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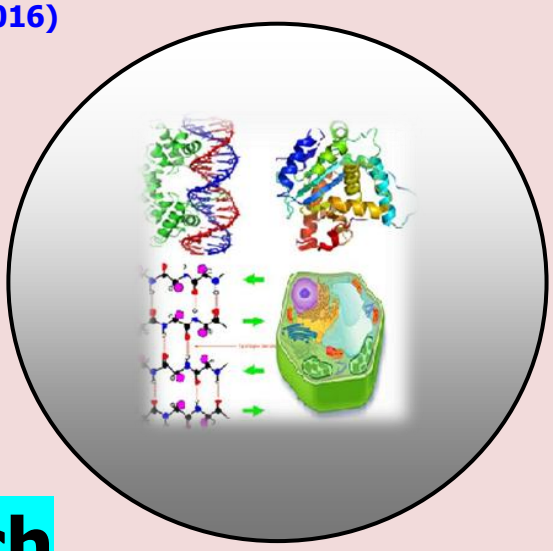

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RESEARCH PAPER

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Impact of Phytochemicals and Phytoelements on Therapeutic Attributes of *A. Esculentus* Leaves: A Libs Based Study

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ABSTRACT

Medicinal plants are pivotal natural source of phytochemicals, possessing therapeutic value which can be used for the development of potentially safe drugs. Along with the phytochemicals, phytoelements are equally important for the therapeutic efficacy of medicinal plants. Thus, the biological attributes associated with these plants are due to the presence of certain set of phytochemicals and phytoelements which act either individually or synergistically. Therefore, the present study is aimed to identify the micro- and macro-elemental distribution in addition to screening of phytochemicals of aqueous extract of Abelmoschus esculentus leaves. Phytochemical screening of major classes of compounds of the extract revealed the presence of alkaloids, carbohydrates, coumarins, flavonoids, glycosides, saponins, steroids, tannins and terpenoids. Whereas, detection of micro- and macro-elements using Laser Induced Breakdown Spectroscopy (LIBS) of A. esculentus leaves, in the spectral range of 200–900 nm, shows the atomic lines of Potassium(K), Sodium (Na), Calcium (Ca), Magnesium (Mg) and Silicon (Si) along with other common elements like Carbon(C), Hydrogen (H), Oxygen (O) and Nitrogen (N). Results of such LIBS based comprehensive elemental analysis clearly reveal the abundance of K, Ca, Na and Mg. While, the elements such as Si, C, H, N and O were found in lesser extents. Moreover, the presence of these major elements in addition to certain phytochemicals, was also very well correlated with the therapeutic potential of extract of A. esculentus leaves with special reference of treating diabetes and its complications viz. oxidative stress etc. Thus, this extract could be developed further as an oral safe Antidiabetic agent as well.
Keywords: Antidiabetic, A. esculentus, LIBS, Phytochemicals and Phytoelements.

INTRODUCTION

Plants have always been used as an exemplary source of phytomedicines since antiquity and hence play a vital role in the human health care. Strong beliefs are associated with the virtue of traditional medical system worldwide (Rai et al. 2014). It has been observed that the specific therapeutic efficacy of any plant is due to presence of certain phytochemicals and a specific elemental composition (Rai et al. 2009, Debrah et al. 2011). Thus, in recent years there is an upsurge of treating various diseases using medicinal plants as curative agents due to the presence of a specific combo of phytochemicals and phytoelements (Akinmoladun et al. 2007).

Phytochemicals are generally of two types viz primary and secondary metabolites. Secondary metabolites such as polyphenolics, flavonoids, tannins, alkaloids and terpenoids etc form basically the backbone of modern medicine (Afolabi et al 2007, Goh et al. 1995)

The presence of certain set of phytoelements play an important role in making these phytochemicals constituents active in medicinal plants. Therefore, to correlate bioactive profile of any medicinal plant with the presence of specific phytoconstituents and phytoelements is an important step which can be used as a key for unfolding the mystery of medicinal plants. In the modern era of food and nutritional research, detection and identification of elements responsible for bioactivity of plants is a thrust area of research throughout the world (Tripathi, et al. 2014a, 2014b, 2012a, 2012b) There is a curiosity also to know their minimum and maximum level of requirement in the prevention of several diseases. It is well known that proper intake of elements such as Calcium (Ca), Magnesium (Mg), Sodium (Na), Potassium (K), could be helpful to retard the individual risk factors of various diseases (Mertz et al. 1982). Hence, a systematic and scientific study of micronutrients availability in medicinal plants is essential through quick, reliable and eco-friendly analytical technique.

