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Research Article

SPECTROSCOPIC TECHNIQUES BASED CHARACTERIZATION OF PHYTOCHEMICALS AND ANTIOXIDANT POTENTIALS OF ETHANOL LEAF EXTRACT OF *LUFFA CYLINDRICA*

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ABSTRACT

Objectives: This study aimed to characterize the bioactive profile and evaluate the antioxidant activity of the ethanol leaf extract of *Luffa cylindrica* using various *in vitro* antioxidant assays.

Methods: The bioactive components of the extract were analyzed using gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS) techniques. Various antioxidant assays, including nitric oxide, superoxide, hydroxyl radical, 2-diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide, and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) scavenging activities, were performed to evaluate the antioxidant potentials of the extract. In addition, the total antioxidant capacity and ferric reducing antioxidant power (FRAP) inhibitory activities were also determined to assess the overall *in vitro* antioxidant capacity of the extract. The results were subjected to appropriate statistical analysis with the degree of significance set at $p<0.05$.

Results: The GC-FID analysis revealed the presence of 22 bioactive compounds, with a total concentration of 217.43072 ppm. The most abundant bioactive compound was silymarin (158.94587 ppm), followed by apigenin (8.412 ppm), catechin (5.72264 ppm), apigenin (4.03142 ppm), artemetin (2.63596 ppm), vanillic (2.26698 ppm), and epicatechin (2.05439 ppm) and others. The GC-MS analysis identified 30 prominent bioactive compounds, including 2,4-Di-tert-butylphenol (8.08%), apigenin (7.73%), dodecane (4.89%), chlorogenic acid (4.71%), carbonic acid, nonyl vinyl ester (3.73%), and limonene (3.38%). The antioxidant properties of the extract were evidenced by its ability to scavenge nitric oxide (2.02–2.47 µg/mL), superoxide (65.74–73.89%), DPPH (93.1–94.8%), and inhibitory activity on FRAP ranges from 33.487–79.345% among others at various concentrations.

Conclusion: The extract exhibited a rich bioactive composition and potent antioxidant properties, suggesting its potential therapeutic applications in the management of oxidative stress-related diseases and other degenerative health conditions.

Keywords: *Luffa cylindrica*, Antioxidant, Silymarin, Catechin, 2,4-Di-tert-butylphenol, Spectroscopy, Characterization.

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INTRODUCTION

Reactive oxygen species (ROS) are characterized as transient molecules, ions, and radicals. Their half-lives range from nanoseconds to hours. They are formed in numerous chemical reactions and during certain biological processes, such as respiration and the electron transport chain [1]. Elevated levels of ROS can induce oxidative stress and result in damaging cellular and molecular functions. Consequently, various forms of diseases, such as neurological disorders, lung conditions, cardiovascular issues, cancer, and inflammation, can arise in living organisms. Oxidative stress and ROS have been recognized as significant environmental threats for various types of chronic illnesses, including cancer, immunodeficiency syndrome, age-related conditions, cardiovascular diseases, arteriosclerosis, diabetes, and obesity [2].

ROS are produced in living organisms during standard cellular metabolism and can be detrimental to essential biomolecules such as proteins, nucleic acids, lipids, and carbohydrates. The antioxidant defense system comprises antioxidant components in addition to antioxidant enzymes. In terms of pharmaceutical products, antioxidants can easily scavenge ROS and reduce the lipid autoxidation of foods and pharmaceutical products during production and storage processes [3]. For food products, antioxidants are characterized as molecules that inhibit oxidation in small quantities or concentrations. Moreover, they readily slow down or prevent the oxidation of substrates. Consequently, recent efforts have focused on discovering effective antioxidants, including phenolic compounds sourced from natural and readily available resources [4]. Plants contain many biologically active phytochemicals, such as phenols and polyphenols, that have structural characteristics exhibiting antioxidant activities. Therefore, there is an increasing demand for safer, natural antioxidants derived from plant sources for use in pharmaceuticals and food. Phenolics are active secondary metabolites that scavenge ROS and mitigate oxidative damage. Their biological effects, including the antioxidant properties of phenols found in medicinal plants, render them vital products for their protective roles against various degenerative disorders, including diabetes, cancer, arteriosclerosis, hypercholesterolemia, and cardiovascular diseases [1].

Antioxidants derived from plants can function as reducing agents, hydrogen donors, free radical scavengers, singlet and triplet oxygen quenchers, or peroxide decomposers, thereby playing a significant role in adsorbing and neutralizing free radicals. In recent years, antioxidants sourced from nature have taken on a prominent role in the pharmaceutical sector due to their nutritional and therapeutic benefits. In addition, there has been an overall inclination to substitute synthetic food additives with natural antioxidants. Natural sources of antioxidants have attracted considerable interest because of their potential health advantages and reduced side effects compared to synthetic alternatives. Antioxidants derived from plants, especially those present in spices and herbs, have been thoroughly investigated for their antioxidant characteristics and therapeutic uses [5].

Bioactive compounds sourced from plants have garnered significant interest due to their possible therapeutic effects and applications across various domains, including pharmaceuticals, nutraceuticals,

and functional foods [6]. These compounds frequently demonstrate antioxidant, anti-inflammatory, antimicrobial, and other advantageous actions, rendering them valuable natural resources for the promotion of human health and wellness.

