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HR-LCMS Analysis for the identification of bioactive compound of *Justicia adhatoda* L. leaf extract and the potential Antioxidant and Anti-lipid peroxidation Assay *In vitro*

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Abstract

Throughout human history, medicinal plants have always been used as medicine to treat various diseases, extracts and active compounds of the medicinal plant most use of the therapies and play a major role in medication since the beginning of human civilization and also contribute to the manufacturing of drugs these days. The purpose of this study was to assess the *Justicia adhatoda* L. butanol (JABE) and methanol (JAME) leaves extracts for potential against lipid peroxidation activity, as well as for several antioxidant assays. A number of characteristics were examined, such as anti-lipid peroxidation, scavenging of free radicals (ferric reducing power potential, iron chelating activity, hydroxyl, hydrogen peroxide, superoxide and nitric oxide radicals). The JABE and JAME extracts was also subjected to HR-LCMS analysis. The HR-LCMS analysis revealed the makeup of a variety of bioactive compounds, such as Andrographolide, Vasicinone, vincamine, Curcumin, Kaempferitrin, 6-Gingerol, Sitagliptin, Kynurenic acid, Oleoyl ethanolamide, Luotonin A and many more, which exhibit numerous pharmacological activities such as antioxidant, anti-inflammatory, anticancer, and antidiabetic, antihypertensive, antimicrobial, antiaging, hepatoprotective, lipid lowering potential. These fractions also demonstrated a strong correlation with the IC_{50} values for the scavenging of hydrogen peroxide radicals, superoxide radicals, anti-lipid peroxidation, nitric oxide, iron chelating and hydroxyl radical efficacy. Additionally, the maximum scavenging activity for the above mention assays and for the reduction of Fe^{3+} to Fe^{2+} was demonstrated by both the fractions. The current findings indicated that *Justicia adhatoda* L. leaves, specifically the JABE and JAME fractions, have therapeutic potential as agent to prevent damage caused by free radicals.

Keywords: Medicinal plant; Free radical; HR-LCMS; Hepatoprotective; Antioxidant; Lipid peroxidation

1. Introduction

The use of plants as medicines is as old as human civilization itself, and more than 10% of the approximately 258,650 species of higher plants known to exist worldwide are used to treat sick population [1]. Plants have been essential to preserving human health and improving the quality of human life for thousands of years. Numerous naturally occurring antioxidants have been identified and extracted from therapeutic plants. Moreover, phytochemicals' antimicrobial, antithrombotic, and vasodilator potential have raised interest in them as complementary and alternative therapies [2]. Reactive oxygen species, including nitric oxide, peroxide, hydroxyl, and superoxide radicals, apart from the dangers oxidative stress poses to biological systems, it is also the cause of chronic illnesses like cancer, heart disease, aging, diabetes, and cataracts [3]. Numerous studies have proposed a link between consuming these phytochemicals through food and preventing a range of abnormalities brought on by stress [4–8]. Given that the majority of people in underdeveloped nations use traditional medicines, it is imperative that medicinal plants be described according to their pharmacological characteristics [8–10].Many medicinal plants and herbs contain high concentrations of antioxidants, such as polyphenols, vitamin C, vitamin E, selenium, β-carotene, lycopene, lutein, and other carotenoids, which act as antioxidants by neutralizing, quenching, reducing, or breaking down peroxides [11]. In order to prevent the possible harm of synthetic antioxidants, recent research is concentrating on substituting naturally occurring antioxidants for

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synthetic ones [12–15]. A synergic effect of phytochemicals from medicinal plants exists to exhibit the observed pharmacological properties [16]. The increasing use of phytochemicals in traditional medicinal systems is because of the current trend toward applying green products [17].

Reactive oxygen species [ROS], are different types of activated oxygen that include non-free radical species like hydrogen peroxide (H_2O_2) and free radicals like superoxide ions (O^{2-}) and hydroxyl radicals (OH^-) [18]. Degenerative or pathological processes like aging, cancer, coronary heart disease, Alzheimer's disease, neurological disorders, atherosclerosis, cataracts, and inflammation are all significantly impacted by these ROS [19]. Numerous ROSs are produced in living organisms by a variety of mechanisms, such as peroxisomes, macrophages, activated polymorphonuclear leukocytes, and regular aerobic respiration. These seem to be the primary endogenous sources of the majority of oxidants that cells generate. Tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides are examples of exogenous sources of free radicals [18]. The action of many vitamins, minerals, and other phytochemicals to prevent damage from ROS is referred to as "antioxidant" [20]. Although they are utilized in processed foods, synthetic antioxidants like butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have negative side effects. Due to safety concerns, plant-based antioxidants are now preferred over synthetic ones [21]. As a result, studies on the antioxidant potential of plants are crucial [22]. Currently interest in antioxidant compounds has increased because they play key function in health and diseases and also have nutritional value. The antioxidant activities of plants are different when different prooxidants are used.

HR-LCMS is an important analytical technique e.g. metabolomics experiments [23]. HR-LCMS-based approaches are expected to be of particular importance in plants, owing to the highly rich biochemistry of plants, which covers many semi-polar compounds, including key secondary metabolite groups, which can best be separated and detected by HR-LCMS approaches [24].

According to estimates from the World Health Organization (WHO), 80% of the world's population rely on traditional medicine for their primary medical need entailed applying plant extract or active ingredients [25]. The selected plant *Justicia adhatoda* L. found in Shillong Meghalaya, India is a member of Acanthaceae locally known as ‘dieng kthang’ in Khasi Hills and ‘devlamach’ in Garo Hills. *Justicia adhatoda* L. is a shrub widespread throughout the tropical region of Southeast Asia. It is a perennial evergreen and highly branches shrubs, 1-1.5 m height, with bitter taste. The tender leaves of this plant are used as vegetables after cooking by the Khasi and Garo tribes. It is also been used traditionally for years as herbal medicine for treating cold, cough, bleeding, skin disease, wounds, headache, fever and for lowering blood pressure. The terms *J. adhatoda* L. is frequently referred to as Malabar nut or Vasaka [26]. It has been widely used in Chinese and Indian medicines. Extracts from various part of the plant has been used for the treatment of a variety of diseases such as leprosy, skin diseases, piles, asthma etc. Vasicine is one of the compounds from the plant which belongs to quinazoline alkaloid class. Vasicine has been demonstrated to be active against tuberculosis [27]. Vasicinone is another compound found which also is classified as quinazoline alkaloid. Both vasicine and vasicinone contribute to the antioxidant activities of the plant extract [28]. In addition, vasicol and vasicinolone are also found in the plant extract [29], extract has been shown to possess anti-inflammatory, antioxidant properties [30-32]. Hence, this study was aimed to determine the composition of bioactive compound by HR-LCMS and *in vitro* antioxidant activities of butanol and methanol extract using different assays.

2. Materials and Methods

2.1. Plant material collection

During the month of September- October 2021, aerial plant parts of *Justicia adhatoda* L. were gathered from Laban-Barik area Shillong, East Khasi Hills District Meghalaya, India, leaves were collected, identified, and after being cleaned with distilled water, the renewed foliage was dried for 14 days at room temperature (25-30 °C), out of direct sunlight, and ground into a powder using an electronic grinder. The crushed sample leaves were kept at room temperature in dry, airtight plastic bags

2.2. Preparation of Butanolic and Methanolic Extract

To aid in the extraction of phytoconstituents, a 25g powdered sample was steeped in 250 ml of butanol (JABE), methanol (JAME) solvents in a conical flask for 48 hours, with continuous stirring. Whatman (No. 1) filter paper were then used to filter the entire mixture. Using a rotary evaporator set to lower pressure and temperature, the filtrate was further evaporated and freeze-dry, the pure crude extracts were then collected and stored under -20°C for further analysis.

2.3. Chemicals

Hydrogen peroxide, Ferric Chloride, Di-Sodium hydrogen Phosphate obtained from Merck Specialties Pvt. Ltd., Mumbai, India, butanol and methanol, gallic acid, ascorbic acid, Potassium persulphate, Potassium ferricyanide and Trichloroacetic acid, ferric chloride, ferrozine, ethylenediaminetetraacetic acid EDTA, N-1-naphthyl ethylene diamine dihydrochloride [NED], sulfanilamide, H_3PO_4, riboflavin, nitro blue tetrazolium (NBT) were was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Sodium phosphate,1,1,3,3-tetraethoxypropane (TEP), thiobarbituric acid (TBA) were procured from Sigma-Aldrich (St. Louis, USA). Other solvent used were of analytical grade.

2.4. *In vitro* antioxidant study

2.4.1. Reducing power assay: Ferric reducing antioxidant power (FRAP) assay

This method is based on the conversion of the Fe^{3+}/ferricyanide complex to its ferrous form to formed a violet-colored solution, whose intensity is proportional to the sample concentration. A higher absorbance of the reaction mixture is indicative of a greater reducing power of the extract [33]. The ferric ion reducing power test was used to measure the reducing power of the JABE and JAME extract of *Justicia adhatoda* L. 2 ml of phosphate buffer (0.2 M) with a pH of 6.6 was mixed with 2 ml of various concentration of plant extracts (µg/ml), and 2 ml of potassium ferricyanide were added. For 20 minutes, the mixture was incubated at 50°C. 2 ml of 10% trichloroacetic acid (w/v) was added to the mixture, it was centrifuge for 10 minutes at 3000 rpm, 4 °C. Then equal volume of the supernatant with distilled water and 0.1 ml of 0.1% ferric chloride was mixed, incubated at room temperature for 10 minutes, absorbance of chromogen formed was read at 700 nm, increased absorbance value indicated high reduction capacity [34]. At each concentration, experiment was carried out in triplicate.

