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# PHYSICOCHEMICAL AND FUNGISTATIC PROPERTIES OF *Hibiscus sabdariffa* SEED OIL EXTRACT ON *Aspergillus niger* IN SOFT ROT OF *Dioscorea alata*

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## ASBTRACT

*Hibiscus sabdariffa*, commonly known as zobo in Nigeria is an annual plant in the family Malvaceae. It is used in the production of blast fiber and as an infusion (herbal tea). All over the world, there is an increased interest in the use of oils of plant origin as Plant Protectants and in Organic farming. Extraction of oil from the seed of *H. sabdariffa*, determination of the physicochemical attributes of this oil as well as the determination of the proximate composition of this seed were all determined using AOAC methods.The antimycotic potential of the extracted oil was investigated on *Aspergillus niger* using growth studies. Results from the analysis of the seed oil showed it has a saponification value of 191.30 MgKOH/Kg, iodine value of 143.60 Wij's, peroxide value of 0.21Meq/Kg and specific gravity of 0.9191g. The results for the proximate composition of the seed showed that it contains 20.20% fat, 24.82% protein, 33.52% carbohydrate, 2.40% ash, 7.32% moisture and 9.88% fibre, The seed oil showed considerable fungistatic properties throughout the experiment by significantly ($P$<0.05) inhibiting the growth of this *A. niger* from between 100% to 63.5% for the first 5days and the last 5days respectively. *H. sabdariffa* seed has a high nutritional value. The result from the present study suggests that this oil has some bioactive compounds that are fungistatic in their mode of action. These findings might pave way for the use of this seed as an alternative nutrient source and for industrial uses, while the oil from the seed may find use as a plant protection compound.

Keywords: Zobo seed, Essential oil, Neglected and underutilized crops, yam disease and Botanicals.

## Introduction

*Hibiscus sabdariffa* L. or Roselle, (commonly known as Zobo plant in Nigeria) is an annual plant in the family Malvaceae. It is believed to have originated, probably from Africa (McClintock and El Tahir, 2011). The stem, which is used in the production of blast fibre (for making cloth, twine, cordage, netting, sacks and rope) has characteristics similar to those of hemp or jute (Ilyas *et al*., 2021). The calyx is used as an infusion or herbal tea (Odigie *et al.*, 2003; Okereke *et al.*, 2015). According to Al-Okbi *et al.* (2017), *H. sabdariffa* seed has been reported to have a yield of between 17% - 20% edible oil. In spite of this potential for the production of oil, *H. sabdariffa* is not reckoned as a player in the league of oil producing seeds (Sharma *et al*., 2011; Codex Alimentarious CXS 210-1999, 2021). The oil from the seed of this plant has been used for cooking in places like Chad, Tanzania and China, while yet in other places, this seed is roasted and eaten as a snack (McClintock and El Tahir, 2011). The physicochemical properties of the oil from this seed suggest that it could potentially also have many important industrial uses (Mohammed *et al.*, 2007). This oil is known to be low in cholesterol and rich in lipid soluble antioxidants such as phytosterols and tocopherols; it has also found uses in the production of biodesiel (Nakpong and Wootthikanokkhan, 2010).

Globally, there is an increased interest in the use of oils of plant origin in Plant Protection (Ben ghnaya *et al*., 2013; Campos *et al*., 2016 and El-Mohamedy, 2017). These oils are otherwise referred to as Essential Oils (EOs). EOs belong to the group of Plant Protection compounds commonly referred to as botanicals. They are so called, because they are of plant origin. Botanicals have been in use for centuries as plant protection compounds (Dara, 2018). The use of secondary metabolites of plant origin as a plant protection agent has been reported to have dated back to more than 3000 years (Pavela, 2016). Commenting on a 17$^{th}$ century record, BPIA (2017) noted that one of the classical documented account of the use of botanicals as a plant protection agent, was when nicotine was used against Plum beetles and other pests. In addition, the use of mineral oils as plant protectants was cited in experiments in the 19th century (BPIA, 2017). In more recent times, literature has been replete with the use of a number of plant or plant parts as plant protection agents. As a matter of fact, the list appears to be consistently on the rise. For instance Behiry *et al.* (2022) reported on the actions of different plant extracts as an antimycotic and antiaflatoxigenic agents on *Aspergillus flavus*. In their work Ngegba *et al.* (2022) listed 44 different plants that have found usefulness as botanicals. This list however does not include *H. sabdariffa* or any of the part of this plant. Although *H. sabdariffa* has been recognized as an economic plant in the Food and Pharmaceutical industries (Sulieman, 2022), its use as a botanical in the protection of economic plants however appears yet to be acknowledged. Botanicals are becoming increasingly acceptable because they are perceived to be eco-friendlier in comparison to the widely used synthetic pesticides (Campos *et al*., 2016).

Cultivated yams belong to the family Dioscoreceae and to the genus *Dioscorea*. It is one of the most important food crops widely cultivated in the tropics (Obidiegwu and Akpabio, 2017). According to FAO (2020) more than 90% of the total global production of yam takes place in West

Africa. It is an important source of calorie to the vast majority of people in many tropical and subtropical countries (Asiedu and Sartie, 2010). *D. alata* is also cultivated in Southeast Asia, where it is believed to have originated from (Acevedo-Rodríguez, 2005). In this region, *D. alata* is the most important yam species and is grown in all countries of this region, namely Indonesia, Malaysia, Papua New Guinea, Philippines, and Vietnam. It is also the most widely distributed species of yam the world over (Acevedo-Rodríguez, 2005). In West Africa, *D. alata* is the 2nd most widely grown species of yam, while in Nigeria, the most cultivated species of yam are the *D. alata, D. cayenesis*, and *D. rotundata* (Amusa *et al*., 2003). In Nigeria, stored yam is believed to be stored wealth because it can be sold all-year-round by the farmer or marketer (Okaka *et al.*, 1991; Okaka and Anajekwu, 1990).

Notwithstanding the huge demand for yam tubers, in Nigeria especially, its demand has always exceeded its supply on account of a number of factors, one of which is the spoilage of this produce by the actions of diseases and pests organisms, both in the field and in the store.
Losses in yam due to the actions of pathological species such as nematodes, fungi and bacteria in storage have been severally reported (Coursey, 1967; Ogunleye and Ayansola, 2014). It has been estimated that an average of over 25% of total yam produced annually is lost to diseases and pests (Ezeh, 1998; FAO, 1998). The losses were estimated to be 10-15% in the first three months (Amusa *et al*., 2003) while F.A.O (1998) estimated the loss to be between 50% and 56% after 6 months in the yam barn. Most of the pathogens of yam tuber are soil borne, but manifestations of the tuber disease are observed mostly during storage. Onayemi (1983) also reported that over 50% of the yam tuber produced and harvested in Nigeria are lost in storage.

