INTRODUCTION
The use of plants for the production of chemical compounds have in a long time been adopted for the promotion of health and fight diseases and some of them are also marketed as a food or herbal medicines [1]. Herbal medicines have long been viewed as a curative remedy both in religious and cultural traditions [2]. There is no denying the fact that the reliance of humans on plants to cure or manage various pathologies is as old as their history while the attraction towards developing the domain of nutrition in the last few decades has led to the discovery of the therapeutic potential of many culinary herbs [3].

Black cumin is an annual herbaceous plant native to (and cultivated in) Southwest Asia and cultivated and naturalized in Europe and North Africa, Saudi Arabia, Turkey, Syria, Pakistan and India [4]. The taxonomic classification [5] of black cumin depicts it as a flowering dicotyledon plant that belongs to family Ranunculaceae, under kingdom Plantae [6] with finely divided, linear leaves. The colors of the flowers are usually pale blue and white, with 5 to 10 petals. The fruit is composed of 3 to 7 united follicles which are trigonally shaped seeds [7]. Scientific investigations of black cumin have depicted its proximate composition [8]. Likewise, pharmacological findings have explored the effectiveness of its essential oil and active ingredient against various maladies caused by reactive oxygen species (ROS) like oxidative stress, cancer, immune dysfunction, hypertension and diabetic complications [9]. Like most herbs, the bioactive components of black cumin varies with the geographic distribution and time of harvest. This project was designed to examine the total phenol content, the Fe²⁺ chelation, OH radical scavenging ability of the aqueous extract of the black cumin seeds and also characterize the bioactive contents using HPLC-DAD.

MATERIALS AND METHODS

Materials
Black cumin seeds were purchased from the central market Kano, Nigeria. The identification and authentication were done at the Department of Biology, Federal University of Technology, Akure, Nigeria. All the chemicals used were of analytical grade, while distilled was used.

Sample Preparation and Aqueous Extraction
The seeds were hand-picked to remove dirt and inedible portions. The edible portions were rinsed, sun-dried and ground into powder. Five gram of the powdered black cumin seeds was soaked overnight in 100 ml distilled water. The supernatant was collected and treated as reported in this research. Thereafter, the mixtures were centrifuged at 358g for 10 mins and supernatants collected were made up to 100 ml and later used for further analysis.

Determination of Total Polyphenols Content
Total polyphenols were determined using Folin-Ciocalteau method and values were expressed as gallic acid equivalent (GAE) [10]. Briefly, 0.5ml of aqueous extracts were oxidized with 2.5ml 10% Folin-Ciocalteau’s reagent (v/v) and neutralized by 2.0ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40min at 45°C and the absorbance was measured at 765nm in the spectrophotometer (JENWAY 6305).

Fe²⁺ Chelation Assay
The ability of the extract to chelate Fe²⁺ was determined using a modified method of Minotti and Aust [11] with a slight modification by Puntel et al. [12]. Freshly prepared 500 mM FeSO₄ (150 mL) was added to a reaction mixture containing 168 mL of 0.1 M Tris–HCl (pH 7.4), 218 mL of saline and the aqueous extract of the pepper (25 mL). The reaction mixture was incubated for 5 min before the addition of 13 mL of 0.25% (w/v) 1,10-phenanthroline. The absorbance was subsequently measured at 510nm in the spectrophotometer (Jenway 6305).

OH Radical-Scavenging Ability
The ability of the black cumin extract to prevent Fe³⁺/H₂O₂ - induced decomposition of deoxyribose was carried out using the method of Halliwell and Gutteridge [13]. Briefly, freshly prepared aqueous extract (100 mL) was added to a reaction mixture containing 120 mL of 20 mM deoxyribose, 400 mL of 0.1 M phosphate buffer, 40 mL of 20 mM hydrogen peroxide and 40 mL of 500 mM FeSO₄ and the volume was made to 800 mL with
distilled water. The reaction mixture was incubated at 37°C for 30 min, and the reaction was stopped by the addition of 0.5 mL of 2.8% TCA; this was followed by the addition of 0.4 mL of 0.6% thiobarbituric acid solution. The tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm in a spectrophotometer (Jenway 6305).

