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**Research Article**

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# Effect of *Sida Acuta* ethanolic Leaf Extract on the Oxidative Stability of Groundnut Oils

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**Abstract** Natural and biosynthesized phytocompounds have gained significant attention in the food industry because of their non-controversial health and safety benefits as compared with chemically synthesized antioxidants. However, no natural biosynthesized phyto-compounds has been commercially available and used in routine food processing. This study evaluates the antioxidant property of *Sidaacuta* plant commonly used by many locals in storing cooking oils. The ethanolic leaf extract of this plant was evaluated for its antioxidant potential, total phenolic (TP) and total flavonoid (TF) contents using standard procedures. The extract was added to groundnut oil at four different concentrations of 100 mg/L, 200 mg/L, 400 mg/L and 800 mg/L and stored over a six-month period, while chemical properties of oil including peroxide value (PV), anisidine value (AnV), acid value (AV) and iodine value (IV) were checked on a monthly basis. The results showed that the extract had significant antioxidant effect for DPPH radical scavenging activity (27.4800±0.0173 $IC_{50}$ % inhibition), β-carotene linoleic antioxidant activity (29.7267±0.0251 $IC_{50}$ % inhibition), ORAC (6.1067±0.0115 μ Mol TE/g) and FRAP (291.0367±0.0305 μ Mol $Fe^{2+}$/L). Total phenolic content expressed in tannic acid equivalents was 27102.365±10.189 μg/ml, while total flavanoid content expressed in quercetine equivalents was 1024.783±5.569 μg/ml. The addition of the leaf extract to oils showed significant retardation of oxidation compared to samples without the extract. The findings reveal that the use of *Sidaacuta* as a natural antioxidant to prevent vegetable oils oxidation if properly harnessed can be a good alternative to synthetic antioxidants.

**Keywords** Lipid peroxidation, groundnut oil, natural antioxidant, chemical property

## Introduction

Groundnut oil like other vegetable oil is determined on the ester which is made up of straight chain higher fatty acids and glycerine. The fatty acids include the unsaturated; palmitic acid and stearic acid, mono unsaturated fatty acids; such as oleic acid, and polyunsaturated fatty acids such as linoleic acid, linolenic acid; docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) [1]. Groundnut oil is characterized by 45.2 % oleic acid (18:1) and 32.4 % linoleic acid (18:2), palmitic (C16:0), and a trace amount of linolenic fatty acid (C18:3), [2-3]. It also contains some stearic acid, arachidic acid, arachidonic acid, behenic acid, lignoceric acid and other fatty acids. At present, the fatty acid composition of groundnuts has become increasingly important with the realization that the compositions of Oleic and Linoleic fatty acids have a large and important bearing on the stability, nutritional quality, and flavor of

groundnut oil and its derived products [4-5]. Oils including fats are recognized as essential nutrients in the human diet. Benefits of oil in any food preparation include provision of concentrated form of energy, provision of essential fatty acids as precursors of certain hormones, contribution to satiety after eating, and as carriers of fat-soluble vitamins among others [6-7]. Unfortunately, these benefits may be compromised during prolonged storage if lipid peroxidation occurs, which is one of the major causes of food deterioration [8]. Oxidation of oil also referred to as rancidity is due to the reactions of fats and oils with molecular oxygen to form free radicals that are capable of initiating chain reaction that can give rise to undesirable characteristics such as change in colour, odour, flavour and nutritive value [10]. Lipid oxidation also reduces the organoleptic characteristics of the foods they are prepared with [11]. All oil is in a state of oxidation, which cannot be completely stopped, but attempts are made to reduce its impact on oil quality at each stage of oil processing.