Soft rot is reputed to be one of the most important postharvest diseases of yam, accounting for over 45% of postharvest losses of yam in store, second only to dry rot, which accounts for over 54% losses under the same storage conditions (Ogunleye and Ayansola, 2014). In a previous study of the different pathogens associated with rot in yam, Ogaraku and Usman (2008) reported *A. niger* to have had the highest percentage frequency of occurrence compared to 5 other pathogenic fungal species that were encountered in their study.
In view of the foregoing, this work aims at contributing to literature on the valuable attributes of the seed, as well as the oil from the seed of *H. sabdariffa*, especially with respect to its potential usefulness as probable plant protection agent.

## Materials and Methods

### COLLECTION OF SAMPLES

Whole mature Roselle *(Hibiscus sabdariffa*) seeds were obtained from Kurmi market (12.0000° N, 8.5167° E) in Kano, Northern Nigeria. The seeds were kept in a plastic container at room temperature for approximately 5 days until analysis. A sample each of yam infected with soft rot disease was obtained randomly from 6 sellers at Afaha Market in Ibesikpo Asutan (4.9703º, 7.9456ºE) Local Government Area of Akwa Ibom State, Nigeria.

### A. EXTRACTION OF OIL AND THE DETERMINATION OF THE PHYSICOCHEMICAL PARAMETERS OF THE SEED OIL

Soxhlet extraction method with petroleum ether (AOAC 996.06 and modified AOAC 996.06) as reported by Akpan *et al*. (2006) was adopted. The seeds were decupled, cleaned and crushed and later dried in the oven (Techmel, TT-9083A) for three hours at 50°C to ensure that moisture content was reduced to the barest minimum, until a constant weight in seeds was obtained. This seed was thereafter ground. A modified cold extraction method was adopted. About 200 g of the ground seed was transferred into a 500 ml beaker to which n-hexane was poured up to the 400 ml mark and swirled using a glass rod to minimize loss of n-hexane. The set up was kept overnight in a cool dry place.
The n-hexane supernatant extract was then carefully decanted and filtered using whatman filter paper no 5. The n-hexane was then removed using a soxhlet setup at 20ºC to obtain a clear oil extract. The filtrate was concentrated under vacuum in a rotary. A small sample was taken from the extracted oil and analyzed for its physical and chemical properties as described below. All reagents used were of analytical grade. The rest of the oil extracted was stored in a freezer.

#### i. Saponification value

Saponification value was analyzed according to the method described by Akpan *et al* (2006), where 0.5M KOH was prepared in 95 % ethanol, 5 g of oil sample was weighed and 50 ml of KOH was added, 50 ml of the blank solution was also measured into a conical flask. The two samples were then connected to a reflux apparatus and allowed to boil for an hour until the reflux is completed. Thereafter, 1 ml of phenolphthalein was added to the mixture and the resulting mixture was titrated while hot against 0.5 M HCL acid solution. The volume of the acid used to attain the end point was recorded, the blank determination was carried out using the same procedure described above until the color changes from blue to transparent white, then the volume of acid used was noted, the Saponification value was determined using the relationship below.

$$\text{SAPONIFICATION VALUE} = \frac{56.1 \times \text{T}(\text{V}_\text{O} - \text{V}_1)}{\text{M}}$$

Where:
T= Morality of the standard KOH solution used (M),
$V_0$ = Volume of acid used for the first titration with oil sample (cm3),
$V_1$ = Volume of acid used for the second titration blank solution (cm3),
M = Mass of the oil sample used (g).

#### ii. Iodine value

The iodine value (IV) of the oils which quantifies their unsaturation level was determined according to AOAC

(2000). Approximately 0.2 g of oil was weighed and placed in a dry and clean flask specially offered for the test. Ten ml of chloroform was used for dissolving the oil. A 25 ml of pyridine sulphate dibromide solutions was added and finally 20 ml of KI (0.1 N) were added to the contents. The flask was then stopped and the mixture was allowed to stand for 10 minutes in a dark place. The stopper and the side of the flask were rinsed with enough amount of distilled water, the content of the flask was thereafter stopped and the mixture was allowed to stand for 10 minutes in a dark place. The stopper and the side of the flask were then shaken and titrated against 0.1N sodium thiosulphate solution using starch liquid as indicator. A blank determination was carried out simultaneously.

$$IODINE\ VALUE = (\mathrm{B} - \mathrm{A}) \times \frac{0.01269 \times 100}{\mathrm{S}}$$

Where:
B: Volume (ml) of sodium thiosulphate in blank solution.
A: Volume (ml) of sodium thiosulphate in test active solution.
S: Weight (gm) of the oil sample.
Iodine factor = 0.01269 (a constant)

**iii. Acid Value**

Acid value content was determined according to the British Standard Institute Method (1958).
Five gramme of the oil was weighed into 250 ml conical flask, 50 ml mixture of 95% alcohol and ether solvent (1:1) were added. The solution was neutralized after addition of 1 ml of phenolphthalein indicator. The contents of the flask were then heated with caution until the oil was completely dissolved. The contents of the flask were then titrated with 0.01N KOH with constant shaking until a pink colour persisted for 15 seconds. The number of ml of 0.1 N KOH recorded as percentage.

$$ACID\ VALUE = \frac{\mathrm{V} \times \mathrm{N} \times 56.1}{\mathrm{W}}$$

Where:
V: Volume of titration (ml).
N: Normality of KOH.
W: weight of sample.

**iv. Peroxide value**

Five gramme of sample was weighed into cleaned dried boiling tube, and 1 g of potassium iodine (KI) powder was added to the oil and 20 $cm^3$ of the solvent mixture (i.e, glacial acetic acid and chloroform in the ratio 2:1). Then, a boiling tube was placed in boiling water bath so that the liquid mixture boils was allowed to boil vigorously for not more than 30 seconds, the content after boiling was quickly poured into a flask containing 20 $cm^3$ of 5 % potassium iodine (KI) solution and the tube was washed out twice with 25 $cm^3$ of water. Then the mixture was titrated with 0.002 M sodium sulphate using fresh 1 % starch solution. A blank titration was carried out on the sample, and the peroxide value was calculated according the method described by Akpan *et* al (2006), using the relationship below.

$$PEROXIDE\ VALUE = \frac{\mathrm{T} \times \mathrm{M} \times 1000}{\mathrm{W}}$$

Where:
T = titer value of $Na_2S_2O_3$ = (Sample titer – Blank titer,)
M = Morality of $Na_2S_2O_3$
W = Weight of sample / (g).

