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Original Article

FREE RADICAL SCAVENGING INDEX OF *CUCURBITA MAXIMA* SEEDS AND THEIR LIBS BASED ANTIOXIDANT ELEMENTAL PROFILE

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ABSTRACT

Objective: To assess the free radical scavenging index and antioxidant capacity of *C. maxima* (*Cucurbita maxima*) seed extract *in vitro* and its correlation with the phytoelemental profile, quantitatively assessed by LIBS (Laser-Induced Breakdown Spectroscopy).

Methods: A number of *in vitro* antioxidant and free radical scavenging assays were performed along with standard as a reference. Reduction capacity and scavenging potential of *C. maxima* seeds were analyzed in addition to their IC_{50} values and were compared with respective standards. Quantitative measurements of phytoelements present which is responsible for antioxidant activity is carried out by LIBS.

Results: Significant Antioxidant power of the extract was validated by high values of Total Phenolics (18.42 mg/g of gallic acid equivalent) and Total Flavonoids (5.53 mg/g of quercetin equivalent). A result of Ferric Reducing Antioxidant Power (163.4 µM Fe^{+2}/g) clearly reflects its reduction capacity. Percentage inhibition of 2,2-diphenyl-1-picrylhydrazyl (69.25), Nitric Oxide (63.63) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (62.09) radicals proves its antioxidant potential undoubtedly, which was further confirmed by their IC_{50} values. Reducing power was found to increase significantly in a dose-dependent manner. Extent lipid peroxidation was also measured by comparing the results of Ferric thiocyanate assay with the Thiobarbituric acid method. LIBS analysis showed the presence of certain phytoelements viz. Mg, Ca and Na in the extract, which could be responsible for its antioxidant capacity.

Conclusion: Thus, the seeds of *C. maxima* having a combination of antioxidant phytochemicals and phytoelements could be explored for developing as a complementary and alternative medicine for managing oxidative stress including diabetes as well as aging.

Keywords: Reactive oxygen species, *Cucurbita maxima*, Elemental analysis, Laser spectroscopy

INTRODUCTION

Natural antioxidants have recently received much attention as they have been found to be useful not only in controlling several diseases caused by ROS (Reactive Oxygen Species) but also in managing the oxidative stress caused by several diseases like diabetes, etc. [1]. A number of plants have significant antioxidant activity due to the presence of certain Natural Products responsible for scavenging the excess free radicals from the system [2]. Such natural antioxidants, either in the form of crude extracts or their pure chemical constituents are extremely valuable for controlling oxidative stress caused by ROS [3]. The presence of set certain elements have also been stated to play a pivotal role in the development of active chemical constituents in medicinal plants . Therefore, it is important to identify the elemental profile of medicinal plants in order to correlate their biological activities with the presence of specific phytochemicals constituents.

LIBS (Laser-Induced Breakdown Spectroscopy) are a real-time, versatile, sensitive and *in-situ* elemental analytic technique for qualitative and quantitative analysis of elements, even in traces, of any material, without prior sample preparation. The ability of LIBS to interrogate solid samples has also been demonstrated for the direct elemental analysis of plant materials without any chemical contaminations [4]. These elements even in traces help in controlling the metabolic process of the biological system.

The plant, *Cucurbita maxima* (family: Cucurbitaceae) commonly known as pumpkin is a large annual or perennial climbing herb. It is used as a vegetable and also in the traditional system of medicine as antidiabetic, antitumor, antihypertensive, antiinflammatory, immunomodulatory, and antibacterial [5]. It is rich in polysaccharides, contains high amounts of amino acids, fatty acids, carotenoids, minerals and vitamin E [6]. Since drug dissolution has been recognized as an important element for enhancing drug bioavailability in the system, therefore, choice of solvent for their better solubility is an important challenge to pharmaceutical scientists. As herbal medicines are gaining importance these days, therefore, water which has been globally accepted as a 'universal solvent', should be preferred for preparing plant extracts. Moreover, a plant extract prepared in an aqueous medium is far effective and safer due to its enhanced bioavailability, non-toxic nature, and greater diffusion ability in the targeted cells. Since non-aqueous extracts of its seeds had already been evaluated for their bioactivities [7], therefore, its aqueous extract was selected for the first time for assessing its antioxidant profile *in vitro* in order to develop a better oral antioxidant agent, with improved efficacy and no toxicity at all, for managing oxidative stress. The most important aspect, which has been explored for the first time through this study, and has added value to the present study is the correlation between antioxidant profile and phytoelemental profile of *C. maxima* seeds. Hence, the novelty and rationality of the present study cannot be denied and ignored.