2.4.2. Iron chelating assay

The ability of antioxidants to stop electron transport by creating a coordination complex with metal ions, which stops oxidation reactions and the production of free radicals. Consequently, the red color of the iron (II)–ferrozine complex is decolorized when other chelating agents are present as they compete with ferrozine for the ferrous ions. The decreased in absorbance at 562 nm indicates the chelating activity of ferrous ions [35]. A common spectrophotometric technique, was used to measure the chelating activity of ferrous ions. 1 ml of 0.125 Mm ferrous sulfate solution, 1 ml of JABE and JAME extracts at different concentrations (µg/ml) and 1 ml of ferrozine (0.3125 mM), was added to start the reaction. Vortexed, and allowed to stand at room temperature for 10 minutes. EDTA was used as a positive control. The absorbance was read at 562 nm using UV-Vis spectrophotometer. At each concentration, tests were performed in triplicates. The tendency of the sample to chelate the ferrous ion was calculated using the following formula:

$$\% \text{ Iron II chelating activity} = [(A_0 - A_1)/A_0] \times 100$$

Where,
A_0 = control absorbance
A_1 = extract or EDTA absorbance

2.4.3. Hydroxyl Radical (OH^-) Scavenging assay

This assay relies on the extract's capacity to prevent the deoxyribose degradation caused by hydroxyl radicals through the Fenton's reaction, which uses a reaction combination of Fe^{3+}-EDTA-ascorbic acid and H_2O_2. [36-37]. Reagent solution was prepared by sequential addition of 50 mM KH_2PO_4-KOH buffer (pH 7.4), 200 mM $FeCl_3$ (1:1 v/v), EDTA (1.04 mM), H_2O_2 (2.0 mM) 200µl, ascorbic acid (1.0 mM), 2-deoxy-2-ribose (28 mM) 100µl, and 100µl the extract at different concentration (µg/ml) were all added to a reaction mixture. For 1 h, the mixture was incubated at 37°C. Following incubation, 1ml of 2.8% trichloroacetic acid (TCA) and 1% thiobarbituric acid (TBA) were added, and the mixture was then incubated for an additional 20 minutes at 100 °C to formed a pink coloured complex. Once cooled, the absorbance at 532 nm was measured. All of the reagents, excluding the extract, were present in the blank solution. Gallic acid was used as positive control. Each experiment was carried out in triplicates. The hydroxyl radical scavenging activity percentage was calculated using the formula below:

$$\% \text{ } OH^- \text{ scavenging activity} = [(A_0 - A_1) / A_0] \times 100$$

Where,
A_0 = control absorbance
A_1 = extract or standard.

2.4.4. Nitric oxide (NO) scavenging assay

Sodium nitroprusside (SNP) generated nitric oxide on its own. This nitric oxide then combines with molecular oxygen to form nitrite ions, which may be measured with the Griess reagent. The extract's nitric oxide scavengers compete with molecular oxygen, which lowers the amount of nitrite ions produced. [38]. Nitric oxide radical scavenging activity of JABE and JAME was determined, Nitric oxide generated from SNP in aqueous solution at physiological pH interacted with oxygen to produce nitrite ions which were measured using Griess reaction method. 3 ml of 10mM SNP in phosphate buffer 0.2M, pH 7.4 was added to 1 ml of different concentrations of extracts preparation (µg/ml). A similar procedure was repeated with methanol as a blank, were incubated at room temperature for 30 minutes. Following incubation, 1.5 ml of sample solution incubated and 1.5 ml of Griess reagent (0.1% N-1-naphthyl ethylene diamine dihydrochloride [NED], 1% sulfanilamide, and 2% H_3PO_4) was added (The extracts mixed with an equal volume of freshly prepared Griess reagent). Nitrite ion diazotization with sulfanilamide and pink chromophore was produced by a subsequent interaction with NED, whose Spectrophotometric measurements of absorbance were made at 546 nm against reagent blank [39]. Three duplicates of each test were performed. Ascorbic acid was used as the positive control. The percentage nitrite radical scavenging activity of the extracts and ascorbic acid were calculated using the following formula:

$$\% \text{ NO scavenging activity} = [(A_0 - A_1)/A_0] \times 100$$

Where
A_0 = absorbance of control
A_1 = absorbance in the presence of extracts / standard.

2.4.5. Hydrogen peroxide (H_2O_2) scavenging assay

The decrease in absorbance that occurs when the antioxidant chemical reduces H_2O_2, is basis of this scavenging assay. The scavenging capacity of extracts for H_2O_2 was established with minor adjustments. H_2O_2 (100 mM) was made into a solution with 40 mM phosphate buffer saline at pH 7.4. [40]. 2 ml of hydrogen peroxide solution with 1 ml of varying concentration of the extracts, were added vortexed, then incubated. After 10 mins, the absorbance of hydrogen peroxide at 230 nm was measured against blank solution that contained phosphate buffer but no hydrogen peroxide. A different blank sample was utilized for background subtraction at every concentration. Gallic acid was employed as positive control. Each test was conducted in triplicate. Percent inhibitory activity was calculated using the following formula:

$$\% \ H_2O_2 \text{ scavenging activity} = [(A_0 - A_1)/A_0] \times 100$$

Where,
A_0 = is the absorbance of the control
A_1 = is the absorbance of the extract/standard,

2.4.6. Superoxide (O^{2-}) scavenging assay

The assay relies on the extract's capacity to scavenge the superoxide radicals produced in the riboflavin-light-nitro blue tetrazolium (NBT) system, thereby preventing the formation of formazan [41], 50 mM sodium phosphate buffer (pH 7.6), 20µg riboflavin, 12 mM ethylenediaminetetraacetic acid (EDTA), 1.22 mM NBT were added. The total volume of reaction mixture was 3 ml which was prepared by sequential addition of 1 ml of plant extracts at varying concentration. The samples of different concentrations were prepared in 50 mM sodium phosphate buffer (pH 7.6.) The photo-induced reactions were initiated by illuminating the reaction mixtures. Illuminating the reaction mixture with varying concentrations of plant extracts for 90 seconds initiated the reaction at room temperature. To ascertain the amount of formazan produced, the absorbance of the reaction mixture at 562 nm was measured right after illumination is completed. Ascorbic acid was used as the positive control, and each test was run in triplicates, the percentage inhibition was determined using the following formula:

$$\% \ O^{2-} \text{ scavenging activity} = [(A_0 - A_1) / A_0] \times 100$$

Where,
A_0 = absorbance of control
A_1 = absorbance of test/ standard

2.4.7. In vitro anti-lipid peroxidation assay

A pink substance with an absorption maximum at 532 nm is formed when malondialdehyde (MDA), a byproduct of lipid peroxidation (the breakdown of polyunsaturated fatty acids), combines with thiobarbituric acid (TBA). MDA was

measured using a slightly modified version as described by Smail Aazza et al., (2011) [42]. 2.0 ml of the TCA HCl reagent 15% (w/v) TCA, 0.375% (w/v) TBA, and 0.25 N HCl and the plant extract at different concentration were present in the reaction mixture with a final volume of 1.0 ml. To remove the TCA precipitate that formed the light pink supernatant (MDA), the reaction mixture was incubated in a water bath at 90 °C for 10 minutes, cooled, and centrifuged at 10,000 rpm for 10 minutes. Ascorbic acid was used as a standard. Absorbance of clear supernatant of MDA generated was measured in each sample at 532 nm against the reagent blank. All tests were performed in triplicate. The percentage of lipid peroxidation inhibition was calculated using the following equation:

$$\% \text{ Lipid peroxidation} = [(A_0 - A_1)/A_0] \times 100$$

Where,
A_0 = control absorbance
A_1 = extract / ascorbic acid absorbance

2.4.8. High Resolution Liquid Chromatography and Mass Spectrometry (HR-LCMS) Analysis

After being prepared in their respective solvent, the JABE and JAME was analyzed using HR-LCMS. Using the Agilent high resolution liquid chromatography and mass spectrometry model-G6550A with 0.01% mass resolution, chemical fingerprints of a chosen medicinal plant extract were created. MS was used as the acquisition method, with a scanning rate of one spectrum per second with a minimum range of 50 (M/Z) and a maximum of 1000 Dalton (M/Z). each spectrum with scanning rate per second, at 250 °C the gas chromatography was kept with a flow of 13 psi/minute. With an auxiliary speed of 100µl/minute, an ejection speed of 100µl/minute, a flush out factor of 5µl and 8µl injection volume used for HR-LCMS, Hip sampler with model- G4226A was used. Within half an hour during the first two minutes of the acquisition time, the solvent composition A:B flow was 95:5. HR-LCMS used a solvent i.e., water 100%, Acetonitrile at 100%.

2.4.9. Statistical analysis

In vitro and other parametric assays were performed and shown as mean ± standard error of mean of the results of three replicates per sample used to express the values of the JABE and JAME extract of *Justicia adhatoda* L. leaves for iron chelating activity, hydroxyl activity, hydrogen peroxide activity, superoxide scavenging activity, nitric oxide scavenging activity, ferric reducing power activity and anti-lipid peroxidation assay. Antioxidant potential of different assays was determined as IC_{50} values by applying Graph Pad Prism 5 and Microsoft Excel 2021 used to examine the results.

