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Phytochemical screening and antioxidant activity of *Elaeocarpus serratus* L. of Assam

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Abstract
Phytochemicals found in plants are known with many potent applications for the well-being of mankind. In the current study, the phytochemical screening of leave extracts (aqueous, ethanol and methnol) of *Elaeocarpus serratus* L. showed positive result with the presence of many significant phytochemicals like saponins, tannins, cardiac glycosides, flavanoids, steroids, etc. While the DPPH antioxidant analysis showed the standard Ascorbic acid with EC_{50} value at 25.53 µg/ml, whereas methanolic extract of *E. serratus* showed EC_{50} at 75.47 µg/ml. the HR-MS analysis revealed the presence of five novel compounds in the methanolic leave extract, *viz.*, clotrimazole, etamiphylline, 2'-O-Methylcytidine, aspidocarpine and leupeptin. On the basis of the overall finding, it can be concluded that the plant *E. serratus* L. has been identified as potential source of medicinally important plants, for that further extensive analyses is required.

Keywords: *Elaeocarpus serratus*, phytochemicals, DPPH, HRMS, Ascorbic acid

1. Introduction
Elaeocarpus serratus L. (*syn Elaeocarpus genitrus* Roxb. ex G. Don) commonly termed as 'rudraksha' in India is an evergreen tree which is well known for its various medicinal and commercial purposes. The tree is reported to be found in Assam and Himalayan region in India. It is used in folk medicine in treatment of stress, anxiety, depression, palpitation, nerve pain, epilepsy, migraine, lack of concentration, asthma, hypertension, arthritis and liver diseases. The beads, leaves and bark of *E. serratus* has immense importance in Ayurveda and has been described to cure ailments like mental disorders, headache, fever, skin diseases, palpitation, insomnia, infertility etc. [4].
Phytochemicals present in the plants are responsible for various activities of medical and therapeutic history which play an important role directly or indirectly to human health. Medicinal plants are the source of such bioactive compounds which play a pivotal role in the pharmaceutical industry. Many phytochemical investigations have been performed from different plant parts by the scientists till now. In the bark, leaves and fruits of *E. ganitrus*, presence of alkaloid, tannins, flavanoids fatty acid have been reported [7, 11, 2, 10]. These chemical constituents make the tree a medicinally important one. So far no work has been done on phytochemical screening of compounds and antioxidant properties of *E. serratus* found in Assam. The present study aims in screening of phytochemical constituents and antioxidant activity of *E. serratus* L.

2. Materials and methods
Collection of plant material
Elaeocarpus serratus L. leaves were collected from the Dr. H. K. Baruah Regional Botanical Resource Centre, Department of Botany, Gauhati University during the month of January, 2016 for the purpose of phytochemical and antioxidant study.

Preparation of plant extract
The plant leaves were thoroughly washed under tap water to avoid dusts and unwanted materials accumulated on the leaves. Then the plant materials were dried for 30 days at the room temperature and were powdered using a grinder. At last fine powder was collected by using a sieve and was used for further analysis.

Maceration with ethanol and methanol solvent
20 gm of leaf powder was dissolved in 150 ml of ethanol and methanol solvent separately in a 250 ml conical flask and plugged with cotton tightly.

The extract was macerated in a rotary shaker (Remi) at 30 ºC with 150 rpm for 24 hrs for thorough mixing. The liquid was filtered by using Whattman filter paper No. 1, filtrated liquid was taken for phytochemical analysis.

Maceration with water

The filtrate residue left in the filter paper was taken and dissolved in distilled water and was again kept in the rotary shaker at same condition (24 hrs, 150 rpm, 30 ºC). Then the aqueous extract was collected by using Whattman filter paper no 1. Then the filtrate extract was then subjected for further analysis.

Qualitative test for phytochemical analysis

The ethanolic, methanolic and aqueous extract of *E. serratus* was subjected to qualitative phytochemical screening for the presence of alkaloids, saponins, tannins, terpenoids, cardiac glycosides, flavanoids, phenols, coumarins, steroids, reducing sugar and anthraquinones by adopting standard procedures [12, 9, 5].

Antioxidant analysis

The antioxidant activity of methanolic extract of leaves was determined by using DPPH [1]. DPPH is monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. The various concentrations (7.5, 15, 22.5, 30µg/ml) of methanolic extract was used and ascorbic acid was used as a standard for antioxidant activity. Methanol was used as blank and methanol with DPPH as control. Then, the sample was incubated at 25 ºC and the decrease in absorbance was calculated at 570 nm using spectrophotometer (Beckman Coulter, DU730). All analysis was done in triplicate form. Radical scavenging activity (%) was calculated by the following formulae:

Radical scavenging activity (%) = (Abs control – Abs sample)/Abs control × 100

Where, Abs control = Absorbance of DPPH + methanol, Abs sample = Absorbance of DPPH radical + sample (*i.e.*, extract or standard).