Luffa cylindrica, also known as sponge gourd, vegetable sponge, or loofah, is a subtropical plant species that belongs to the Cucurbitaceae family. It is extensively grown in numerous areas, such as Asia, Africa, and the Mediterranean, for its distinctive fibrous interior and potential medicinal benefits [7]. The identification and characterization of these bioactive compounds are vital for comprehending the plant's medicinal attributes and investigating its potential within the pharmaceutical, nutraceutical, and functional food sectors. *L. cylindrica* has indicated that the leaf extracts comprise sterols, saponins, flavonoids, alkaloids, and phenols, whereas resins, tannins, terpenes, balsams, and anthraquinones were in low concentrations [8]. Another initial phytochemical screening of the aqueous methanol extract of *L. cylindrica* leaves uncovered sugar molecules such as glucose, fructose, and galactose, alongside amino acids such as phenylalanine, glycine, and tyrosine. Furthermore, phytate and oxalate were discovered in the methanol extract of the flowers and leaves of *L. cylindrica*. The seeds of *L. cylindrica* contain a high quantity of saponins, alkaloids, and phlobatannins [9].

Antioxidants are essential in counteracting damaging ROS and averting oxidative harm to cellular elements, which can result in different chronic diseases. *L. cylindrica* extract contains a variety of bioactive substances, including phenolic compounds and flavonoids, which are thought to enhance its antioxidant capabilities. This research utilized several *in vitro* antioxidant assays, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical cation decolorization, and ferric reducing antioxidant power (FRAP) and other antioxidant assays, to assess the antioxidant effectiveness of *L. cylindrica*. Identifying and characterizing the bioactive compounds found in the ethanol leaf extract of *L. cylindrica* may offer valuable information regarding the plant's medicinal attributes and possible uses across various sectors. Evaluating the antioxidant activity of the leaf extract can also aid in the creation of natural antioxidant sources, which possess numerous advantages in the food, pharmaceutical, and cosmetic fields.

METHODS

Sample collection and extract preparation

'The leaves of *Luffa cylindrica* (Fig.1) were gathered from healthy, mature plants located in Ishieke, Abakaliki in Ebonyi State. A taxonomist from the Department of Applied Biology, Ebonyi State University, Nigeria, identified and authenticated the plant. The leaves were washed and allowed to dry at room temperature for 20 days. The dried sample was ground using a mechanical blender to produce fine powder, which was subsequently weighed. Extraction was performed by soaking 400 g of the powdered sample in 1000 mL of 98% absolute ethanol for 72 h with periodic shaking. Following this, the extract was filtered and concentrated to dryness with the help of a rotary evaporator.

Extraction of phytochemicals

Exactly 1 g of the extract was measured and moved into a test tube, then 25 mL of ethanol was added. The test tube was permitted to react on a hotplate at 60°C for 90 min. Following the reaction duration, the reaction product present in the test tube was moved to a separatory funnel. The tube was thoroughly rinsed with 20 mL of ethanol, 10 mL of cold water, 10 mL of hot water, and 3 mL of hexane, all of which were transferred to the funnel. These extracts were mixed and washed 3 times with 10 mL of a 10% v/v ethanol aqueous solution. The solution was dried using anhydrous sodium sulfate, and the solvent was removed through evaporation. The sample was dissolved in 1000 µL of pyridine, from which 200 µL was transferred to a vial for analysis.

Identification of chemical constituents

Bioactive substances obtained from various extracts were recognized according to the gas chromatography retention time on the HP-5MS column and the comparison of the spectra with the computer software data for standards (Replib and Mainlab data of gas chromatography-mass spectrometry [GC-MS] systems).

Quantification by GC-MS

The examination of phytochemicals was conducted using a BUCK M910 Gas chromatography system outfitted with an HP-5MS column (30 m in length×250 µm in diameter×0. 25 µm in thickness of film). GC-MS spectroscopic detection employed an electron ionization setup that used high-energy electrons (70 eV). Pure helium gas (99.995%) served as the carrier gas with a flow rate of 1 mL/min. The starting temperature was established at 50°C with an increasing rate of 3°C/min and a hold period of approximately 10 min. Ultimately, the temperature was raised to 300°C at a rate of 10°C/min. One microliter of the prepared 1% extracts diluted in acetonitrile was introduced in a splitless mode. The relative amount of the chemical compounds found in each of the extracts was represented as a percentage based on the peak area generated in the chromatogram.

Quantification of phytochemicals of the extract by gas chromatography-flame ionization detector (GC-FID)

The phytochemicals analysis was conducted using an Agilent 6890 Gas chromatography system that features a flame ionization detector. A RESTEK 15 m MXT-1 column (15 m×250 µm×0. 15 µm) was utilized. The injector's temperature was set to 280°C with a splitless injection of 2 µL of the extract and a linear velocity of 30 cm^{-1}. The carrier gas was helium 5.0 pa.s, operating at a flow rate of 40 mL/min. The oven began at a temperature of 200°C, then was increased to 330°C at a rate of 3°C min^{-1} and maintained at this temperature for 5 min. The temperature of the detector was set at 320°C. Phytochemicals were quantified by the ratio of the area and mass of the internal standard to the area of the detected phytochemicals. The concentrations of the various phytochemicals were expressed in µg/g.

