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International Journal of Current Research
Vol. 5, Issue, 04, pp.992-997, April, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

PHYSICOCHEMICAL ANALYSIS, PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES OF SOME VEGETABLE OILS FROM OGUN STATE, NIGERIA

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ARTICLE INFO

Article History:
Received 16th January, 2012
Received in revised form
11th February, 2013
Accepted 29th March, 2013
Published online 13th April, 2013

Key words:
Physico-chemical,
Phytochemical Sreening,
Antimicrobial Analysis,
Ogun, Nigeria.

ABSTRACT

The study was carried out to assess the physicochemical properties, phytochemical screening and antimicrobial activities of twenty five different edible vegetable oil samples collected from Ota, Ogun state in Nigeria for their domestic and commercial applications. The moisture contents (0.02 – 0.13%), acid values (0.46 – 1.73 mg KOH/g), saponification values (226.19 – 310.19 mg KOH/g), iodine values (0.06 – 1.17 mg I_2/g) and the low pH values (0.0 – 2.49) generally make these vegetable oils to be more useful in industrial applications than consumed directly or indirectly as ingredients in food. The results of the phytochemical screening of these oil samples indicated the presence of terpenoids, deoxysugar, steroids and cardenolides. The microbial sensitivity tests showed that most of the vegetable oil samples exhibited no inhibitory power against *candida*, *Klebsiella pneumonia*, *Escherichia coli* but only sample 25 (soya bean oil) was sensitive to these three microbes and its inhibition of these microbes was concentration dependent. The mineral contents of these oils showed detectable levels of Zn (2.57 – 21.43 ppm), Mn (0.12 – 0.98 ppm), Fe (2.21 – 97.41 ppm), Cu (0.49 – 61.70 ppm), Cd (0.42 – 0.74 ppm) and Pb (0.14 – 31.09 ppm) which were found to be above the WHO permissible limits for these metals in drinking water.

INTRODUCTION

The nutritive and calorific values of seeds make them good sources of edible oils and fats diet (Akubugwo *et al*., 2008; Odoemelam, 2005). Edible seed oils have been extracted and used as food ingredients since ancient times (Parry *et al*., 2005). Vegetables oils are derived from plant sources like soya beans, melon, groundnut, corn, oil palm, shea butter, coconut, etc. The term "vegetable oil" can be narrowly defined as referring only to substances that are liquid at room temperature (Parwez, 2011), or broadly defined without regard to a substance's state of matter at a given temperature (Robin, 1999). For this reason, vegetable oils that are solid at room temperature are sometimes called vegetable fats. Some of these vegetable oils are used for domestic (edible) and industrial purposes (Gurr, 1991). The domestic use of vegetable oil began in the early 1900s when new chemical processes allowed them to be extracted. Unlike butter or coconut oil, these vegetable oils can't be extracted just by pressing or separating naturally. They must be chemically removed, deodorized and altered. Vegetable oils are composed of triglycerides which are the ester of one molecule of glycerol and three molecules of fatty acids. Fatty acids are primary nutritional components found in edible seed oils. Vegetable oils are mainly classed as Oleic-Linoleic acid oils since they contain a relatively high proportion of unsaturated fatty acids, such as the monounsaturated oleic acid and the polyunsaturated linoleic acid (Nkafamiya *et al*., 2010; Musa *et al*., 2012). They are characterised by a higher ratio of polyunsaturated fatty acids to saturated fatty acids. Vegetable oils contain a very high concentration of Omega 6 fatty acids and polyunsaturated fats. Nutritionally, vegetable oils are usually preferred to animal fat as evidence linking health benefits to the consumption of vegetable oils continues to grow (Wardlaw, 1999; Parry *et al*., 2005) even when recent researches (Ravnskov, 1998; Golomb, 1998; Wallstrom *et al*., 2007; Crowe *et al*., 2008; Sieri *et al*., 2008; Alexander *et al*., 2009) disapproves this notion. Vegetable oils also contain additional health beneficial phytochemicals such as saponin, phlobatanin, flavonoid, tanin, terpenoid, anthraquinone, etc. Majority of the phytochemicals have been known to bear valuable therapeutic activities such as insecticidals (Kambu *et al*., 1982), antibacterial, antifungal (Lemos *et al*., 1990), anticonstipative (Ferdous *et al*., 1992), spasmolytic (Sontos *et al*., 1998), antiplasmodial (Benoitvical *et al*., 2001) and antioxidant (Vardar-unlu *et al*., 2003) activities etc. Vegetable oils also contain some metals. Some of these metals like Na, K, Ca, Mg, Fe, Cu, Zn and Mn are essential nutrients for human growth while certain forms of these metals can be toxic. The presence of trace metals is an important factor as far as the quality of edible oil is concerned.