A LIB (Laser-Induced Breakdown Spectroscopy) is an ideal tool to detect and identify a wide range of elements present in any plant. This technique is gaining popularity for qualitative and quantitative analysis of trace elements present in any material without any complicated sample preparation in addition it is portable and easy to use, with high reproducibility. The ability of LIBS to interrogate solid samples has also been demonstrated for the direct elemental analysis of plant materials without any chemical contaminations (Rai et al.2010).

These elements even in traces help in controlling the metabolic process of the biological system. *Abelmoschus esculentus* is a flowering plant commonly known as 'Bhindi' in Hindi, belongs to the family Malvaceae, have already been reported for biological attributes (Sabitha et al. 2014). Thus, the present study deals with the phytochemical and phytoelemental evaluation of *Abelmoschus esculentus* leaves in order to define their impact on its therapeutic efficacy. The most important aspect, which has been explored through this study, and has added value to the present study is the correlation between phytochemical and phytoelemental profile of *A. esculentus* leaves and their role in managing its bioactivity. Hence, the novelty and rationality of the present study cannot be denied and ignored.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Abelmoschus esculentus* (500g) were collected from the local area of Allahabad U.P. (India) and authenticated by Professor Satya Narayan, Taxonomist Department of Botany, University of Allahabad, India. Collected leaves of *A. esculentus* were washed with distilled water and dried completely under shade. The shade dried leaves (150g) were mechanically crushed, powdered and then extracted with 500ml distilled water in soxhelt apparatus for 8 hrs at 24˚C. The collected aqueous fraction was concentrated using a rotatory vaccum evaporator for drying sample .Dried extract of *A. esculentus* was preserved in bottles and kept in refrigerator for further experimental analysis.

Chemicals

All the chemicals and solvents used in these assays were of high purity (99%). Hager's reagent, lead acetate, picric acid, potassium iodide, conventional solvents viz. hexane, benzene, ethyl acetate, dichloromethane, chloroform, ethanol, methanol, ammonia, isoamyl alcohol and other solvents viz. ammonium hydroxide, sodium hydroxide, acetic acid, conc.H_2SO_4, HCl , $FeCl_3$, α-naphthol, etc. were purchased from Sigma Aldrich, New Delhi, India.

Chemical test for Screening of Phytoconstituents

Screening for the phytoconstituents in *Abelmoschus esculentus* leaves extract (AMLE) were carried out by using standard methods (Yadav et al.2014) as given below:

Experimental Setup for LIBS analysis

The present experimental arrangement for LIBS contains frequency doubled (532nm) Q- switched Nd: YAG high power pulsed laser (Continuum Surelite III-10) of pulsed width 4ns (FWHM) and varying rate of repetition up to 10Hz having maximum deliverable energy of 425mJ. For LIBS analysis of sample we have prepared it in pellet form. The pellet is placed on sample stage and laser beam is focused with lens of 15cm focal length to produce plasma on sample surface.

On cooling plasma the spectral emission are collected through collimator and 5000, Andor) which is imbedded with intensified charged Coupled Device (ICCD, iStar 334,Andor technology) detector system and synchronized with digital Delay Generator (DDG,DG535) and connected with computer system which is installed with Andor Solis Software (Tiwari et al. 2018).

