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Food as medicine: Assessment of antioxidant potential of methanolic leaf extracts of *Coriandrum sativum*, *Mentha arvensis* and *Leucas aspera*

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ABSTRACT

The delineation of therapeutic potentials of various herbal plants has been investigated from the past several decades due to their excellent medicinal properties, low toxicity and economic viability.Various studies account that there is an inverse relationship between antioxidative status and occurrence of human diseases such as cancer, aging, neurodegenerative disease, and atherosclerosis. As plants produce significant amount of antioxidants to prevent the oxidative stress, they represent a potential source of new compounds with antioxidant activity. It will be more advantageous, if the edible plants possess such antioxidant activities, since they provide both the nutritional and therapeutic values. Therefore, in the present study, the antioxidant properties of methanolic leaf extracts of*C. sativum, M. arvensis* and *L. aspera* was evaluated through various antioxidant assays.The results suggest that all three leaf extracts exhibited excellent radical scavenging activity, when verified through hydrogen peroxide scavenging assay, where the percentage of inhibition at 500 µg/mL for methanolic leaf extract of *C. sativum*, *M. arvensis* and *L. aspera* was 95.05±0.01 %, 82.45 ±0.15 % and 50.19 ± 0.53 % respectively. Similarly, the results of reducing power assay which is a significant reflection of the antioxidant activity displayed absorbance values of0.4032 ± 0.15 for *C. sativum*,0.4522 ±0.16 for *M. arvensis* and 0.703± 0.17 for *L. aspera* at the concentration of 500µg/mL. The results of DNA nicking assay also suggests that the plant extracts mitigate the oxidative stress on biomolecules such as DNA. In addition to that, all the three extracts were found to possess high amount of polyphenols, which might be responsible for the above mentioned antioxidant potentials. Hence the results suggest that these edible plants possess excellent antioxidant activities and may hold promising protective potentials against oxidative stress mediated diseases.

INTRODUCTION

Reactive oxygen species (ROS), which is mainly composed of hydroxyl radicals, super-oxide, nitric oxide are formed in human cells either through endogenous factors or exogenously causing extensive oxidative damage[1]. ROS are highly reactive and charged metabolic intermediates that attack DNA, RNA, protein and lipids to leave oxidized cellular components that are genotoxic or cytotoxic, and unable to perform their normal biological functions[2]. Additionally, ROS and RNS have also been analyzed as a causative agent for various diseases such as inflammation, atherosclerosis, and aging[3].Various studies suggests that oxidative stress is one of the critical causative factors in major neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis[4]. Plants are one of the most important sources of medicines. Today large numbers of drugs in use are derived from plants[5]. According to a survey (1993) of World Health Organization (WHO), the practitioners of traditional system of medicine treat about 80% of patients in India, 85% in Burma and 90% in Bangladesh (WHO survey.)Medicinal plants provides a significant source of synthetic and herbal drugs and havealso been used for the treatment or prevention of diseases since classical times. Secondary metabolites produced by plants are tremendous resources in developing new drugs and exhibit numerous biological activities like antifungal, anticancer, and antibacterial and antioxidant properties[6]. However, the past few years have seen a renewed interest in the use of natural compounds and, more importantly, their role in drug development[7]. Thus, the useof these natural antioxidants has been increased considerably, when

compared to the synthetic compounds[8].

Coriandrum sativum L. is a culinary and medicinal herb of the Apiaceae family commonly known as coriander. The roots and leaves of *C. sativum* are enhanced with aromatic flavour and are popularly used Thai and other Asian cooking. Traditionally, the plant parts are used to relieve spasms, gastric complaints, bronchitis, gout and giddiness[9]. Coriander seed is a popular spice and finely ground seed is a major ingredient of curry powder.The seeds possess medicinal uses and coriander leaves have been used as a drug for indigestion (against worms), rheumatism and pain in the joints[10]. Numerous studies reveal that it also possesses the ability to inhibit lipid peroxidation[11] and were found to have antioxidant qualities[12]. Previous studies on this herb displayed various medicinal properties, including antidiabetic, antioxidant, hypocholesterolemic, antihelmintic, antibacterial, hepatoprotective, anticancer and anxiolytic activities[13].

