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Full Length Research Paper

Physiochemical and phytochemical analysis of oil from *Persea american, Garcinia kola,* and *Annona muricata* seeds

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ABSTRACT: Oils from *Annona muricata* (soursop), *Persea americana* (avocado pear), and *Garcinia kola* (bitter kola) seeds were extracted using n-hexane as solvent. The different oils were analyzed and evaluated to determine their physicochemical characteristics using only the n-hexane extract. The results of the physicochemical analysis showed the following values for *Annona muricata* (soursop), *Persea americana* (avocado pear) and *Garcinia kola* (bitter kola) oils respectively: pH: 4.10, 6.80, 5.40; Peroxide: 5.4, 8.40, 5.60 (meq/kg); Iodine: 72.74, 102.54,52.18; Specific gravity:1.79, 9.26, 0.84; Saponification: 49.09, 61.26, 16.28 (mg); Unsaponification: 51.16, 61.26, 16.28. However, three extracts (n-hexane, aqueous, and ethanol) were screened for phytochemicals such as saponins, tannins, and alkaloids and analyzed. Results of the phytochemical analysis revealed the absence of tannins and alkaloids in n-hexane extracts of the three seed oils including the aqueous and ethanol extracts of soursop but present in the aqueous extracts of both bitter kola and avocado pear. The infra-red (IR) analysis however revealed several peaks with a specific frequency of absorption for these oils, though similarities and variations between the functional groups were present.

Keywords: Physicochemical, phytochemical, solvent extracts, infra-red

INTRODUCTION

The need for alternative source of oil from non-conventional sources is inevitable in order to augment the available ones to meet the demand of oil for both industrial and nutritional use. To this end, numerous researchers (Akubugwe et al., 2017; Abakaka et al., 1989, Deferne and Pate, 1998) have carried out several analyses on various seed oils for both their physicochemical characteristics and phytochemical contents. Phytochemical components of plants are responsible for protecting the plants against microbial infections by pests (Mason, 1981; Obadomi and Ochuko, 2001; Van-Burden and Robinson, 1981).

Although, these oils may not be for table requirement, their potentials are exploitable, particularly in pharmaceuticals, oleochemicals, and cosmetics and also in the formulation of fast drying surface coatings and stabilizers etc.

Avocado pear (*Persea americana*) of the family lauraceae is a well-known plant in West Africa. The fruits are edible and the bark, leaves, stem and roots are used as local medicine (Doughari, 2012). Previous studies have shown that the seeds from these fruits contain oil which has considerable nutritional value. They could provide a useful supplement to animal feeds (Doughari, 2012) and the fruits are rich in lipid (Sarkar and Li, 2006).

It is important to note that 80-85% of the free fatty acid composition of Avocado oil is unsaturated fatty acids according to analysis carried out by Messrs McLachian and Lazar Ltd, (Consulting Industrial Chemists) in Johannesburg RSA.

Horace Pearce, (1959), wrote an article on the nutritional value of Avocado oil in the Avocado year book of 1959 and stated as follows. “It has been found that the oil of Avocado is one of the most unsaturated fatty acids, being fifth on the list of the most desirable oils known as an anti-cholesterol agent” (Obadomi, and Ochuko, 2001; Boam and Kocipai, 1994).

Soursop is the fruit of *Annona muricata* and is mostly grown in the Eastern, Western and Southern parts of Nigeria due to their high humidity (Sakar and Li, 2006; Sofowa, 1993 and Trease, 1989). The anti-cancer capabilities of soursop were first discovered in the mid-1970s, and were kept hidden from the public until recently (Makkar et al., 1998).

Bitter kola is a type of kola *(Garcina kola*) found in some parts of Nigeria and the nuts are widely traded and eaten as stimulant (Sarkar and Li, 2006; Paquot and Hauffeme, 1987). Bitter kola is also rich in caffeine and theobomine and is believed to be an aphrodisiac, unlike other kola nuts (Obadomi and Ochuko, 2001).Thus this study is aimed at determining the medicinal and nutritional values of the oils from these seeds.