Phytoconstituents	**Colour Tests**	**Observation**
Alkaloids (Hager's Test) (Wagner's test) (Mayer's test)	2ml extract + few drops of Hager's reagent 2 ml extract+ 2 drops HCl (1.5%) +3 drops Wagner's reagent 2 ml extract (EtOH) + few drops Mayer's reagent	Yellow precipitate Brown precipitate Yellow precipitate
Anthraquinone (Borntrager's Test)	3ml extract + 3ml Benzene + 5ml NH_3 (10%)	Pink, Violet or Red colour
Anthocyanins	2ml extract + 2ml HCl (2N) + NH_3	Pinkish Red to Bluish Violet colour
Carbohydrate (Molisch's Test) (Fehling's test)	2ml extract (EtOH) + 10ml H_2O + 2 drops ethanolic α-naphthol (20%) + 2ml conc.H_2SO_4 2 ml extract + 1 ml of Fehling's solution A and B + heat	Reddish Violet ring at the junction Red precipitate
Coumarins	2ml extract + 3ml NaOH (10%)	Yellow colour
Emodins	2ml extract + 2ml NH_4OH + 3ml Benzene	Red colour
Flavonoids	1ml extract + 1ml $Pb(OAc)_4$ (10%)	Yellow precipitate
Glycosides (Liebermann's Test) (Salkowski's Test)	2ml extract + 2ml $CHCl_3$+ 2ml CH_3 COOH 2ml extract + 2ml $CHCl_3$ + 2ml conc. H_2SO_4	Violet to Blue to Green colour Reddish Brown ring at the Junction
Leucoanthocyanins	5ml extract + 5ml Isoamyl alcohol	Organic layer turns into Red colour
Phlobatannins (Precipitate test)	2ml extract + 2ml HCl (1%) + boil	Red Precipitate
Proteins (Xanthoproteic test) (Biuret's test)	1ml extract + 1ml conc.H_2SO_4 1 ml extract+ 5-6 drops w/v NaOH + 2 drops $CuSO_4$ (30%w/v)	White precipitate to Yellow on heating Violet Red colour
Saponins (Foam Test) (Emulsion test)	5ml extract + 5ml H_2O+heat 5ml extract + Olive oil (few drops)	Froth appearance Emulsion formation
Steroids (Salkowski's Test)	2ml extract + 2ml $CHCl_3$+ 2ml conc. H_2SO_4	Reddish Brown color at interface
Tannins (Braymer's Test)	2ml extract + 2ml H_2O +few drops of $FeCl_3$ (5%)	Green color
Terpenoids	2mlextract+EtOH+2ml$CHCl_3$+ Δ (2 mint.) 3 drops conc. H_2SO_4	Deep red color

RESULT AND DISCUSSION

Table 1, shows the presence of the phytochemicals present in *Abelmoschus esculentus* aqueous leaf extract (AMLE). The data reveals the significant presence of alkaloids, carbohydrate, coumarins, flavonoids, terpenoid and tannins in comparison to saponin, steroid and glycosides which were present in lesser extent whereas anthocyanins, anthraquinones, emodins, leucoanthocyanins, phlobatannins and proteins were totally absent.

Table 1. Screened Phytoconstituents in *A.esculentus* leaves.

Phytoconstituents	***A. esculentus* leaves**
Alkaloids	++
Carbohydrate	++
Coumarins	++
Flavonoids	++
Glycosides	+
Saponins	+
Steroids	+
Tannins	++
Terpenoids	++
Anthocyanins	-
Anthraquinones	-
Emodins	-
Leucoanthocyanins	-
Phlobatannins	-
Protein	-

LIBS based elemental analysis of *A. esculentus* leaves

Figure 1, show the LIBS spectra of the AMLE in the spectral ranges of 200-900nm. Relative concentrations of elements present in AMLE have been evaluated by measuring the intensity of the selected lines in triplicates from the LIBS spectra of sample. Table1 indicate the relative intensity ratios of different phytoelements of the sample (AMLE) with respect to C (247.8nm). Thus, the results of Table1 shows the relative intensities of elements with respect to C (247.8 nm) in descending order viz. K>Ca>Na>Mg along with the presence of Si, H, C, N and O.

Preliminary phytochemical analysis made for the leaf part of *A. esculentus* revealed the presence of specific groups of phytochemicals which are reported to have many biological and therapeutic properties (Tiwari et al. 2016). In results presence of Alkaloids represent a class of compound which are known to posses diuretic properties and also affects the central nervous system and reduces appetite. Presence of carbohydrate and coumarins are associated for increasing the immunity and body strength hence could be served as dietry supplements whereas Tannins are known for their stringent and anticancer properties. Terpenoids have been reported for their various therapeutic efficacies, flavonoids are also correlating with their antioxidant activities (Rabi et al.2009, Wagner et al 2003, National Academy Press, USA, 2010).

