyanogenetic glycosides, alkaloids, saponins and polyphenols were carried out as described by Ojiako and Akubugwo, (1997) while the total sugar content was analysed by the methods of A.O.A.C, (2000). The oil content of the seed flour was extracted by Soxhlet extraction method of AOAC, (2005) using n- hexane, methanol and a mixture of n-hexane: methanol (ratio 1:3) separately as extracting solvents. At the end of each extraction, the solvent was distilled off to obtain the oil. Specific gravity and refractive index were determined by the methods described by Idris et al., (2020) while the other physicochemical properties of the extracted oils were done using AOAC, (2005) methods except the oxidative stability index (OSI) for the samples that was done using the method of Ardabili et al., (2011). The fatty acid profiles of the seed oil was determined using gas chromatographic analyser (SP-2500) of the fatty methyl esters (FAMEs) by following the methods documented by El-Hamdani and Fdil, (2015). Antioxidants and the elemental contents of the n-hexane and methanolic oil extracts were determined using the methods described by A.O.A.C, (2000) while the FT-IR analysis of the n-hexane: methanol (ratio 1:3) extracted seed oil was done by the method of Pakkirisamy et al., (2017).

3. Results and Discussion

3.1 Results

The results of the research work on phytochemical screening, ascorbic acid, total sugar of the seed flour and physicochemical properties as well as the fatty acid profile of the extracted oil from seed flour are presented on Tables 1, 2, 3, 4 and 5.

Table 1. Ascorbic acid and total sugar of the *T. cucumerina* seed flour

Parameter	Observation
Vitamin C (mg/100g)	0.24 ± 0.02
Total sugar (mg/100g)	0.12 ± 0.01

n = 3

Table 2. Phytochemical screening of the *T. cucumerina* seed flour

Parameter	Observation
Cyanogenetic glycosides	-
Alkaloids	-
Saponins	+
Polyphenols	+

Note: - is absent

+ is present

Table 3. Physicochemical properties of n-hexane and methanolic oil extracts

Parameter	n-hexane	methanol
Specific gravity	0.90	0.96
Refractive index	1.48	1.40
State of oil at room temperature	Liquid	Liquid
Acid value (mgKOH/g)	3.45 ± 0.00^{a}	3.30 ± 0.01^{b}
Peroxide value (mEq O_2/Kg)	7.78 ± 0.00^{a}	6.50 ± 0.01^{b}
Iodine value (mgKOH/g)	36.72 ± 0.01^{a}	28.30 ± 0.00^{b}
Saponification value (mgKOH/100g)	260.54 ± 0.01^{a}	295.20 ± 0.01^{b}
Oxidative stability index (h)	5.72 ± 0.01^{a}	5.56 ± 0.01^{b}

n =3. Results are expressed as mean standard deviation. Data having different superscripts across the row are significantly different ($p< 0.05$).

Table 4. Selected fatty acids profile of n-hexane and methanolic oil extracts (%)

Parameter	n-hexane	methanol
Lauric acid (C12:0)	10.28 ± 0.01^{a}	8.20 ± 0.01^{b}
Myristic acid (C14:0)	6.54 ± 0.01^{a}	15.62 ± 0.01^{b}
Palmitic acid (C16:0)	11.87 ± 0.01^{a}	4.50 ± 0.00^{b}

Stearic acid (C18:0)	15.54 ± 0.01[a]	18.25 ± 0.01[b]
Oleic acid (C18:1)	26.51 ± 0.00[a]	19.47 ± 0.01[b]
Linoleic acid (C18:2)	0.75 ± 0.01[a]	1.80±0.00[b]
Palmitoleic acid (16:1)	0.45 ± 0.01[a]	0.48 ± 0.01[a]

n =3. Results are expressed as mean standard deviation. Data having different superscripts across the row are significantly different ($p< 0.05$).