**v. Specific gravity determination**

The specific gravity bottle was cleaned with acetone, ether and dried in an oven at 60ºC. The weight of the empty bottle was taken, after which the bottle was filled with the oil sample and properly covered. The weight was then recorded using a weighing balance, after which the sample was removed from the bottle. The bottle was properly washed and filled with distilled water, after which the weight was taken and finally, the specific gravity was computed according the method described by Akpan *et al.*, (2006), using the relationship below.

$$SPECIFIC\ GRAVITY = \frac{(\mathrm{W}_0 - \mathrm{W})}{(\mathrm{W}_1 - \mathrm{W})}$$

Where:
W = Weight of empty bottle (g),
$W_0$ = Weight of the bottle and oil content (g).
$W_1$ = Weight of bottle and water content (g).

B. **Proximate analysis of the seed**

The moisture, ash and crude fiber contents were analyzed according to the methods described in AOAC (1997). All the proximate analyses for each of the parameter were carried out in triplicate and the results expressed as percentage of the sample analyzed.

**i. MOISTURE**

The dry seed (5g) was weighed into a clean dry aluminum dish with a known weight. The sample was dried in vacuum oven at a temperature of 105ºC for 6 hours, cooled in desiccators and weighed. And weighing was repeated twice until there was no difference in the two successive weights. The moisture content was calculated following the method of AOAC (1997).

$$MOISTURE\ CONTENT = \frac{(\mathrm{W}_2 - \mathrm{W}_1) - (\mathrm{W}_3 - \mathrm{W}_1)}{(\mathrm{W}_2 - \mathrm{W}_1)} \times 100$$

**Where:**
**$W_1$**= weight of empty crucible.
**$W_2$**= weight of crucible with the sample.
**$W_3$**= weight after drying.

ii. **CRUDE FIBRE**

Crude fibre was analyzed using the method described in AOAC (1997), where 2 g of Roselle seed powder was transferred into a 200 ml beaker after which 1.25 % Sulphuric acid (50 ml) and distilled water (150 ml) were added. The sample mixture was then boiled for 30 minutes under reflux flask and later treated with 1.33% potassium hydroxide (50 ml) and 150ml water. The solution was re-boiled again for 30 minutes and registered using vacuum crucible filtrate on system. The sample in the crucible was rinsed with water followed by acetone. The samples was put

into a pre-weighed crucible and transferred to the oven to dry for 4 hours, cooled in desiccators and weighed. The weighed sample was kept in the furnace set at 660 °C for 5 hours until it became grey ash which was cooled in the desicator and weighed. The weight of ash was then calculated as follows:

$$CRUDE\ FIBRE = \frac{(W_1 - W_2)}{W_S} \times 100$$

Where:
W1= weight of crucible with sample before ashing.
W2= weight of crucible with sample after ashing.
Ws= weight of sample.

### iii. TOTAL ASH

The dry seed sample (5 g) was placed in a dry clean porcelain dish and heated progressively for 6 hours at 550°C until, grey -reddish ash was obtained according to AOAC (1997). The sample was cooled in a dessicator, weighed and total ash calculated using the following formula:

$$Total\ Ash = \frac{(\text{Weight of dish and ash}) - (\text{Weight of dish})}{(\text{Weight of sample})} \times 100$$

### iv. CRUDE PROTEIN

Crude Protein was determined by the method of AOAC (1997) through the following stages:

**a.** Digestion stage**:**
The dry Roselle seed powder (2g) was placed in kijeldahl tube and a 4g mixture (catalyst; sodium sulphate and copper sulphate) was added to the mixture and digested with concentrated sulphuric acid (25 ml) for 2 hours in fume hood until the solution became clear to light green.

b. Distillation stage:
Distilled water (120 ml) was added to the solution and allowed to cool. Sodium hydroxide (45%) was also added without agitation. The flask was then connected to the distillation bulb with the tip of the condenser immersed in a standard acid solution (boric acid 2%) containing 5 drops of the indicator. The flask was then heated to release ammonia into the indicator solution.

c. Titration stage
The excess standard acid in the distillate was titrated with 0.1N standard HCL. The conversion factor of 6.25 was used (AOAC, 1997) and % of Nitrogen calculated as below:

$$CRUDE\ PROTEIN = \frac{(T - B) \times N \times 14 \times 100 \times 6.25}{W_S \times 1000}$$

Where:
T= Titration reading.
T= Blank titration reading.
N= HCl normality.
$W_S$= sample weight.

### v. Total carbohydrate

Total carbohydrates were determined by difference using the method in (AOAC, 1997).

$$TOTAL\ CARBOHYDRATE = 100 - (MC + AC + FC + CF + CP)$$

Where:
**MC**= moisture content.
**AC**= Ash content.
**FC**= fat content.
**CF**= crude fiber.
**CP**= crude proteins.

## C. GROWTH AND INHIBITION STUDIES

### a. Media preparation

The work surface and apparatus were sterilized using the method described by Sanyaolu (2016). Thereafter, 10 g of Potato Dextrose Agar (PDA) was poured into 250 ml of distilled water in a conical flask and mixed properly with a stirring rod after which it was made air tight using aluminum foil. The conical flasks containing the solution and distilled water were put into an autoclave and sterilized at a pressure of 121mmHg for 20 minutes, depressurized and allowed to cool before they were taken out. Chloramphenicol (200 mg) was added to inhibit the growth of Bacteria. About 15 ml of the molten medium was poured into each plate. The plates were swirled gently for even distribution and allowed to solidify.

### b. Isolation of fungi

Surface sterilization of diseased yam tuber was done following the method of Sanyaolu *et al.* (2014) wherein the diseased yam tubers were cut to about 3mm-4mm pieces with a sterile knife and dropped into sodium hypochlorite in a beaker for about 60 seconds and later rinsed in several changes of sterile distilled water. The disinfected cut pieces of the yam were inoculated onto the PDA medium. All the plates were sealed with masking tape, stored in the incubator at room temperature (28-32°C) and observed daily for growth of fungi.

### c. Sub-culturing

After 5 days fungal growths were seen in the Petri dishes. The process of PDA preparation was repeated aseptically and poured into new sterile petri dishes. Inoculation loop and Cork borer were surface sterilized over the flame and used in picking individual (visually distinct) fungal strain/species from the mixed cultured plate and transferred each into the freshly prepared plates. All the plates were sealed with masking tape, stored in the incubator at room temperature (28-32°C) and observed daily for growth of pure cultures of single species isolate.