3. Results

3.1. *In vitro* antioxidant study

3.1.1. Ferric reducing antioxidant power (FRAP) Assay

Depending on each compound's reducing capability, the test solution's yellow color in this assay changes to a variety of green and blue hues. The presence of radicals (i.e. antioxidants) causes the conversion of the Fe^{3+} / ferricyanide complex utilized in this approach to the ferrous form indicated by the production of brilliant Prussian blue. Figure 1 displays the findings of the FRAP assay of the JABE and JAME at 700 nm with reference to the standard ascorbic acid. The standard curve was plotted using OD value against various concentrations of both the extracts and ascorbic acid standard. Additionally, it has been shown that when the concentration of study plant extracts in the test sample increases, the absorbance values continue to increase significantly. The ferric reductive activity increased in proportion to the five tested concentrations of the JABE and JAME. In terms of their ability to reduced, all of the concentrations were statistically comparable to the ascorbic acid standard concentration.

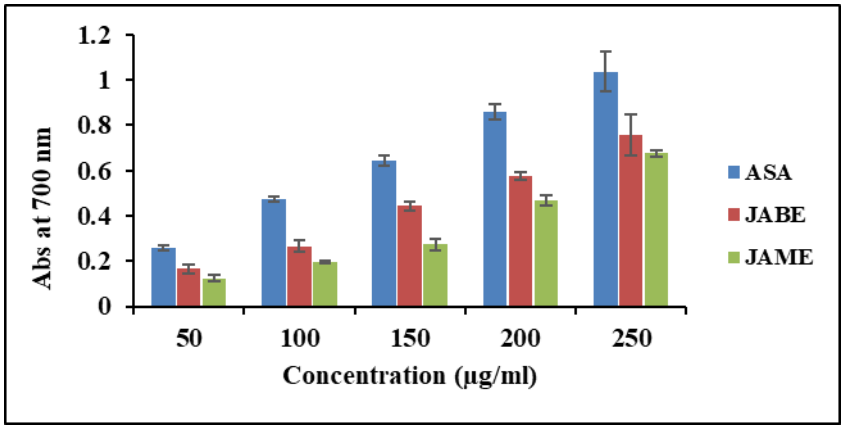


Figure 1 *In vitro* ferric-reducing antioxidant power of JABE and JAME. Results are expressed as means ± SD for replicate measurements, n = 3; JABE: *Justicia adhatoda* L. butanolic extract; JAME: *Justicia adhatoda* L. methanolic extract; ASA: Ascorbic acid

3.1.2. Hydrogen peroxide (H_2O_2) radical scavenging activity

Table 1 displays the findings of the hydrogen peroxide scavenging activity of the JABE and JAME at 230 nm with reference to the standard. The standard curve used to determine each sample's IC_{50} (µg/ml) was plotted using the percentage of inhibition against various concentrations of both extracts and gallic acid. The results showed that the IC_{50}s for the JABE extract, JAME extract, and gallic acid were 170.59 ± 0.20, 187.58 ± 0.18, and 26.32 ± 0.25, respectively. Hydrogen peroxide, although it is not very reactive by itself, it can occasionally be harmful to cell because it can produce hydroxyl radical inside the cells. Consequently, elimination of H_2O_2 is crucial for safeguarding the systems.

3.1.3. Nitric oxide (NO) scavenging activity

Mammalian cells produce the free radical nitric oxide (NO), which regulates a number of physiological functions. However, a number of disorders may be initiated and developed as a result of excessive NO generation [43]. Antioxidant potential in JABE, JAME may compete with oxygen to react with nitric oxide [44], so inhibiting the production of nitrite. This might explain the nitric oxide scavenging activity that was reported to be concentration dependent. The quantity of nitrous acid drops when a scavenger, is present. In comparison to the standard ascorbic acid, which had respective IC_{50} values of 34.59 ± 0.36 µg/m, the percentage inhibition of JABE and JAME of triplicate readings revealed IC_{50} values of 250.31 ± 0.25 and 602.33 ± 0.20 µg/ml respectively.

3.1.4. Superoxide (O^{2-}) scavenging activity

The first reduction product of oxygen that is quantified by the suppression of O_2 production is superoxide anion [45]. In aerobic organisms, the superoxide radical is ubiquitous in nature. Despite being slightly reactive with biological molecules, the superoxide radical might change into an extremely harmful and toxic hydroxyl radical. The dismutation of reactive superoxide anion to oxygen and hydrogen peroxide is catalyzed by superoxide dismutase. Plant extract's ability to scavenged anions may be attributed to its suppression of superoxide production. The JABE and JAME was shown to have this effect, the findings indicate that the possibilities for scavenging potential of JABE and JAME were discovered to be comparable to the ascorbic acid that was used as favorable control. The superoxide radical scavenging activity of JA extracts shown in Table 1. The scavenging activities was comparable to the standard in concentration dependent manner. The IC_{50} value was found out to be 31.38 ± 0.64 for ascorbic acid, 81.12 ± 0.29 for JABE, 63.82 ± 0.25 for JAME respectively.

3.1.5. Hydroxyl (OH^-)radical scavenging activity

At all extract concentrations, the JABE and JAME showed strong hydroxyl radical scavenging action. *Justicia adhatoda* L. leaf extract's capacity to scavenge hydroxyl radicals was observed to be in a concentration dependent manner. The hydroxyl radical scavenging activity of JABE and JAME was statistically comparable to that of a standard reference gallic acid. The hydroxyl radical scavenging activity varied the extract's ability to scavenge hydroxyl radicals elevated drastically with increasing concentrations, with the highest extract concentration showing the highest activity. Findings of the present study also revealed that the JABE and JAME IC_{50} value of 275.56 ± 6.38 and 344.71 ± 7.85 µg/mL comparable to that of the standard, gallic acid, whose IC_{50} value was 234.78 ± 8.54 µg/mL as illustrates in table 1.

3.1.6. Iron-Chelating activity

The investigation indicates that there was a concentration related increase in iron chelating activity of the JABE and JAME. The potential to inhibit the formation of iron (II)–ferrozine complex was significantly different among all the concentrations. The highest extract concentration showed significantly higher activity than those of the lower extract concentrations. The iron-chelating activities exhibited by JABE and JAME were statistically comparable to that of the standard reference, EDTA. Furthermore, it was observed that the IC_{50} value of the JABE and JAME showed 280.51 ± 3.45 and 380.39 ± 1.89 µg/mL, whereas the IC_{50} value for the standard, EDTA, was 194.55 ± 2.54 respectively (table 1).

3.1.7. Lipid Peroxidation Inhibition activity

Lipid peroxidation inhibition increased with concentration in the JABE and JAME leaf extract. The *Justicia adhatoda* L. leaf's ability to inhibit lipid peroxidation extract varied considerably, the increase in extract concentration demonstrated substantially higher inhibition compared to the inhibition at the lowest extract concentration. JABE and JAME suppression lipid peroxidation at various concentrations were statistically comparable to the standard ascorbic acid. The JABE and JAME displayed an IC_{50} value of 110.78 ± 2.58 ,238.37 ± 8.25 which is comparable to standard ascorbic acid 105.78 ± 5.23 respectively.

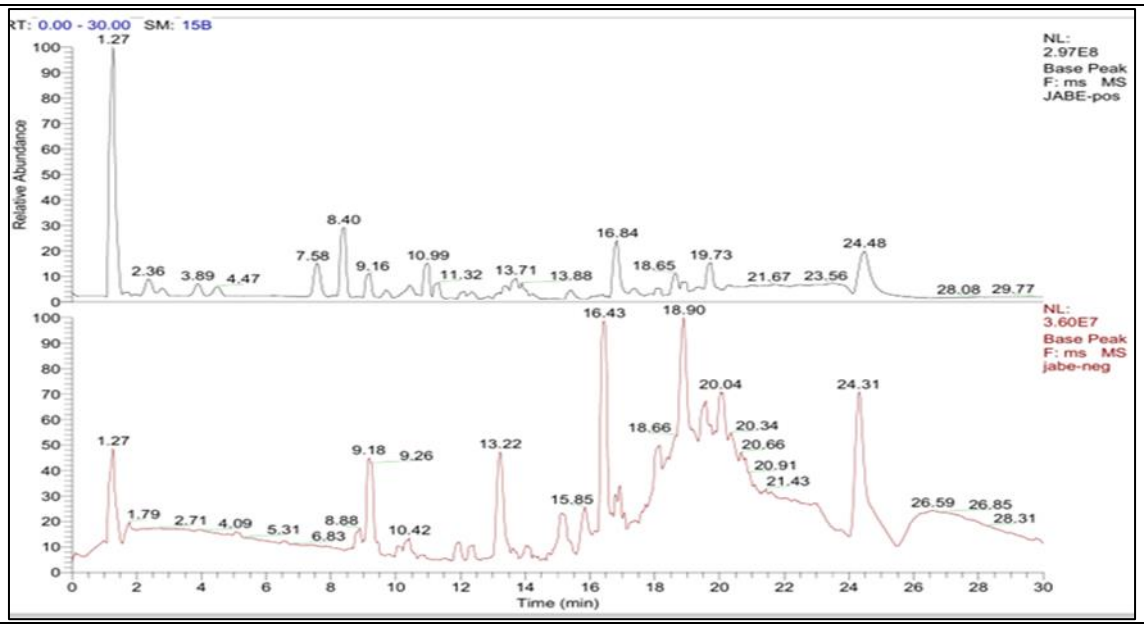
Table 1 Results of all the % Inhibition and IC_{50} studies. Values are presented as M ± SEM (n = 3) M: Mean; SEM: Standard error of mean; JABE: Butanolic extract; JAME: Methanolic leaves extract; IC_{50}, half maximal inhibitory concentration; MDA: malondialdehyde; EDTA :ethylenediaminete traacetic acid.