HR-MS analysis

HR-MS was used for identification of few major bioactive compounds from the crude methanolic extract of *E. serratus* (Model-Agilent Mass Hunter Z400) system. The analytical column was maintained at 40 °C, while the sample manager at 15 °C.

3. Results

Phytochemical screening of the plant material

In phytochemical analysis of alcoholic, aqueous and methanolic extract of *E. serratus* L., many positive results were obtained for different phytochemical constituents. In aqueous extract, 9 out of 11 tests showed positive result. In the test performed, saponins, tannins, cardiac glycosides, flavanoids, and steroids were detected in high intensity while anthraquinone and alkaloids were not detected. In ethanolic extract, 7 out of 11 tests were found to be positive. In the test performed tannin, cardiac glycosides, phytosterol, phenol was detected in high intensity while saponin, coumarin, steroid and anthraquinone were not detected. In methanolic extract, 6 out of 11 tests were found to give positive result in which cardiac glycosides and reducing sugar was detected in high intensity, while alkaloids, flavanoides, coumarin, steroids and anthraquinone were not detected in this analysis (Table 1).

***In vitro* antioxidant activity**

It was observed that the methanolic extract have demonstrated the dose dependent increase in the DPPH radical scavenging activity. Ascorbic acid (Standard) has shown EC_{50} at 25.53 µg/ml concentration obtained by equation (Y = 0.8336*X +28.71) whereas methanolic extract of *E. serratus* showed EC_{50} at 75.47 µg/ml concentration obtained by equation (Y = 0.508*X +11.66) [Table 2-3; Fig. 1].

HR-MS analysis

HR-MS analysis of methanolic leaf crude extract of *E. serratus* L. showed the presence of eight novel compounds as presented in the graph obtained. To perform the on-line identification of each compound, LC gradients were developed to better separate solutes. Mass spectrometry is a powerful tool which provides structural information on molecules. However, ionization parameters have to be appropriate to the physicochemical properties of the compounds analyzed. The type of ionization source and mode of ionization were therefore optimized so as to be adapted to extract polarity. The electrospray ionization source (ESI) is well suited to polar compound. Thus, ESI was used to analyze the polar molecules present in the methanolic extract (Table 4; Fig. 2-4). Further, APCI mode of scanning was also used for probable detection of other novel molecules not seen in ESI scanning (Table 5; Fig. 5-8).

However, in order to obtain the most exhaustive fingerprint possible, generic ionization parameters were applied in order to detect the majority of compounds properly. Here, only positive mode was tested. Then, high resolution mass spectrometry gave access to the accurate mass of compounds. The corresponding molecular formula was established with Data Analysis software and probable structures were assigned by searching in the Chemspider database. Finally fragmentation analysis confirmed the proposed identifications. To characterize the methanolic extract, the positive ionization mode was more appropriate for flavonoids detection. Molecules were detected mainly as de protonated molecule ions [M+H].

Table 1: Preliminary phytochemical screening of extract of *E. serratus*

Sl. No.	Secondary metabolites	Water extract	Ethanol extract	Methanol extract
1	Alkaloids	-	++	-
2	Saponins	+++	-	++
3	Tannin	+++	+++	+
4	Cardiac glycosides	+++	+++	+++
5	Phytosterol	+	+++	+
6	Flavanoids	+++	++	-
7	Phenol	++	+++	++
8	Coumarin	++	-	-
9	Steroids	+++	-	-
10	Reducing sugar	++	++	+++
11	Anthraquinone	-	-	-

[(+++) strong intensity reaction, (++) medium intensity reaction, (+) weak intensity reaction, (-) not detected]

Table 2: DPPH radical scavenging activity of methanolic extract of *E. serratus* (MEES)

Concentration (µg/ml)	Scavenging (%)	
	Ascorbic acid	MEES
7.5	33.80±4.4	16.04±9.32
15.0	48.58±0.98	23.4±5.38
22.5	57.06±3.52	25.87±5.55
30.0	58.76±1.95	32.15±5.92

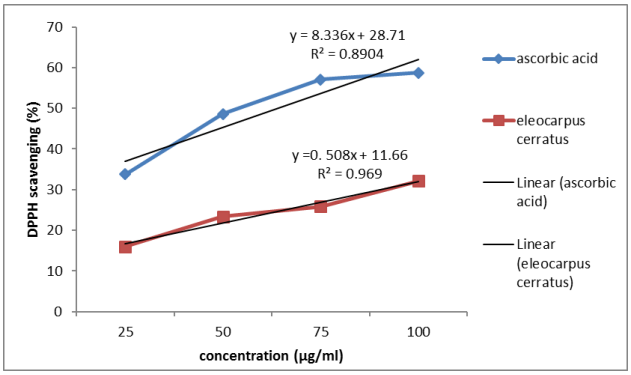

Fig 1: DPPH scavenging activity (%) of ascorbic acid and methanolic leave extract of *E. serratus* L.