### d. Inhibition studies

The process of PDA preparation was repeated aseptically, after which 1 ml of the freshly extracted roselle seed oil was aseptically introduced into 6 empty sterile petri dishes and prepared PDA was poured inside the petri dishes. After the

solidification of the PDA, Inoculation loop and 10 mm sized Cork borer was surface sterilized over the flame, allowed to cool, and thereafter used in picking the dominant fungal strain (*A. niger*) from its pure culture plate and transferred into the freshly prepared plates. An equal number of sterile PDA plates that does not contain the extracted roselle oil, but instead 1 ml of sterile distilled water, were also inoculated with *A. niger*. These represented the Control samples for the inhibition studies. All the plates were sealed with masking tape, stored in the incubator at room temperature (28-32°C) and observed daily for the period of 14 days; records of mycelia growth were also taken daily during the 14 day period by measuring the radial growth of the mycellia from the middle of the plate using a ruler, following the method of Nkang and Sanyaolu (2018).

e. **Statistical analysis of data**

Proximate analyses were carried out in three triplicates, and the results reported are mean value of the replicates. Also, the experimental design for the growth studies was Complete Randomized Design (CRD). The measurements of the mycelia growth in the incubation studies were carried out in 6 replicates, and values reported were the mean for these replicates. A paired T-test was used to determine the variation between the effects of *Hibiscus sabdariffa* seed oil on fungal growth compared to the control, while the mean values were separated using LSD at 95% confidence interval. The Statistical analysis was carried out using SPSS package.

## Results

### A. Physicochemical Characteristics of Oil from *Hibiscus sabdariffa* Seed

The results showed that the oil from the seed of *Hibiscuss sabdariffa* have saponifinication value of 191.30 MgKOH/Kg, iodine value of 143.60 Wij's, peroxide value of 0.21Meq/Kg and specific gravity of 0.9191g (Table 1)

### B. Proximate composition of *H. sabdariffa* seed

From Table 2 the results showed that *H. sabdariffa* seed contains 20.20% fat, 24.82% protein, 33.52% carbohydrate, 2.40% ash, 7.32% moisture and 9.88% fibre.

### C. Growth Inhibition studies

The results for the growth inhibition of *A. niger* (associated with soft rot in yam) as presented in Table 3 shows that the oil from the seed of *H. sabdariffa* caused a significant ($P<0.05$) reduction in the growth of this fungus for the entire duration of this study. From days 1-5, this oil achieved a 100% inhibition in the growth of this soft rot fungus. From day 6-14 however, the rate of inhibition of this fungus by the extracted seed oil, though significantly ($P<0.05$) higher than in the control, was however not 100% compared to what obtained in the first 5 days of study, but was between 95.15 – 63.35% (Table 3).

Table 1: Physicochemical properties of Oil from *H. sabdariffa* seed

| Fat (%) | Protein (%) | Carbohydrate (%) | Ash (%) | Moisture (%) | Crude Fibre (%) |
|---|---|---|---|---|---|
| 20.20 | 24.82 | 33.52 | 2.40 | 7.32 | 9.88 |

Table 2: Proximate composition of *H. sabdariffa* seed

| Saponification Value (mgKOH/g) | Acid Value (mg/g) | Iodine Value (wij's) | Peroxide Value (meq/kg) | Specific gravity(g) |
|---|---|---|---|---|
| 191.30 | 0.66 | 143.60 | 0.21 | 0.9191 |

Table 3: Effect of Treatment on the Growth of the Mycellia of *A. niger*

| Day | Daily growth rate (cm) | | Percentage control in mycelia growth | P value |
|---|---|---|---|---|
| | Treatment | Control | | |
| 1 | $0.000\pm0.000^a$ | $0.933\pm0.033^b$ | 100 | < 0.0001 |
| 2 | $0.000\pm0.000^a$ | $4.330\pm0.000^b$ | 100 | < 0.0001 |
| 3 | $0.000\pm0.000^a$ | $4.330\pm0.000^b$ | 100 | < 0.0001 |
| 4 | $0.000\pm0.000^a$ | $4.330\pm0.000^b$ | 100 | < 0.0001 |
| 5 | $0.000\pm0.000^a$ | $4.330\pm0.000^b$ | 100 | < 0.0001 |
| 6 | $0.210\pm0.210^a$ | $4.330\pm0.000^b$ | 95.15 | < 0.0001 |
| 7 | $0.260\pm0.260^a$ | $4.330\pm0.000^b$ | 93.99 | < 0.0001 |
| 8 | $0.850\pm0.850^a$ | $4.330\pm0.000^b$ | 80.36 | 0.010924 |
| 9 | $1.400\pm1.400^a$ | $4.330\pm0.000^b$ | 67.66 | 0.011939 |
| 10 | $1.500\pm1.500^a$ | $4.330\pm0.000^b$ | 65.35 | 0.013225 |
| 11 | $1.500\pm1.500^a$ | $4.330\pm0.000^b$ | 65.35 | 0.013225 |
| 12 | $1.500\pm1.500^a$ | $4.330\pm0.000^b$ | 65.35 | 0.013225 |
| 13 | $1.500\pm1.500^a$ | $4.330\pm0.000^b$ | 65.35 | 0.013225 |
| 14 | $1.500\pm1.500^a$ | $4.330\pm0.000^b$ | 65.35 | 0.013225 |

Values carrying different superscripts along the same row are significantly different at $P<0.05$

## DISCUSSION

Sulieman (2022) described the oil from *H. sabdariffa* seed as being rich in anthocyanins. It thus exhibits a high level of antimicrobial activity. He further described the oil as stable, not being affected much by enzymatic degradation, and that it is characterized by fairly high resistance to oxidation and spoilage. In spite of its obvious potential for the production of good quality edible and Industrial grade oil, *H. sabdariffa* seed is yet to be reckoned as a player in the league of oil

producing seeds (Sharma *et al*., 2011; Codex Alimentarious CXS 210-1999, 2021).

The determination of free fatty acid (FFA) content is an important analysis for evaluating the quality of oils and their degradation during storage, as well as throughout the shelf life (Vicentini-Polette *et al*., 2021). The oxidation process of oil in store and during its shelf life may give rise to different quality, smell, flavour and sensorial attributes (Franco *et al*., 2018). An important attribute governing all the above attributes in oil is the acid value. Comparing the acid value of 0.66 mg/g obtained in this study with the much higher value of 5.447 mg/g and 4.40 mg/g from palm oil and groundnut oil respectively reported by Pandurangan *et al*., (2014), it is evident that the oil from *H. sabdariffa* seed has a higher food value than palm oil and groundnut oil because it contains lesser amount of free fatty acids and oxidation products. Acid value measures the presence of corrosive free fatty acids and oxidation products, which is an important variable in considering the quality of oil. The lower the free fatty acid amount therefore, the better the quality of the oil. Although, the maximum permissible limit of free fatty acid for edible oils is ≤10 (Codex Alimentarious CXS 210-1999, 2021), but its value in the present study is much lower than this maximum permissible limit, thus confirming its stability and quality as edible oil.