Result of Radical Generated	Samples	IC_{50} (µg/ml) from standard curve
Hydroxyl (OH^-)	Gallic acid	234.78 ± 8.54
	JABE	275.56 ± 6.38
	JAME	344.71 ± 7.85
Iron (II)-ferrozine complex	EDTA	194.55 ± 2.54
	JABE	280.51 ± 3.45
	JAME	380.39 ± 1.89
Hydrogen peroxide (H_2O_2)	Gallic acid	26.32 ± 0.25
	JABE	170.59 ± 0.20
	JAME	187.58 ± 0.18
Nitric oxide (NO)	Ascorbic acid	34.59 ± 0.36
	JABE	250.31 ± 0.25
	JAME	605.33 ± 0.20
Superoxide (O^{2-})	Ascorbic acid	31.38 ± 0.64
	JABE	81.12 ± 0.29
	JAME	63.82 ± 0.25

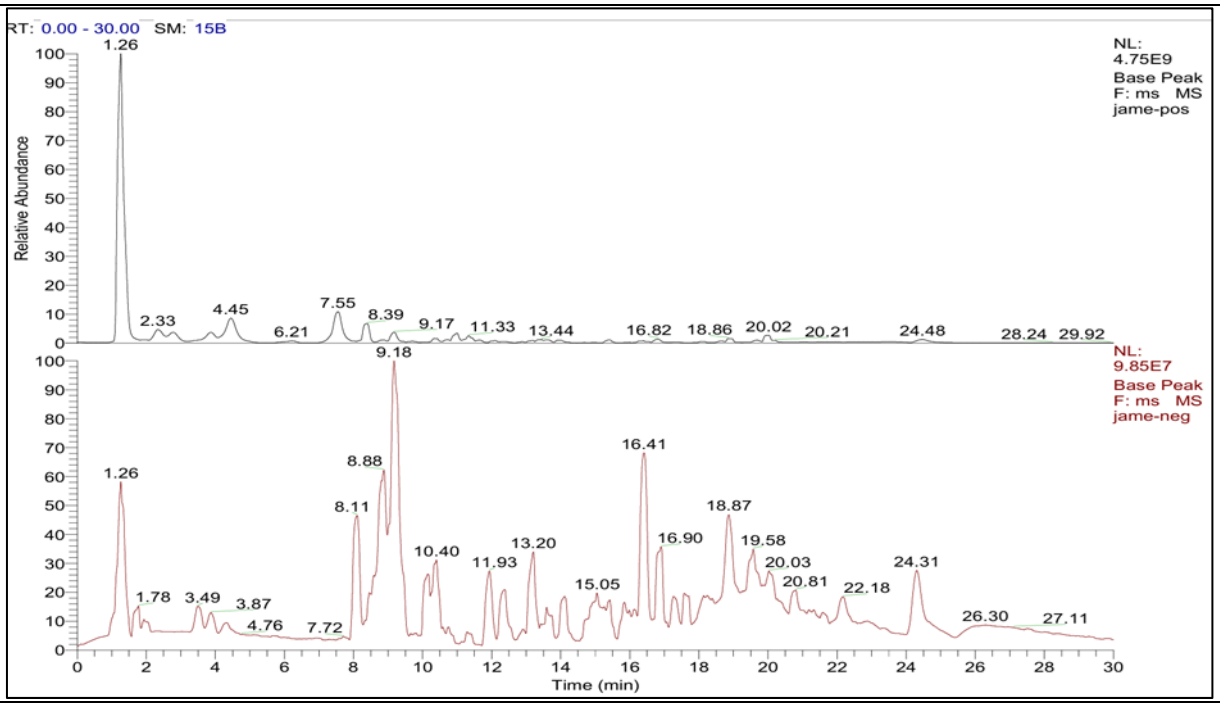
MDA	Ascorbic acid	105.78 ± 5.23
	JABE	110.78 ± 2.58
	JAME	238.37 ± 8.25

3.1.8. HR-LCMS Analysis

Major phytoconstituents were chromatographically identified from the *Justicia adhatoda* L. butanolic and methanolic extracts producing spectra with more than 500 compounds and their corresponding retention times in both positive and negative ionization modes. The reference library was used to identify and compare these peaks. Every peak in Figure 2 (a) and (b) chromatograms represented a distinct compound found in the JABE and JAME. The medicinal significance and distinctive details of the bioactive chemicals isolated by HR-LCMS analysis were shown in Table 2 (a) and (b), were among the main bioactive compounds identified. These compounds have strong antioxidants with bactericidal, anti-inflammatory, anti-aging, anti-diabetic, anti-cancer, neuroprotective, and hepatoprotective properties. Furthermore, phenolics and flavonoids serve as protective substances [46]. Due to their antimicrobial qualities, plant metabolites act as chemical barriers against infections and are utilized in a variety of food sectors as flavouring, colouring, and texturizing [47].



2 (a)



2 (b)

Figure 2 HR-LCMS chromatograms of (a) JABE, (b) JAME based on retention time plotted against relative abundance. Peaks are the fingerprints of bioactive components in both positive and negative modes

Table 2 (a) Major bioactive compounds identified by HR-LCMS Analysis: JABE positive and negative ionization mode

Sr No.	Bioactive compound	Molecular Mass (g/mol)	RT min)	Pharmacological Significance
1.	Andrographolide	350.2093	11.90	Antioxidant, anticancer, antihypertensive, anti-inflammatory [48].
2.	Luotonin A	285.0902	12.231	Antioxidant, antihyperlipidemic, antidiabetic, anticancer, antimicrobial [49].
3.	Oleoyl ethanolamide	325.2981	19.40	Anti-inflammatory, anti- antioxidant [50].
4.	vasicinone	202.0742	8.366	Anti-inflammatory, antioxidant, anticancer, antiaging, antimicrobial, antidiabetic, hepatoprotective [51].
5.	Kynurenic acid	189.042	8.726	Anti-inflammatory, antidiabetic, antihyperlipidemic, Antioxidant [52]
6.	Vincamine	354.1943		Antioxidant, antidiabetic, antihyperlipidemic [53]
7.	Betaine	117.0791	1.215	Antioxidant, anti-inflammatory [54]
8.	Sitagliptin	407.1181	10.221	Antioxidants, anti-inflammatory [55]
9.	6-Gingerol	294.3859	13.49	Antioxidant, lipid-lowering, antihyperglycemic [56]
10	Curcumin	368.1260	13.889	Antioxidative, anti-inflammatory, hypolipidemic effect [57].
11.	Benzoic acid [Negative]	122.0368	6.20	Antioxidants, antidiabetic, anti-inflammatory [58].
12.	(±)9-HpODE	312.2301	14.91	Antioxidant, antidiabetic [59].

13.	Azelaic acid	188.1049	10.41	Antidiabetic, anticonvulsant, anti-helminthic [60].
14.	Furfuryl sulfide	194.0408	1.208	Antioxidant [61].
15.	Ciproxifan	270.1367	13.204	Antioxidant [62].
16.	2-Furoylglycine	169.0375	1.23	Antidiabetic, lipid-lowering, antioxidant [63].
17.	D-(-)-Quinic acid	192.0634	1.24	Antioxidant [64].
18.	13(S)-HOTrE	294.2195	15.45	Antioxidant, anti-diabetic antioxidant, anticancer, anti-inflammatory, antidiabetic [65].
19.	12-Hydroxydodecanoic acid	216.1725	15.45	Antioxidant, anti-diabetic [66].
20.	Galactonic acid	196.0583	1.20	Neuroprotective, antioxidant, anti-inflammatory [67].