Synthetic antioxidants have been tested for safety and approval for use in oils at low concentrations on the basis of complex toxicity studies in many countries; however, there is still doubt about their safety as many reports have documented their health concerns [12]. As previously reported, many natural antioxidants like ginger extract, raspberry leaves, pussy willow extract, olive leaf juice have been used to protect oxidizable constituents of essential oils from oxidation and known as shelf life enhancers [13-14]. However, natural antioxidants have not been used on large commercial scale owing to its high input cost, seasonal availability of their sources and a lack of scientific and technological data in favors of their safe, nutritious, and sensory parameters. So, a common, all-inclusive available and cheap source of phyto-compounds is the prime interest of the researchers currently [15]. Although some research has been carried out that show many properties of *Sidaacuta* relating to medicine [16] and physiological remedy for oxidative stress, there is no documented evidence of its antioxidant potential for the purpose oxidative stabilisation of edible oils storage. Consequently, this research work was designed to evaluate the antioxidant potential of the plant extracts using groundnut oil as an oxidation substrate stored over a specified period of time.

## Methodology

### Sample Collection

Mature *Sidaacuta* (SA) leaves were collected from Makurdi metropolis and were properly identified by an expert botanist at the Department of Biological Sciences of Benue State University. The leaves were washed under running tap water and air-dried for 3 days and subsequently crushed mechanically to obtain a suitable surface area. The resulting samples were then dried at room temperature in the shade to avoid exposure to sunlight for two hours before being stored for further analysis. Groundnut oil samples were collected from local processors in Makurdi, Benue State and kept in tightly sealed MacCakney bottles prior to storage.

### Extraction of *Sidaacuta* leaf extracts

Extraction of leaf extracts was done by cold maceration according to the method described by Hossain *et al.* [17] and Evbuomwan *et al*. [18]. About 500 g of ground leaves were weighed into 4000 mL of ethanol and the solution was macerated for about 24 h with gentle shaking at 360 rpm until the soluble matter had properly dissolved. After extraction, the solution was clarified by filtration under vacuum using Whatman filter paper and the ethanol solvent evaporated completely using a rotary evaporator. The solvent free ethanol crude extract was suspended in diethyl ether to purify the extract after which it was exposed to the atmosphere for a while to ensure elimination of the solvent odour. The extracts obtained were then weighed and refrigerated for further analysis.

### Phytochemical Analysis

**Total Phenolic Content**: The total phenolic content of the leaf extracts of the samples was determined by taking 20 µL of the extract in a screw capped 11-mL test tube, together with 1.6 mL distilled water and Folin-Ciocalteu reagent (100 µL). All components were mixed with each other, then 300 µL of 20% $Na_2CO_3$ solution added and well shaken in a shaking water bath at 40°C for 30 min. Total phenolic content was determined from the standard curve plotted by using gallic acid as standard at 760 nm according to the method of Anwar *et al*. [19].

**Total Flavonoid Content**: The total flavonoid (TF) content of the leaf extracts was quantified according to the method described by Dewanto *et al.* [20] and the results determined as catechin equivalents (mg/100 g of dry weight). At a concentration of 1 mg/mL, the extracts were diluted with 4 mL of water in a 10 mL volumetric flask. Initially, 0.3 mL of 5% $NaNO_2$ solution was added to each volumetric flask; at 5 min, 0.3 mL of 10% $AlCl_3$ is added; and at 6 min, 2 mL of 1.0 mol/L NaOH was added before 2.4 mL Water was added to the reaction flask and mixed well. Absorbance of the reaction mixture was read at 510 nm.

**Tannins:** 0.2 g of sample was measured into a 50 ml beaker. 20 ml of 50% methanol added and covered with paraffin and placed in a water bath at 77-80oC for 1 h and stirred with a glass rod to prevent lumping. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper into a 100 ml volumetric flask using 50% methanol to rinse. This was then made up to mark with distilled water and thoroughly mixed. 1 ml of sample extract was then pipette into 50 ml volumetric flask, 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% $Na_2C0_3$ then added and mixed properly. The mixture was made up to mark with distilled water, mixed well and allowed to stand for 20 min till a bluish-green colouration developed. Standard Tannic Acid solutions of range 0-10 ppm was treated similarly as 1 ml of sample above. The absorbances of the Tannic Acid Standard solutions as well as samples are read after colour development on a Spectrophotometer at a wavelength of 760 nm. Percentage tannin was calculated using the formula:

$$\text{Tannin (\%)} = \frac{\text{Absorbance of sample x Average gradient x Dilution factor}}{\text{Weight of sample x 10,000}}$$