Iodine value is the most important analytical characteristic of oils, and it is indicative of the relative degree of unsaturation (He and Liu, 2019). The greater the iodine value of any oil, the more its unsaturation, susceptibility to oxidation, instability and rancidification (Sanders, 2003). The result as obtained in the present study shows that the iodine value of *H. sabdariffa* seed oil is higher than that of palm oil (55.11) and groundnut oil (95.30) as reported by Pandurangan *et al*., (2014). Vegetable oils can be divided into four major categories depending on their iodine value: saturated oils (iodine value between 5 and 50), so-called semi-siccative mono-unsaturated oils (50 and 100), di-unsaturated oils, also called semi-siccative (100 and 150) and tri-unsaturated oils called siccative (over 150).

Peroxide value is a measure of the degree to which rancidity have taken place while oils are in store, and it is regarded as one of the attributes that determines the quality and stability of fats and oils (Abd EL-Razek Mohdaly *et al*., 2017). In other words, it is an indicator of the degree of oxidation of the oil, and it gives an insight into the level of its deterioration (Okechalu *et al*., 2011). According to the Codex Alimentarious (CXS 210-1999, 2021) the permissible peroxide value in any edible oil must not be higher than 10meq/kg. The result obtained for this parameter in this study thus confirms the suitability of this oil for human consumption. In addition, the value for this parameter in *H. sabdariffa* seed oil was also lesser than in palm oil (7.48meq/kg) and groundnut oil (1.97meq/kg) as reported by Pandurangan *et al*., (2014). A lower peroxide value indicates a good quality of oil and a good preservation status.

Saponification number is the number of milligrams of potassium hydroxide or sodium hydroxide required to neutralize the fatty acids from the complete hydrolysis of 1g of fat. In other words, it is the hydrolysis of fats or oils under basic conditions to afford glycerol and the salt of the corresponding fatty acid. Saponification literally means "soap making", and the saponification value of any oil or fat gives information concerning the character of the fatty acids. The longer the carbon chain, the less acid is liberated per gram of fat hydrolyzed. The saponification value obtained for the *H. sabdariffa* seed oil in this work was higher than that groundnut oil (189. 90) and lower than palm oil (197.30) as was reported by Pandurangan *et al*., (2014). The higher the saponification numbers of the oil, the more soluble the soap that can be made from it (Alyas *et al.*, 2006). From the Industrial perspective of the usefulness of oils/fats for soap making, it therefore means that the oil extracted from the seed of *H. sabdariffa* is of higher value than groundnut oil, but of a lower value compared to palm oil.

Specific gravity refers to the heaviness of a substance compared to that of water, and it is expressed without units. According to Pandurangan *et al*. (2014), the specific gravity of palm oil was 0.937 and that of groundnut oil was 0.911. Thus, the result from the present study shows that the oil from the seed of *H. sabdariffa* is less dense than palm oil, but denser than groundnut oil. Lower specific gravity may be due to the removal of some polar compounds from the oil by alkali refining (Abd EL-Razek Mohdaly *et al*., 2017).

Result from this study showed that *H. sabdariffa* seed has a low moisture content (7.32%), a feature that confers high stability and long shelf life on the seeds during storage (Demir *et al*., 2021), thereby protecting it from moulds and ensuring a high yield of dry weight. Seeds with low moisture content (15 or 50% RH and below) have been reported to be able to survive significantly higher temperatures (Tangney *et al*., 2018).

Available literatures suggest that *H. sabdariffa* seed is yet to be acknowledged as a veritable source of protein for both man and his livestock. Result from the present study however shows that the protein content of *H. sabdariffa* seed compares favourably with those of some well-known and commercially exploited oil seeds. In this regard, *H. sabdariffa* seed has a protein value higher than the 10.03 and 8.86 percent reported by Sanyaolu *et al.* (2014) for healthy and diseased seeds respectively of *Irvingia gabonensis*; 10.23 and 22.6 for *Bombax glabrum* and *Arachis hypogea* seeds respectively reported by Oladimeji and Kolapo (2008). However, it has a lower protein content compared to *Cucumis melo* – 34.4 to 39.8 (Petkova and Antova, 2015) and soybean – 34.1 (Oladimeji and Kolapo. 2008). This shows that the seed can serve as a protein source to augment the deficiency of this nutrient in both humans and animals.

In different studies involving *H. sabdariffa* seed, Rimamcwe and Chavan (2016) reported a slightly higher value of

23.80% of fats compared to 20.20% in the present study. Mohamed *et al.*, (2007) reported 17%; Nzikou *et al.*, (2011) reported 21.8%, while Nady (2014) reported 21.6% of fat for this seed. The high percentage of fat in this seed thus confirms it as an oil seed. In addition, the relatively high fat and protein content of this seed also indicate that it can become an excellent economic source for edible oil production.

Ash is the inorganic residue arising the ignition or complete oxidation of organic matter in any food sample (Ismail, 2017). It is an indicator of the quantity and nature of minerals in a food sample. Mineral content is an important factor in a seed's nutrition and quality; and like water, it influences the ability of microorganisms to thrive (Nwajinka *et al*., 2020). Ash content of *H. sabdariffa* seed found in the present study was lower than 6.8% reported by Nady (2014).

Although crude fibre has little food value, it nevertheless provides the bulk necessary for proper peristaltic action in the intestinal tract (Chandaka Madhu *et al*., 2017). Crude fibre is also important in preventing and or treating constipation, hemorrhoids, diverticulosis, some heart diseases and cancers, obesity and some gastrointestinal diseases. Crude fiber consists of indigestible cellulose (60-80%) and lignin (4-6%) and mineral matter (Chandaka Madhu *et al*., 2017). The amount of crude fibre reported in this study was lower than 16.3% and 16.4% reported by Alwandawi *et al.,* (1984) and Nzikou *et al.,* (2011) respectively in their own proximate determination of crude fibre in *H. sabdariffa* seed. However, *H. sabdariffa* seed has a higher crude fibre content compared to 5 other oil seeds namely: soybean, melon, cashew, groundnut and coconut reported by Oladimeji and Kolapo (2008).