Table 5. Elemental contents of the n-hexane and methanolic oil extracts (mg/g)

Parameter	n-hexane	methanol
Sodium (Na)	13.50 ± 0.00[a]	9.90 ± 0.01[b]
Potassium (K)	26.90 ± 0.01[a]	32.60 ± 0.00[b]
Calcium (Ca)	2.17 ± 0.00[a]	2.51 ± 0.00[b]
Magnesium (Mg)	3.00 ± 0.01[a]	1.80 ± 0.01[b]
Iron (Fe)	0.21 ± 0.00[a]	0.27 ± 0.00[b]
Zinc (Zn)	0.41 ± 0.00[a]	0.20 ± 0.01[b]
Lead (Pb)	0.03 ± 0.01[a]	0.04 ± 0.00[b]
Copper (Cu)	0.12 ± 0.01[a]	0.12 ± 0.00[b]
Manganese (Mn)	0.02 ± 0.00[a]	0.02 ± 0.00[b]
Nickel (Ni)	0.04 ± 0.01[a]	0.03 ± 0.00[b]

n=3. Results are expressed as mean standard deviation. Data having different superscripts across the row are significantly different ($p< 0.05$).

Table 6. Flavonoid and terpenoid contents of the n-hexane and methanolic oil extracts (mg/g)

Parameter	n-hexane	methanol
Flavonoid	0.20 ± 0.07	0.29 ± 0.01
Terpenoid	5.25 ± 1.99	7.26 ± 0.05

n = 3

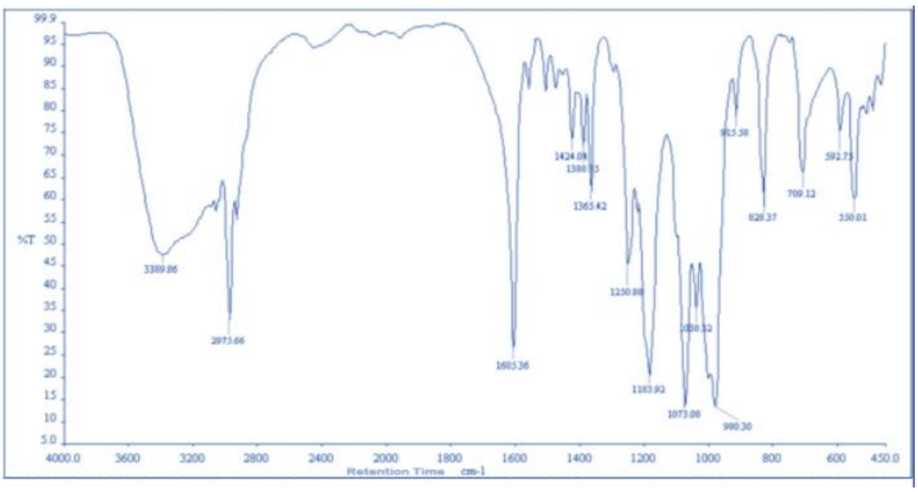

Figure 1. FT-IR spectra of the n-hexane: methanol (1:3) oil extract

4. Discussion

The vitamin C and total sugar contents in mg/100g of the seed were 0.24 ± 0.02 and 0.12 ± 0.01 respectively (Table 1). This vitamin C content is low compared to 0.24 ± 0.05 mg/100g reported for *T. anguina* seed by Ojiako and Igwe, (2008) while the total sugar content is lower than 12.4 ± 1.7 % and 9.0 ± 1.7 % reported for the whole and water processed seeds of *Acacia colei* seeds (Adewusi et al., 2003). Vitamin C has anti-infective properties which helps to fight diseases, promotes wound healing and essential for production and stabilization of collagen. The total sugar content obtained revealed the seed as good source of the energy to the body.

All the phytochemicals screened except saponins and polyphenols were found to be absent in the seed (Table 2). Their absence will help the body to resist the development of substances that may cause oxidative damage and reduce the risk of developing cancer. The absence of these toxicants especially glycosides is of particular importance because its toxic effects include decreased in heart rate, sympathetic activity and systemic vascular resistance (Jimoh and Oladiji, 2005). The presence of saponins in the seed flour however is beneficial as it will aid in inhibiting the Na^+ efflux from the cell which invariably will enhance higher Na^+ concentration in the cells and activates the Na^+ - Ca^+ antiporter in cardiac muscle (Madziga et al., 2010). The saponins presence will also make the seed flour useful in soap production and therapeutically for anticancer activity (Onuekwusi et al., 2014).