**Evaluation of Antioxidant Activity**

**DPPH radical scavenging activity (DRSA)**: Initial DPPH assay on TLC plate was done and then the antioxidant activity of the different crude extracts was evaluated as described by Hossain *et al.* [17] with modification. Four milliliter (4mL) of each concentration was placed in a working test tube and then DPPH (2,2-diphenyl-1-picrylhydrazyl) (1mL, 0.1mM, methanol) added to the test tube and shaken vigorously. After shaking, all the test tubes were allowed to stand at 27 $^{o}$C in a dark place for 45 min. A control sample was prepared according to the same procedure without any extract. The absorbance of the tested samples was then be measured by UV spectrophotometer at the wavelength 517 nm. The antioxidant activity of each sample was expressed in terms of concentration required to inhibit DPPH radical formation by 50% ($IC_{50}$ µg/ml) and calculated from the log-dose inhibition curve.

$$\%DPPH = \frac{(Ac - At)100}{Ac}$$

Where Ac=Absorbance of Control (defined as absorbance in absence of standards or extracts)

At= Absorbance of Sample (defined as absorbance in presence of standards or extracts.

**Oxygen Radical Absorbance Capacity (ORAC)**

The method employed by Girgih *et al*., [21] was used. The samples were dissolved in sodium phosphate buffer (75 mM, pH 7.4) and then mixed with 300 nM fluorescein in a 96-well microplate followed by incubation of the mixture in the dark at 37°C for 15 min (final peptide concentration of 1mg/mL). Thereafter, a 50µL aliquot of 80mM 2,2$^{l}$-azobis (2-amidinopropane) dihydrochloride (AAPH) was added to the mixture and the change in fluorescence due to AAPH-induced oxidation of fluorescein measured at 1 min intervals for 90min at excitation and emission wavelengths of 485 nm and 528 nm, respectively, using a fluorescence microplate reader. Different concentrations of Trolox (5–80 µM) was used to prepare a standard curve and the ORAC values of the samples calculated as follows:

$$1 + = \sum_{i=1}^{i-100} \frac{\int i}{\int 0}$$

ORAC values was expressed as µ MT trolox Equivalent (TE)/g of sample.

**Ferric Reducing Antioxidant Power (FRAP)**

The method employed by Girgih *et al*. [21] was used. 250μL of the sample was dissolved in 0.2M sodium phosphate buffer at pH 6.6 and blank (250μL of buffer) was mixed with 250μL of same buffer followed by addition of 250 μL of 1% (w/v) potassium ferricyanide solution. Thereafter, 250μL of peptide/TCA mixture was combined with 50μL of 0.1% (w/v) ferric chloride and 200μL of double distilled water and allowed to stand at room temperature for 10min.The solution was then centrifuged at 10,000×*g* and 200μL of the clear supernatant transferred to a 96-well plate for determination of the absorbance of the supernatant at 700nm.

**β-Carotene Bleaching Antioxidant Assay**

In this assay the antioxidant capacity of *Sidaacuta* was determined in emulsion by the β-carotene bleaching method of Farag *et al*. [14] consisting in a coupled oxidation of linoleic acid and β-carotene. A stock solution of β-carotene/linoleic acid (Sigma–Aldrich) was prepared as follows. β-carotene (0.5 mg) was dissolved in 1 ml of chloroform (HPLC grade), then 25 μl of linoleic acid and 200 mg of Tween 40 (Merck) were added. The chloroform was subsequently evaporated, then distilled and oxygenated water (100 ml) was added with vigorous shaking. Aliquots (2.5 ml) of the stock solution were transferred to test tubes, and 300 ml portions of the extracts (1 g/l in methanol) were added before incubating for 48 h at room temperature. The antioxidant activity was evaluated by absorbance measurement at 470 nm against a blank containing emulsified linoleic acid without β-carotene.

**Preparation of Oil Samples for Storage**

The leaf extracts of *Sidaacuta* was added to the groundnut oil samples in four concentrations of 100 mg/L, 200 mg/L, 400 mg/L and 800 mg/L. The acceptable maximum concentration of 100 mg/L of butylated hydroxyl toluene (BHT) was added to another portion of the oils to act as standard while a sample without extract or BHT was used as the control. All samples were measured into 100 mL transparent bottles to the filled and cork screwed. They were then kept in a dark cupboard from where samples were taken for analysis each month.