Table 2(b) Major bioactive compound identified by HR-LCMS Analysis: JAME positive and negative ionization mode

Sr No	**Bioactive compound**	**Molecular Mass(g/mol)**	**RT (min)**	**Pharmacological Significance**
1.	Luotonin A	285.0902	12.22	Anticancer, anti-inflammatory [68].
2.	Nicotinic acid	123.0320	1.69	Antioxidant, anti-inflammatory antihyperglycemic [69].
3.	Vasicinone	202.0742	10.34	Antioxidant, anticancer, anti-aging, antimicrobial antidiabetic, hepatoprotective, antihyperlipidemic [70].
4.	Andrographolide	350.2093	14.11	Antioxidant, anticancer, anti-inflammatory, antihypertensive [71]
5.	(11E,15Z)-9,10,13 trihydroxyoctadeca 11,15-dienoic acid	328.2250	12.47	Antioxidant, anti-inflammatory [72]
6.	Trigonelline		1.24	Antioxidant, cardiovascular Vascular protective, anticancer, anti-obesity, antibacterial, antioxidant [73].
7.	Piperine	285.1365	14.21	Antihyperglycemic, antioxidant [74 - 75].
8.	Kynurenic acid	189.0426	8.72	Neuroprotective, anti-inflammatory [76].
9.	curcumin	368.1260	13.88	Anti-oxidant, anti-inflammatory, antihyperglycemia [77].
10.	D-Glucosamine	179.0794	1.12	Antioxidant, anti-aging, anti-inflammatory, antimicrobial, antitumor, antihyperglycemic [78].
11.	Hexadecanedioic acid	286.2144	18.23	Antioxidant, antidiabetic [79].
12.	Kaempferitrin	578.1636	9.32	Antioxidant, hypolipidemic, anti-lipid peroxidation [80].
13.	Prothionamide	180.0725	1.225	Antidiabetic, neuroprotective, immune stimulant, anti-inflammatory, analgesic, anti-carcinogenic, antioxidant [81].
14.	Azelaic acid	188.1049	10.40	Anti-inflammatory, anti-melanoma [82].
15.	Ferulic acid	194.0579	10.68	Antihyperglycemic, antioxidant [83]

16.	Gentisic acid	154.0266	7.02	Antioxidant anti-inflammatory [84].
17.	Indole-3-acetic acid	175.0633	9.67	Antioxidant, anti-inflammatory, anti-hyperglycemic and anti-hyperlipidemic [85].
18	Protocatechuic acid	154.0266		Anticoagulatory, anti-inflammatory, antioxidative [86].
19.	Theophylline	180.0647		Antioxidant, anti-inflammatory, cardiovascular vasodilator [87].
20.	2-Hydroxycinnamic acid	164.0473	9.32	Antioxidant, anti-inflammatory [88-89].

4. Discussion

More than 434 bioactive compounds have been identified in JABE and in JAME over 500 bioactive compounds in both positive and negative ionization modes, through phytochemical Alkaloids, steroids, flavonoids, phenolic acids, organic acids, and other chemical compounds including naphthalene, alkenes, acetylene, alkylamine, hydrocarbon alkanes, and naphthoquinone are some examples of these. The presence of these bioactive phytochemicals in large quantities gives *J. adhatoda* L. a variety of pharmacological activities, including antimicrobial, antioxidant, anti-inflammatory, antipyretic, insecticidal, hepatoprotective, anti-diabetic, anti-tubercular, anti-cancer and radioprotective, anti-ulcer, and anti-respiratory properties [90-91]. Some of the best match compounds of J. adhatoda L. are shown in table 2(a & b). Basic details regarding a plant extract's potential pharmacological value are provided by phytochemical screening. The JABE and JAME was examined using HR-LCMS, using this method, the phytochemical makeup of the plant extract was chromatographed and fingerprinted. It is a complex combination of many chemicals, which are frequently found to be conjugated to one or more carbohydrate moieties and other polar groups, as shown by the chromatograms' base peak compositions, to discover and isolation of these chemicals using elemental analysis and other analytical techniques like NMR spectroscopy should be consider. [92-93].

The majority of oxidizing molecules, including singlet oxygen, and other free radicals linked to a number of illnesses are effectively scavenged by flavonoids. Hydrogen peroxide (H_2O_2) is a non-radical, rather stable reactive oxygen species (ROS) that may permeate biological membranes. Dismutation of the superoxide anion radical and 2-electron reduction of molecular oxygen either way leads to generation of H_2O_2 [94-96]. Finding indicate that both the plant extracts had higher radical scavenging effect which are comparable with the reference standard. The bioactivity of these crude extracts is caused by their high phenolic and flavonoid content.

Nitric oxide (NO) is a free radical that is produced in mammalian cells and plays a regulatory role in a number of physiological processes. However, excessive production of NO can cause the onset and progression of several diseases [97]. When oxygen is present, nitric oxide is a very unstable species. It produces the stable product when it interacts with O_2. nitrates and nitrite via the NO_2-mediated intermediates N_2O_4 as well as N_3O_4 estimated by Griess reagent. The antioxidant potential of JABE, and JAME may be responsible for the dose-dependent nitric oxide scavenging activity, which may compete with oxygen to react with nitric oxide [98] and thereby inhibit the generation of nitrite. Hydroxyl radicals are the main active oxygen species that cause lipid peroxidation, DNA damage, and protein damage [99]. When the scavenger is present, the Nitrous acid content falls. The degree of decline shows the degree of scavenging.

The first reduction product of oxygen is superoxide anion, which is quantified by the inhibition of O_2 generation [100]. Reactive superoxide anion is dismutated to oxygen and hydrogen peroxide by superoxide dismutase [101]. The superoxide anion radical scavenging assay was used to assess the fractions of JABE and JAME's ability to scavenge the O^{2-} radical from the PMS-NADH coupling system, the decrease in absorbance at 560 nm with an increase in concentration indicated the consumption of superoxide anion in the reaction mixture.

The glycation and protein-modifying processes that free radicals cause in cellular components are known as lipid peroxidation. An important biomarker of lipid peroxidation, MDA has been linked to the pathophysiology of a number of disorders, including cancer, atherosclerosis, diabetes mellitus, Alzheimer's as well as lysosome deterioration, mitochondrial enlargement, and disintegration. Lipid hydroperoxides in biological systems break down more quickly into peroxyl and alkoxyl radicals, which in turn spread the chain reaction in lipids. Lipid peroxidation is started by the generation of hydroxyl and superoxide radicals [102], a number of aldehyde compounds are created, with MDA being the most significant byproduct. The hallmark of the changes in the structure and function of cellular membranes caused by an increase in membrane permeability and a decrease in membrane lipid fluidity [103]. In this investigation, JABE

and JAME extract's suppression of hydroxyl ion-induced lipid peroxidation led to a concentration-dependent reduction in MDA formation, as determined by the TBA reaction, with a 532 nm maximum absorption. This capability may result from the extracts' capacity to scavenge the hydroxyl radicals produced during hydrogen peroxide [104].

The most powerful ROS in biological systems' free radical pathology is the hydroxyl radical, which can destroy every single cellular component.[105]. When metal cations like Fe2+ and Cu+ are present, hydrogen peroxide and superoxide anion typically combine to form hydroxyl radicals. Proteins, lipids, and DNA are all oxidatively damaged by these very reactive radicals [106]. Lipid peroxidation is started when hydroxyl radicals oxidize the polyunsaturated fatty acid moieties of the phospholipids that make up the cell membrane [105]. By breaking polynucleotide strands and changing the structure of DNA bases, the hydroxyl radical also damages nucleic acids, which contributes to cytotoxicity, mutagenicity, carcinogenesis [106-107]. The ability of fractions to scavenge hydroxyl radicals and thereby inhibit lipid peroxidation is directly linked to its antioxidant potential [108]. The significant buildup of hemoglobin and polyunsaturated fatty acids makes the erythrocytes inherently more vulnerable to peroxidation because of the significant buildup of hemoglobin and polyunsaturated fatty acids. Erythrocytes are constantly exposed to high oxygen tension during respiration, which can cause oxidative damage [109-110]. Additionally, the experiment's fractions showed evidence of scavenging hydroxyl radicals, indicating the presence of primary antioxidants with anti-lipid peroxidation potential.

Antioxidants provide one electron in the reducing power assay to stabilize the radicals and stop the free radical chain reaction [111]. The availability of antioxidant phytochemicals may have an impact on the fractions' capacity to demonstrate reducing power in this study. According to the study's findings, JABE and JAME, ferric reducing capacity at different concentrations complied with Beer's law at 700 nm. It was observed that the extract's capacity to donate electrons is indicated by the reduction of Fe^{3+} to Fe^{2+}. A direct proportionality between the observed absorbance at 700 nm and the quantity of Fe^{2+} - complex generated showed the extracts reductive ability. Consequently, a stronger reductive ability shown by an increase in optical density. [112]

Ferrozine and Fe^{2+} can react quantitatively to produce a crimson complex. The ferrozine- Fe^{2+} complex cannot form if a chelating agent is present in the reaction mixture. As the concentration of the chelating agent increases, the intensity of the red hue that formed decreases [113]. This study showed that the plant extracts inhibited the formation of a ferrozine-Fe^{2+} complex by competing with ferrozine for the ferrous ions, which resulted in a concentration-dependent decreased in color change. The JABE and JAME demonstrated chelating activity by preventing the formation of the ferrous and ferrozine complex, indicating that it captures ferrous ions prior to the ferrous and ferrozine complex formation was prevented by the extracts, indicating that it exhibits chelating action and binds ferrous ions before ferrozine.

5. Conclusion

In the present investigation, the JABE and JAME extracts of *Justicia adhatoda* L. demonstrated significant and substantial free radical scavenging activity in the evaluation of *in vitro* antioxidant activity in both of the plant extracts that are on par with the typical reference standard, particularly in an assay of nitric oxide, superoxide, and hydrogen peroxide, iron chelating, hydroxyl radical and lipid peroxidation respectively. The butanolic extract's as well as methanolic extract shows a significant % inhibition activity and their IC_{50} value almost comparable with the standard, in respective assays methods for evaluating antioxidants. This showed that both the extracts' strong antioxidant activity could be caused by the fact that it has higher concentration of flavonoids, phenolics, tannins, and terpenoids, may be linked to the extract's and its derived fractions' more active in antioxidant evaluation method and may be the cause of their notable and strong pharmacological activities. These findings were further supported by using HR-LCMS analysis, indicating that *Justicia adhatoda* L. leaves extracts may find application in phytotherapy. Nevertheless, additional research is required to identify each biologically active metabolites from *Justicia adhatoda* L. and determine its exact mechanisms of action.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] Shinwari ZK. Medicinal plants research in Pakistan. J Med Plants Res. 2010 Feb 4;4(3):161-76.