Carbohydrate is the nitrogen free extract comprising of sugar, starch and organic acids. They provide the body with the energy that is used for work and produces heats that is used to maintain body temperatures. Also, glycogen, a byproduct of carbohydrate serves as the food reserve for the mammalian body amongst many other varied uses in plant and animal systems. The result obtained in the present study shows that *H. sabdariffa* seed has a higher carbohydrate level when compared to the values reported by Oladimeji and Kolapo (2008) for some known oil seeds such as soybean, melon, cashew, groundnut and coconut. Also, the carbohydrate value reported in this work was higher than 26.6% reported by Alwandawi *et al.* (1984) and 21.2% reported by Nzikou *et al.* (2011) for the same *H. sabdariffa* seeds. This variation may probably be due to difference in the variety, genetics, environment, ecology and harvesting and postharvest processing conditions of this seed.

**Anti-fungal properties of the oil**

Many previous studies have demonstrated the effectiveness of essential oils (Eos) - oils of plant origin- in the control of plant diseases. In their own work, Peighami-Ashnaei *et al*. (2009) investigated the effectiveness of oils extracted from *Syzygium aromoticum, Foeniculum vulgare, Cuminum cyminum* and *Mentha piperita* against grey mould of apple caused by the fungus *Botrytis cinerea*. In this work, they showed that both the oils extracted from *S. aromoticum,* and *F. vulgare* caused a remarkable inhibitory effect on the PDA culture of *B. cinerea*. In another work, Souza-Júnior (2009) found that EOs from *Lippia sidoides, Ocimum gratissimum, Cymbopogon citratus, Lippia citriodora* inhibited the germination and mycelial growth of the fungus, *Colletotrichum gloeosporioides*. Results from the present study shows that the EO extracted from the seed of *H. sabdariffa* significantly ($P<0.05$) inhibited the growth of the mycelia of *A. niger* associated with soft rot in *D. alata* for the entire duration of the study. The effectiveness as shown from the results was such that this oil caused a 100% inhibition in the growth of the mycelia of this fungus within the first 5 days of the application of the treatment. The inhibition rate of this oil on this yam rot fungus for the 6$^{th}$, 7$^{th}$ and 8th, day was 95.15, 94.00 and 80.36% respectively. From the 9$^{th}$ to the 14$^{th}$ day however, the Treatment caused a static growth in this fungus, by effectively inhibiting the mycelia growth by 67.66%, thus clearly showing its fungistatic property against *A. niger* in soft rot of *D. alata*. This finding aligns with the findings of Krzyśko-Łupicka *et al*. (2020), where they reported the fungistatic effect of 8 EOs against 4 species of *Fusarium* associated with Cereal grains. They further reported that the fungistatic potency of some of these oils, from Lemon grass, thyme, verbena and *Litsea cubeba* produced effects comparable to that of the synthetic pesticide, Funaben T. In another work, Nazzaro *et al*. (2017) explained the mechanism of action of Eos as fungistatic agent via their role in blocking cell communication mechanisms, fungal biofilm formation, and mycotoxin production. Seifeldin *et al*. (2012) reported that extract from the calyx of *Hibiscus sabdariffa* inhibited aflatoxins production and mycelia growth in *Aspergillus flavus* and *Aspergillus parasiticus*. Also Al-shayeb and Mabrook (1984) reported that the extract of dried leaves of *Hibiscus sabdariffa* reduces aflatoxin production in fungi. The fungal inhibitory properties shown by *Hibiscus sabdariffa* seed oil suggests that this seed oil can be explored industrially, as a biologically safe control additives against soft rot disease in *D. alata* and probably in other agricultural products.

**Conclusion**

*H. sabdariffa*, in spite of its enormous potential and usefulness is a neglected and underutilized crop. Evidence from the present study clearly shows that *H. sabdariffa* seed and the Eo from this seed represents a veritable source of nutrient for man and his livestock, a potentially useful industrial raw material for soap making and also a probable safer and cheaper alternative to many of the ecotoxic fungicides. It is therefore suggested that further investigations be carried out on how to improve on the production of *H. sabdariffa*, as well as elucidate the active components of this oil that proves it a useful fungistatic agent.

**References**

Abd EL-Razek Mohdaly, A., Abd El-Hameed S.K., Abd EL-Moneam, M.A.E. and Abdel Tawab, A. M. (2017) Effect of Refining Process on the Quality Characteristics of Soybean and Cotton seed Oils. *International Journal of Current Microbiology and Applied Science*, **6(1):** 207-222. http://dx.doi.org/10.20546/ijcmas.2017.601.026

Acevedo-Rodriguez, P. (2005). *Vines and climbing plants of Puerto Rico and the Virgin Islands.* Contributions from the United States National Herbarium, 51: 483pp

Akpan, U.G.; Jimoh, A. and Mohammed, A.D. (2006): Extraction, Characterization and Modification of Castor Seed Oil. *Leonardo Journal of Sciences*, **8**:43-52.

Al-Okbi, S.Y., Abdel-Razek, A.G., Mohammed, S.E. and Ottai, M.E. (2017). Roselle Seed as a Potential New Source of Healthy Edible Oil. *Journal of Biological Sciences*, **17**: 267-277. DOI: 10.3923/jbs.2017.267.277

Al-Shayeb, N.M. and Mabrook, S.S. (1984). Utilization of some edible and medical plants to inhibit aflatoxins formation. *Nutrition Report International*, **29**: 273-282.

Al-Wandawi, H., Al-Shaikhly, K. and Abdul-Rahman, M. (1984). Roselle seed: A new protein source. *Journal of Agricultural Food Chemistry*, **32**: 510-512.

Alyas, S. A., Abdulah, A. and Idris, N. A. (2006). Changes of beta-carotene content during heating of red palm olein. *Journal of Oil Palm Res*earch (Special Issue): 99-102

Amusa, N.A., Adegbite, A.A., Muhammed, S. and Baiyewu, R.A. (2003). Yam Diseases and its Management in Nigeria. *African Journal of Biotechnology*, **2(12):** 497-502

AOAC (1997) *Official Methods of Analysis. 20th Edition*, The Association of Official Analytical Chemists, Washington, DC.

AOAC (2000) *Official Methods of Analysis. 17th Edition*, The Association of Official Analytical Chemists, Gaithersburg, MD, USA. Methods 925.10, 65.17, 974.24, 992.16.