Table 3: Concentration of extract at DPPH radical scavenging activity (50%)

Sample extract	(IC_{50}) (µg/ml)
Ascorbic acid	25.53
Elaeocarpus serratus	75.47

Table 4: Metabolites, calculated mass and detected m/z ratios (ESI-scan)

Sl. No.	Metabolite	Ion Type	Mass to Ion Ratio (m/z)	
			Calculated	Detected
A	Clotrimazole	$[M+H]^+$	345.1153	344.1080
B	Etamiphylline	$[M+H]^+$	280.17746	279.16952
C	2'-O-Methylcytidine	$[M+H]^+$	258.1499	257.101171

Table 5: Metabolites, calculated mass and detected m/z ratios (APCI- scan)

Sl. No.	Metabolite	Ion Type	Mass to Ion Ratio (m/z)	
			Calculated	Detected
A	Aspidocarpine	$[M+H]^+$	371.1543	370.225643
B	Leupeptine	$[M+H]^+$	427.2169	426.29545

4. Discussion

Qualitative phytochemical analysis of leaf extract of *E. serratus* L. has established the presence of secondary metabolites which might be responsible for their established medicinal properties [3]. Ethanolic extract of *E. serratus* showed the presence of tannins, cardiacglycosides, phenols, phytosterols in high quantity [6, 8] while alkaloids, reducing sugar and flavonoids was detected in moderate quantity. Aqueous extract of *E. serratus* showed positive results for almost all test except for alkaloid and anthraquinone. Methanolic extract of *E. serratus* showed positive results for saponin, tannin, cardiac glycosides, phenol and reducing sugar while alkaloid, coumarin, and steroid could not be detected. In none of the extract athraquinone was detected.

Through LC-HRMS analysis, individual components were identified by comparison of their m/z in the total ion count (TIC) profile, with those of the selected compounds described in literature or by matching their MS/MS spectra with those in *E. serratus* L. Five major compounds were identified *viz.*, Clotrimazole, Etamiphylline, 2'-O-Methylcytidine, Aspidocarpine and Leupeptin respectively.

The antioxidant activity of the crude methanolic extract of *E. serratus* L. leaves in our study has shown dose dependent antioxidant property. *In vitro* DPPH antioxidant assay showed IC_{50} of *E. serratus* as 75.47 µg/ml while the IC_{50} of standard ascorbic acid was found to be 25.53 µg/ml.

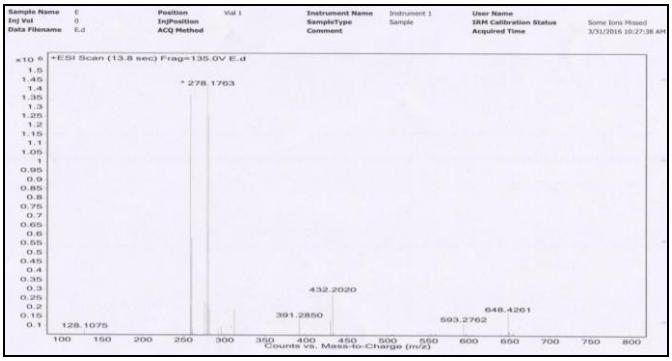

Fig 2: HR ion chromatograms, based on accurate mass of each analyte, and experimental and calculated MH^+ isotopic spectra ranging from 100 – 800 m/z (ESI scan)

Fig 3: HR ion chromatograms, based on accurate mass of each analyte, and experimental and calculated MH^+ isotopic spectra ranging from 258 – 282 m/z (ESI scan)