Thus, the present study was carried out to assess the free radical scavenging index and antioxidant potential of *C. maxima* aqueous seed extract *in vitro*. The identified efficacy was correlated with the phytoelemental profile of the extract as evident from the LIBS spectra. This is the first reporting of the role of elements involved in antioxidant efficacy of the aqueous extract of *C. maxima* seeds.

MATERIALS AND METHODS

Material

The seeds of *C. maxima* plant were procured from the local market of Allahabad, India and authenticated by Prof. Satya Narayan,

Taxonomist, Department of Botany, University of Allahabad, Allahabad, India. A voucher specimen has been submitted to the University herbarium (No. MRL/CM/01). The seeds were washed well with water and dried in the shade. The shade dried seeds were powdered and extracted with hot distilled water. The extract obtained was filtered, concentrated and lyophilized till constant weight. The dry powder so obtained of CMSE (*C. maxima* seed extract) was stored at-40 °C for further use.

Chemicals

The chemicals, as follows, were purchased from Merck Chemicals, New Delhi, India**:** Gallic acid, Folin-Ciocalteu's phenol reagent, Na_2CO_3, Quercetin, $AlCl_3$, Ascorbic acid, $K_3[Fe(CN)_6]$, TCA (trichloroacetic acid), $FeCl_3$, Na_2HPO_4, NaH_2PO_4, TPTZ (2, 4, 6-tripyridyl-s-triazine), HCl, $FeSO_4$, linoleic acid, NH_4SCN (ammonium thiocyanate), $FeCl_2$, BHT (butylated hydroxytoluene), C_2H_5OH, TBA (thiobarbituric acid), FTC (ferric thiocyanate), DPPH (1,1-diphenyl-2-picrylhydrazyl), SNP (sodium nitroprusside), sulphanilamide, phosphoric acid, NED [N-(1-naphthyl) ethylenediamine dihydrochloride], KCl, NaCl, KH_2PO_4, ABTS (2,2'-azino-bis (3-ethyl-benzthiazoline-6-sulfonic acid), $K_2S_2O_8$ and CH_3OH. All these chemicals and solvents used were of analytical grade.

Evaluation of antioxidant potential *in vitro*

Estimation of total phenolic contents

Total phenolic contents were determined by the method of Wolfe et al. [8], using Folin-Ciocalteu reagent. Na_2CO_3 (2 %) was added to the different concentrations (5-25 µg) of the extract. After 2 min, Folin-Ciocalteu reagent (1:1) was added, and the mixture was allowed to stand in the dark at room temperature. The absorbance was measured at 750 nm after half an hour. The assay was carried out in triplicate. A standard curve was made using Gallic acid as a reference. The total phenolic contents were expressed as mg GAE (Gallic acid equivalent) per g of dried sample.

Estimation of total flavonoid contents

Total flavonoid contents were determined by the method of Ordonez et al. [9]. Ethanolic $AlCl_3$ (2 %) was added to the different concentrations (5-25 µg) of the extract. and the mixture was kept at room temperature. The absorbance was measured at 420 nm after one hour. The appearance of yellow colour indicated the presence of flavonoids. The assay was carried out in triplicate. A standard curve was made using Quercetin as a reference. The total flavonoid contents were expressed as mg QE (Quercetin equivalent) per g of dried sample.

Reducing power estimation

Reducing power was determined by the method of Oyaizu [10]. Different concentrations (50-200 µg) of the extract, were mixed with 0.2 M phosphate buffer (pH 6.6) and 1 % K_3 $[Fe(CN)_6]$ and was incubated at 50 °C for 20 min. TCA (10 %) was added to the mixture and centrifuged for 10 min. The upper layer of the solution was mixed with distilled water and $FeCl_3$ (0.1 %) followed by measurement of absorbance at 700 nm. The assay was carried out in triplicate. Ascorbic Acid was used as a reference.