Vegetable oils are a very important ingredient in many manufactured products. Vegetable oils are used in various industrial applications such as emulsifiers, lubricants, plasticizers, surfactants, plastics, solvents, and resins. Vegetable oils are reusable. They are used for animal feed and pet food. More recently, waste vegetable oils have become known for their ability to be refined into biodiesel, which can be used like conventional diesel fuel in diesel engines. Vegetable and edible oils had made an important contribution to the diet of people in many countries, serving as a good source of protein, lipid and fatty acids for human nutrition including the repair of worn out tissues, new cells formation as well as a useful source of energy (Atasie *et al*., 2009). In Nigeria, the major sources of edible oils are groundnut, palm and soybeans. Vegetable oils, mostly groundnut oil is of high quality and can withstand higher temperature without

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burning or breaking down. It has neutral flavour and odour. It does not absorb odours from other foods (Passera, 1981; Musa *et al*., 2012). These make it the most preferred oil in most parts of Nigeria. It is therefore necessary to analyse these vegetable oils that are preponderant in our market places to determine its edibility, suitability for a given purpose and its overall impact on public health. Therefore, the aim of this study is to investigate the physicochemical properties of the sampled edible oils, determine the presence and actions of some phytochemicals in the vegetable oils and also determine the susceptibility pattern of micro-organisms found in the vegetable oils.

MATERIALS AND METHODS

Sample Identification and Collection

The vegetable oil samples were bought in neat bottles from an open market and supermarket in Ota, Ogun State, Nigeria. The samples gotten from the open market involved both branded and unbranded oil from different spots while the samples from the supermarket were branded oil. The samples were labeled 1-25 and were stored in a 400ml sterile capped glass bottles at room temperature in cupboard to prevent exposure to light which can also affect its properties.

Phytochemical Screening, Physicochemical analysis and Mineral Contents

The vegetable oil samples were screened for phytochemical constituents using standard procedure to identify the constituents as described by Harborne (1973), Sofowora (1993) and Trease and Evans (1989). The phytochemicals tested were alkaloids, anthraquinones, flavonoids, saponins, steroids, tannins, terpenoids, phlobatanin, alkaloid, cardenolides and deoxysugars. The vegetable oil samples were analysed for moisture, iodine value, saponification value, acid value, pH and refractive index using the methods described by AOAC (1997). The metal contents of zinc, manganese, iron, cadmium, copper and lead were determined using the method as described by Kingston and Jessie, 1998.

Microbial Sensitivity test

Test Organism

Staphylococcus aureus, *Escherichia coli*, *Klebsiella pneumoniae*, *candida, Aspergillus niger* and *Aspergillus flavus* were obtained from National Institute of Medical Research, Yaba, Lagos state, Nigeria. The identity and confirmation of these test microorganisms were carried out in the collection centres. Fresh plates of the test bacteria were made from the isolate cultures obtained on agar slants. Colonies of fresh cultures of the different bacterial isolates were picked and suspended in 5ml nutrient broth in well labelled sterile bijou bottles. They were incubated for 24 hours at 30^0C. The stock solution of the samples was prepared by dissolving 2g of each sample in 2ml of sterile distilled water to give a concentration of 1000mg/ml. The stocks were labeled appropriately and refrigerated at 4^0C until required for use.

Determination of Antimicrobial Activity of Extracts

The Agar well dilution method as described by Lino and Deogracious (2006) was used. Standardized inoculums (0.5 McFarland turbidity standard equivalent to 5 x 10^8cfu/ml) (NCCLS, 1999) of each test bacterium was spread onto sterile Mullar hinton Agar plates so as to achieve even growth. The plates were allowed to dry and a sterile cork borer (3.0 mm diameter) was used to bore wells in the agar plates. The samples were prepared by double dilution method. This was done by adding 5ml of the stock solution to the test-tube containing 2ml of sterile distilled water to achieve a concentration of 200mg/ml. This concentration (500ml/ml) was further diluted to concentrations of 100mg/ml and 50mg/ml. Subsequently, 0.1ml of each sample was introduced in wells earlier bored in the agar plate cultures. Chloramphenicol (100mg/ml) was used as a positive control and 20% Mullar hintor Agar as negative control. The plates were then incubated at 37^0C for 24h. Antimicrobial activity of the samples was determined after incubation period by measurement of zones of inhibition. The sensitivity test was done in triplicate and mean zone of inhibition was taken.