Mentha arvensis (Lamiaceae) is one of the large plant families used as a framework to evaluate the occurrence of some typical secondary metabolites. The genus Mentha includes 2530 species that grow in the temperate regions of Asia, Eurasia, Australia and South Africa. The mint species have a substantial importance, in terms of both medicinal and commercial values. Leaves, flowers and stems of Mentha spp. are frequently used in herbal teas to offer aroma and flavour. Furthermore, Mentha spp. has been used as a conventional method for treatment of nausea, bronchitis, flatulence, anorexia, ulcerative colitis, and liver complaints due to its anti-inflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue, and anticatharrhal activities[14].

Leucas aspera(Labiatae) is a common aromatic herb and grows abundantly in Bangladesh and also in the wide area of South Asia. *Leucas aspera* is commonly called thumbai in tamil and dandokolos in Bangladesh. Conventionally, the whole plant is taken orally for analgesic-antipyretic, anti-rheumatic, anti-inflammatory, and antibacterial treatment[15]. Detailed investigation suggests that since these plant exhibit antioxidant properties, the following study aims at evaluating the antioxidant property of methanolic extracts of *C. sativum, M. arvensis and L. aspera*.

MATERIALS AND METHODS

Preparation of crude extracts:

Extracts were prepared accordance with [1]. Fresh leaves of *C. sativum*, *M. arvensis*and *L. aspera*was purchased from local market. Leaves werewashed with alcohol, water and dried under shade. The air dried leaves were powdered and subjected to cold extraction with methanol (MeOH) at 37°C in orbital shaker for 72 h. The extracts were dried and the percentage of yield was calculated. The dried extract was dissolved in distilled water and used for further analysis. The yield of the extract was calculated as below:

$$\text{Yield of the extract} = \frac{\text{Weight of the beaker with extract} - \text{Weight of the empty beaker}}{\text{Weight of the sample in grams}} \times 100$$

Hydrogen peroxide (H_2O_2) scavenging activity:

The ability of the methanolic leaf extract of *C. sativum, M. arvensis* and *L. aspera* to scavenge $H_2O_2$was determined according to [16]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations of *C. sativum, M. arvensis* and *L. aspera* (100500 µg/mL) in distilled water were added to H_2O_2solution (0.6 mL, 40 mM). Ascorbic acid(100-500 µg/mL) was used as standard antioxidant. Absorbance of H_2O_2at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without H_2O_2.The percentage of scavenging activity of $H_2O_2$was calculated as follows:

$$\% \text{ Scavenged } [H_2O_2] = [(A_o - A_1)/A_o] \times 100$$

Where, A_o was the absorbance of the control

A_1 was the absorbance in the presence of the sample of extract and standard

Determination of total phenolics:

Total soluble phenolic compounds in methanolic leaf extracts of *C. sativum, M. arvensis* and *L. aspera*were determined with FolinCiocalteu reagent according [17]using Gallic acid as standard. 100 µL of the sample (1 g of dry sample in10 mL of acetone) in duplicates was incubated with 1 mL of diluted Folin Ciocalteu's reagent (1:2 with water) at RT for 5 min. To the reaction mixture, 1 mL of 7% $Na_2CO_3$was added and incubated at RT for 90 min and the absorbance was read at 750 nm. The total phenolic content (TPC) was expressed as gallic acid equivalent(GAE) in milligram per gram of dry sample.

Total reducing power:

Total reducing capacity of seaweeds was determined according [18].Different doses of methanolic extracts of *C. sativum, M. arvensis* and *L. aspera* (100-500 µg/mL) in 0.25 mL of distilled water were mixed with phosphate buffer (0.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (0.5 mL, 1%) and the mixture was incubated at 50°C for 20 min. Then, 0.5 mL of 10% TCA was added to the reaction mixture and centrifuged at 1000 × g for 10 min. The upper layer of solution (0.5 mL) was mixed with distilled water (0.5 mL) and $FeCl_3$(0.1 mL, 0.1%) and the absorbance was measured at 700 nm. Ascorbic acid (100500 µg/mL) was used as positive control. The higher the absorbance of the reaction mixture the greater is the reducing power.