**Analysis of Physico-chemical Quality Parameters**

**Peroxide Value**

Peroxide value was determined according to the method described by Morris [22]. 5g of oil was dissolved in 30 mL of solvent mixture consisting of 60% glacial acetic acid and 40% chloroform and 0.5 mL of saturated solution of potassium iodide (KI). The flask was shaken until clear by giving a rotary motion to the flask. After exactly 2 minutes from time of the KI, 30 mL of water was added and the liberated iodine was titrated with 0.1 N sodium thiosulphate solution. The flask was shaken vigorously to remove any traces of iodine from the chloroform layer. A blank titration was also done. The result was expressed in terms of miliequivalents per 1000g of oil using the following formula:

$$Milliequivalents\ per\ 1000g = \frac{mL\ \times N}{g} \times 1000$$

Where, mL = mL of sodium thiosulphate solution, N = normality of sodium thiosulphate solution, g = gram of oil

**Anisidine value**

Anisidine value (AnV) was determined according to AOAC ISO Method 6885. The oil (5 g) was weighed directly into a 25 mL volumetric flask, dissolved, and made up to the mark with isooctane. 5 mL of the oil solution was then pipetted to a clean screw capped test tube, and 1 mL of anisidine reagent (0.25% solution of anisidine in glacial acetic acid) added. Thereafter, the tube was closed, vortexed and kept in the dark at room temperature for 10 min after which the solution was transferred to a clean, dry spectrophotometer cell, and the absorbance read at 350 nm on a JENWAY UV-spectrophotometer. The experiment was repeated with an adjusted amount of oil whenever the measured absorbance was outside of the range 0.2 to 0.8. Unreacted test solution was similarly prepared but instead

of anisidine reagent, glacial acetic acid (1 mL) was added. For the blank, the oil solution was replaced with isooctane (5 mL). The anisidine value was calculated using the following formula:

$$AV = \frac{25}{M}[1.2 \times (A1 - A2 - A0)]$$

Where: $A_0$ is the absorbance of the unreacted test solution, A1is the absorbance of the reacted solution, A2 is the absorbance of the blank, and M is the mass of the oil in grams.

**Acid Value**

2g of sample was weighed and placed in a 250mL flask. 50mL of a mixture of equal volumes of ethanol and ether, which has been neutralized by 0.5N of potassium hydroxide, was then added. The resulting mixture was heated for 10 minutes to allow for complete dissolution of the sample and then cooled. 1mL of phenolphthalein indicator was then added while shaking the contents vigorously. The mixture was titrated with 0.5N potassium hydroxide until a pink colour was obtained as described by AOAC [23]. The entire procedure was repeated for a blank analysis. The acid value was then calculated using the formula:

$$Acid\ Value = \frac{TD \times N \times 56.1}{M}$$

Where;

TD= Titre Difference = B – S, B= Titre value blank; S= Titre value with sample

N= Normality of titrating solution (KOH used herein), M= Mass of sample (g)

The free fatty acid value is usually regarded as half the acid value of the oil.

**Iodine Value**

The method described by Nadeem *et al*. [24] was used. 0.2g of oil sample was weighed and placed in a 250mL flask and 20mL of chloroform was then added to the sample. 25mL of Wijs reagent was then added with the aid of a pipette and the resulting mixture stirred and stored in a dark place at 25°C for 30 minutes before 10mL of 30% potassium iodide was then added to the mixture as well as 100mL of distilled water. The mixture was then titrated with 0.1N sodium thiosulphate until the yellow colour is almost disappeared. 1mL of starch solution was then added and the mixture titrated further until the blue starch-iodine colour disappeared. A blank titration was also carried out and the Iodine value calculated using the formula below

$$Iodine\ value = \frac{\mathrm{TD} \times 1.269}{\mathrm{M}}$$

Where: TD = titre difference, M = weight of oil measured

**Statistical analysis**

All experiments were performed in triplicate and data is reported as mean ± standard deviation. One-way ANOVA with *post hoc* Duncan Multiple Range testwas conducted by using SPSS software (version 23.0).