FRAP (Ferric reducing antioxidant power) assay

FRAP Assay was determined by the method of Benzie and Strain [11]. Different concentrations (50-200 µg) of the extract, were mixed with the FRAP reagent which was prepared by mixing acetate buffer (25 ml, 300 mM/l, pH 3.6), 10 mM/l TPTZ solution in 40 mM/l HCl and 20 mM/l $FeCl_3$ solution (2.5 ml) in proportions of 10:1:1 (v/v), respectively. The absorbance of the reaction mixture was then recorded at 593 nm after 4 min. The assay was carried out in triplicate. The standard curve was constructed using $FeSO_4$ solution (0.5-10 mg/ml). The results were expressed as µM Fe^{+2}/g dry weight of the extract. Ascorbic Acid was used as a reference.

FTC and TBA assays

FTC and TBA Assays were determined by Kikuzaki and Nakatani [12].

FTC assay

This method is used for evaluating the amount of peroxides formed before lipid peroxidation. A mixture, containing 4 mg of extract in 4 ml of 99.5 % ethanol, 4.1 ml of 2.51 % linoleic acid in 99.5 % ethanol, 8 ml of 0.05 M phosphate buffer (pH 7.0) and 3.9 ml of distilled water was placed in a vial with screw cap and then placed in an incubator at 40 °C in the dark. To 0.1 ml of this mixture 9.7 ml of 75 % ethanol and 0.1 ml of 30 % NH_4SCN were added. Precisely after 3 min 0.1 ml of 0.002 M ferrous chloride in 3.5 % HCl was added to the reaction mixture. The absorbance of red colour so obtained was measured at 500 nm for every 24 h until the absorbance of the control reached the maximum. The assay was carried out in triplicate. BHT was used as a reference.

TBA assay

This method is used for evaluating the extent of lipid peroxidation. At low pH and high temperature, malonaldehyde binds TBA to form a red complex. To 1 ml of the above solution, 2 ml of 20 % TCA and TBA solutions were added and placed in a boiling water bath for 10 min. Absorbance was measured at 532 nm. Antioxidant activity obtained was based on absorbance on the final day. The assay was carried out in triplicate.

Assessment of free radical scavenging index

DPPH radical scavenging assay

Scavenging of DPPH• was determined by the method of Liyana-Pathiranan & Shahidi [13]. 0.1 mM DPPH solution was mixed with different concentrations of the methanolic extract and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured at 517 nm. The assay was carried out in triplicate. Ascorbic Acid was used as a reference. The ability to scavenge DPPH• was calculated by the following formula:

$$\text{Scavenging rate} = [(Ac–As)/Ac] \times 100$$

Where, as ***Ac*** is absorbance of control, and ***As*** is the absorbance of the sample.

NO• (nitrosyl radical) scavenging assay

Scavenging of NO• was determined by the method of Green *et al* [14]. 2 ml SNP solution was mixed with different concentrations of the extract at 25 °C. After 2.5 h, 0.5 ml of the above solution was mixed with 1 ml of sulphanilamide. Then after 5 min, 1 ml of NED was added. The absorbance of the solution was measured at 540 nm after 30 min. The assay was carried out in triplicate. The level of NO• scavenging was calculated using the above-mentioned formula for DPPH•.

ABTS•+(ABTS radical) scavenging assay

Scavenging of ABTS•+ was determined by the method of Re *et al.* [15]. The ABTS•+ was pre-generated by mixing 7 mM ABTS solution and 2.45 mM potassium persulfate. The solution was incubated for 12 h in the dark. 1 ml of above solution was added in 1 ml of different concentrations of extract and absorbance was measured at 734 nm. The assay was carried out in triplicate. The ability to scavenge ABTS•+was calculated using the above-mentioned formula for DPPH•.

Experimental setup for LIBS

In order to identify the presence of the best set of elements responsible for the antioxidant efficacy of an aqueous extract of *C. maxima* seeds, LIBS spectra were recorded using laser wavelength of 532 nm with pulse duration of 4 nanoseconds. The sample was focused with a pulsed laser beam from a Q-switched Nd: YAG (Neodymium**:** Yttrium-Aluminum-Garnet) laser (Continuum Sure-Lite III-10) using a Quartz converging lens of 30 cm focal length. Consequently, the temperature of the locally heated region rose rapidly which led to plasma formation on the sample surface. The light emitted from micro-plasma was collected using an optical fiber tip placed in the vertical plane at 45 ° with respect to the laser beam and finally fed into an entrance slit of the spectrometer (Ocean Optics LIBS2000+) equipped with CCD (charge-coupled device) and 4 gratings. The initial three gratings had the resolution of 0.1 nm covering the wavelength range from 200–310 nm, 310–400 nm and 400–510 nm, respectively, while the fourth grating, called as broadband grating, covered the wavelength range from 200–1100 nm and had a resolution of 0.75 nm. For recording the LIBS spectra