DPPH scavenging assay

The capacity of the leaf extracts to scavenge the DPPH radical was evaluated in a quick dot-plot screening [10] and measured using a spectrophotometric method. Portions of the extract were carefully spotted onto thin layer chromatography plates and allowed to dry for 3 min. The plates with the dried spots were turned upside down for 10 s in a 0.4 mm DPPH solution, after which the layer was dried. The colored silica layer displayed a purple background with yellow spots, indicating radical scavenging ability. The scavenging capacity of the natural antioxidants from the leaves against the stable free radical DPPH was quantified spectrophotometrically [11]. The extracted sample (20 µL) was mixed with 0.5 mL of a 0.1 mm methanolic solution of DPPH and 0. 48 mL of methanol. The mixture was allowed to react at room temperature for 30 min. Methanol acted as the blank, and DPPH in methanol, without the sample, served as the positive control, while butylated hydroxytoluene (BHT) was used as a reference. After 30 min of incubation, the reduction of the purple color was assessed at 518 nm using a spectrophotometer (Genesys 10-S, USA).

ABTS scavenging effects

The antioxidant properties of the leaf extract were examined using the ABTS radical cation decolorization assay [12]. ABTS radical cations ($ABTS^{+}$) were generated by reacting an ABTS solution (7 mm) with 2.45 mm ammonium persulphate. This mixture was permitted to stand in the dark at room temperature for a duration of 16 h before its application. Aliquots (0.5 mL) from the various samples were combined with 0.3 mL of ABTS solution, and the total volume was adjusted to 1 mL of ethanol. The absorbance was measured at 745 nm using a spectrophotometer (Genesys 10-S, USA), and the percentage inhibition was calculated.

Hydrogen peroxide scavenging effects

The capability of the leaf samples to eliminate hydrogen peroxide was evaluated. Exactly 40 ml solution of H_2O_2 was prepared in a phosphate buffer. The extract at a concentration of 10 mg/10 µL was combined

with the H_2O_2 solution (0.6 mL), and the total volume was adjusted to 3 mL. The absorbance of the reaction mixture was measured at 230 nm with a spectrophotometer (Genesys 10-S, USA). A blank solution consisting of a phosphate buffer without H_2O_2 was created. The degree of H_2O_2 scavenging by the leaf samples was determined [13].

Measurement of superoxide scavenging activity

The ability of the samples to scavenge superoxide was evaluated. This assay relies on the inhibition of nitroblue tetrazolium formazan production by the superoxide ion, which is quantified spectrophotometrically at 560 nm [14]. Superoxide anions were produced in samples that included 3.0 mL, 0.02 mL of the extract (20 mg), 0.2 mL of ethylenediaminetetraacetic acid (EDTA), 0.1 mL of nitro blue tetrazolium (NBT), 0.05 mL of riboflavin, and 2.64 mL of phosphate buffer. Control tubes were also prepared, where dimethyl sulfoxide was substituted for the sample. Each tube was vortexed, and the initial optical density was recorded at 560 nm using a spectrophotometer (Genesys, 10-S, USA). The tubes were exposed to a fluorescent lamp for 30 min. Absorbance was measured at 560 nm. The change in absorbance before and after illumination was representative of the superoxide anion scavenging activity.

Measurement of nitric oxide scavenging activity

The degree of inhibition of nitric oxide radical production *in vitro* was monitored. The process commenced by incorporating 2.0 mL of sodium nitroprusside, 0.5 mL of phosphate-buffered saline, and 0.5 mL of the extract (50 mg) and was kept at 25°C for 30 min. Griess reagent (0.5 mL) was added and allowed to incubate for an additional 30 min. Control tubes were made without including the sample. The absorbance was measured at 546 nm against the reagent blank using a spectrophotometer (Genesys 10-S, USA) [15].

Measurement of hydroxyl radical scavenging activity

The degree of hydroxyl radical scavenging from the Fenton reaction was assessed using 2'-deoxyribose oxidative degradation [16]. The reaction mixture consisted of 0.1 mL of deoxyribose, 0.1 mL of $FeCl_3$, 0.1 mL of EDTA, 0.1 mL of H_2O_2, 0.1 mL of ascorbate, 0.1 mL of KH_2PO_4-KOH buffer, and 20 mL of samples in a total volume of 1.0 mL. The mixture was incubated at 37°C for 1 h. Upon completion of the incubation, 1.0 mL of TBA was introduced and heated at 95°C for 20 min to allow for color development. After cooling, the formation of thiobarbituric acid reactive substances (TBARS) was evaluated spectrophotometrically (Genesys 10-S, USA) at 532 nm compared to a suitable blank. The hydroxyl radical scavenging activity was calculated by contrasting the absorbance of the control with that of the samples. The percentage of TBARS production for the positive control (H_2O_2) was established at 100%, and the relative percentage of TBARS was determined for the sample-treated groups.