MATERIALS AND METHODS

The materials for this study includes; Avocado pear (*Persea americana*), Soursop (*Annona muricata*), Bitter kola (*Garcina kola)*, n-hexane solvent, sulphuric acid, Mayer’s reagent, Wij’s solution, phenolphthalein, potassium hydroxide, carbon tetrachloxide, potassium iodide, HCl, sodium thiosulphate, ammonia, ferric chloride, mercuric chloride etc. These chemicals and reagents were used to carry out the physicochemical analysis of the various oils extracted from the seeds. The extraction was carried out from each of the seed with the aid of Soxhlet apparatus using n-hexane as solvent. The oils were separated from the solvent after extraction using rotary evaporator. The oils were subjected to physiochemical characteristics and phytochemical screening of the n-hexane extracts of the oils from the three seeds (*Annona muricata, Garcina kola and Persea americana*), using standard methods (Trease, 1989; Obadomi and Ochuko, 2001; Boam and Kocipai, 1994; Makkar et al., 1993).

Physicochemical characteristics

The percentage of oil content and moisture content of seeds were determined using IUPAC standard method (Wollenberg, 1990).

The percentage of oil content: % oil content extracted was calculated as shown below:

$$\% \text{ of oil} = \frac{\text{Weight of oil obtained in grams}}{\text{weight of seeds in grams}} \text{ x } \frac{100}{1}$$

Determination of moisture content of seeds

5g of the various seeds were placed in a crucible and heated at 105^{o}C in an oven for at least 3hours and cooled to a room temperature and re-weighed and heats until constant readings were gathered.

$$\% \text{ of moisture} = \frac{W1 - W2}{W1} \text{ x } \frac{100}{1}$$

Where W1 = original weight of sample before drying
W2 = Weight of sample after drying.

The other physicochemical properties were determined as follows; Iodine value (AOAC method 1990; 920-159), Acid value (AOAC method 1990; 933-08), Specific gravity (AOAC method 1990; 920-212), Saponification value (AOAC method 1990; 920-160), Unsaponifiable matter (AOAC method 1990; 933-08) and Peroxide value (AOAC method 1990; 965-33).

Phytochemicals analysis

The phytochemicals screening of the extracts (n-hexane, ethanol and aqueous/water) of the different seeds were analyzed using standard procedure as described by (Gottlieb, 1990; Trease, 1989; Sofowora, 1993; Obadomi, 2001) to identify the phytochemical constituents. The phytochemicals properties such as alkaloids, saponins and tannins were determined using IUPAC standard methods (Paquaot and Hauffeme, 1987).

Alkaloids

2g of each extract was warmed with water and 10ml of 2% H_2SO_4 in a test tube on a water bath for two minutes and filtered. The pH of the filtrate was adjusted with ammonia to about 6-7, and few drops of Mayer’s reagents (mixture of 1.36g mercuric chloride in 60ml distilled water and 5g potassium Iodide in 20ml distilled water with the total volume adjusted to 100ml with distilled water) was added separately to about 0.5ml of each filtrate in the different test tube and the colour observed, creamy white precipitate or turbidity in the resulting precipitate indicate the presence of alkaloids (Gottlieb, 1990; Trease, 1989; Sofowora, 1993; Obadomi, 2001).

Saponins

1g of each extract was with 10ml of distilled water in a test tube and it was shaken vigorously for two minute to froth, and it was also observed on standing for 5-20

minutes for stable foam indicating the presence of saponins (Gottlieb, 1990; Trease, 1989; Sofowora, 1993; Obadomi, 2001).

Tanins

2g of each extract were separately boiled with 20ml distilled water for five minutes in a water bath and was filtered while hot.1ml of the cooled filtrate was increased to 5ml with distilled water and a few drops of 10% ferric chloride were added and observed . Transient greenish to black color indicate the presence of tannin (Gottlieb, 1990; Trease, 1989; Sofowora, 1993; Obadomi, 2001).