at 1 Hz laser frequency and 100mJ laser energy, all the four gratings were used simultaneously. The sample solution was prepared by dissolving 1.0 g of lyophilized material in 10 ml of distilled water. Several experiments were carried out by varying the experimental parameters such as laser power, lens to sample distance, the position of collection emission optics with respect to plasma plume to get best S/N (Signal to Noise) and S/B (Signal to Base) ratio. In order to get better S/N ratio we have taken the average spectra, whereas for enhanced S/B ratio, continuum background was reduced. The optimum results were found at laser energy 100mJ; a lens to sample distance 30 cm, tip of fiber bundle at 45° with respect to the laser beam. Each LIBS spectrum is average of 100 laser shots.

Statistical analysis

The entire group of data was statistically evaluated using one-way ANOVA, followed by a post hoc Scheffe's test using the SPSS computer software, version 7.5. The values were considered significant when $P<0.05$. Experiments were done in triplicate, and the mean value was reported as mean ± S.D.

RESULTS

Estimation of total phenolic contents

Since, polyphenols are natural antioxidants responsible for the antioxidant activity of plants; therefore the obtained amount of total polyphenols present in the extract indicates the extent of its antioxidant activity [16]. Total phenolic contents of CMSE was found to be 18.42±0.02 mg/g of GAE with reference curve (Y = 0.009X, R^2 = 0.995) as shown in fig. 1.

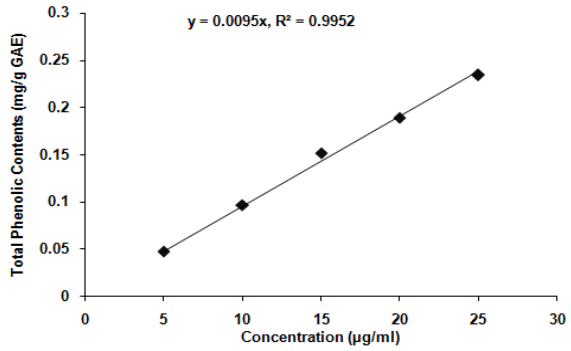

Fig. 1: Total phenolic contents of *Cucurbita maxima* aqueous seed extract

All values are expressed as mean±SD, n=3

Error bars have been omitted for simple presentation

Estimation of total flavonoid contents

As antioxidants, flavonoids have been reported to be able to interfere with the biochemical pathway involved in the generation of ROS, quenching free radicals, chelating transition metals and rendering them redox inactive in the Fenton reaction [16]. Total flavonoid contents of CMSE was found to be 5.53±0.03 mg/g of QE with reference curve (Y = 0.041X, R^2 = 0.994) as shown in fig. 2.

Error bars have been omitted for simple presentation

Reducing power estimation

The reducing properties are generally associated with the presence of reductions [17], which have been shown to exert antioxidant action by backing the free radical chain by donating a hydrogen atom. Reductions are also reported to react with certain precursors of peroxides, thus preventing peroxide formation [18]. The dose response curve for the reducing power of CMSE and reference, Ascorbic Acid (AA) is shown in fig. 3. It was found that the reducing powers of both the samples increased with each concentration. The highest reducing power was attained with the concentration of 200µg/ml of both the samples. However, AA exhibited a significantly higher reducing power than CMSE.