Determination of Minimum Inhibition Concentration (MIC)

A current definition of MIC is the lowest concentration which resulted in maintenance or reduction of inoculums viability (Carson *et al*., 1995). The MIC of the potent sample was determined according to the broth dilution technique (Junaid *et al*., 2006). Standardization suspensions of the test organisms (1.0 x 10^8cfu/ml) were inoculated into a series of sterile tubes of Mullar hinton broth containing two-fold dilutions (400mg/ml, 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml) of sample and incubated at 37^0C for 24h. The MIC of the extracts was determined for each of the test organisms in triplicates. The MIC was read as the least concentration that inhibited the growth of the test organisms.

RESULTS AND DISCUSSION

Physico-Chemical analysis

The results of the physicochemical analysis of the vegetable oil samoles are presented in Table 1.

The moisture contents (ranging between 0.02 – 0.13%) of all the vegetable oil samples analysed were very low compared to the amounts obtained by Kershaw and Hackett (1987) from edible oil seeds such as cottonseeds (6.46%), peanuts (4.58%), palm kernel (5.31%), sesame (4.60%), soybean (11.07%) and sunflower seeds (6.58%). Moisture content is a widely used parameter in the processing and testing of food. It is an index of the water activity of many foods (Andzouana and Mombouli, 2012). A major aim of processed food item is to keep its moisture content very low so that it can be stored or preserved for long periods of time; a reason that might be responsible for the low moisture contents of the vegetable oil samples analysed. The acid values of the vegetable oil samples ranged between 0.46 and 1.73 mg KOH/g and are lower than the acid values of groundnut oil samples (1.88 and 6.83 mg KOH/g) reported by Musa *et al*., 2012. Acid value determination is often used as a general indication of the condition and edibility of the oil. This is because an increase in acid value is accompanied by development of objectionable flavours and odours (Hariod, 1990; Nkafamiya *et al*., 2006). The saponification values (ranged between 226.19 and 310.19 mg KOH/g) obtained for the vegetable oil samples are comparable with the values obtained for groundnut oil by Musa *et al*., 2012 and palm oil samples by Akinola *et al*., 2010. Saponification number is an indication of the amount of fatty saponifiable material in oil or fat. It gives information concerning the character of the fatty acids of the oil or fat and in particular regarding the solubility of their soaps in water. The higher the saponification number of the oil, the more soluble the soap that can be made from it (Alyas, 2006). The saponification values of the vegetable oil samples analysed indicate that the oils may be used industrially for making soap and other cosmetic products.

The iodine values of the vegetable samples analysed were very low compared to the results obtained by Musa *et al*., 2012 for groundnut oil samples and Akinola *et al*., 2010 for palm oil samples. Iodine value is an indicator of double bindings in the molecular structure, which influences the long term stability properties of the oil (i.e. important for storage). It has been reported that lowering the iodine value improves the stability and good yield of the liquid oil (Tan *et al*., 2002). The pH of the samples was carried out to determine the acidity or alkalinity of the oil samples. pH values greater than 7 are alkaline while pH values less than 7 are said to be acidic. The results obtained for acid values from table 1 shows that all the oil samples are highly acidic. This implies that they contain large amount of fatty

Table 1. Physicochemical Analysis of Vegetable Oil Samples

Samples	Moisture content (%)	Acid value (mg KOH/g)	Saponification value (mg KOH/g)	Iodine value (mg I_2/g)	pH	Refractive index
1	0.10	0.46	267.72	0.18	0.00	1.45
2	0.04	0.50	248.40	0.12	0.32	1.45
3	0.07	0.91	247.72	0.06	0.41	1.45
4	0.12	1.23	310.19	0.06	0.84	1.45
5	0.11	0.74	215.97	0.37	1.02	1.45
6	0.10	1.73	306.16	0.25	0.87	1.45
7	0.13	0.97	283.30	0.19	0.00	1.45
8	0.11	0.48	245.28	0.63	0.54	1.45
9	0.09	1.47	226.19	0.88	0.89	1.45
10	0.09	1.00	258.46	1.17	1.81	1.45
11	0.09	0.70	272.71	0.31	1.90	1.45
12	0.09	0.75	242.29	1.11	2.08	1.45
13	0.02	0.74	289.10	1.17	2.49	1.45
14	0.08	0.73	284.27	0.31	2.13	1.45
15	0.12	0.49	274.36	1.11	0.00	1.45
16	0.08	0.97	278.90	1.00	1.19	1.45
17	0.12	0.99	285.13	0.31	1.74	1.45
18	0.11	1.00	285.29	0.44	1.62	1.45
19	0.08	0.98	253.97	0.13	1.65	1.45
20	0.05	0.98	266.18	0.13	0.00	1.45
21	0.06	0.73	259.58	0.67	0.00	1.46
22	0.03	0.70	259.98	0.61	0.00	1.46
23	0.07	0.70	258.88	1.00	0.00	1.45
24	0.06	0.49	226.69	0.57	0.00	1.45
25	0.04	0.93	256.86	0.06	0.00	1.46

*The results displayed are the mean results of triplicate determinations.