[2] Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food chemistry. 2006 Jan 1;99(1):191-203.

[3] Aruoma OI, Cuppett SL, editors. Antioxidant methodology: in vivo and in vitro concepts. The American Oil Chemists Society; 1997.

[4] Le Marchand L. Cancer preventive effects of flavonoids—a review. Biomedicine & pharmacotherapy. 2002 Aug 1;56(6):296-301.

[5] Khan MR, Rizvi W, Khan GN, Khan RA, Shaheen S. Carbon tetrachloride-induced nephrotoxicity in rats: Protective role of Digera muricata. Journal of ethnopharmacology. 2009 Feb 25;122(1):91-9.

[6] Khan RA, Khan MR, Sahreen S. Evaluation of Launaea procumbens use in renal disorders: A rat model. Journal of ethnopharmacology. 2010 Mar 24;128(2):452-61.

[7] Khan MR, Haroon J, Khan RA, Bokhari J, Rashid U. Prevention of KBrO3-induced cardiotoxicity by Sonchus asper in rat. J Med Plants Res. 2011 Jun 18;5(12):2514-20.

[8] Sahreen S, Khan MR, Khan RA. Evaluation of antioxidant activities of various solvent extracts of Carissa opaca fruits. Food chemistry. 2010 Oct 15;122(4):1205-11.

[9] Sahreen S, Khan MR, Khan RA. Phenolic compounds and antioxidant activities of Rumex hastatus D. Don. Leaves. J Med Plants Res. 2011 Jul 4;5(13):2755-65.

[10] Bursal E, Köksal E, Gülçin İ, Bilsel G, Gören AC. Antioxidant activity and polyphenol content of cherry stem (Cerasus avium L.) determined by LC–MS/MS. Food research international. 2013 Apr 1;51(1):66-74.

[11] Topal F. Pomological Features, Nutritional Quality, Polyphenol Content Analysis, and Antioxidant Properties of Domesticated and 3 Wild Ecotype Forms of Raspberries (Rubus idaeus L.).

[12] Middleton E Jr, Kandaswami C, Theoharides TC: The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. Pharmacol Rev 2000, 52:673–751.

[13] Velioglu YS, Mazza G, Gao L, Oomah BD: Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J Agri Food Chem 1998, 46:4113–4117.

[14] Gulcin I, Kufrevioglu OI, Oktay M, Buyukokuroglu ME: Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (Urtica dioica L.). J Ethnopharmacol 2004, 90:205–215.

[15] Gulcin I, Tel AZ, Kirecci E: Antioxidant, antimicrobial, antifungal and antiradical activities of Cyclotrichium niveum (Boiss.) Manden and Scheng. Int J Food Prop 2008, 11(2):450–471.

[16] Borquaye LS, Doetse MS, Baah SO, Mensah JA. Anti-inflammatory and anti-oxidant activities of ethanolic extracts of Tamarindus indica L.(Fabaceae). Cogent Chemistry. 2020 Jan 1;6(1):1743403.

[17] Rojas-Armas JP, Arroyo-Acevedo JL, Ortiz-Sánchez JM, Palomino-Pacheco M, Herrera-Calderon O, Calva J, Rojas-Armas A, Justil-Guerrero HJ, Castro-Luna A, Hilario-Vargas J. Cordia lutea L. Flowers: a promising medicinal plant as chemopreventive in induced prostate carcinogenesis in rats. Evidence-Based Complementary and Alternative Medicine. 2020;2020(1):5062942.

[18] Yildirim A, OKTAY M, BİLALOĞLU V. The antioxidant activity of the leaves of Cydonia vulgaris. Turkish Journal of Medical Sciences. 2001;31(1):23-7.

[19] Huang DongJiann HD, Chen HsienJung CH, Lin ChunDer LC, Lin YawHuei LY. Antioxidant and antiproliferative activities of water spinach (Ipomoea aquatica Forsk) constituents.

[20] Khlifi S, El Hachimi Y, Khalil A, Es-Safi N, Belahyan A, Tellal R, El Abbouyi A. In vitro antioxidant properties of Salvia verbenaca L. hydromethanolic extract. Indian Journal of Pharmacology. 2006 Jul 1;38(4):276-80.

[21] Jain A, Singhai AK, Dixit VK. In vitro evaluation of Tephrosia purpurea Pers for antioxidant activity. Journal of natural remedies. 2006 Jun 1;6(2):162-4.

[22] Akinmoladun AC, Ibukun EO, Afor E, Akinrinlola BL, Onibon TR, Akinboboye AO, Obuotor EM, Farombi EO. Chemical constituents and antioxidant activity of Alstonia boonei. African journal of biotechnology. 2007;6(10).

[23] Tautenhahn R, Böttcher C, Neumann S. Highly sensitive feature detection for high resolution LC/MS. BMC bioinformatics. 2008 Nov 28;9(1):504.

[24] De Vos RC, Moco S, Lommen A, Keurentjes JJ, Bino RJ, Hall RD. Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. Nature protocols. 2007 Apr;2(4):778-91.

[25] Schuhly W. Pharmacognosy: Phytochemistry, medicinal plants. Phytomedicine: International Journal of Phytotherapy & Phytopharmacology. 2004 Jan 1;11(1):90-1.

[26] Chakraborty A, Brantner AH. Study of alkaloids from Adhatoda vasica Nees on their antiinflammatory activity. Phytotherapy Research. 2001 Sep;15(6):532-4.

[27] Grange JM, Snell NJC. Activity of Bromhexine and ambroxol, semi-synthetic derivatives of vasicine from the Indian shrub Adhatoda vasica, against Mycobacterium tuberculosis in vitro. J Ethnopharmacol. 1996;50(1):49-53. doi: 10.1016/0378-8741(95)01331-8, PMID 8778507.

[28] Roja G, Vikrant BH, Sandur SK, Sharma A, Pushpa KK. Accumulation of vasicine and vasicinone in tissue cultures of Adhatoda vasica and evaluation of the free radical-scavenging activities of the various crude extracts. Food Chem. 2011;126(3):1033-8. doi: 10.1016/j.foodchem.2010.11.115.

[29] Kumar Gangwar A, Ghosh AK. Medicinal uses and Pharmacological activity of Adhatoda vasica. ~ 88 ~ Int J Herb Med. 2014;2(1):88-91.

[30] Roja G, Vikrant BH, Sandur SK, Sharma A, Pushpa KK. Accumulation of vasicine and vasicinone in tissue cultures of Adhatoda vasica and evaluation of the free radical-scavenging activities of the various crude extracts. Food chemistry. 2011 Jun 1;126(3):1033-8.

[31] Gangwar AK, Ghosh AK. Medicinal uses and pharmacological activity of Adhatoda vasica.

[32] Zhang S, Liu X, Bawa-Khalfe T, Lu LS, Lyu YL, Liu LF, Yeh ET. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. Nature medicine. 2012 Nov;18(11):1639-42.

[33] Gupta A, Naraniwal M, Kothari V. Modern extraction methods for preparation of bioactive plant extracts. Int J Appl Nat Sci. 2012; 1:18-26.

[34] Oyaizu M. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. The Japanese journal of nutrition and dietetics. 1986;44(6):307-15.

[35] Dinis TC, Madeira VM, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of biochemistry and biophysics. 1994 Nov 1;315(1):161-9.

[36] Harsha SN, Latha BV. In vitro antioxidant and in vitro anti inflammatory activity of Ruta graveolens methanol extract. Asian J Pharm Clin Res. 2012;5(1):32-5.

[37] Bajpai A, Verma AK, Srivastava M, Srivastava R. Oxidative stress and major depression. Journal of clinical and diagnostic research: JCDR. 2014 Dec 5;8(12):CC04.

[38] Bajpai VK, Agrawal P. Studies on phytochemicals, antioxidant, free radical scavenging and lipid peroxidation inhibitory effects of Trachyspermumammi seeds. Indian J. Pharm. Educ. Res. 2015 Jan 1;49:58-65.

[39] Farhan H, Malli F, Rammal H, Hijazi A, Bassal A, Ajouz N, Badran B. Phytochemical screening and antioxidant activity of Lebanese Eryngium creticum L. Asian Pacific Journal of Tropical Biomedicine. 2012 Jan 1;2(3):S1217-20.

[40] Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989 Jun 1;10(6):1003-8.

[41] Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical biochemistry. 1971 Nov 1;44(1):276-87.

[42] Halliwell B, Gutteridge J. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochemical journal. 1984 Apr 1;219(1):1.

[43] Gibanananda R, Sayed AH. Oxidants, antioxidants and carcinogenesis. Indian J. Exp. Biol. 2002;40:1213-32.

[44] Ialenti A, Moncada S, Di Rosa M. Modulation of adjuvant arthritis by endogenous nitric oxide. British journal of pharmacology. 1993 Oct;110(2):701-6.

[45] Kamalakkannan N. Effect of Aegle marmelos Correa.(Bael) fruit extract on tissue antioxidants in streptozotocin diabetic rats. Indian Journal of Experimental Biology. 2003 Nov 1;41(11):1285-8.