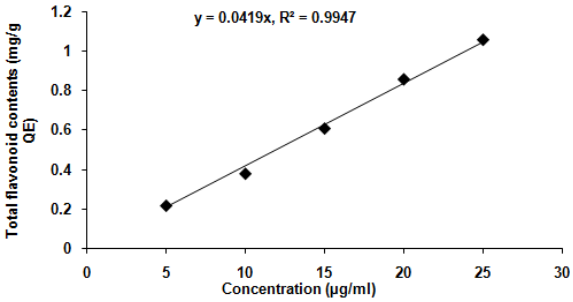

Fig. 2: Total flavonoid contents of *Cucurbita maxima* aqueous seed extract

All values are expressed as mean±SD, n=3

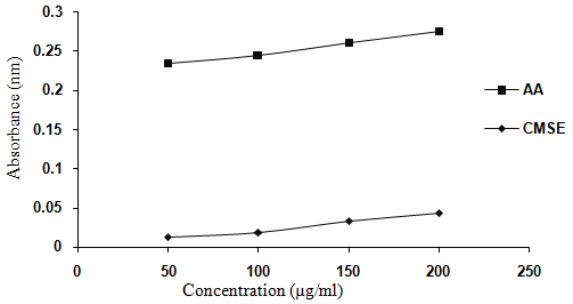

Fig. 3: Reducing power of *Cucurbita maxima* seed extract and Ascorbic Acid

All values are expressed as mean±SD, n=3

Error bars have been omitted for simple presentation

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay is widely used in the evaluation of antioxidant components in dietary polyphenols. The antioxidant activity increases proportionally to polyphenol contents and according to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in numerous plant species [19]. The ability of CMSE and the reference, AA to reduce ferric ions was determined by FRAP assay. The change in absorbance at 593 nm owing to the formation of a blue coloured Fe^{+2} tripyridyltriiazine (TPTZ) compound from the colourless oxidized Fe^{+3} forms was by the action of electron-donating antioxidants [20]. The FRAP value of CMSE was found to be significantly high (163.4±0.28 µM Fe^{+2}/g of extract). Since FRAP assay is easily reproducible and linearly related to the molar concentration of the antioxidant present. Thus, it can be reported that CMSE may act as a potent free radical scavenger, capable of transforming reactive free radical species into stable non-radical products. The antioxidant potential of CMSE was estimated from its ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) at 593 nm and its antioxidant activity increased proportionally with the polyphenol content.

Ferric thiocyanate (FTC) assay

FTC method was used to measure the amount of peroxide formed at the primary stage of linoleic acid peroxidation. The peroxide reacts with ferrous chloride to form a reddish ferric chloride pigment [21]. In this assay, the concentration of peroxide decreases as the antioxidant activity increases. The control showed an increase in absorbance values gradually and reached a maximum and then dropped. This reduction is due to the increased level of malonaldehyde compounds from linoleic acid oxidation, which is not stable [22]. Higher absorbance values indicate a lower level of

antioxidant activity of the sample. Control was found to exhibit increasing absorbance values from 0th day until the absorbance reached the maximal level on 8th day after which, the absorbance value dropped on 9th day, as shown in fig. 4. The absorbance of CMSE and reference, AA was found to be 0.241 and 0.216, respectively on the 8th day. These absorbance values of CMSE and AA were found to be comparable thereby, exhibiting comparable antioxidant efficacy.

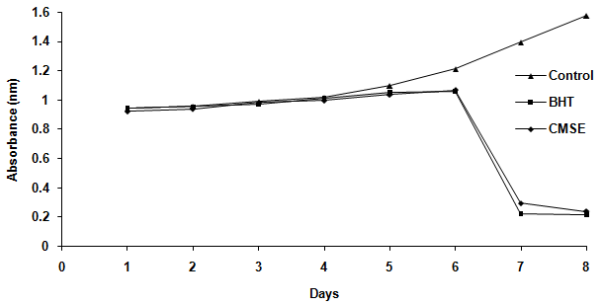

Fig. 4: FTC Assay of *Cucurbia maxima* seed extract and Butylated Hydroxytoluene

All values are expressed as mean±SD, n=3

Error bars have been omitted for simple presentation

Thiobarbituric acid (TBA) assay

TBA assay is used to measure the secondary product of oxidation such as aldehyde and ketone [23]. The TBA analysis of CMSE on the 8th day is shown in fig. 5. The absorbance values of CMSE and reference, BHT were found to be 0.419 and 0.081, respectively on the 8th day. However, the absorbance value of control was found to be 0.703, which was much higher than CMSE and BHT. The TBA results were in accordance with the obtained FTC results. CMSE thus showed low absorbance value, which indicated a high level of antioxidant activity.