Asiedu, R. and Sartie, A. (2010). Crops that feed the world 1. Yams: yams for income and food Security. *Food Secur*ity, **2**: 305-315 https://www.cabi.org/isc/datasheet/19293 Accessed Nov. 4, 2022

Behiry, S.I.; Hamad, N.A.; Alotibi, F.O.; Al-Askar, A.A.; Arishi, A.A.; Kenawy, A.M.; Elsamra, I.A.; Youssef, N.H.; Elsharkawy, M.M.; Abdelkhalek, A. and Heflish, A.A. (2022). Antifungal and Antiaflatoxigenic Activities of Different Plant Extracts against *Aspergillus flavus*. *Sustainability*, **14**: 12908. Accessed on 25 October 2022 at https://doi.org/10.3390/ su141912908

Ben ghnaya, A., Hanana, M., Ismael, A., Balti, H., Gargouri, S., Jamoussi, B. and Hamrouni, L. (2013): Chemical composition of *Eucalyptus erythrocorys* essential oils and evaluation of their herbicidal and antifungal activities. *Journal of Pest Science*, **86(1)**: 571-577 DOI: 10.1007/s10340-013-0501-2

Biological Products Industry Alliance (BPIA), (2017). History of biopesticides. http://www.bpia.org/history-of-biopesticides/

Campos, E.V.R., de Oliveira, J.L., Pascoli, M., de Lima, R. and Fraceto, L.F. (2016). Neem Oil and Crop Protection: From Now to the Future. *Frontiers in Plant Science*, **7**:1494. doi: 10.3389/fpls.2016.01494

Codex Alimentarius – International Food Standards (2021). *Standard for Named Vegetable Oils CXS 210-1999.* Accessed 4 November 2022 at https://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/

Yadilal, G., Manisha, M., Pavani, E., Navya, M., Alekhya, C., Chandini, G.H., and Chandaka Madhu (2017). Estimation of Crude Fibre Content in Spices and Fruits. *Indo American Journal of Pharmaceutical Sciences*, **4(10)**: 3864-3868.

Coursey, D. G. (1967). *Yams. An account of the nature, origins, cultivation and utilisation of the useful members of the* Dioscoreaceae. Longmans, Green and Co. Ltd, London: 244 pp

Dara, S.K. (2018). Brief history of botanical and microbial pesticides and their current market. *Ucanr e-journal of Entomology and Biologicals*. Retrieved on October 17, 2022 at https://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=26249

Demir, I., Gokdas, Z., Mavi, k. and Kose, E. (2021). Longevity of Cabbage, Leek and Marrow Seeds in Relation to Storage at Ambient Regional Storage Temperatures with Various Seed Moisture Content. *alatarim*, **20(1)**: 1-11

El-Mohamedy, R.S.R. (2017): *Plant Essential Oils for Controlling Plant Pathogenic Fungi*. In: Volatiles and Food Security, Role of Volatiles in Agro-ecosystems : Devendra K. Choudhary · Anil K. Sharma Prachi Agarwal · Ajit Varma Narendra Tuteja (eds). :392pp. DOI: 10.1007/978-981-10-5553-9_9

Ezeh, N.O. (1998). *Economics of production and Post-harvest Technology* In: Orkwor, G.C., Asiedu, R and Ekanayake, I.J. (eds.) Food yam: Advances in research. IITA and NRCRI, Nigeria: 187-214.

FAO (1998). Food and Agriculture Organization Production Year Book FAO Rome.

F.A.O. (2020). FAOSTAT Crop Production Data. 2020. Accessed: 29 October 2022 at http://www.fao.org/faostat/en/#data/QC

Franco, D., Rodríguez-Amado, I., Agregán, R., Munekata, P.E.S., Vázquez, J.A., Barba, F.J. and Lorenzo, J.M. (2018). Optimization of antioxidants extraction from peanut skin to prevent oxidative processes during soybean oil storage. *LWT - Food Science and Technology*,**88**:1–8. doi: 10.1016/j.lwt.2017.09.027.

He, D. and Liu, L. (2019). *Analytical Aspects of Rice Bran Oil* In: Rice Bran and Rice Bran Oil – Chemistry, Processing and Utilization Ling-Zhi Cheong and Xuebing Xu (eds.). Academic Press and AOCS Press, Elsievier: 301pp

Ilyas, R.A., Asyraf, M.R.M., Sapuan, S.M., Afiq, T.M.N., Suhrisman, A., Atikah, M.S.N. and Ibrahim, R. (2021). *Development of Roselle Fiber Reinforced Polymer Biocomposites Mug Pad using Hybrid Design for Sustainability and Pugh Method*. In Roselle: Production, Processing, Products and Biocomposites; Sapuan, S.M., Razali, N., Radzi, A.M., Ilyas, R.A.,(eds.). Elsevier Academic Press: Amsterdam, The Netherland, 2021; pp. 197–213. ISBN 978-0323852135.

Ismail, B.P. (2017). *Ash Content Determination*. In: Food Analysis Laboratory Manual. Food Science Text Series. Springer, Cham. Accesed on 30 September 2022 at https://doi.org/10.1007/978-3-319-44127-6_11

Krzyśko-Łupicka, T., Sokół, S. and Piekarska-Stachowiak, A. (2020). Evaluation of Fungistatic Activity of Eight Selected Essential Oils on Four Heterogeneous *Fusarium* Isolates obtained from Cereal Grains in Southern Poland. *Molecules*, **25(2):** 292

McClintock, N.C. and El Tahir, I.M. (2011). *Hibiscus sabdariffa* L. [Internet] Record from PROTA4U. Brink, M. and Achigan-Dako, E.G. (eds). PROTA (Plant Resources of Tropical Africa / Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. Accessed 26 October 2022 at http://www.prota4u.org/search.asp.

Mohamed, R., Fernandez, J., Pineda, M. and Aguilar, M. (2007). Roselle (*Hibiscus sabdariffa*) Seed oil is a rich source of gamma-tocopherol. *Journal of Food Science*, **72(3):** 207-211.

Nady, A. (2014). Comparative Studies on Egyptian and Libyan Roselle Seeds as a Source of Lipid and Protein. *Food and Nutrition Sciences*, **5**: 22372245

Nazzaro, F., Fratianni, F., Coppola, R. and Feo, V.D. (2017). Essential Oils and Antifungal Activity. *Pharmaceuticals (Basel)*, **10(4):** 86. doi: 10.3390/ph10040086

Nakpong, P. and Wootthikanokkhan, S. (2010). Roselle (*Hibiscus sabdariffa* L.) oil as an alternative feedstock for biodiesel production in Thailand. *Fuel*, **89**: 1806-1811.

Ngegba, P.M., Cui, G., Khalid, M.Z. and Zhong, G. (2022). Use of Botanical Pesticides in Agriculture as an Alternative to Synthetic Pesticides. *Agriculture*, **12**: 600. Accessed on 30 September 2022 at https://doi.org/10.3390/ agriculture12050600.