DNA nicking assay:

DNA nicking assay was performed using super coiled pUC 18 plasmid DNA according to [19]. Plasmid DNA (0.5 µg) was added to Fenton's reagents (30 mM H_2O_2, 50µM ascorbic acid, 80µM $FeCl_3$) containing 10 µL (1 mg/mL) of methanolic extract of *C. sativum , M. arvensis* and *L. aspera* and the final volume of the mixture was brought up to 20 µL. The mixture was then incubated for 30 min at 37°C and the DNA was analyzed on a 1% agarose gel electrophoresis followed by ethidium bromide staining

RESULTS

The present study was carried out to analyze the antioxidant properties of different methanolic leaf extracts of *C. sativum , M. arvensis* and *L. aspera*.

Hydrogen peroxide scavenging activity:

The ability of methanolic leaf extract of *C. sativum*, *M. arvensis* and *L. aspera* to scavenge hydrogen peroxide was calculated as percentage inhibition which was found to be 12.39 ±0.03%, 29.99± 0.08% and 18.43±0.14% respectively at the concentration of 100 µg/mL. When the concentration was

Table 1 : Total phenolic content for methanolic leaf extracts of *C. sativum, M. arvensis* and *L. aspera*

S.NO	Sample	Concentration (µg/mL)	Total phenolic content/mg of extract (µg Gallic acid Equivalent/ mg)
1.	*Coriandrum sativum*	500	0.8476 ±0.15
2.	*Mentha arvensis*	500	0.6706 ± 0.21
3.	*Leucas aspera*	500	0.6391 ± 0.140

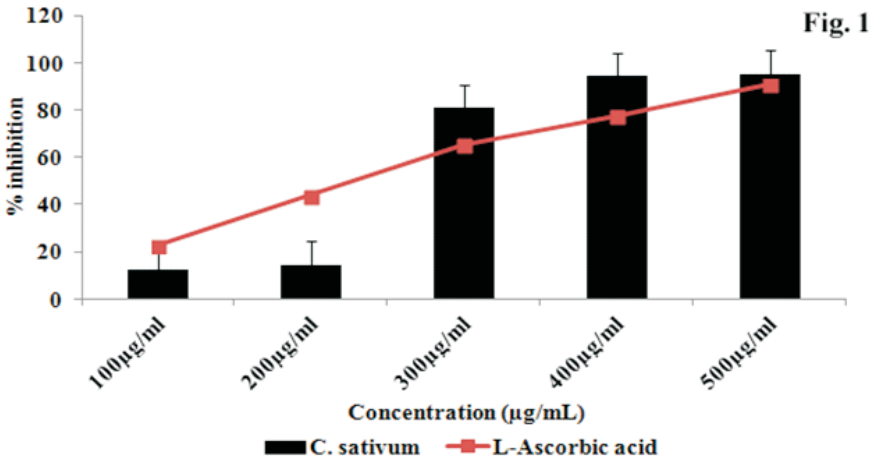

Fig. 1 : Hydrogen peroxide scavenging activities of methanolic leaf extract of *Coriandrum sativum* (100-500 µg/mL) in comparision with L- Ascorbic acid (100-500 µg/mL) . Results are expressed as mean ± SD of three parallel measurements.

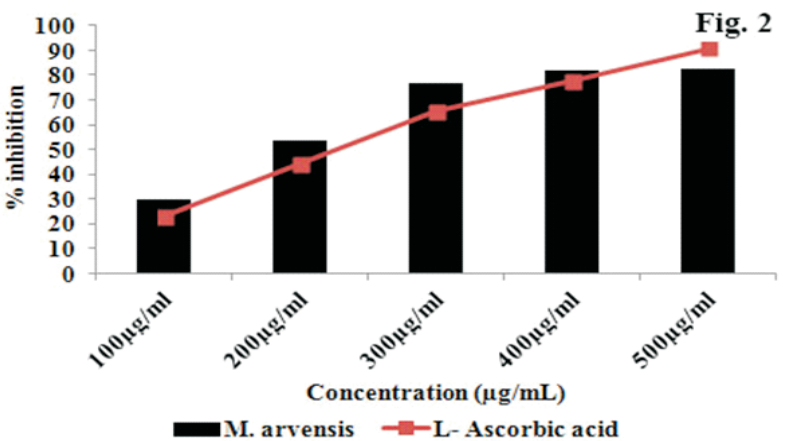

Fig. 2 : Hydrogen peroxide scavenging activities of methanolic leaf extract of *Mentha arvensis* (100-500 µg/mL) in comparision with L- Ascorbic acid (100-500 µg/mL). Results are expressed as mean ± SD of three parallel measurements.

increased gradually from 100 µg/mL to 500 µg/mL, the percentage inhibition of H_2O_2 also escalated to 95.05±0.01%, 82.45 ±0.15% and 50.19 ± 0.53% for *C. sativum, M. arvensis* and *L. aspera* respectively (Figure 1-3). At 500 µg/mL,the % inhibition by L-ascorbic acid (standard) was 90.83± 0.03%.