Thus the presence of these secondary metabolites in AMLE provides diversity in its biological effects, including antimicrobial, antiinflammatory, analgesic, antipyretic and antiproliferative (Lemos et al. 1990, Gaikwad Switi et al. 2014, Kahkonen et al. 1999). There has been a reporting of antioxidant properties of AMLE due to presence of certain phytoconstituents present in the plant extract.

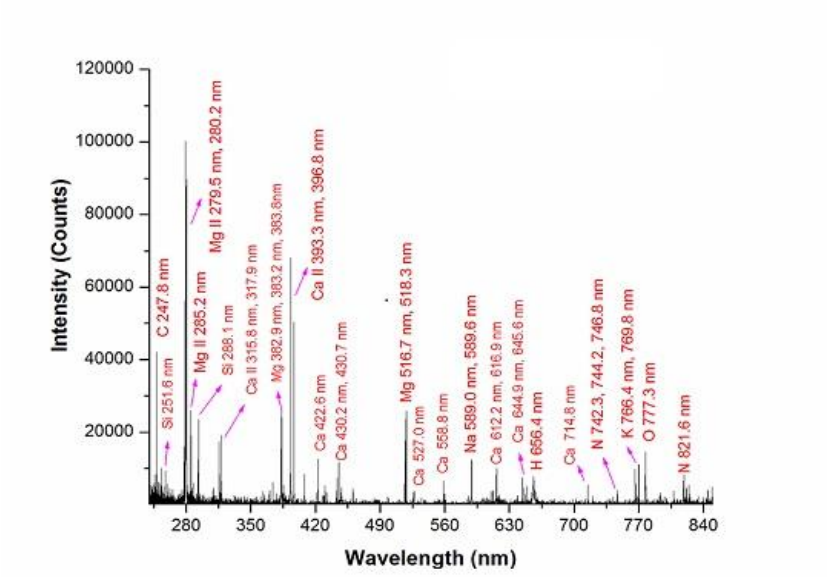

Figure 1. LIBS spectra of the *A. esculentus* leaf extract in the spectral range of 200-900nm

Table 2. Intensity ratio of different elements of *A. esculentus* with respect to C (247.8nm) Spectral range (200-900nm)

Elements	Wavelength (nm)	Element/Ref	*Intensity ratio by C (247.8nm)*
Mg	518.3	Mg/C (**247.856)**	0.19
Ca	422.6	Ca/C (**247.856**	0.58
K	769.7	K/C (**247.856**	0.97
Na	589.0	Na/C (**247.856**	0.47
Si	288.1	Si/C (**247.856)**	0.13
H	656.2	H/C (**247.856)**	0.61
O	777.1	O/C (**247.856)**	0.84
N	746.8	N/C (**247.856)**	0.09

The elemental analysis of the aqueous extract of *A. esculentus* showed the abundance of Potassium (K) followed by Ca, Na and Mg. While, the elements such as Si, C, H, N and O were found in lesser extents. Among these elements Na and K are well known for their important role in Sodium-Potassium pump which are active transporter, use to pump ions against the gradient. Whereas Sodium is also very important to maintain the electrolytic balance in the body and Mineral elements like potassium, calcium, and magnesium has already been reported for the release of insulin hormone from the beta cells of islets of langerhans thus these phytoelements are helpful in maintaining the normal glucose level. (Gayathri et al.2010, Kar et al. 1997). Ca is also play very important role in human diet as it reduces the risk of hypertension and osteoporosis and also reduces the serum cholesterol level in human body. Hence in the leaves of *A. esculentus* presence of Calcium may attribute to its hypolipedimic property and Magnessium could be used as a therapeutic weapon to improve sensitivity towards insulin and manage diabetes and its related complications (Vaskonen et al. 2002, MA Brenna et al 1999). Thus the present study reveals with the Phytochemical and Phytoelemental indexing of *Abelmoschus esculentus* leaves and provide evidence in support of the medicinal importance of selected plant which could also be served as a supplementary source of these nutritional elements in human diet and may be used as a alternative of synthetic antioxidants and antidiabetic agents.

CONCLUSION

Conclusively it could be stated that *A. esculentus* leaves with the identified phytochemicals and phytoelemental profile could serve as a therapeutic agent for treating various diseases.

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