Nkang, Y.C. and Sanyaolu, A.A.A. (2018). Impact of Dimethoate on the Mycelia Growth of Some Soil Inhabiting Fungi. *World Journal of Applied Science and Technology*,**10(1B)**: 231-236

Nwajinka, C.O., Okonjo, E.O., Amaefule, D.O. and Okpala, D.C (2020). Effects of microwave power and slice thickness on fiber and ash contents of dried sweet potato (*Ipomoea batatas*). *Nigerian Journal of Technology (NIJOTECH)*, **39(3):** 932 – 941 dx.doi.org/10.4314/njt.v39i3.36

Nzikou, J.M., Matos, G.L., Ganongo-Po, F.B. and Mboungou-Mboussi, P.S. (2011). Lipid and Protein. *Food and Nutrition Sciences*, 5: 22372245. Accessed Online 13 November 2022 in SciRes at http://www.scirp.org/journal/fns.

Obidiegwu, J.E. and Akpabio, E.M. (2017). The geography of yam cultivation in southern Nigeria: Exploring its social meanings and cultural functions. *Journal of Ethnic Foods*, **4(1)**: 28-35

Odigie, I.P., Ettah, R.R. and Adigun, F. (2003). Chronic administration of aqeous extract of *Hibiscus sabdariffa* attenuates hypertension and reverses cardiac hypertrophy in hypertensive rats. *Ethnopharmcology*, **86**:181-185.

Ogaraku, A.O. and Usman, H.O. (2008). Storage Rot of Some Yams (Dioscorea Spp) In Keffi and Environs, Nasarawa State, Nigeria. *PAT 2008*, **4(2):** 22-27. Accessed online on 28 October 2022 at www.patnsukjournal.com/currentissue

Ogunleye, A.O. and Ayansola, O. T. (2014). Studies of Some Isolated Rot-Causing Mycoflora of Yams (*Dioscorea* Spp.). *American Journal of Microbiology and Biotechnology*, **1(1):** 9-20. Accessed 10 November 2022 at https://www.researchgate.net/publication/282442735.

Okaka, J.C. and Anajekwu, B. (1990). Preliminary studies on the production and quality evaluation of a dry yam snack. *Tropical science*, **30(1):** 65-72

Okaka, J.C.O., Okorie, P.A. and Ozon, O.N. (1991). Quality Evaluation of sundried yam chips. *Tropical Science*, **31(3):** 265-275.

Okechalu, J.N., Dashen, M.M., Lar, M.P., Okechalu, B. and Tongriyang, G. (2011). Microbiological Quality and Chemical Characteristics of Palm Oil Sold within Jos Metropolis, Plateau State, Nigeria. *Journal of Microbiology and Biotechnology Research*, **1(2):** 107-112.

Okereke, C.N, Iroka, F.C. and Chukwuma, M.O. (2015). Phytochemical analysis and medicinal uses of *Hibiscus sabdariffa. International Journal of Herbal Medicine*, **2(6):** 16-19.

Oladimeji, G. R. and Kolapo, A. L. (2008). Evaluation of proximate changes and microbiology of defatted residues of some Nigerian oil seeds. *African Journal of Agricultural Research*, **3(2):**126-129.

Onayemi, O. (1983). *Observation on the dehydration characteristics of different varieties of yam and cocoyam* . Abstract 6th symposium of the Int. Soc. For Trops. Peru February, 1983.

Pandurangan, M. K., Murugesan, S. and Gajivaradhan, P. (2014). Physico-chemical properties of groundnut oil and their blends with other vegetable oils. *Journal of Chemical and Pharmaceutical Research*, **6(8):**60-66.

Pavela, R. (2016) History, Presence and Perspective of Using Plant Extracts as Commercial Botanical Insecticides and Farm Products for Protection against Insects - a Review. *Plant Protection Science*, **52(4):** 229 -241. DOI: 10.17221/31/2016-PPS

Peighami-Ashnaei, S., Farzaneh, M., Sharifi-Tehrani, A. and Behboudi, K. (2009). Effect of essential oils in control

of plant diseases. *Communications in Agricultural and Applied Biological Sciences*, **74(3)**: 843-847.

Petkova, Z. and Antova, G. (2015). Proximate composition of seeds and seed oils from melon (*Cucumis melo* L.) cultivated in Bulgaria. *Cogent Food and Agriculture*, **1(1):** 1018779 DOI: 10.1080/23311932.2015.1018779

Rimamcwe, K.B. and Chavan, U.D. (2016). Properties and Nutritional Potentials of Indian Roselle (*Hibiscus sabdariffa* l) seeds. *International Journal of Current Research*, **8(09)**: 38644-38648.

Sanders, T.H. (2003). *Ground nut oil*. Encyclopedia of Food Sciences and Nutrition (Second Edition): 2967-2974. Accessed online on 25 October 2022 at https://doi.org/10.1016/B0-12-227055-X/01353-5

Sanyaolu, A.A.A., Adekunle, A.A. and Osuntoki, A. (2014). The Effects of Post Harvest Mycodeterioration on the Proximate compostion of *Irvingia gabonensis* seeds. *International Journal of Phytopathology*, **3(1):** 41-48.

Sanyaolu, A.A.A. (2016). Postharvest fungal deterioration of tomato (Lycopersicum esculentum mill) and pepper (Capsicum annum l): the "esa" Connection. *Science World Journal*, **11(3)**: 1-10

Saifeldin, A.F. E., Al-Bahry, S.N , Elshafie ,A.E. and AlHilali, S. (2012). Effect of *Hibiscus sabdariffa* extract and *Nigella sativa* oil on the growth and aflatoxin B1 production of *Aspergillus flavus* and *Aspergillus parasiticus* strains. *Food Control*, **25**: 59-63

Sharma, M., Mondal, A.K. and Gupta, S.K. (2011). *Production and trade of major world oil crops*. In: Technological Innovations in Major World Oil Crops, Springer: New York, USA: 1–15

Souza-Júnior, I.T. (2009). Fungitoxic essential oils effect on *Colletotrichum gloeosporioides*, isolated from the yellow passion fruit. *Biotemas*, **22(3)**: 77-83.

Sulieman, A. M.E. (2022): *Antimicrobial activity of Roselle (Hibiscus sabdariffa L.) seed oil*. In: Multiple Biological Activities of Unconventional Seed Oils. Abdalbasit Adam Mariod (Ed). Academic Press, 436pp. Accessed on 25 October 2022 at http:// 91-100 doi.org/10.1016/B978-0-12-824135-6.00003-9

Tangney, R., Miller, B.P., Merritt, D.J. and Fontaine, J.B. (2018). Seed moisture content as a primary trait regulating the lethal temperature thresholds of seeds. *Journal of Ecology*, **107(3)** DOI: 10.1111/1365-2745.13095

Vicentini-Polette, C.M., Ramos, P.R. Cintia Bernardo Gonçalves, C.B. and De Oliveira, A.L.

(2021). Determination of free fatty acids in crude vegetable oil samples obtained by high-pressure processes. *Food Chemistry*, **12**: 100166