Total phenolic content:

The TPC for the different extracts was measured spectrophotometrically by FolinCiocalteau method and the results are expressed as µg of gallic acid equivalents (GAE)/mg of dry extract. TPC for 500 µg/mL of *C. sativum, M. arvensis* and *L. aspera* was found to be 0.8476 ±0.15, 0.6706 ± 0.21, 0.6391 ± 0.140 µg/mg of dry extract respectively. From the table given (Table 1), it is observed that all the methanolic leaf extracts exhibited the presence of phenolic content at the concentration of 500 µg/mL.

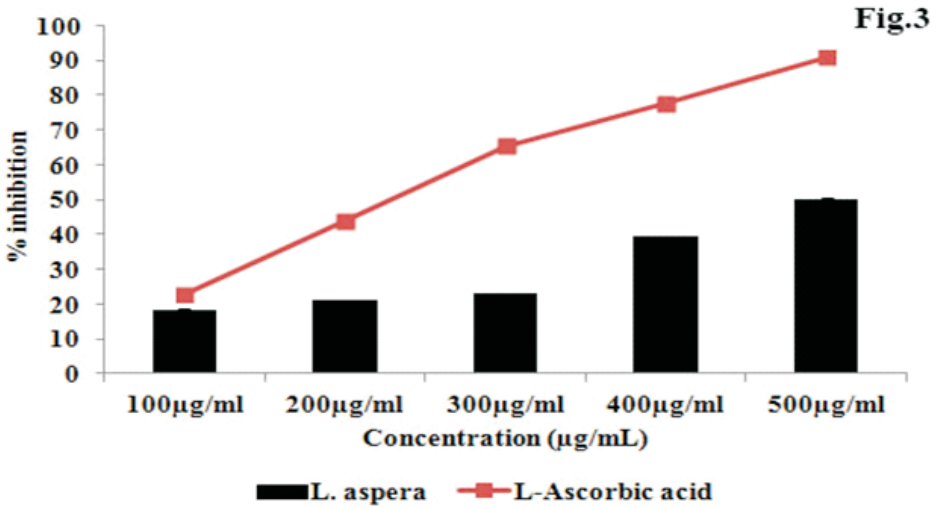

Fig. 3 : Hydrogen peroxide scavenging activities of methanolic extract of *Leucas aspera* (100-500 µg/mL) in comparision with L- Ascorbic acid (100-500 µg/mL) .Results are expressed as mean ± SD of three parallel measurements.

Total reducing power:

The reducing power of methanolic leaf extracts of three different plants *C. sativum, M. arvensis* and *L. aspera* (100 µg/mL-500 µg/mL) was analysed and compared with the standard L-ascorbic acid. Results suggested that at the concentration of 100 µg/mL, the absorbance at 700 nm was found to be 0.0281 ± 0.005 for *C. sativum*, 0.038 ± 0.016 for *M. arvensis*, 0.026 ± 0.016 for *L .aspera* . As the concentration was increased to 500 µg/mL the absorbance recorded at 700 nm was 0.4032 ± 0.15 for *C. sativum* , 0.4522 ± 0.16 for *M. arvensis* and 0.703 ± 0.17 for *L. aspera.* At the concentration of 500 µg/mL of L-ascorbic acid, the absorbance at 700nm was recorded to be 0.728 ±0.16 (Figure 4-6).