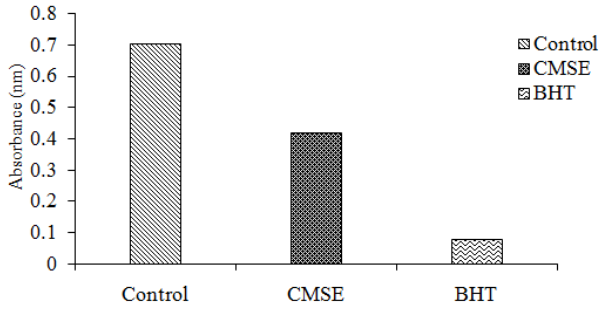

Fig. 5: TBA Assay of *Cucurbita maxima* seed extract and Butylated Hydroxytoluene

All values are expressed as mean±SD, n=3

Error bars have been omitted for simple presentation

DPPH radical scavenging assay

DPPH• scavenging assay, considered as one of the standard and easy colorimetric methods [13], is consistently used to assess the free radical scavenging potentials of antioxidant molecules. CMSE was assessed for its DPPH• scavenging activity, with AA taken as a reference. CMSE was found to exhibit a relatively lower inhibition of 39.7 % in comparison to the reference, AA showed higher inhibition of 59.13 % at a concentration of 50 µg/ml, as illustrated in fig. 6. Antioxidant activity of both the samples was found to increase in a concentration-dependent manner wherein, inhibition of 69.25±0.27 and 92.01±0.58 % was found in the case of CMSE and AA, respectively at the highest evaluated concentration of 50 µg/ml. Moreover, the IC_{50} value for CMSE was found to be 108.48±0.35. Whereas, the reference, AA gave a much lower IC_{50} value of 9.23±0.05.

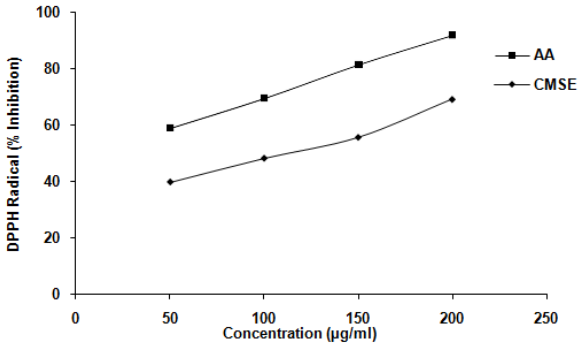

Fig. 6: DPPH radical scavenging assay of *Cucurbita maxima* seed extract and Ascorbic Acid
All values are expressed as mean±SD, n=3

Error bars have been omitted for simple presentation

NO radical scavenging assay

NO• is an effective pleiotropic mediator of physiological processes such as neuronal signalling, inhibition of platelet aggregation, smooth muscle relaxation and regulation of cell-mediated toxicity [24]. CMSE was assessed for its NO• scavenging activity, with AA taken as a reference. CMSE was found to exhibit a relatively lower inhibition of 23.23 % in comparison to the reference, AA showing higher inhibition of 48.48 % at a concentration of 10 µg/ml, as illustrated in fig. 7. Antioxidant activity of both the samples was found to increase in a concentration-dependent manner wherein, almost comparable inhibition of 54.54±0.42 and 63.63±0.31 % was found in the case of CMSE and AA, respectively at the highest evaluated concentration of 50 µg/ml. Moreover, a significantly low IC_{50} value of 43.18±0.19 was found in the case of CMSE. Whereas, the reference, AA gave an even lower IC_{50} value of 12.94±0.47.

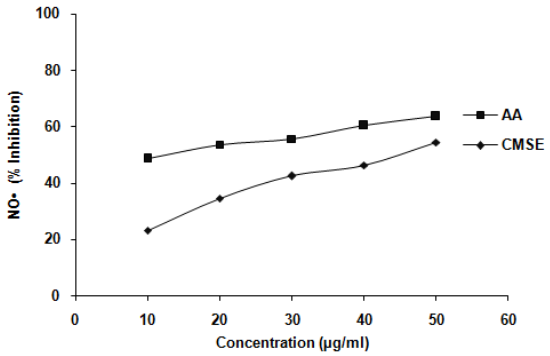

Fig. 7: NO radical scavenging assay of *Cucurbita maxima* seed extract and Ascorbic Acid
All values are expressed as mean±SD, n=3

Error bars have been omitted for simple presentation

ABTS radical scavenging assay

ABTS•+ is a well-known reactive radical that can lead to cell damage. In this assay, ABTS•+ oxidizes to ABTS•+ chromophore on reaction with potassium per sulphate and reduced by the antioxidant sample. Results justify that CMSE has ABTS•+ scavenging activity and proved that plants may be used for the treatment of ABTS•+ related with oxidative stress due to their ABTS•+ quenching ability [3]. CMSE was assessed for its ABTS•+ scavenging activity, with AA taken as a reference. CMSE and the reference, AA was found to exhibit a

comparable inhibition of 17.07 % and 19.06 %, respectively at a concentration of 25 µg/ml, as illustrated in fig. 8. Antioxidant activity of both the samples was found to increase in a concentration-dependent manner wherein, noteworthy inhibition of 62.09±0.58 and 94.13±0.06 % was found in the case of CMSE and AA, respectively at the highest evaluated concentration of 125 µg/ml. Moreover, IC_{50} value of 92.97±0.51 was found in the case of CMSE. Whereas, the reference, AA gave a lower IC_{50} value of 65.73±0.18.