**Results**

**Table 1:** Quantitative Phytochemical composition of *Sidaacuta* leaf extracts

| | **SA** |
|---|---|
| **Tannins (µg/mL)** | 9040.142±8.794 |
| **Phenolics (µg/mL)** | 27102.365±10.189 |
| **Flavonoids (µg/mL)** | 1024.783±5.569 |

Values are Mean ± Standard deviation of three determinations.

**Table 2:** Antioxidant assay results of *Sidaacuta* leaf extracts

| | *Sidaacuta* | Butylated Hydroxyl Toluene (BHT) |
|---|---|---|
| **DPPH Scavenging Activity ($IC_{50}$ % inhibition)** | 37.4800±0.0173 | 82.5333±0.0611 |
| **B-Carotene Linoleic Acid Oxidation ($IC_{50}$ % inhibition)** | 29.7267±0.0251 | 61.0633±0.0404 |
| **ORAC (μ Mol TE/g)** | 6.1067±0.0115 | 11.8333±0.0351 |
| **FRAP (μ Mol $Fe^{2+}$/L)** | 291.0367±0.0305 | 314.1267±0.0305 |

Values are Mean ± Standard deviation of three determinations

**Table 3:** Peroxide values (meq/Kg) of groundnut oils treated with leaf extracts of *Sidaacuta*

| | Month 1 | Month 2 | Month 3 | Month 4 | Month 5 | Month 6 |
|---|---|---|---|---|---|---|
| **Gs100** | 3.123±0.002$^{c}$ | 4.150±0.002$^{d}$ | 5.064±0.000$^{c}$ | 8.370±0.006$^{c}$ | 10.845±0.063$^{c}$ | 10.078±0.005$^{d}$ |
| **Gs200** | 2.921±0.001$^{b}$ | 3.507±0.055$^{b}$ | 4.684±0.006$^{b}$ | 8.085±0.001$^{b}$ | 8.725±0.035$^{b}$ | 9.394±0.004$^{b}$ |
| **Gs400** | 3.214±0.000$^{d}$ | 3.878±0.033$^{c}$ | 5.092±0.028$^{c}$ | 8.790±0.001$^{e}$ | 8.763±0.002$^{b}$ | 9.966±0.005$^{c}$ |
| **Gs800** | 3.421±0.012$^{e}$ | 4.159±0.008$^{d}$ | 5.273±0.002$^{d}$ | 8.887±0.010$^{j}$ | 8.860±0.014$^{b}$ | 10.062±0.007$^{d}$ |
| **GBHT** | 1.421±0.012$^{a}$ | 1.461±0.004$^{a}$ | 1.572±0.003$^{a}$ | 1.877±0.002a | 1.945±0.000a | 2.091±0.005$^{a}$ |
| **GCONT** | 3.521±0.005$^{f}$ | 4.655±0.004$^{e}$ | 6.791±0.045$^{e}$ | 8.486±0.000$^{d}$ | 11.385±0.021$^{d}$ | 14.378±0.062$^{e}$ |
| **ANOVA** | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |

Values are Mean ± Standard deviation of three determinations

Values with same superscript are not statistically significant (Duncan Multiple range test) at $p \leq 0.05$