[46] Diwan A, Ninawe A, Harke S, et al. Gene editing (CRISPR-Cas) technology and fisheries sector. Can J Biotech. 2017;1(2):65-72. Güneş A, Kordali Ş, Turan M, Bozhüyük AU. Determination of antioxidant enzyme activity and phenolic contents of some species of the Asteraceae family from medicanal plants. Industrial crops and products. 2019 Oct 1;137:208-13.

[47] Güneş A, Kordali Ş, Turan M, Bozhüyük AU. Determination of antioxidant enzyme activity and phenolic contents of some species of the Asteraceae family from medicanal plants. Industrial crops and products. 2019 Oct 1;137:208-13.

[48] Adiguna SB, Panggabean JA, Swasono RT, Rahmawati SI, Izzati F, Bayu A, Putra MY, Formisano C, Giuseppina C. Evaluations of andrographolide-rich fractions of Andrographis paniculata with enhanced potential antioxidant, anticancer, antihypertensive, and anti-inflammatory activities. Plants. 2023 Mar 7;12(6):1220.

[49] Nicolaescu OE, Ionescu C, Samide A, Tigae C, Spînu CI, Oprea B. Advancements in Cyclodextrin Complexes with Bioactive Secondary Metabolites and Their Pharmaceutical Applications. Pharmaceutics. 2025 Apr 11;17(4):506.

[50] Spanoudaki M, Chrysafi M, Papadopoulou SK, Tsourouflis G, Pritsa A, Giaginis C. Naturally Occurring Compounds Targeting Peroxisome Proliferator Receptors: Potential Molecular Mechanisms and Future Perspectives for Promoting Human Health. Applied Sciences. 2024 Nov 1;14(21):9994.

[51] Banik B, Das S, Das MK. Medicinal Plants with Potent Anti-inflammatory and Anti-arthritic Properties found in Eastern Parts of the Himalaya: An Ethnomedicinal Review. Pharmacognosy Reviews. 2020 Jul 1;14(28).

[52] Mohammed A, Pandey KB, Rizvi SI. Effect of Phytochemicals on Diabetes-Related Neurological Disorders. Neuroprotective Effects of Phytochemicals in Neurological Disorders. 2017 Feb 28:283-98.

[53] Bouhenna MM, Bendif H, Segueni N, Khalfa H, Djadoudi A, Tahraoui S, Benslama A, Ksouri A, Boumechhour A, Miara MD, Rebbas K. Phytochemical profiling, LC-MS analyses, cytotoxicity, antioxidant, and antimicrobial activities of ruta Montana: an ethnomedicinally important plant in Algeria. Current Bioactive Compounds. 2024 May 1;20(4):90-9.

[54] Arumugam MK, Paal MC, Donohue TM, Ganesan M, Osna NA, Kharbanda KK. Beneficial effects of betaine: a comprehensive review. Biology. 2021 Jun;10(6):456.

[55] Chhabria S, Mathur S, Vadakan S, Sahoo DK, Mishra P, Paital B. A review on phytochemical and pharmacological facets of tropical ethnomedicinal plants as reformed DPP-IV inhibitors to regulate incretin activity. Frontiers in Endocrinology. 2022 Nov 11;13:1027237.

[56] Alharbi KS, Nadeem MS, Afzal O, Alzarea SI, Altamimi AS, Almalki WH, Mubeen B, Iftikhar S, Shah L, Kazmi I. Gingerol, a natural antioxidant, attenuates hyperglycemia and downstream complications. Metabolites. 2022 Dec 16;12(12):1274

[57] Boarescu PM, Boarescu I, Bocşan IC, Gheban D, Bulboacă AE, Nicula C, Pop RM, Râjnoveanu RM, Bolboacă SD. Antioxidant and anti-inflammatory effects of curcumin nanoparticles on drug-induced acute myocardial infarction in diabetic rats. Antioxidants. 2019 Oct 22;8(10):504.

[58] Bouknana S, Rherabi AE, Abdnim R, Berraaouan A, Bnouham M. Medicinal Plants and bioactive compounds with potential anti-inflammatory and antidiabetic activities: a review. Letters in Drug Design & Discovery. 2024 Sep 1;21(11):1985-2007.

[59] Pertami SB, Yunitasari E, Budiono B, Yulifah R, Astuti NP, Herawati T, Arifah SN, Atho'illah MF. Bioactive Compounds and Antioxidant Activity in Greek-style Yoghurt Infused with Shield Aralia Leaves. Natural and Life Sciences Communications. 2024 Jul 1;23(3):e2024045.

[60] Chan EW, Wong SK, Chan HT. A review on the phytochemistry and pharmacology of two Hibiscus species with spectacular flower colour change: H. tiliaceus and H. mutabilis. Int. J. Pharmacogn. Phytochem. Res. 2016;8:1200-8.

[61] Nadiveedhi MR, Nuthalapati P, Gundluru M, Yanamula MR, Kallimakula SV, Pasupuleti VR, Avula VK, Vallela S, Zyryanov GV, Balam SK, Cirandur SR. Green synthesis, antioxidant, and plant growth regulatory activities of novel α-Furfuryl-2-alkylaminophosphonates. ACS omega. 2021 Jan 21;6(4):2934-48.

[62] Julie, A.O.J., Bébé, N.O., Sandrine, M.N.Y., Emmanuel, O.P., Antoine, K.K., Dupon, A.A.B., Cicilien, N.P.Q., Emmanuel, N.N.F., Laure, N.J., Claude, B.D. and Désiré, D.D.P., 2025. Phytochemical, In Silico, In Vitro, and In Vivo Research on Piptadeniastrum africanum (Fabaceae) Unveiling Anti-Stereotypic, Anxiolytic, and Analgesic Effects in a Sodium Valproate-Induced Autistic Disorders Model. Brain and Behavior, 15(3), p.e70408.

[63] Zhang J, Li H, Yang H, Zhu L, Zhang Y, Huang C, Wang S, Duan Y, Jiang Z, Liu M, Zhao S. Prevention of high-fat/high-sugar diet-induced type 2 diabetes mellitus-associated non-alcoholic fatty liver disease in rats with fermented and raw Rosa roxburghii Tratt (Cili) juice. Frontiers in Nutrition. 2025 May 19;12:1584551.

[64] Benali T, Bakrim S, Ghchime R, Benkhaira N, El Omari N, Balahbib A, Taha D, Zengin G, Hasan MM, Bibi S, Bouyahya A. Pharmacological insights into the multifaceted biological properties of quinic acid. Biotechnology and Genetic Engineering Reviews. 2024 Nov 1;40(4):3408-37.

[65] Faseela KV, Berin P, Jayadeep A, Biju S, Jiji J, Abida PS, Deepu M. Unlocking Nutraceutical Potential: Metabolomic Analysis Reveals Therapeutic Compounds in Rice. Journal of Pharmacology and Pharmacotherapeutics. 2025:0976500X241309437.

[66] Lin CH, Kuo YH, Shih CC. Antidiabetic and hypolipidemic activities of eburicoic acid, a triterpenoid compound from Antrodia camphorata, by regulation of Akt phosphorylation, gluconeogenesis, and PPARα in streptozotocin-induced diabetic mice. RSC advances. 2018;8(37):20462-76.

[67] Mekky RH, Abdel-Sattar E, Segura-Carretero A, Contreras MD. Phenolic compounds from sesame cake and antioxidant activity: A new insight for agri-food residues' significance for sustainable development. Foods. 2019 Sep 22;8(10):432.

[68] Radwan AA, Alanazi FK. Biological activity of quinazolinones. InQuinazolinone and Quinazoline Derivatives 2020 Jan 29. IntechOpen.

[69] Rashid U, Khan MR, Sajid M. Antioxidant, anti-inflammatory and hypoglycemic effects of Fagonia olivieri DC on STZ-nicotinamide induced diabetic rats-in vivo and in vitro study. Journal of ethnopharmacology. 2019 Oct 5;242:112038.

[70] Banik B, Das S, Das MK. Medicinal Plants with Potent Anti-inflammatory and Anti-arthritic Properties found in Eastern Parts of the Himalaya: An Ethnomedicinal Review. Pharmacognosy Reviews. 2020 Jul 1;14(28).

[71] Adiguna SB, Panggabean JA, Swasono RT, Rahmawati SI, Izzati F, Bayu A, Putra MY, Formisano C, Giuseppina C. Evaluations of andrographolide-rich fractions of Andrographis paniculata with enhanced potential antioxidant, anticancer, antihypertensive, and anti-inflammatory activities. Plants. 2023 Mar 7;12(6):1220.

[72] Shah SL, Bashir K, Rasheed HM, Rahman JU, Ikram M, Shah AJ, Majrashi KA, Alnasser SM, Menaa F, Khan T. LC-MS/MS-based metabolomic profiling of constituents from Glochidion velutinum and its activity against cancer cell lines. Molecules. 2022 Dec 17;27(24):9012.

[73] Sekhar MG, Shanmugam KR, Chakrapani IS. Trigonelline, a fenugreek bioactive compound protects heart tissue against alcohol intoxication: An in-vivo study focusing on antioxidant perspective. Journal of Ayurveda and Integrative Medicine. 2024 Jul 1;15(4):100963.

[74] Haq IU, Imran M, Nadeem M, Tufail T, Gondal TA, Mubarak MS. Piperine: A review of its biological effects. Phytotherapy research. 2021 Feb;35(2):680-700.