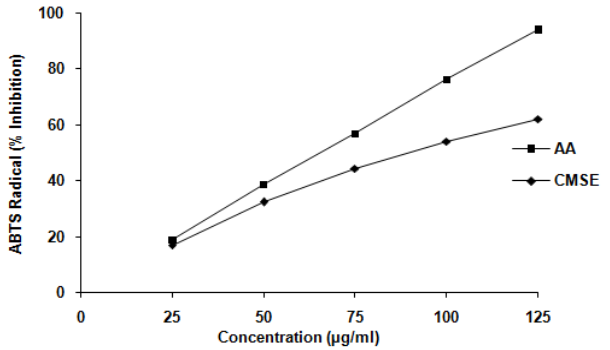

Fig. 8: ABTS radical scavenging assay of *Cucurbita maxima* seed extract and Ascorbic Acid
All values are expressed as mean±SD, n=3

Error bars have been omitted for simple presentation

Analysis of trace mineral elements responsible for *in vitro* antioxidant potential of *C. maxima*

LIBS spectra of the aqueous extract (Figs. 9a and 9b) reveal that it comprises of essential elements like Mg, Ca and Na in the spectral ranges of 200–500 nm and 600–850 nm besides C, P, O, H and N. Minerals are involved in structural components of human tissues, resources of acid-base balance and maintain the body fluids, transport of gases and muscle contractions [25]. Calcium is an important element for bones, nail, hair, teeth development and nerve, muscle and heart functions. It activates prothrombin to thrombin, blood coagulation, enzyme activation, membrane permeability, muscle contraction, normal transmission of nerve impulses and muscles by regulating endo-exoenzymes and blood pressure [26-27]. Calcium deficiency causes rickets, back pain, osteoporosis, indigestion, irritability, premenstrual tension and cramping of the uterus [28]. Magnesium is an essential element for activation of many enzymes (cofactors) such as phosphate-transferring enzyme myokinase, diphophopyridinenucleotide kinase and creatine kinase, act as a cofactor for thymine pyrophosphate [26]. Magnesium prevents heart diseases, the requirement in plasma, extracellular fluid, maintaining osmotic equilibrium, nucleotide participation, etc [29]. Sodium plays a primary source for extracellular fluids, maintains acid-base balance, osmotic pressure and plasma volume. It prevents irritability of muscles, cell permeability, nerves activation, muscle function, membrane potential maintenance and nerve pulse transmission, absorption process for monosaccharides, amino acids, pyrimidines and bile salts [26, 30]. Na is diuretic in nature and plays an important role in the transport of metabolites. The Na/K ratio for food is an important factor in the prevention of hypertension arteriosclerosis, where Na enhances, and K depresses blood pressure [31].

To ascertain the concentration of Mg, Ca and Na in CMSE, we have evaluated the relative concentration of these essential elements by measuring the intensity of the selected lines in triplicate from the LIBS spectra of the sample. The relative intensity ratios of different constituent elements of the sample (CMSE) are tabulated in Tables 1a and 1b. Table 1a shows the relative intensities of elements with respect to C (247.8 nm) in descending order viz. Mg>Ca>C>P. While table 1b shows the relative intensities of elements with respect to O (777.4 nm) in descending order viz. H>N>Na>O>K.