Total antioxidant capacity (TAC) assay

This assay was conducted to measure the inhibition of NBT formation resulting from the superoxide ion by the plant extract. The TAC of the extract in various extracting solvents (absolute ethanol, 70%, and 50% ethanol) was evaluated using the phosphomolybdate method [17]. An aliquot (30 mL) of different concentrations (20, 40, 60, 80, and 100 mg) of the test extract was combined with 3 mL of the reagent solution (0.6 m sulfuric acid, 28 mm sodium phosphate, and 4 mm ammonium molybdate) placed in test tubes. The tubes were covered with aluminum foil and incubated in a boiling water bath at 95°C for a duration of 90 min. The reaction mixture was permitted to cool to room temperature, and the absorbance was recorded spectrophotometrically at 695 nm against a blank that contained 3 mL of the reagent solution and the corresponding volume of the dissolving solvents. The blank was incubated under the same conditions as the test samples, with ascorbic acid serving as the standard reference compound to compare the activities of the extracts.

FRAP inhibitory activity assay of the *L. cylindrica* extract

FRAP solution (3.6 mL) was added to distilled water (0.4 mL) and incubated at 37°C for 5 min. Following this, the solution was mixed with a specific concentration of the plant extract (80 mL) and incubated at 37°C for 10 min. The absorbance of the reaction mixture was assessed using a spectrophotometer at 593 nm. To create the calibration curve, five concentrations of $FeSO_4$, $7H_2O$ (0.1, 0.4, 0.8, 1, 1.12, 1.5 mm) were utilized, and the absorbance values were recorded as done for sample solutions [18].

Statistical analysis

The results were subjected to appropriate statistical analysis. The averages were compared using one-way analysis of variance (ANOVA), and considerable variations among sets were determined by the Ducan multiple range test using the Statistical Package For The Social Sciences for Windows version 20. The degree of significance was set at $p<0.05$.

RESULTS

Bioactive composition of *L. cylindrica*

GC-FID based bioactive profile of ethanol leaf extracts of L. cylindrica

The results indicated the existence of 22 bioactive compounds in the extract, accumulating to a total concentration of 217.43072 ppm. Of these, the bioactive compound that had the highest concentration was silymarin (158.94587 ppm), followed closely by apigenin (8.412 ppm), catechin (5.72264 ppm), apigenin (4.03142 ppm), artemetin (2.63596 ppm), vanillic (2.26698 ppm), and epicatechin (2.05439). In addition, other bioactive phytocompounds such as retusin, daidzein, naringenin, hesperidin, isorhamnetin, myricetin, and others were found in lower concentrations, under 2 ppm, as illustrated in Fig. 2, and the chromatogram in Fig. 3.

Phytochemical profile of ethanol leaf extract of L. cylindrica based on GC-MS

The GC-MS analysis revealed the presence of 30 significant bioactive compounds. The compound with the greatest concentration was 2,4-di-tert-butylphenol (8.08%), followed by apigenin (7.73%), dodecane (4.489%), chlorogenic acid (4.51%), carbonic acid, nonyl vinyl ester (3.73%), and limonene (3.38%). Other compounds include undecane (2.93%), 1-octadecene (2.66%), z-8-hexadecene (2.35%), tricosane, 2-methyl- (2.26%), and 1-hexadecanesulfonic acid (2.07%), along with 18 additional bioactive compounds with concentrations below 2%, as illustrates in Fig. 4.

Nitric oxide scavenging activity of L. cylindrica ethanol extract

The findings noted the nitric oxide scavenging ability of the extract at various concentrations, which ranged from 2.02 to 2.47 µg/mL at concentrations of 40 mg/mL and 10 mg/mL, respectively. There was a significant difference ($p<0.05$) in the nitric oxide scavenging abilities of the various extract concentrations, although the effect did not depend on concentration (Fig. 5).

Fig. 1: *Luffa cylindrica*

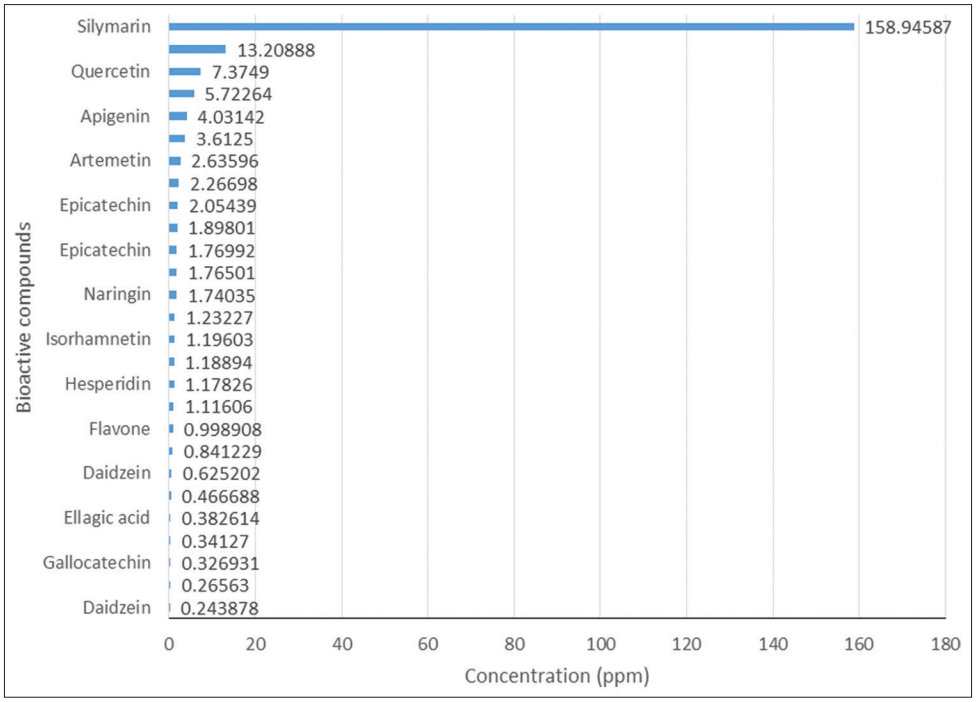

Fig. 2: Gas chromatography-flame ionization detector bioactive content of ethanol leaf extracts of *Luffa cylindrica*