Chemical, Apparatus and General Procedures

All chemical were of analytical grade. Gallic acid, chlorogenic acid, caffeic acid and rosmarinic acid were purchased from Merck (Darmstadt, Germany). Quercetin, rutin and kaempferol were acquired from Sigma Chemical Co. (St. Louis, MO, USA). High performance liquid chromatography (HPLC-DAD) was performed with a Shimadzu Prominence Auto Sampler (SIL-20A) HPLC system (Shimadzu, Kyoto, Japan), equipped with Shimadzu LC-20AT reciprocating pumps connected to a DGU 20A5 degasser with a CBM 20A integrator, SPD-M20A diode array detector and LC solution 1.22 SP1 software.

Quantification of compounds by High-Performance Liquid Chromatography (HPLC) Coupled Diode Array Detector Analyses.

Chromatographic analyses were carried out under gradient conditions using C 18 column (4.6 mm A 150 mm) packed with 5 mm diameter particles; the mobile phase was water containing 1% formic acid (A) and acetonitrile (B), and the composition gradient was: 13% of B until 10 minutes and changed to obtain 20%, 30%, 50%, 60%, 70%, 20%, and 10% B at 20, 30, 40, 50, 60, 70, and 80 minutes, respectively, following the method described by Kamdem et al. [14] with slight modifications. Black cumin extract and mobile phase were filtered through 0.45 mm membrane filter (Millipore) and then degassed by ultrasonic bath prior to use, bitter gourd leaf extracts were analyzed at a concentration of 20 mg/mL.

Table 1: The total phenol (mg/gGAE), Fe2+ chelation (%) and OH radical scavenging ability (%) of black cumin seed extract

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total-phenol</th>
<th>Fe2+ chelation (%)</th>
<th>OH-radical scavenging ability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black cumin seeds extract</td>
<td>2.1±0.85</td>
<td>40.06±0.63</td>
<td>82.64±0.77</td>
</tr>
</tbody>
</table>

Values represent mean ± standard deviation of triplicate experiments

Table 2: Phenolics and flavonoids composition of the black cumin seed extract

<table>
<thead>
<tr>
<th>Parameters</th>
<th>mg/g</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic acid</td>
<td>37.09 ±0.02</td>
<td>3.70</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>25.47 ±0.01</td>
<td>2.54</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>48.15 ±0.04</td>
<td>4.81</td>
</tr>
<tr>
<td>Rutin</td>
<td>5.28 ±0.03</td>
<td>0.52</td>
</tr>
<tr>
<td>Rosmarinic acid</td>
<td>31.57 ±0.05</td>
<td>3.15</td>
</tr>
<tr>
<td>Quercetin</td>
<td>109.42 ±0.01</td>
<td>10.94</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>13.25 ±0.03</td>
<td>1.32</td>
</tr>
</tbody>
</table>

Values represent mean ± standard deviation of triplicate experiments

Fig. 1: Representative of high-performance liquid chromatography profile of black cumin seeds extract. Gallic acid (peak 1), chlorogenic acid (peak 2), caffeic acid (peak 3), rosmarinic acid (peak 4), rutin (peak 5), kaempferol (peak 6), quercetin (peak 7)
DISCUSSION

In a normal situation in the body system, the generation of Reactive Oxygen species (ROS) results from the consequence of aerobic metabolism. Apart from hydroxyl radicals (HO.) formation, other common ROS types are superoxide anions (O$_2^-$) hydrogen peroxide (H$_2$O$_2$) which are produced by biological reduction of molecular oxygen [16]. It has also been observed that under hypoxic conditions, the mitochondrial respiratory chain also produces nitric oxide (NO) which can generate reactive nitrogen species (RNS) [17]. According to an earlier report, tissue injury can in itself cause ROS generation by initiating the activation of phagocytes or releasing transition metal ions from a damaged cell which may contribute to the further degeneration of the injury [18].

Medicinal plants according to research have been considered to be the richest bio-resource of drugs in the area of nutraceuticals, various food supplements, modern medicines, pharmacy and chemical entities for synthetic drugs production [19]. Suggestions from the evidence produced in various researches have hereby continued to point to the fact that the use of medicinal plant based drugs contains least or no side effects which have been considered to be of great importance to the health of individuals and communities [20]. Plant products derived from leaves, stems, and pods have been researched and included to form part of ingredients used in phytomedicines [21] with various extraction methods used to harvest plant bioactive substances to be tested and recommended for human good use. Many active compounds have been isolated, identified and reported so far in different varieties of black seeds [22]. The fact that there is no limitation to research has deemed it fit to continue to dig out for more of the other bioactive compounds that black cumin is endowed with. Polyphenolics are one of the largest and the most widely studied group of phytochemicals. They have been widely reported in the recent time to possess remarkable medicinal properties [23].