RESULTS AND DISCUSSION

From (Table 1), the percentage oil content from these seeds are 10.2, 40, and 20 for *Persea americana, Annona muricata and Garcina kola* respectively. This indicates that *Annona muricata* seeds are oilier when compared with *Garcina kola* and *Persea americana*. Also, the moisture content of these seeds are 65.6, 33.7 and 15% for *Persea americana, Garcina kola* and *Annona muricata* respectively. The higher moisture contents of *Persea americana* when compared to Annona muricata and Garcina kola, may be due to the bigger size of its seed when compared to the other seeds (*Annona muricata and Garcina kola*) and the moisture content of seeds depends also to a large extend on the freshness of the seeds. The iodine values are 72.74, 102.54 and 52.18 for bitter kola, soursop and avocado pear respectively. From (Table 1), the higher iodine value of soursop than that of bitter kola and avocado is an indication that the soursop is a better drying oil than bitter kola and avocado oils. The iodine value gives an indication of the degree of unsaturation of fatty acid. Therefore while the soursop oil can be classified as a semi drying oil, the bitter kola and avocado pear oils can be classified as nondrying oil and so may not be used as alkyl resins for paint formulation or as varnishes, but may however be used in conjunction with amino resin as finishers for certain application such as plasticizers. The relative low iodine values of both avocado pear and bitter kola seed oils may be indicative of the presence of few unsaturated bonds and hence they are expected to have low oxidative rancidity potentials (Ajiwe et al., 1997), a property which makes it possible to store them. Saponification values (SV) are 49.09, 248.51 and 232.82 for bitter kola, soursop and avocado pear oil respectively. The high SV for soursop and avocado pear oil make them useful for soap making just like that of coconut oil (SV 255), palm kernel oil (SV 247) and butter fat (SV 225), and also contains a high proportion of the lower fatty acids (sterols, phospholipids, waxes, terpenes). Also from (Table 1), the observed higher unsaponifiable values of 51.16% and 61.26% for bitter kola and soursop than avocado pear (16.28%) indicate that bitter kola and soursop can yield more sterols than avocado pear. These sterols are used in the production of drugs in pharmaceutical industries (Mason, 1981). The phospholipids extract can also be used in the production lecithin, used in manufacturing of margarine, confectionery, shortenings, etc. (Cary et al., 2000). Peroxide values are used as indicators of deterioration of oils. From the above it shows that these oils cannot easily get rancid, because high peroxide values are indicative of high level of oxidative rancidity.

The low peroxide values of these oils suggest that these oils can be stored. The acid values of the oils which are 4.50, 8.40 and 5.60mg NaOH/g for bitter kola, soursop and avocado pear respectively shows that soursop has higher fatty acid value than avocado pear and bitter kola. The acid value of this seed oil is in agreement with that reported for *pentacle thramacrophylla* and *Treculia Africana* (Akubugwe et al., 2007; Wollenberg, 1990). However, these values are lower when compared to that of *persea gratesima* (11.46mg NaOH/g), *cucumeropsis edulis* (9.36mg NaOH/g) and *prunus mygdalus* (9.66NaoH/g) (Idigo, 1989; Sarkar and Li, 2006) and as well as some other conventional oils sold in the market. The low acid value of these oils may be attributed to their low levels of free fatty acids which are also within the acceptable limits for edible oils (Liu, 2004). From the results, the pH values are 4.10, 6.80, and 5.40 for bitter kola, soursop and avocado pear respectively. Both avocado and bitter kola is acidic, while soursop is mildly acidic or close to neutral. Figure 1 shows the healthy human body pH range.

Research has shown that unless the human body's pH level is slightly alkaline, it cannot heal itself, no matter what means you choose to take care of the body, it would not be effective until the pH level is balanced.

The pH level of the body has the ability to affect every single cell of the body. When the blood has an alkaline pH, it will have a positive effect on how every bodily system functions.