**Table 4: Anisidine values of groundnut oils treatedwith leaf extracts of *Sidaacuta***

| | Month 1 | Month 2 | Month 3 | Month 4 | Month 5 | Month 6 |
|---|---|---|---|---|---|---|
| **Gs100** | 4.213±0.015$^{c}$ | 5.227±0.019$^{b}$ | 6.445±0.021$^{bc}$ | 6.756±0.138$^{bc}$ | 9.371±0.487$^{bc}$ | 12.418±0.135$^{c}$ |
| **Gs200** | 4.102±0.025$^{b}$ | 5.283±0.002$^{c}$ | 6.316±0.059$^{b}$ | 6.546±0.157$^{b}$ | 9.483±0.548$^{bc}$ | 11.675±0.051$^{b}$ |
| **Gs400** | 4.515±0.013$^{d}$ | 5.563±0.004$^{d}$ | 7.610±0.042$^{de}$ | 7.185±0.071$^{de}$ | 9.627±0.520$^{bc}$ | 12.534±0.654$^{c}$ |
| **Gs800** | 4.531±0.011$^{e}$ | 5.788±0.001$^{e}$ | 7.885±0.063$^{f}$ | 7.619±0.049$^{f}$ | 10.167±0.133$^{c}$ | 13.113±0.131$^{d}$ |
| **GBHT** | 3.500±0.010$^{a}$ | 3.646±0.001$^{a}$ | 3.740±0.155$^{a}$ | 4.055±0.155$^{a}$ | 4.192±0.084$^{a}$ | 4.634±0.050$^{a}$ |
| **GCONT** | 5.231±0.052$^{f}$ | 7.619±0.001$^{f}$ | 11.735±0.275$^{g}$ | 12.853±0.125$^{g}$ | 13.955±3.656$^{d}$ | 15.146±0.152$^{e}$ |
| **ANOVA** | 0.001 | 0.001 | 0.001 | 0.001 | 0.007 | 0.001 |

Values are Mean ± Standard deviation of three determinations

Values with same superscript are not statistically significant (Duncan Multiple range test) at $p \leq 0.05$

**Table 5:** Totox values for groundnut oils treated with *S. acuta* leaf extract

| | Month 1 | Month 2 | Month 3 | Month 4 | Month 5 | Month 6 |
|---|---|---|---|---|---|---|
| **Gs100** | 10.459 | 13.527 | 16.573 | 23.496 | 31.061 | 32.574 |
| **Gs200** | 9.944 | 12.297 | 15.684 | 22.716 | 26.933 | 30.463 |
| **Gs400** | 10.943 | 13.319 | 17.794 | 24.765 | 27.153 | 32.466 |
| **Gs800** | 11.373 | 14.106 | 18.156 | 25.393 | 27.887 | 33.237 |
| **GBHT** | 6.342 | 6.568 | 6.884 | 7.809 | 8.082 | 8.816 |
| **GCONT** | 12.273 | 16.929 | 25.317 | 29.825 | 34.725 | 43.902 |

Values are Mean ± Standard deviation of three determinations

Values with same superscript are not statistically significant (Duncan Multiple range test) at $p \leq 0.05$

**Table 6:** Acid values (meq/100 g) of groundnut oils treated with leaf extracts of *Sidaacuta*

| | Month 1 | Month 2 | Month 3 | Month 4 | Month 5 | Month 6 |
|---|---|---|---|---|---|---|
| **Gs100** | 4.002±0.005$^{e}$ | 4.582±0.003$^{c}$ | 4.644±0.000$^{d}$ | 4.942±0.010$^{c}$ | 5.257±0.000$^{d}$ | 6.024±0.000$^{c}$ |
| **Gs200** | 3.852±0.001$^{b}$ | 4.537±0.003$^{b}$ | 4.611±0.000$^{c}$ | 4.856±0.005$^{b}$ | 5.157±0.000$^{b}$ | 5.885±0.006$^{b}$ |
| **Gs400** | 3.895±0.001$^{c}$ | 4.557±0.024$^{b}$ | 4.622±0.001$^{c}$ | 4.940±0.007$^{c}$ | 5.163±0.000$^{b}$ | 6.023±0.001$^{c}$ |
| **Gs800** | 3.901±0.003$^{d}$ | 4.674±0.034$^{d}$ | 4.736±0.001$^{e}$ | 5.007±0.008$^{e}$ | 5.177±0.001$^{c}$ | 6.161±0.001$^{e}$ |
| **GBHT** | 3.820±0.001$^{a}$ | 4.177±0.003$^{a}$ | 4.566±0.001$^{a}$ | 4.637±0.003$^{a}$ | 4.987±0.000$^{a}$ | 5.587±0.000$^{a}$ |
| **GCONT** | 4.132±0.003$^{f}$ | 5.015±0.006$^{d}$ | 4.585±0.000$^{b}$ | 4.969±0.006$^{d}$ | 5.298±0.001$^{e}$ | 6.139±0.001$^{d}$ |
| **ANOVA** | 0.009 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |

Values are Mean ± Standard deviation for three determinations

Values with same superscript are not statistically significant (Duncan Multiple range test) at $p \leq 0.05$

**Table 7:** Iodine values ($I_2$/100 g) of groundnut oils treated with leaf extracts of *Sidaacuta*

| | Month 1 | Month 2 | Month 3 | Month 4 | Month 5 | Month 6 |
|---|---|---|---|---|---|---|
| **Gs100** | 98.025±0.030$^{b}$ | 96.447±0.074$^{bc}$ | 95.648±0.004$^{cd}$ | 93.383±0.051$^{d}$ | 88.865±0.120$^{cd}$ | 86.382±0.132$^{a}$ |
| **Gs200** | 98.111±0.018$^{c}$ | 96.692±0.081$^{bc}$ | 95.750±0.000$^{d}$ | 93.585±0.073$^{e}$ | 88.965±0.077$^{de}$ | 87.211±0.044$^{b}$ |
| **Gs400** | 98.153±0.081$^{c}$ | 95.785±0.283$^{a}$ | 95.679±0.000$^{cd}$ | 93.077±0.038$^{c}$ | 88.440±0.141$^{b}$ | 86.500±0.065$^{a}$ |
| **Gs800** | 98.321±0.141$^{d}$ | 95.744±0.121$^{a}$ | 95.579±0.012$^{bc}$ | 92.907±0.015$^{b}$ | 88.340±0.127$^{b}$ | 86.412±0.061$^{a}$ |
| **GBHT** | 98.502±0.122$^{e}$ | 96.794±0.223$^{bc}$ | 95.161±0.005$^{a}$ | 95.904±0.067$^{f}$ | 91.945±0.148$^{f}$ | 90.415±0.070$^{c}$ |
| **GCONT** | 97.921±0.052$^{a}$ | 95.873±0.160$^{a}$ | 95.488±0.007$^{b}$ | 91.176±0.082$^{a}$ | 88.015±0.120$^{a}$ | 86.543±0.106$^{ab}$ |
| **ANOVA** | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |

Values are Mean ± Standard deviation for three determinations

Values with same superscript are not statistically significant (Duncan Multiple range test) at $p \leq 0.05$

Key:

GS100 = groundnut oil + 100 mg/L *S. acuta* leaf extract

GS200 = groundnut oil + 200 mg/L *S. acuta* leaf extract

GS400 = groundnut oil + 400 mg/L *S. acuta* leaf extract

GS800 = groundnut oil + 800 mg/L *S. acuta* leaf extract

GBHT = groundnut oil + 100 mg/L Butylated Hydroxyl Toluene

GCONT= groundnut oil Control (no extract)

**Discussion**

This study revealed that ethanolic extracts of *Sidaacuta* contain phytochemical compounds such as tannins, phenolics and flavonoids with phenolics occurring in the highest amount recorded as 27102.365±10.189 µg/ml. The phenolic composition as determined in this study is in conformity with other findings in which their presence correlated with extension of oil shelf life [25-26] as well as free radical scavenging ability [27]. Similarly, the ability of phenolic compounds to enhance oxidative stability of oils has been reported [28]. Although the interest in phenolic compounds is related primarily to their antioxidant activities, they also show an important biological activity *in vivo* and may be beneficial in combating diseases arising from exposure to excessive oxygen radical formation exceeding the antioxidant defense capacity of the human body. The DPPH radical scavenging assay showed that *Sidaacuta* had an $IC_{50}$ % inhibition of 37.4800±0.0173. This value was lower than the standard antioxidant used, which was 82.5333±0.061 $IC_{50}$ % inhibition, but comparable with the value obtained for Rosemary extract used in stabilizing soyabean oil by Sayyad *et al.,* [29]. There is also the possibility of improving the radical scavenging property of the extracts if it undergoes refinement before usage. The B-carotene Linoleic antioxidant assay showed that the extract had about half the value of the synthetic antioxidant but the health concerns associated with the latter would make the former a preferable choice. The Ferric reducing antioxidant power (FRAP) test result of the plant extract, which was 291.0367±0.0305 µ Mol $Fe^{2+}$/L compares well with 314.1267±0.0305 µ Mol $Fe^{2+}$/L for standard synthetic antioxidants. The FRAP test is a useful assay to determine the potential of plant extract to protect oils from oxidative damage and the result found through this method here is similar to those documented for different plant extracts used in stabilizing groundnut oil [30].