Table 2. Phytochemical analysis of the vegetable oil samples

Saponin	Phlobatanin	Tanin	Anthraquinone	Alkaloid	Flavonoid	Terpenoid	Deoxysugar	Steroids	Cardenolides
-	-	-	-	-	-	++	+	-	+
-	-	-	-	-	-	+	+	++	+
-	-	-	-	-	-	++	+	+	+
-	-	-	-	-	-	++	+	-	+
-	-	-	-	-	-	++	+	-	+
-	-	-	-	-	-	-	+	+	+
-	-	-	-	-	-	++	+	-	+
-	-	-	-	-	-	++	+	+	+
-	-	-	-	-	-	++	+	-	+
-	-	-	-	-	-	++	+	-	+
-	-	-	-	-	-	++	+	+	+
-	-	-	-	-	-	++	+	-	+
-	-	-	-	-	-	++	+	-	+
-	-	-	-	-	-	++	+	-	+
-	-	-	-	-	-	++	+	-	+
-	-	-	-	-	-	++	+	+	+
-	-	-	-	-	-	++	+	-	+
-	-	-	-	-	-	++	+	+	+
-	-	-	-	-	-	++	+	-	+
-	-	-	-	-	-	++	+	+	+
-	-	-	-	-	-	++	+	+	+
-	-	-	-	-	-	++	+	-	+
-	-	-	-	-	-	++	+	-	+
-	-	-	-	-	-	++	+	++	+
-	-	-	-	-	-	++	+	++	+

+ = Present ++ = Present in high Concentration= Absent

acids making them unfit for edible purposes. High concentrations of free fatty acids are undesirable in vegetable oils because they can reduce the palatability and the shelf-life of the oil. The refractive index was carried out on the samples to check purity and also measure the speed of light in the oil samples. There was no significant difference between the refractive index of the different oil samples. The range is 1.4500 – 1.4578.

Phytochemical analysis

The results of the phytochemical compounds analysed quanlitatively are shown in Table 2. The results of the phytochemical screening of these oil samples indicated the presence of terpenoids, deoxysugar, and cardenolides. Steroids were present in some of the samples and absent in others while saponins, phlobatanins, tannins, anthraquinones, alkaloids and flavonoids were absent in the samples. These phytochemicals exhibit diverse pharmacological and biochemical actions when ingested by animals (Amadi *et al*., 2006). Stereoidal compounds are of importance due to their relationship with some compounds such as sex hormones (Okwu, 2001). Steroids, Glycosides, terpenoids and alkaloids have been reported to exert inhibiting activity against most bacteria (Camacho-Corona *et al*., 2008; Al-Bayati and Suleiman, 2008).

Microbial sensitivity test

The results of the antimicrobial activities of the vegetable oil samples are shown in Table 3 and Table 4 gives the Minimum Inhibitory Concentration of the most potent sample (sample 25).

Table 3. Zone of inhibitions of the vegetable oil samples

Organisms / Oil Samples	Zone of Inhibition in mm					
	Staphylococcus aureus	*E. coli*	*Klebsiella pneumonia*	*Yeast*	*Aspergillus niger*	*Aspergillus flavus*
1	R	R	R	R	R	R
2	R	R	R	R	R	R
3	R	R	R	R	R	R
4	R	R	R	8	R	R
5	R	R	R	R	R	R
6	R	R	R	R	R	R
7	R	R	R	R	R	R
8	R	R	R	R	R	R
9	R	R	R	R	R	R
10	R	R	R	R	R	R
11	R	R	R	R	R	R
12	R	R	R	R	R	R
13	R	R	R	R	R	R
14	R	R	R	R	R	R
15	R	R	R	R	R	R
16	R	R	R	R	R	R
17	R	R	R	R	R	R
18	R	R	R	R	R	R
19	R	R	R	R	R	R
20	R	R	7	R	R	R
21	R	R	R	R	R	R
22	R	R	R	R	R	R
23	R	R	R	R	R	R
24	R	R	R	R	R	R
25	R	11	16	17	R	R
Control	R	R	R	R	R	R