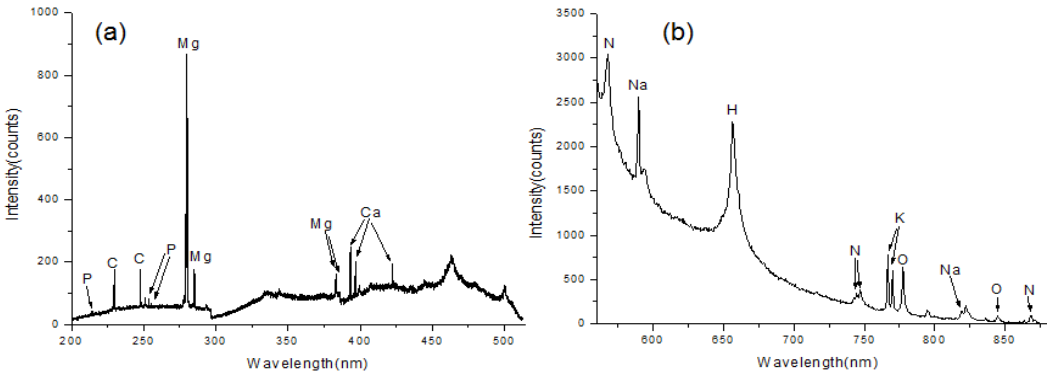

Fig. 9: Laser-Induced Breakdown Spectroscopy of *Cucurbita maxima* seeds in the spectral range of (a): 200–500 nm and (b): 600–850 nm

Table 1a: Intensity ratio of different elements of *Cucurbita maxima* with respect to C (247.8 nm) spectral range (200-500 nm)

Element	Wavelength (nm)	Element/Ref	Intensity Ratio by C (247.8 nm)
Ca	393.366	Ca/C (247.856)	2.12952
Mg	279.553	Mg/C(247.856)	6.11145
P	253.561	P/C(247.856)	0.21837
C	247.856	C/C(247.856)	1

Table 1b: Intensity ratio of different elements of *Cucurbita maxima* with respect to O (777.4 nm) Spectral range (600-850 nm)

Element	Wavelength (nm)	Element/Ref	Intensity Ratio by O (777.4 nm)
Na	589.592	Na/O(777.417)	1.33342
K	766.49	K/O(777.417)	0.75596
N	567.956	N/O(777.417)	1.85389
H	656.271	H/O(777.417)	9.2443
O	777.417	O/O(777.417)	1

DISCUSSION

Several organic solvents have already been used for preparing the extracts of the *C. maxima* seeds which had also been explored for assessment of their bioactivities viz. antidiabetic, antihyperglycemic, etc. [7]. However, the aqueous extract of the seeds of *C. maxima* has not been evaluated so far for any biological activity and hence, this is the first reporting of its kind. In addition to it, this is a value added study as it also deals with the identification of phytoelements responsible for its antioxidant profile. There are several reports which deal with its phytochemicals analysis but there are no reports till date on its phytoelemental analysis and specifically on their correlation. Moreover, micro-minerals viz. Mg, Ca, Na, K and P, not only act as structural components of different tissues but are also important components of body fluids. They are reported to play a pivotal role in the normal functioning of cells [31, 1]. The optimal intake of such elements can reduce individual risk factors as reported in several studies carried out in human beings and animals. The mineral elements are essential regulators of physiological processes. Ca, Mg and Na are important as cofactors in enzymatic processes, mainly in the structure of the DNA repair system [33]. Magnesium is also important in the change of energy in the mitochondria. Some minerals are components of important enzymes such as Ca and Mg for superoxide dismutase [34, 35]. Both these enzymes protect the cell membranes from oxidative damage. The high concentration of these mineral elements in CMSE may be responsible for promoting the enzyme activity, and as a consequence provide antioxidant capacity of CMSE. In the present study, CMSE was used for determination of its intrinsic elements responsible for its antioxidant potential *in vitro*. Different *in vitro* antioxidant and free radical scavenging assays were carried out and elemental detection was performed by LIBS technique. Analytical findings from LIBS data of CMSE exhibit that higher concentration of Mg, Ca and Na in CMSE may be concurrently responsible for its *in vitro* antioxidant and free radical scavenging activities.

CONCLUSION

This is an exciting era for bio-LIBS research. Highly encouraging and intriguing results have been obtained from the present study. It may therefore be conclusively summarized from our findings that definite concentrations of essential elements such as Mg, Ca and Na in CMSE act collectively to bring about the free radical scavenging potential *in vitro* in the aqueous seed extract. Moreover, results noticeably signify the efficiency of LIBS as a potent and fast analytical device for ascertaining the presence of antioxidant elements in an unknown sample. Hence, higher antioxidant potential and essential elements of CMSE indicates that the extract may be used as a replacement source of synthetic antioxidants. Thus, CMSE can be used as possible food supplement for natural antioxidants. The results indicate the importance of trace elements present in plants, which can be used in exploiting the medicinal value of plants for treating various biological disorders.

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CONFLICT OF INTERESTS

Declare none

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