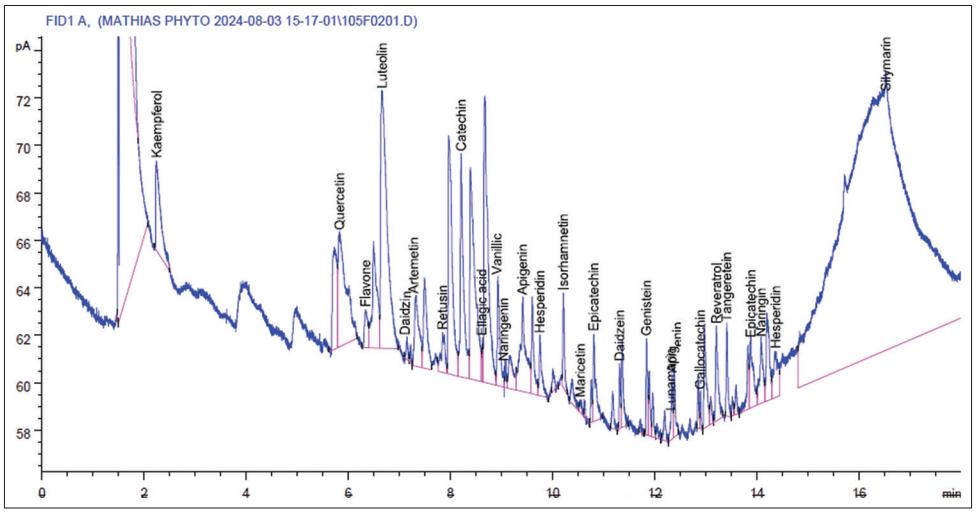

Fig. 3: Gas chromatography-flame ionization detector chromatogram of *Luffa cylindrica* ethanol leaf extracts

Superoxide scavenging activities of L. cylindrica ethanol leaf extract

The outcome indicated that the superoxide scavenging ability of the extract was greatest at 20 mg/mL (73.89%), followed by 40 mg/mL (72.09%) and 10 mg/mL (71.9%), but it was lowest at the extract concentration of 80 mg/mL (65.74%), as illustrated in Fig. 6. The ANOVA results revealed a significant difference ($p<0.05$) in the superoxide scavenging activity among the different concentrations of the extract.

Hydroxyl radical scavenging activities of L. cylindrica ethanol leaf extract

The hydroxyl radical scavenging ability of the extract varied between 61.1 and 68.9% at 10 mg/mL and 20 mg/mL extract concentrations, respectively. At concentrations of 40 and 80 mg/mL, the hydroxyl radical scavenging effectiveness was 63.5% and 65.6%, respectively (Fig. 7). A significant difference ($p<0.05$) existed in the hydroxyl radical scavenging abilities among the varying extract concentrations.

DPPH scavenging activity of L. cylindrica ethanol leaf extract

The DPPH scavenging activity of various extract concentrations varied from 93.1 to 94.8% (concentrations 10 mg/mL and 80 mg/mL, respectively). The control (BHT) exhibited a significantly greater DPPH scavenging activity (98.5%) in comparison to the extract concentrations. The DPPH scavenging activity of the extract was dependent on the concentration (Fig. 8). The ANOVA results indicated a highly significant difference ($p<0.05$) in the DPPH scavenging activities of the extract.

Hydrogen peroxide scavenging activity of L. cylindrica ethanol leaf extract

The results indicated that the hydrogen peroxide scavenging activity of the extract varied between 65.96 and 70.51%. The 50 mg/mL concentration demonstrated a significantly higher ($p<0.05$) hydrogen peroxide scavenging activity (70.51%), followed by 10 mg/mL (69.45%) and 5 mg/mL (67.44%), while the 100 mg/mL concentration exhibited the least hydrogen peroxide scavenging activity (65.96%).