On the other hand, when the pH of the body is acidic, it is susceptible to many diseases and problems. Research has shown that the oils from these seeds, suorsop, biter kola and avocado pear as part of their medicinal values have the ability to keep the pH human body akaline (Obadomi and Ochuko, 2001; Boam and Kocipai, 1994). From the results, the specific gravities are 1.79, 9.29 and 0.84 for bitter kola, soursop and avocado pear respectively.

The results of the phytochemical screening of the extracts of n-hexane, water and ethanol to ascertain the presence of saponins, tanins and alkaloids in *Annona muricata*, *Garcina kola* and *persea Americana* seed oils are shown in (Table 2). The screening shows that saponins were present in all the three types of seeds from the n-hexane extracts, including the aqueous and

Table 1: Results of physiochemical characteristics of the various seed oils.

Physiochemical Characteristics	BITTER KOLA	SOUR SOP	AVOCADO PEAR
% Moisture content	33.7	15	65.6
% Oil extracted	20	40	10.5
pH	4.10	6.80	5.40
Acid value (mg KOH/g)	4.50	8.40	5.60
Peroxide value (Meq/kg)	3.00	3.20	4.40
Iodine value	72.74	102.54	52.18
Specific gravity	1.79	9.26	0.84
Saponification value (mg HOH)	49.09	248.51	232.82
Unsaponifiable value (%)	51.16	61.26	16.28

Table 2: Results of phytochemical screening of n-hexane, ethanol and water extract of *Garcina kola, Annona muricata, and Persea americana* oils.

Seed oil	N –hexane extract			Aqueous extract			Ethanol extract		
	Saponins	Tanins	Akaloids	Saponins	Tanins	Akaloids	Saponins	Tanins	Akaloids
Soursop	+	-	-	+	-	-	+	-	-
Bitter kola	+	-	-	-	+	-	-	+	+
Avocado pear	+	-	-	-	+	-	+	+	-

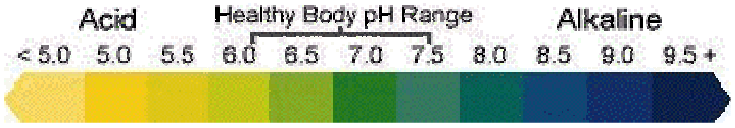

Figure 1: Healthy Body pH Range.

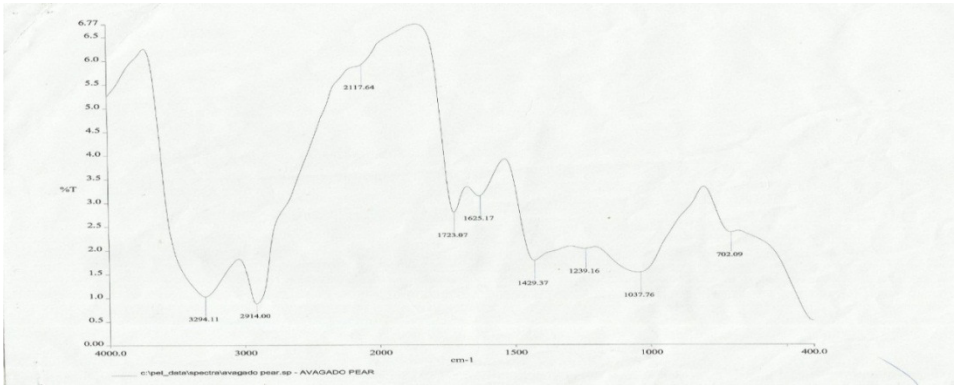

Figure 2: Infra- red spectroscopy of avocado pear.