The physicochemical properties of the extracted oils from the seed flour using n-hexane and methanol as solvents were presented on Table 3. The saponification and peroxide values were higher while the acid value was lower than the values obtained for *Ocimum basilicum* seed oil (Idris et al., 2020). The specific gravity of (0.9 and 1.48) and refractive index values of (0.96 and 1.40) were obtained for the oils. The value of specific gravity obtained for the n-hexane oil is similar to 0.97 ± 0.01 reported for *Lavandula bipinnata* by Hosakatte et al., (2014). The specific gravity and refractive index of the methanolic oil extract were not within the range of values obtained for *Momordica charantia* by Ali et al., (2008). The refractive index is an indication of the level of saturation of the oil (Oderinde et al., 2009). The acid values are considered low relative to 5.76 ± 0.36 mgKOH/g obtained for *L. bipinnate* seed (Hosakatte et al., 2014) and 13.73 ± 1.40 reported for *Piliostigma thonningii* seeds (Jimoh and Oladiji, 2005). The acid values for the extracted oils were lower than 4.02 mg KOH/g and 4.66 mg KOH/g reported for seed oils from boiled and fermented *Hibiscus sabdariffa* (Chukwu et al., 2019). The low acid values for the two samples pointed to the edibility as well as their stability to rancidity. The observed lower acid value for n-hexane oil extract relative to the methanolic extracted oil indicates that it will contain fewer free fatty acids which will make it more or less exposed to rancidity than the methanolic extracted seed oil.

The peroxide values (mEq O_2/Kg) were found to be lower than 11.60 ± 0.80 and 12.00 ± 0.50 obtained by Oderinde et al., (2008) for *C. pulcherrima* and *A. lebbeck* seeds respectively. The peroxide values obtained were within the limit stipulated by Codex Alimentations Commission that reported a permitted maximum peroxide level of not more than 10 mequivalent of peroxide oxygen/kg of oil (Jimoh & Oladiji, 2005). The peroxide value is a measure of auto-oxidation and deterioration of oils. The methanolic extracted seed oil with high peroxide value indicates that it is more unsaturated than the n- hexane extracted seed oil. Highly unsaturated oils are expected to absorb more oxygen and develop a higher peroxide value which makes them prone to rancidity (Onuekwusi et al., 2014).

The methanolic and n hexane extracted oils gave iodine values of 36.72 ± 0.02 mg KOH/g and 28.30 ± 0.01 mg KOH/g which was higher than the value reported for *Annona senegalensis* by Adebisi, (2009). The iodine values for the seed oils are comparable with those reported by Oderinde et al., (2008) for *C. pulcherrima* and *A. lebbeck* seeds, thus reflecting a lower degree of unsaturation and may therefore not contain a high amount of essential acids. The iodine value of < 100 pointed to the importance of the oils as non-drying in nature which makes them extensively useful in production of lubricants and hydraulic brake fluids. The saponification values in mg KOH/100g of the seed oils were found to be 260.54 ± 0.50 and 295.20 ± 0.20 in methanolic and n hexane seed oils respectively. The values were higher than 210 ± 0.90 mg KOH/100g reported for baobab seed oil (Magdi et al., 2004). A high saponification value is a relevant parameter to the usage of oils for soap making. The oxidative stability index (OSI) values for the n hexane and methanolic extracted seed oils were 5.56 and 5.71 (at 120 ºC and 20 I/h). These OSI values were close to the range of 5.99 - 7.40 h reported for rice bran oil (Anwar et al., 2005) but less than 6.57 ± 0.09 h documented for pumpkin seed oil (Ardabili et al., 2011).

From the data regarding the selected fatty acids (FA) profile of the oil (%), an increase was observed in the amount of myristic, linoleic stearic and palmitoleic acids contents of the n hexane extracted seed oil (Table 4). This trend was in agreement with the findings of Chukwu et al., (2019) for *Hibiscus sabdariffa*. The values obtained for palmitic, linoleic and palmitoleic acids in the two extracted oils are lower than the values obtained by Idris et al. (2020) for the same fatty acids in *Ocimum basilicum* seed oil. Lauric, myristic, palmitic, stearic and oleic acids values in the methanolic extracted seed oil were higher than those reported for the same acids by Hosakatte et al. (2014). Onuekwusi et al. (2014) echoed it that the lauric acid contents and the saponification values of oils could be a useful consideration in determining oils for soap making. The presence of myristic acid in both oils could be utilized in cosmetic products to serve as cleaning, opacifying and emulsifying agents. The palmitic acid values of 11.87 % and 4.30 % in the methanolic and n hexane extracted seed oils are considered high relative to a range of 0.01 - 0.03 % reported for *Brachystegia eurycoma* (Ajayi et al., 2014). Palmitic acid helps in lowering high blood cholesterol, surface tension of oils and can also as surfactants form any industrial applications.