DNA nicking assay:

To analyse the scavenging effect of methanolic leaf extracts of *C. sativum*, *M. arvensis* and *L. aspera* on Fe^{3+}dependent hydroxyl radicals, the ability of the extract to reduce Fe^{3+}dependent DNA nicking was investigated. As seen in the figure 7, when pUC18 plasmid DNA was exposed to Fenton's reaction mixture, a loss of supercoiled native DNA (Form I) and generation of Form III (linear plasmid DNA) and Form II (single-stranded, nicked circular plasmid DNA) was observed in lane 2. Shielding against hydroxyl radical-mediated DNA nicking was observed towards the methanolic leaf extracts of C. *sativum, M. arvensis* and *L. aspera* (lane 3-5). All the three methanolic extracts showed protection against hydroxyl radicals.

DISCUSSION

Hydrogen peroxide scavenging activity:

Hydrogen peroxide is the most stable form of ROS. H_2O_2itself is not highly reactive but it has the ability to react with superoxide radicals to form more toxic hydroxyl radicals in the presence of transition metals [20] and it is also capable of rapid diffusion

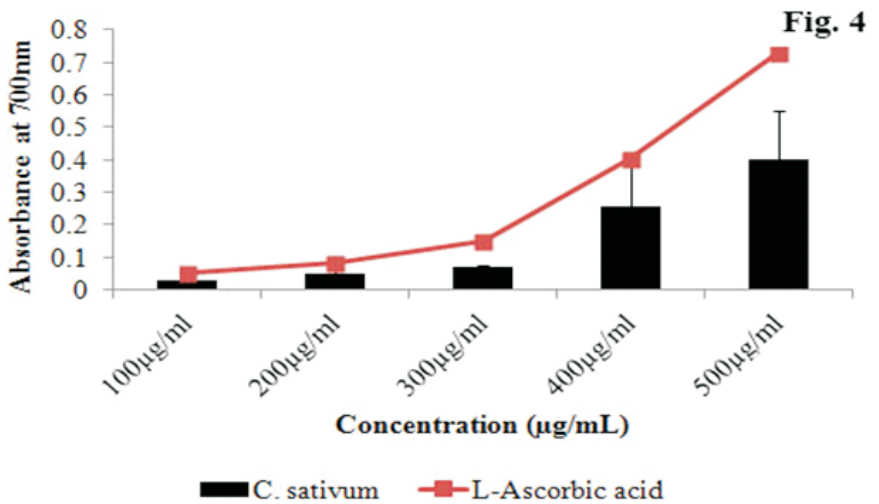

Fig. 4 : Reducing power of methanolic leaf extract of Coriandrum sativum(100-500 µg/mL) in comparison with standard l-Ascorbic acid . Results are expressed as mean ± SD of three parallel measurements.

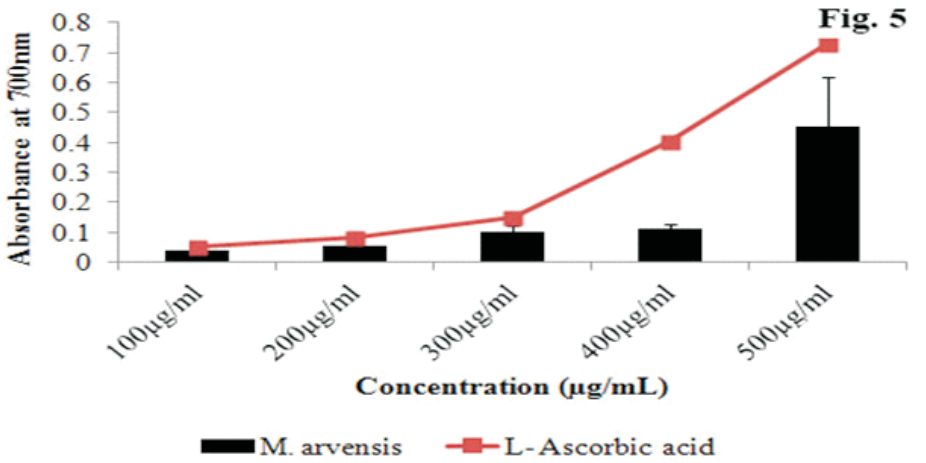

Fig. 5 : Reducing power of methanolic leaf of *M. arvensis* (100-500 µg/mL) in comparison with standard L-Ascorbic acid (100- 500 µg/mL). Results are expressed as mean ± SD of three parallel measurements.