ethanol extracts of soursop. Tanins were absent from the n-hexane extracts of the three seed oils including the aqueous and ethanol extracts of soursop and present in the aqueous extracts of both bitter kola and avocado pear. In addition to the ethanol extracts of bitter kola and avocado pear, alkaloids were absent from both the n-hexane and aqueous extracts of avocado pear, soursop and bitter kola including the ethanol extracts of soursop and avocado pear. The results of the Fourier Transform Infra-Red (FTIR) analysis of the oils are presented in (Figures 2, 3 and 4) for Avocado pear, bitter kola and soursop oil respectively. From the FTIR analysis, several peaks with their specific frequency of absorption were observed for each oil though with similarities and

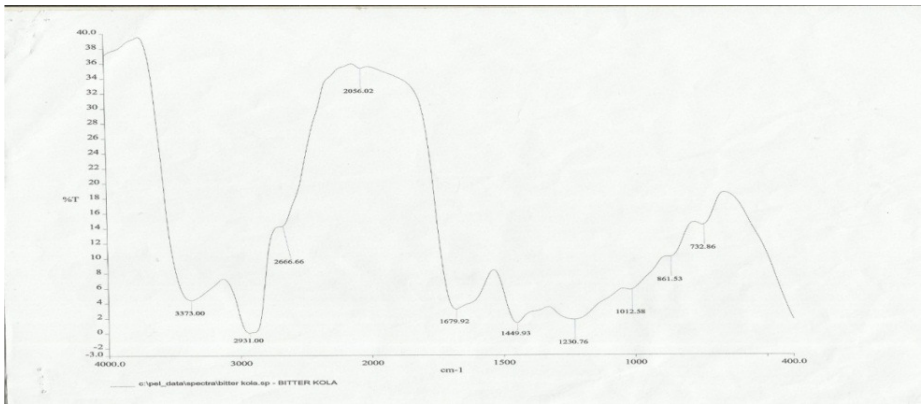

Figure 3: Infra- red spectroscopy of bitter kola.

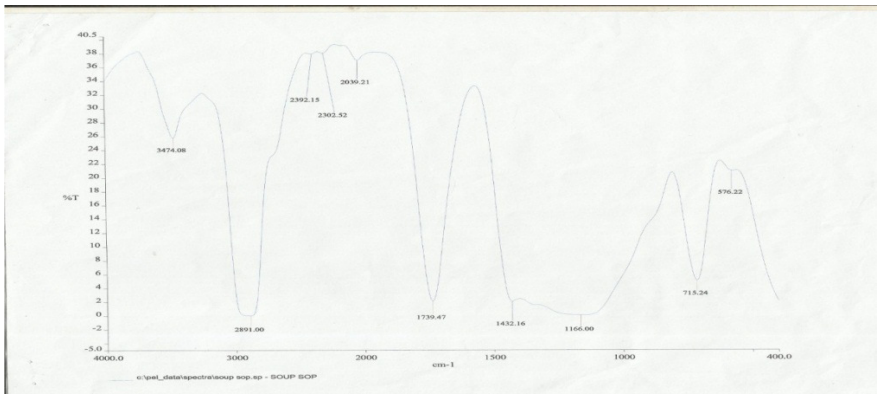

Figure 4: Infra- red spectroscopy of soursop.

variations between the functional groups found in them. The oils contain a hydroxyl group which is either an alcohol or phenyl, and other functional group.

Conclusion

Extraction, characterization and comparative assessment of *Annnona muricata* (soursop), *Garcina kola* (bitter kola) and *Persea americana* (avocado pear) seeds oils were evaluated in this study. The oils from the seeds are classified as non-drying due their low iodine values, coupled with their few unsaturated bonds. The oils have low susceptibility to oxidative rancidity as confirmed by their low peroxides values. Though the oils are non-drying and cannot be used as alkyl resins for paints formulation or vanishes, they may however be found useful in conjunction with amino resins as finishes for certain appliances, and in this case, as plasticizers as well as biodiesel. The results also revealed that *Annona muricata* and *Persea americana* oils have great potentials in soap manufacturing industries due to their high saponification values. Phytochemical screening of these oils place them as a source of raw materials for drugs due to the inbuilt of some phytonutrient or bioactive substances in them

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