Oleic acid (an unsaturated fatty acid) was the dominant FA in the seed oils; this is similar to the report of Eleyinmi et al. (2006) for bitter cola seed oil. The oleic acid value of 20.51 % and 21.47 % for both oils gave lower values compared to 24.89 ± 0.13 % reported for *Hylocerus polyrhizus* (Suganya et al., 2013) and is used as an emulsifying agent in soap. The presence of oleic and palmitoleic acids being monounsaturated fatty acids helps to increase the amount of high density lipoprotein cholesterol which are useful in the prevention of diseases related to coronary arteries. Linoleic acid values of 0.75 % in methanolic and 1.80 % in n- hexane extracted seed oils were considered lower than the range of 47.95 - 50.91 % and 66.25 ± 1.04 % reported for *B. eurycoma* and *L. bipinnata* seed oils (Ajayi et al., 2014). Linoleic acid helps in minimizing waste disposing problem in the body. The palmitic acid values of 0.45 % and 0.48 % in the extracted seed oils were higher than the amount reported for *L. bipinnata* seed oil by Hosakatte et al. (2014).

The macro and micro elements composition of the n-hexane and methanolic extracts of the snake tomato seed oils (Table 5) showed a trend of K > Na > Mg > Ca for macro and Zn > Fe > Cu > Ni > Pb > Mn for micro elements in n-hexane extracted oil while methanolic extracted oil gave K > Na > Ca > Mg for macro and Fe > Zn > Cu > Pb > Ni > Mn for micro elements. The macro elements composition of the extracts revealed a similar trend except the change in position of Ca and Mg while that of the micro elements do not follow any definite pattern. The high amount of K in the two extracted oils is in line with the findings of Lawal et al., (2017) on the relatively high abundance of K in Nigeria agricultural food products. The relatively high values of Na and K is desirable because they are required to maintain osmotic balance and the P^H of the body fluids, regulates the muscle and nerve irritability, control glucose absorption and enhance normal retention of protein during growth (Ajayi et al., 2013). High amounts of K in the two extracts is desirable because low amount of K has been reported to be associated with the risk of high blood pressure, heart disease, stroke, arthritis, cancer, digestive disorder and infertility (Morakinyo et al., 2016). In addition, calcium, magnesium and zinc presence in the extracts are required as cofactors in enzymatic processes that usually participate in the structure of the DNA self-repair system (Blank et al., 2017).

The flavonoid and terpenoid levels in n-hexane and methanolic extracted seed oils were (0.204 ± 0.007 and 0.291 ± 0.013) mg/g and (5.253 ± 0.199 and 7.260 ± 0.053) mg/g respectively (Table 6). The contents of flavonoid in the two oil extracts was found to be low while that of terpenoid was higher than the values reported by Sudha et al., (2017) for *T. cucumerina* seed oil. Flavonoids are group of plant metabolites that are linked with reduced risk of cancer, asthma and stroke while terpenoids are commonly therapeutically used due to their aromatic qualities as anti-inflammatory and skin permeation enhancer.

The FT-IR analyses of the seed oil extract using a mixture of n-hexane: methanol (1:3) as the extracting solvent (Figure 1). The functional groups identified from the spectra revealed the following important peaks (cm^{-1}) and their possible uses. These peaks include 3389.86 cm^{-1} assigned to O-H stretching vibration and presence of phenolic group related to aromatic compounds that is useful as disinfectant, 2975.66 cm^{-1} assigned to C-H stretching vibration and presence of alkanes related to aliphatic compounds useful as major component of fuel and antiseptic and 1183.92 cm^{-1} assigned to C-N stretching vibration and presence of aliphatic amines in amino acids useful as an important part of amino acids (Sayani, 2019). The finger print peaks at 592.75 cm^{-1} and 550.01 cm^{-1} are assigned to C-H stretching of aromatic rings and C=O stretching of carboxylic acids.

5. Conclusion

The study showed that *T. cucumerina* seed and seed oils could be useful in solving socioeconomic problems in terms of availability of quality oils among the low income earners of the world especially in the developing countries of the world such as Nigeria. The elemental and FT-IR analyses for functional groups revealed the presence of important components that can be domesticated for food, medicinal and industrial uses. The results presented useful information on promising opportunities for improving health attributes of *T. cucumerina*.

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