[75] Kumar S, Sharma S, Vasudeva N. Screening of antidiabetic and antihyperlipidemic potential of oil from Piper longum and piperine with their possible mechanism. Expert opinion on pharmacotherapy. 2013 Sep 1;14(13):1723-36.

[76] Özdemir M, Abusoglu S, Baldane S, Kıraç CO, Unlu A, Onmaz DE, Çelik M, Abusoglu G. Analyzing serum tryptophan metabolites in patients with gestational diabetes mellitus. Revista Romana de Medicina de Laborator. 2023 Oct 28;31(4):251-62.

[77] Meng B, Li J, Cao H. Antioxidant and antiinflammatory activities of curcumin on diabetes mellitus and its complications. Current pharmaceutical design. 2013 Apr 1;19(11):2101-13.

[78] Ghaly IS, Ahmed ES, Booles HF, Farag IM, Nada SA. Evaluation of antihyperglycemic action of oyster mushroom (Pleurotus ostreatus) and its effect on DNA damage, chromosome aberrations and sperm abnormalities in streptozotocin-induced diabetic rats. Global Veterinaria. 2011;7(6):532-44.

[79] Adepoju AJ, Adepoju AA, Olawoore IT, Ayodele ET. Phytochemical profiling, antioxidant, and antidiabetic activities of defatted ethanol aerial extract of Heliotropium indicum: Insights from GC-MS and in vitro studies. Tropical Journal of Phytochemistry and Pharmaceutical Sciences. 2025 Sep 7;4(8):320-8.

[80] de Sousa E, Zanatta L, Seifriz I, Creczynski-Pasa TB, Pizzolatti MG, Szpoganicz B, Silva FR. Hypoglycemic Effect and Antioxidant Potential of Kaempferol-3, 7-O-(α)-dirhamnoside from Bauhinia f orficata Leaves. Journal of natural products. 2004 May 28;67(5):829-32.

[81] Singh K, Jain D, Chaitanya MV, Kumar S. A review of molecular investigations on traditional Chinese medicinal plant-based therapies in multidrug-resistant tuberculosis. Pharmacological Research. 2024;13(10052):1..

[82] Ismail M, Hassan MH, Mohamed EI, Azmy AF, Moawad A, Mohammed R, Zaki MA. New insights into the anti-inflammatory and anti-melanoma mechanisms of action of azelaic acid and other Fusarium solani metabolites via in vitro and in silico studies. Scientific Reports. 2024 Jun 22;14(1):14370.

[83] Ou S, Kwok KC. Ferulic acid: pharmaceutical functions, preparation and applications in foods. Journal of the Science of Food and Agriculture. 2004 Aug 30;84(11):1261-9.

[84] Razliqi RN, Ahangarpour A, Mard SA, Khorsandi L. Gentisic acid protects against diabetic nephropathy in nicotinamide-streptozotocin administered male mice by attenuating oxidative stress and inflammation: the role of miR-200a/Keap1/Nrf2 pathway, renin-angiotensin system (RAS) and NF-κB. Chemico-Biological Interactions. 2023 Aug 1;380:110507.

[85] Eze PM, Nnanna JC, Okezie U, Buzugbe HS, Abba CC, Chukwunwejim CR, Okoye FB, Esimone CO. Screening of metabolites from endophytic fungi of some Nigerian medicinal plants for antimicrobial activities. Eurobiotech j. 2019 Jan 1;3(1):10-8.

[86] Lin CY, Huang CS, Huang CY, Yin MC. Anticoagulatory, antiinflammatory, and antioxidative effects of protocatechuic acid in diabetic mice. Journal of Agricultural and Food Chemistry. 2009 Aug 12;57(15):6661-7.

[87] Abubakar KS, Lubabatu MJ. GC-MS Profiling and Secondary Metabolite Analysis of Selected Plant Extracts for Potential Anti-Hyperglycemic Activity. African Journal of Advances in Science and Technology Research. 2025 Apr 4;18(1):94-110.

[88] Sova M, Saso L. Natural sources, pharmacokinetics, biological activities and health benefits of hydroxycinnamic acids and their metabolites. Nutrients. 2020 Jul 23;12(8):2190.

[89] Adisakwattana S. Cinnamic acid and its derivatives: mechanisms for prevention and management of diabetes and its complications. Nutrients. 2017 Feb 21;9(2):163.

[90] Shoaib A. A systematic ethnobotanical review of Adhatoda vasica (L.), Nees. Cell Mol Biol. 2022;67(4):248–63. https://doi.org/10.14715/cmb/2021.67.4.28.

[91] Kumar M, Dandapat S, Kumar A, Sinha MP. Pharmacological screening of leaf extract of Adhatoda vasica for therapeutic efficacy. Global Journal of Pharmacology. 2014;8(4):494-500.

[92] Sahreen S, Khan MR, Khan RA. Evaluation of antioxidant activities of various solvent extracts of Carissa opaca fruits. Food chemistry. 2010 Oct 15;122(4):1205-11.

[93] Yang ZG, Sun HX, Fang WH. Haemolytic activities and adjuvant effect of Astragalus membranaceus saponins (AMS) on the immune responses to ovalbumin in mice. Vaccine. 2005 Oct 25;23(44):5196-203.

[94] Shukla VK, Doyon Y, Miller JC, DeKelver RC, Moehle EA, Worden SE, Mitchell JC, Arnold NL, Gopalan S, Meng X, Choi VM. Precise genome modification in the crop species Zea mays using zinc-finger nucleases. Nature. 2009 May 21;459(7245):437-41.

[95] Agati, G., Azzarello, E., Pollastri, S., & Tattini, M. (2012). Flavonoids as antioxidants in plants: location and functional significance. Plant science, 196, 67-76.

[96] Gibanananda R, Sayed AH. Oxidants, antioxidants and carcinogenesis. Indian J. Exp. Biol. 2002;40:1213-32.

[97] Ialenti A, Moncada S, Di Rosa M. Modulation of adjuvant arthritis by endogenous nitric oxide. British journal of pharmacology. 1993 Oct;110(2):701-6.

[98] Padmanabhan P, Jangle SN. In-vitro antioxidant potential of a herbal preparation containing four selected medicinal plants. Journal of Krishna Institute of Medical Sciences University. 2012 Jul 1;1(2):52-63.

[99] Stoilova I, Krastanov A, Stoyanova A, Denev P, Gargova S. Antioxidant activity of a ginger extract (Zingiber officinale). Food chemistry. 2007 Jan 1;102(3):764-70.

[100] Kamalakkannan N. Effect of Aegle marmelos Correa.(Bael) fruit extract on tissue antioxidants in streptozotocin diabetic rats. Indian Journal of Experimental Biology. 2003 Nov 1;41(11):1285-8.

[101] Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958 Apr 26;181(4617):1199-200.

[102] Babu BH, Shylesh BS, Padikkala J. Antioxidant and hepatoprotective effect of Acanthus ilicifolius. Fitoterapia. 2001 Mar 1;72(3):272-7.

[103] Geetha RK, Vasudevan DM. Inhibition of lipid peroxidation by botanical extracts of Ocimum sanctum: in vivo and in vitro stud ies. Life Sci. 2004;76:21-28.

[104] Gülçin İ, Elias R, Gepdiremen A, Chea A, Topal F. Antioxidant activity of bisbenzylisoquinoline alkaloids from Stephania rotunda: cepharanthine and fangchinoline. Journal of enzyme inhibition and medicinal chemistry. 2010 Feb 1;25(1):44-53.

[105] Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. The international journal of biochemistry & cell biology. 2007 Jan 1;39(1):44-84.

[106] Balu M, Sangeetha P, Haripriya D, Panneerselvam C. Rejuvenation of antioxidant system in central nervous system of aged rats by grape seed extract. Neuroscience letters. 2005 Aug 5;383(3):295-300.

[107] Sharififar F, Dehghn-Nudeh G, Mirtajaldini M. Major flavonoids with antioxidant activity from Teucrium polium L. Food chemistry. 2009 Feb 15;112(4):885-8.

[108] Orabi KY, Al-Qasoumi SI, El-Olemy MM, Mossa JS, Muhammad I. Dihydroagarofuran alkaloid and triterpenes from Maytenus heterophylla and Maytenus arbutifolia. Phytochemistry. 2001 Oct 1;58(3):475-80.

[109] Babu BH, Shylesh BS, Padikkala J. Antioxidant and hepatoprotective effect of Acanthus ilicifolius. Fitoterapia. 2001 Mar 1;72(3):272-7.

[110] Chakraborty D, Verma R. Ameliorative effect of Emblica officinalis aqueous extract on ochratoxin-induced lipid peroxidation in the kidney and liver of mice. Int. J. Occup. Med. Environ. Health. 2010 Jan 1;23(1):63-73.

[111] Farhan H, Malli F, Rammal H, Hijazi A, Bassal A, Ajouz N, Badran B. Phytochemical screening and antioxidant activity of Lebanese Eryngium creticum L. Asian Pacific Journal of Tropical Biomedicine. 2012 Jan 1;2(3):S1217-20.

[112] Naskar S, Mazumder UK, Pramanik G, Bala A, Haldar PK, Islam A, Gupta M. Comparative in vitro antioxidant activity of different parts of Cocos nucifera (Linn.) on reactive oxygen and nitrogen species. Int j pharm pharm sci. 2011;3(Suppl 3):104-7.

[113] Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of botany. 2012;2012(1):217037.