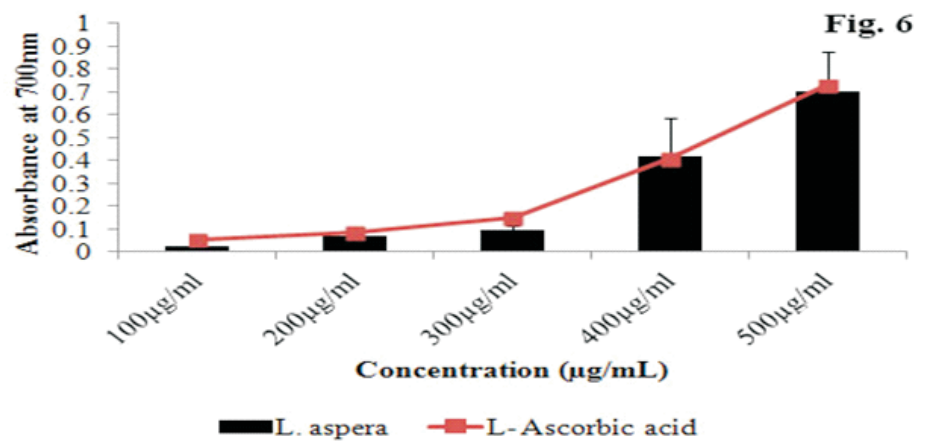

Fig. 6 : Reducing power of methanolic leaf extract of *Leucas aspera* (100-500 µg/mL) in comparison with standard L-Ascorbic acid (100- 500 µg/mL). Results are expressed as mean ± SD of three parallel measurements.

across cell membrane [21].Hence, the presence of hydrogen peroxide acts as a signal of oxidative stress and induces an antioxidative response by altering the redox level in the surrounding environment. The scavenging ability of different methanolic leaf extracts of *C. sativum, M. arvensis* and *L. aspera* on hydrogen peroxide was analysed and was compared with the standard L-ascorbic acid. The graph indicates the respective increase in % inhibition of hydrogen peroxide in accordance with the concentration of the methanolic extracts of the plants. When comparing the scavenging ability of methanolic leaf extracts of all the three plants with L-ascorbic acid (standard), *C. sativum* showed significantly higher % inhibition at 500 µg/mL, higher than that of the standard at the same dosage whereas, *M. arvensis* displayed higher % inhibition but not stronger than L- ascorbic acid at 500 µg/mL[22 &23]. *L. aspera* showed least % inhibition.The result suggests that methanolic leaf extract of *C. sativum* and *M. arvensis* showed higher hydrogen peroxide scavenging potential thereby acting as a better antioxidant. This can be related to presence of high phenolic content which acts as an electron donor thereby quenching the hydrogen peroxide.

Total phenolic content:

Groups of secondary plant metabolites (antioxidant phenolics), which are commonly found in various fruits, vegetables and herbs have shown to provide an effective defence against oxidative stress from oxidizing agents and free radicals[24]. Phenolic compounds have redox properties, which allow them to act as antioxidants. As their free radical scavenging ability is facilitated by their hydroxyl groups, the total phenolic content could be used as a basis for rapid screening of antioxidant activity[25]. *C. sativum* displayed highest TPC followed by *M. arvensis* and *L. aspera* [23& 26].Presence of high polyphenol content increases the antioxidant activity. Hence, from the table it is clear that the methanolic extract of *C. sativum* acts as a better antioxidant because of the high phenol content.

Total reducing power:

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates and can act as

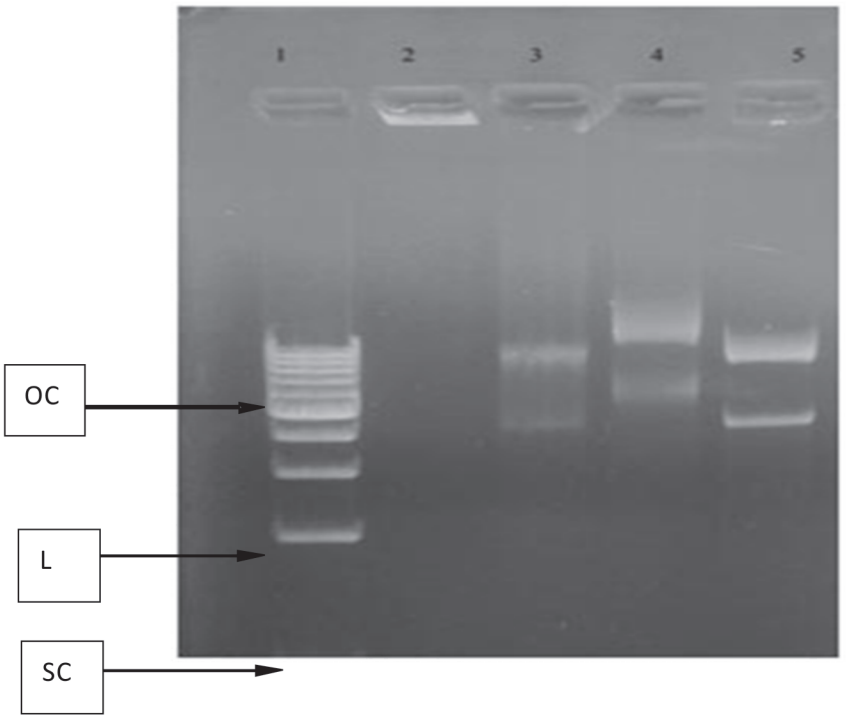

Fig. 5 : Inhibitory effects of methanolic leaf extracts of *C. sativum*, *M. arvensis* and *L. aspera* on DNA nicking caused by hydroxyl radicals. The DNA nicking reaction was initiated by adding 0.5 µg of pUC18 plasmid DNA to Fenton's reaction solution in the absence (Lane 2) or presence of different methanolic leaf extracts of *C. sativum* (1 mg/mL) for 30 min at 37◦C (lane 3), *M. arvensis* (Lane 4) and *L. aspera* (Lane 5) . Lanes 1 shows the DNA molecular marker.

primary and secondary antioxidants [27] The transformation of Fe^{3+} into Fe^{2+} in the presence of various fractions was measured to determine the reducing power ability. Increased absorbance of the reaction mixture indicated the increase in reducing power. From the above graphs, it can be concluded that the reducing power of methanolic leaf extract of *C. sativum, M. arvensis, L. aspera* increases in a concentration dependent manner. *L. aspera* showed significantly higher reducing power ability but less compared to the standard (L- ascorbic acid).*C. sativum* and *M. arvensis* showed moderate reducing power capabilities at 500 µg/mL but not stronger than the standard [28 &26]. The antioxidant properties present in the methanolic leaf extract of all three plants caused the reduction of Fe^{3+} ferricyanide complex to the ferrous form thereby demonstrating its antioxidant ability. Results indicate that *L. aspera* exhibited better reducing power thereby rendering protection against free radicals.

DNA nicking assay:

Reactive nitrogen species (RNS) and Reactive oxygen species (ROS) are implicated in DNA damage causing neurodegenerative diseases. Among the various existing *in vitro* DNA nicking assays, the DNA nicking assay based on the Fenton reaction mimics the *in vivo* biological situation, with the production of hydroxyl free radicals from endogenous entities like intracellular iron [29] Results from Figure 7 depicts that the pUC 18 plasmid DNA when exposed to Fenton's reagent, loses its native supercoiled DNA (Lane 2). The methanolic leaf extract of *C. sativum, M. arvensis* and *L. aspera* protected the Form II of the pUC 18 DNA thereby preventing it from complete digestion (Lane 3-5). Hence, from the above result, it can be concluded that the methanolic extract of all three plants acts as an effective scavenger for hydroxyl radicals. These results indicate that these methanolic extract diminishes the oxidative stress on susceptible biomolecules, such as DNA.

CONCLUSION

The results from this study clearly indicate that the methanolic leaf extracts of *C. sativum*, *M. arvensis* and *L. aspera* have powerful antioxidant qualities against hydrogen peroxide. The most probable reason for their potential as free radical scavengers might be associated with their phenol content. The methanolic extracts also displayed protection against hydroxyl radical-mediated DNA nicking. The findings of this research work could be further used to identify, isolate and characterize bioactive compounds which are responsible for the antioxidant abilities of these plant extracts. These compounds may serve as a major breakthrough in drug development for treating various diseases related to oxidative stress.

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DECLARATION OF INTERESTS

The authors declare that there is no conflict of interest

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