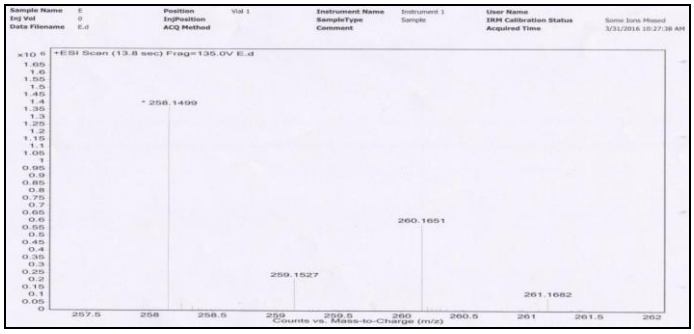

Fig 4: HR ion chromatograms, based on accurate mass of each analyte, and experimental and calculated MH^+ isotopic ranging from 257.5 - 262m/z (ESI scan)

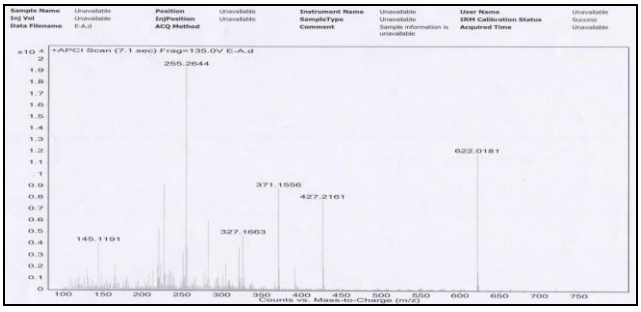

Fig 5: HR ion chromatograms, based on accurate mass of each analyte, and experimental and calculated MH^+ isotopic, spectra ranging from 100-750 m/z (7.1 sec), (APCI- scan)

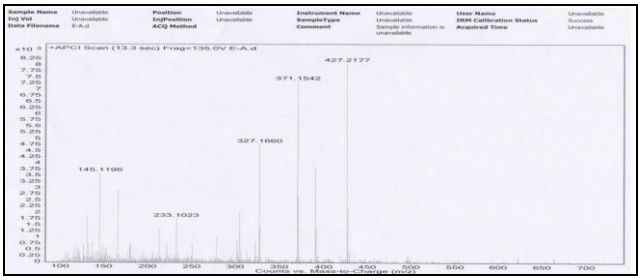

Fig 6: HR ion chromatograms, based on accurate mass of each analyte, and experimental and calculated MH^+ isotopic spectra ranging from 100-700m/z (13.3 sec), (APCI- scan)

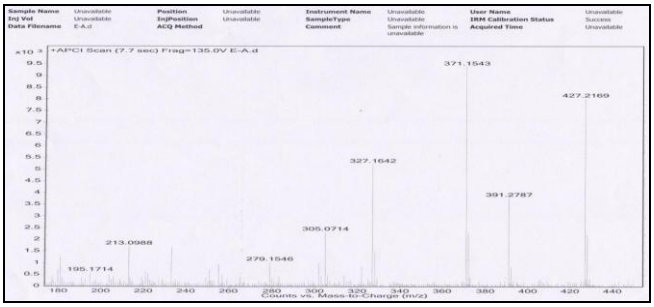

Fig 7: HR ion chromatograms, based on accurate mass of each analyte, and experimental and calculated MH^+ isotopic ranging from 180-440m/z (7.7sec) (APCI- scan)

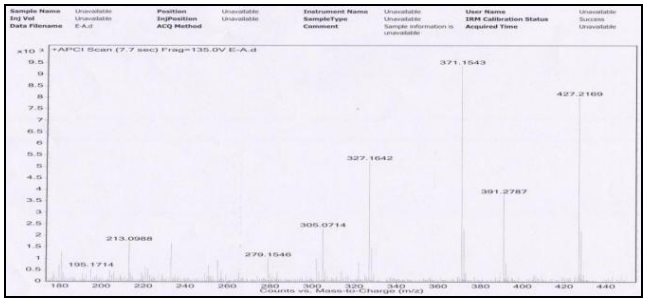

Fig 8: HR ion chromatograms, based on accurate mass of each analyte, and experimental and calculated MH^+ isotopic ranging from 180-440m/z (10.7 sec) (APCI- scan)

5. Conclusion

Exploration, documentation and characterization of the medicinally and economically important plants of Assam are still very limited. Northeast India in general and Assam in particular is one of the mega biodiversity hotspot which supports unique types of vegetation patterns with different flora. Plants are an important source of medicine and play important role in world health. Thus keeping this point in mind the following study of phytochemical indexing was conducted on the medicinally important plant *E. serratus* L.

On the basis of the overall finding, it can be concluded that the plant *E. serratus* L. has been identified as potential source of medicinally important plants. Thus, further studies of this species could unfold much other information, leading to bioprospection for the welfare of mankind.

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