The trend of the total phenol content is in line with what has been reported by [7]. The area of this field will continue to ignite the interest of researchers based on the observation that many natural products have beneficial effects in scavenging reactive oxygen species which are detrimental to human health. The quantity of flavonoid forms a component of the total phenolic content measured in an analysis. Flavonoids such as quercetin, kaempferol and rutin act by exerting antioxidant effects such as anti-inflammatory, antiallergic, antiviral and anticancer activity. They have also been reported to protect against maladies such as liver diseases, cataracts, and cardiovascular diseases. The scavenging action of the hydroxyl (OH) group on the B-ring of flavonoids has also been reported to be responsible for the control of α-amylase and α-glucosidase activities [24] thereby preventing diabetes. Further reports have shown that flavonoids could exert a better protection by preventing the degeneration of pancreatic beta-cell function due to oxidative stress hence preventing the occurrence of type 2 diabetes. This could be achieved by the lowering of the lipid level and postprandial blood glucose thereby increasing the insulin sensitivity with little or no side effect [23]. Caffeic acid had been reported to exert the total radical-trapping antioxidative ability and can also partake in hydrogen and electron transfer reactions [25]. However, the present research under study identified some considerable quantity of caffeic acid in the black cumin seeds.

The participation of Iron (Fe$^{2+}$) in Fenton reaction in the breaking down of hydrogen peroxide to form hydroxyl radicals (OH*), which in turn snatches an electron from polysaturated fatty acids initiates lipid peroxidation reaction [26]. The study of the quantity of H$_2$O$_2$ radical scavenging activities from one of the designated methods of characterizing the ability of antioxidants to reduce the level of pro-oxidants (Oboh and Rocha, 2008). Hydrogen peroxide (H$_2$O$_2$) is not very reactive all by itself, but an increase in its concentration could be toxic to cells over time [27]. The quantity of the Fe$^{2+}$ chelation activity displayed by the aqueous extract of black cumin seeds under study is an indication that it can simultaneously prevent lipid peroxidation originating from iron influx in the cell. The observed OH radical scavenging abilities in the black cumin seeds could be attributed to the quantity and quality of the phenolic compounds that have dissolved in its aqueous extract. In addition, the synergistic antioxidant activities of medicinal plants especially Nigella sativa might contribute its therapeutic effects in restoring cellular energy that is already depleted due to disrupted metabolisms [28] in the human body. This finding is in agreement with the result obtained by Rezaeezadeh et al. [29] which suggests that scavengers of reactive oxygen species (ROS) can prevent and manage some degenerative diseases in both human and animal cells. Gryszczynska et al. [30] also reported that polyphenols such as gallic acid, chlorogenic acid and caffeic acid are capable of forming complexes with Fe$^{3+}$ that can prevent the oxidation of Fe$^{3+}$ to Fe$^{2+}$ which could be initiated by hydrogen peroxide. With these findings, it could be stated that the administration and recorded success of exogenous antioxidants seem to be salutary. Nowadays, a great deal of effort is channelled towards finding effective antioxidants for the treatment or prevention of free radical-mediated deleterious effects [31]. Nevertheless, phenolic compounds have been assessed to contain ideal structural chemistry for free radical-scavenging activities and metal chelation. Available literature has shown them to be more effective antioxidants in vitro than vitamins E and C on a molar basis [32]. Nevertheless, the in vivo experiment of the supplementation of black cumin seeds in animal feed had been carried out using broilers [33]. The finding, however, indicated beneficial effects on body weight gain, feed conversion ratio, and carcass weight by increasing their feed intake. Also, a desirable result was recorded when an approach was adopted to maintain host (broilers) health by increasing the number of desirable bacteria in order to inhibit colonization of invading pathogens [34].

CONCLUSION

The current global interest in the characterization of antioxidant components from plants natural sources and their uses continues to increase and more people tend to tilt towards them due to their suggested pharmacological potency and low or no side effect in protective medicine and food industry. No doubt, further exploration, identification and isolation of these phytocompounds are simultaneously presenting a wider scope for their better therapeutic application for treatment of human diseases. Therefore, the time is now to explore and characterize our traditional therapeutic knowledge and channel their use in the area of recent advancement to fight against oxidative stress.

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DECLARATION OF CONFLICTING INTERESTS

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REFERENCES


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