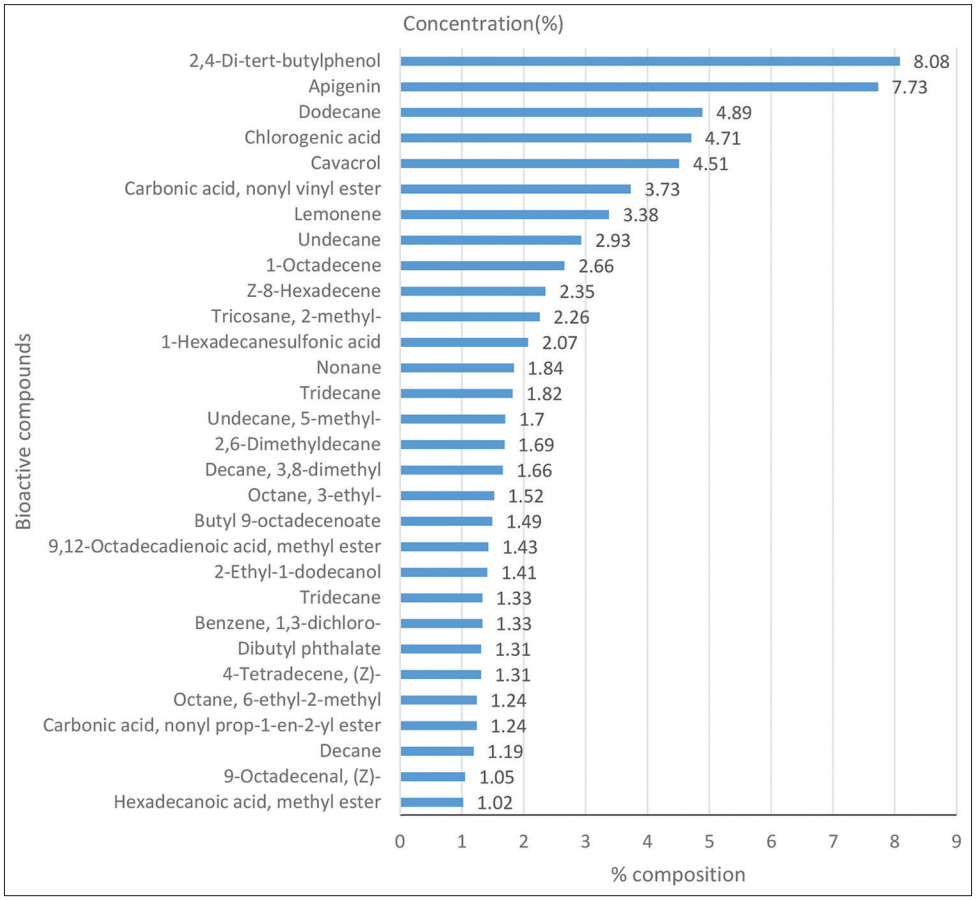

Fig. 4: Gas chromatography-mass spectrometry-based bioactive profile of ethanol leaf extract of *Luffa cylindrica*

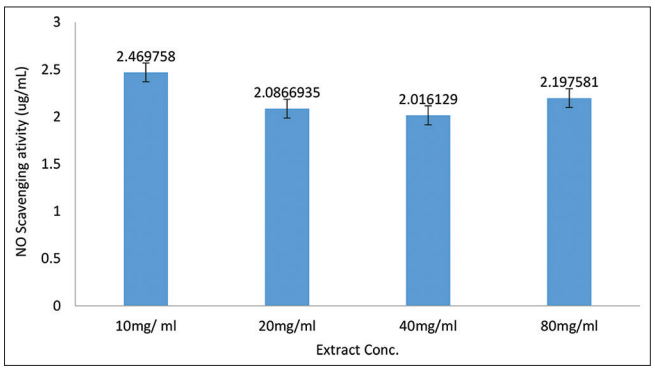

Fig. 5: Nitric oxide scavenging activities of *Luffa cylindrica* ethanol leaf extract

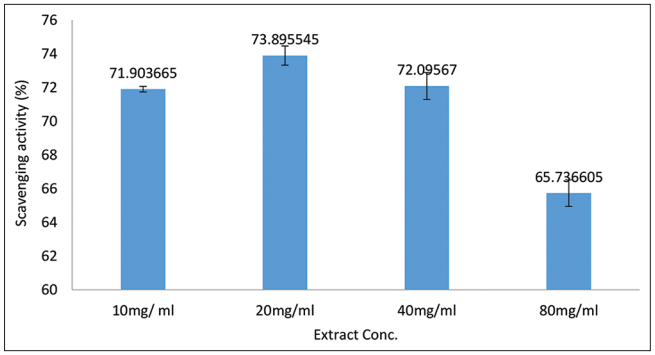

Fig. 6: Superoxide scavenging activities of *Luffa cylindrica* ethanol leaf extract

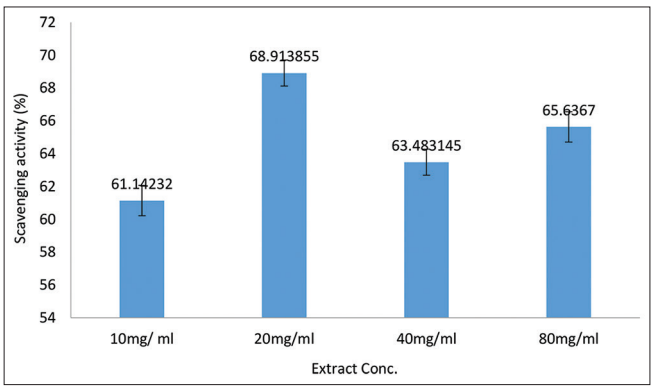

Fig. 7: Hydroxyl radical scavenging activities of *Luffa cylindrica* ethanol leaf extract

The hydrogen peroxide scavenging activity of the varied extract concentrations seemed to be dependent on concentration from 5 to 50 mg/mL but diverged from this pattern at 100 mg/mL. A significant difference was observed in the hydrogen peroxide scavenging activities among the different extract concentrations (Fig. 9).