A measure of oxidative stability of the oil samples was determined through the measurement of peroxide values, anisidine value as well as iodine values. There was a steady increment in peroxide value as storage time increased. Minimum peroxide values for the extracts ranged from 2.921±0.001 meq/Kg to 3.421±0.012 meq/Kg while the maximum was from 9.394±0.004 to 10.078±0.005 meq/Kg. It was observed that minimum peroxide value was evident at a lower concentration of 200mg/kg (sample GS200) and the peroxide value increased as concentration increased. These values are higher than those of Kozlowska and Gruczynska [31], which may be due to the use of different plant extracts. The peroxide values recorded for samples treated with synthetic antioxidant (GBHT) were lower than the samples treated with the plant extract but higher than the control samples, indicating the potential of the plant extract to improve the oil quality upon storage. Although the increase in peroxide values was in a concentration-dependent manner, it appears the samples treated with the extract at the concentration of GS200 was more effective evidenced by lower peroxide values during the storage period. There were observable variations with

respect to anisidine values in treated samples, which were higher than those recoded for samples treated with the synthetic antioxidant. Sample GS100-GS800 recorded a minimum anisidine value of 4.102±0.025 mmol/Kg and a maximum of 13.113±0.131 mmol/Kg. There seemed to be a slight drop in the anisidine value at lower concentrations of extract from 100mg/L to 200mg/L concentration which later went up indicating that higher concentrations of the *S. acuta* extract raised the level of anisidine value and as such had a prooxidant effect. Anisidine levels increased noticeably but not sharply as storage time extended. In all samples GBHT was lower than other values indicating that it had a better keeping power than the extracts with a range twice its value. Samples with extracts however showed promise recording lower anisidine values compared to the control. According to White [32] an acceptable AV for well-refined oils is between 1 and10 mmol $kg^{-1}$ depending on the level of unsaturation of composite fatty acids, thus the values recorded in the present study are within this defined limit. For Totoxvalues, Sample GBHT showed the least totox value which ranged from 6.342 by the first month to 8.816 in the sixth month and a value of 13.866 under accelerated storage. The control GCONT remained the highest all through increasing exponentially over the storage period from an initial 12.273 in the first month to 43.902 by the sixth month. For samples GS100 to GS800, the least totox vale was recorded in GS200 ranging from 9.944 to 30.463 while the highest value was recorded in sample GS800 ranging from 11.373 to 33.237 in the sixth month. With respect to acid values of samples, there was a general trend of increase among samples treated with both the extract and synthetic antioxidant indicative of slight hydrolysis of the triglycerides during the storage period but falling within the prescribed confines defined by CODEX Alimerius. Another important observation was the gradual decline in acid values as concentration of *S. acuta* extract increased similar to the findings of Gretel *et al*., [33]. There was a slight reduction in the iodine values for samples treated with the plant extract in a concentration-dependent fashion similar to the findings of Gretel *et al.,* [33], however, the range of the iodine value recorded falls within the acceptable range for groundnut oil.

## Conclusion

From the present study, it was concluded that *Sidaacuta* ethanolic leaf extract can stabilize groundnut oil effectively at a concentration of 200 mg/L of groundnut oil. It inhibits the oxidative deterioration of oil by improving its hydrolytic stability, inhibit the lipid oxidation and reduce the loss of polyunsaturated fatty acids (PUFAs). *Sidaacuta* leaf extract at concentration of 200 mg/L of oil has stabilization efficacy comparable to the common synthetic antioxidant BHT at its legal limit. Therefore, *Sidaacuta* leaf extract can be recommended as apromising source of natural antioxidant for the stabilization of food and food product, especially edible vegetable oils rich in unsaturated fatty acids

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