R = Resistance

The results in Table 3 show that most of the vegetable oil samples exhibited no inhibitory power against the microbes used in this research work, therefore the organisms were said to be resistant to the oil samples. Sample 4 had antimicrobial sensitivity on *candida* but no activity on the other organisms. *Klebsiella pneumonia* was sensitive to sample 20 at a zone of 7mm while other organisms were resistant to the sample. For sample 25, *yeast*, *Klebsiella pneumonia*, *Escherichia coli* were sensitive to the samples at a zone of 11, 16 and 17mm respectively while the others were resistant. Therefore, sample 25 show some promise as an antimicrobial agent against infections and diseases resulting from *Escherichia coli, Klebsiella pneumonia and candida.* The results of the Minimum Inhibitory Concentration (MIC) of sample 25 show that the inhibitory activities of sample 25 are concentration dependent. At 10^{-1} and 10^{-2} dilution, the three microbes were sensitive to sample 25 with the microbes showing greater sensitivity in the 10^{-1} dilution. At 10^{-3} and 10^{-4} dilution, only *Escherichia coli* showed resistance. At 10^{-5} dilution, all the three microbes were resistant to sample 25.

Table 4. Minimum Inhibitory Concentration (MIC) in mm of sample 25

Oil Dilutions	Microbes		
	Escherichia coli	*Klebsiella pneumoniae*	*Yeast*
10^{-1}	10	14	15
10^{-2}	5	12	12
10^{-3}	R	8	9
10^{-4}	R	4	4
10^{-5}	R	R	R

R = Resistance

Mineral contents analysis

The results of the mineral compositions of the vegetable oil samples are given in Table 5. Determination of heavy metals in edible seed oil is of importance, as heavy metals are useful micronutrients for plants, humans and animals but become toxic for them when their concentration exceeds a limit (Khemnani *et al.*, 2012). From the result obtained for the mineral contents analysis, all the samples showed detectable levels of Zn (2.57 – 21.43 ppm), Mn (0.12 – 0.98 ppm), Fe (2.21 – 97.41 ppm), Cu (0.49 – 61.70 ppm), Cd (0.42 – 0.74 ppm) and Pb (0.14 – 31.09 ppm). The concentrations reported for most of the metals analysed in the samples exceeded the WHO (2008) permissible limit for Zn (0.10 ppm), Cu (1.50 ppm), Cd (0.003 ppm) and Pb (0.01 ppm) in drinking water. The toxicity of these metals even at low concentrations is well documented hence making these edible seed oils analysed unsafe for consumption.

Table 5. Mineral composition of the vegetable oil samples

SAMPLES	Zn	Mn	Fe	Cd	Cu	Pb
1	ND	0.64	14.46	0.47	10.61	ND
2	11.65	1.54	82.17	0.47	40.62	7.45
3	18.62	1.75	13.42	0.54	4.16	3.39
4	ND	0.83	14.15	0.70	5.11	0.14
5	ND	0.48	16.60	0.70	2.55	2.79
6	ND	0.83	9.50	0.60	2.30	ND
7	13.09	1.13	97.41	0.65	13.64	31.09
8	ND	0.53	13.23	0.57	3.21	ND
9	ND	0.77	11.54	0.56	1.99	ND
10	ND	0.98	2.21	0.60	8.80	2.30
11	ND	0.97	3.47	0.62	2.76	ND
12	ND	1.17	44.25	0.58	5.07	ND
13	2.57	0.69	40.98	0.52	10.91	21.54
14	ND	0.57	59.99	0.71	8.54	1.02
15	ND	0.81	9.71	0.60	1.44	ND
16	16.71	0.59	20.22	0.60	22.23	8.47
17	21.43	1.94	40.52	0.74	ND	17.16
18	ND	0.66	31.55	0.46	61.70	10.33
19	ND	0.20	9.75	0.59	56.25	ND
20	ND	0.39	7.88	0.66	0.49	ND
21	ND	0.24	10.15	0.63	2.22	ND
22	ND	0.12	12.59	0.51	2.08	ND
23	ND	0.55	11.13	0.44	2.47	ND
24	ND	0.67	11.76	0.48	19.27	ND
25	ND	0.27	4.64	0.42	1.08	ND

*The results displayed are the mean results of triplicate determinations for each mineral and sample. The results are in ppm. ND = NOT DETECTED

Conclusion

These results imply that the vegetable seed oils have greater potentials as industrial raw materials than to be consumed directly or indirectly as ingredients in food. The soya bean oil is most beneficial to human health having a potential to be used for medical purposes and as an antimicrobial agent against infection and diseases

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