ABTS scavenging activities of L. cylindrica leaf extract

The outcome showed a concentration that depended on the ABTS scavenging activities of the extract. The extract concentration of 300 mg/L demonstrated the highest ABTS scavenging activity (71.67%), followed by 200 mg/L (67.97%), while the lowest activity was noted at the 100 mg/L extract concentration (67.65%). A significant difference ($p<0.05$) was found in the ABTS scavenging activities among the various extract concentrations (Fig. 10).

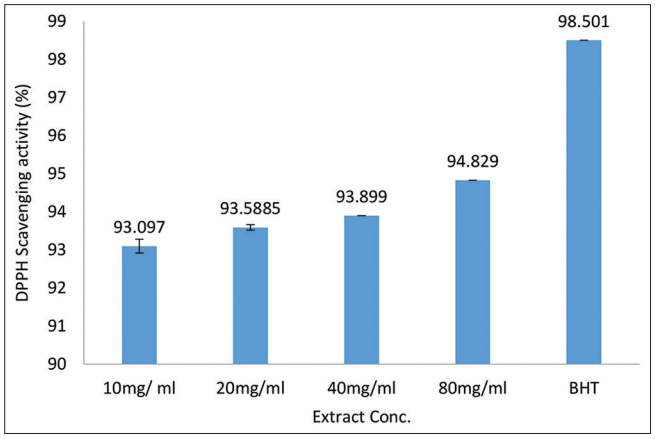

Fig. 8: 2-Diphenyl-1-picrylhydrazyl scavenging activity of *Luffa cylindrica* ethanol leaf extract

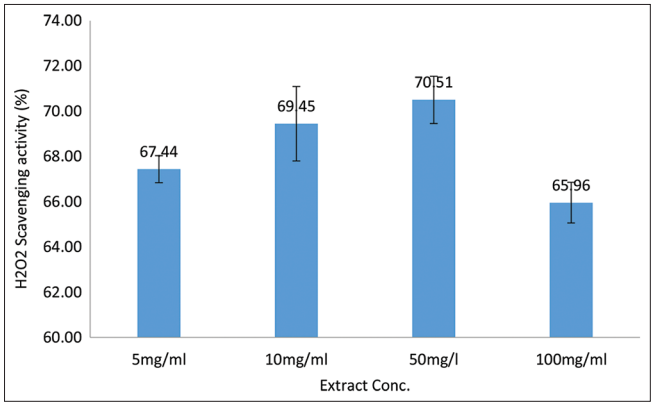

Fig. 9: Hydrogen peroxide scavenging activity of *Luffa cylindrica* ethanol leaf extract

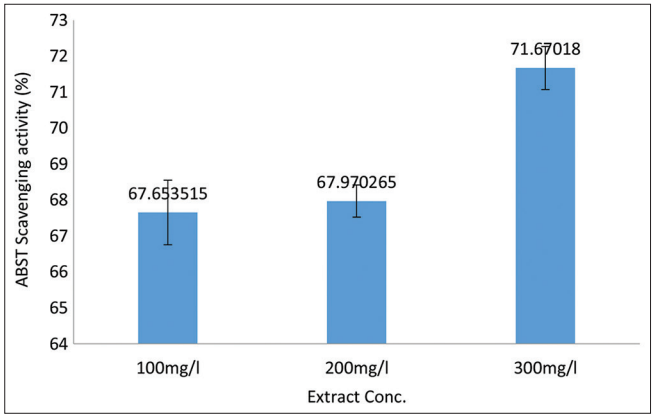

Fig. 10: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) scavenging activities of *Luffa cylindrica* leaf extract

TAC content of L. cylindrica leaf extract

The outcome showed that the TAC content of the extract was greatest at 80 mg/mL (13.356 mg/mL), followed by 40 mg/mL extract concentration (12.576 mg/mL), 10 mg/mL (10.429 mg/mL), and 20 mg/mL (9.844 mg/mL), while it was lowest at 0 mg/mL (0.137 mg/mL), as illustrated in Fig. 11. A notable difference ($p<0.05$) was observed in the TAC content among the various extract concentrations. The TAC activity of the extract was reliant on its concentration.

FRAP inhibitory activity of the L. cylindrica leaf extract

The results demonstrated that the FRAP inhibitory activity of the extract peaked significantly ($p<0.05$) at 80 mg/mL (79.345%), trailed closely by 40 mg/mL (76.898%), 20 mg/mL extract concentration (61.490%), and 10 mg/mL (33.487%), while it was least at 0 mg/mL (0%), as illustrated in Fig. 12. A significant difference ($p<0.05$) was observed in the FRAP inhibitory activity among the various extract concentrations. The FRAP inhibitory activity of the extract was dependent on its concentration.

DISCUSSION

The GC-FID analysis identified 22 bioactive compounds in the ethanol leaf extract of *L. cylindrica*, while the GC-MS results indicated the existence of 30 notable bioactive compounds. The most prevalent bioactive compound was silymarin, a flavonoid recognized for its hepatoprotective and antioxidant characteristics. This result is in agreement with a recent study that identified silymarin as a primary bioactive compound in the leaf extract of *Silybummarianum*, with concentrations from 120 to 180 ppm [19]. The elevated concentration of silymarin, a flavonoid complex in the extract, indicates possible therapeutic use in liver diseases and conditions related to oxidative stress. Flavonoids, a class of secondary metabolites, are responsible for a wide variety of uses in the healthcare industry [20]. The second most abundant compound was apigenin, a flavonoid with potential anti-inflammatory, antioxidant, and anticancer capabilities. The finding supports a previous report that recognized apigenin as one of the key bioactive compounds in the leaf extract of *Solanum nigrum*, with a concentration of 9.2 ppm [21].

Other significant bioactive compounds found in the *L. cylindrica* leaf extract comprise catechin, a flavonoid with antioxidant and cardioprotective benefits, and artemetin, a flavonoid with potential antimalarial and anticancer roles. Vanillic, a phenolic compound with antioxidant and anti-inflammatory attributes, and epicatechin, a flavonoid with possible neuroprotective and cardioprotective properties **(**Figs. 2 and 4). Plant phenolics are a diverse group of secondary metabolites and are utilized as wound and infection protectants. Phenolics have been identified to have several biological activities, such as antioxidants, antimutagenic, anticarcinogenic, anti-inflammatory, and antimicrobial activities in humans [22]. These results were aligned with other recent studies that explored the bioactive profiling of plant extracts. For example, the presence of catechin, vanillic acid, and epicatechin was observed in the leaf extract of *Moringa oleifera* [23], while artemetin was highlighted as a significant bioactive compound in the leaf extract of *Artemisia annua* [24]. A similar content of flavonoid group (neohesperidin, isorhamnetin, astragalin, quercetin, and quercetin-3β-D-glucoside) was also reported in methanol extract of *Zanthoxylum acanthopodium* fruits [25] The detection of these bioactive compounds in substantial amounts corresponds with another author's report who found similar phytochemical profiles in their investigation on *L. cylindrica* extracts [26]. These findings suggest that the extract may serve as an effective remedy for health issues related to oxidative stress.

The antioxidant activities demonstrated by the *L. cylindrica* leaf extract, as assessed through various assays, were consistent with the results of several studies on plant-based extracts, especially from the Cucurbitaceae family. The nitric oxide scavenging activity seen in this study aligns with previous studies that examined the antioxidant properties of *L. cylindrica* leaf extracts [27]. They discovered that the extracts displayed notable nitric oxide scavenging activity, attributing it to the existence of bioactive compounds such as flavonoids and polyphenols. The nitric oxide scavenging activity of the *L. cylindrica* leaf extract was not influenced by concentration, contrasting another report that indicated a concentration-dependent nitric oxide scavenging activity of plant extracts [28]. Nevertheless, the extract showed significant ($p<0.05$) nitric oxide scavenging activity, implying

its potential in alleviating oxidative stress and associated conditions. The superoxide scavenging activity recorded in this study concurs with the results observed by investigating the antioxidant potential of *L. cylindrica* leaf extracts [29]. They noted that the extracts displayed strong superoxide scavenging activity, with higher concentrations demonstrating more robust effects akin to what was observed in the present study. The hydroxyl radical scavenging activity revealed in this study coincides with the findings reported earlier in a study that explored the antioxidant properties of *L. cylindrica* leaf extracts [30]. The research indicated that the extracts exhibited significant ($p<0.05$) hydroxyl radical scavenging activity, associating it with the presence of bioactive compounds such as polyphenols and flavonoids. The DPPH scavenging activity noted in this study aligns with the observations of research that examined the antioxidant potential of *L. cylindrica* leaf extracts [27]. The report said that the extracts demonstrated strong DPPH scavenging activity, although this was less than that of the synthetic antioxidant BHT, which was also consistent with the findings of the present study. The author reported that *Luffa cylindrica* extracts exhibited potent DPPH scavenging activity which can stop peroxidation of biomolecule [31]. The hydrogen peroxide scavenging activity of the extract was also noted in this study to be in agreement with a previous report on the antioxidant properties of *L. cylindrica* leaf extracts [28]. The report indicated that the extracts showed considerable hydrogen peroxide scavenging activity, which exhibited concentration dependence, which is relatively similar to the current study.

The ABTS scavenging activity of the *L. cylindrica* leaf extract was concentration-dependent, which is in agreement with earlier research that recorded a similar trend in plant extracts [32]. The significant ($p<0.05$) ABTS scavenging activity indicates its potential to mitigate oxidative stress-related diseases. TAC and FRAP inhibitory activity of the *L. cylindrica* leaf extract were concentration-dependent, which supports the earlier findings, which reported similar trends in their study on the plant extracts [33]. The reducing powers of the ferric ions were also reported for the hydroethanolic and aqueous extracts of *Flacourtia indica,* another author [34]. The extract exhibited significant ($p<0.05$) TAC and FRAP inhibitory activity, suggesting its potential to mitigate oxidative stress-related conditions.

CONCLUSION

The present study revealed the presence of various bioactive compounds, including silymarin, apigenin, catechin, artemetin, vanillic, and epicatechin, in the ethanol leaf extract of *L. cylindrica.* The extract exhibited potent antioxidant properties, as evidenced by its ability to scavenge nitric oxide, superoxide, hydroxyl radicals, DPPH, hydrogen peroxide, and ABTS. In addition, the extract demonstrated significant TAC and FRAP inhibitory activity. These findings suggest the potential therapeutic applications of *L. cylindrica* leaf extract in the management of oxidative stress-related diseases and the treatment of degenerative diseases.

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AUTHORS CONTRIBUTIONS

The research author contributed to all aspects of the research.

CONFLICT OF INTEREST

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