Asian Journal of Chemistry; Vol. 26, No. 12 (2014), 3675-3678

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2014.17052

GC-MS Analysis and *in vitro* Cytotoxicity and Antioxidant Studies on *Leucas aspera*†

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Published online: 5 June 2014; AJC-15322

Leucas aspera Spreng. is a common Laminaceous member whose leaf and its juice is traditionally used as medicine for various ailments. The aim of the present work is to prove the antioxidant and cytotoxic efficacy of various parts of *Leucas aspera* by *in vitro* methods. *Leucas aspera* commonly called "Thumbai" is collected from in and around Thanjavur. After proper identification and authentication the different parts of plant were separated and decoction prepared, filtered, lyophilized and subjected to *in vitro* and chemical studies. The preliminary phytochemical analysis of the test drug revealed the presence of sterols, flavonoids, terpenoids and reducing sugars. GC-MS analysis helped to identify the fragments of the chemical constituents present in the plant. In the present study, antioxidant and cytotoxic efficacy of the selected drug was established through *in vitro* methods. The efficacy confirmed is further supported by chemical evidences derived from GC-MS analysis.

Keywords: *Leucas aspera*, GC-MS, Antioxidant, Cytotoxicity.

INTRODUCTION

Leucas aspera Spreng. (LA) commonly called as "thumbai" is a small erect branched annual herb growing up to 60 cm in height. Leaves sub sessile, linear- lanceolate, obtuse, entire, or crenate, flowers white, in dense axillary whorls. Whorls are large, about 2.5 cm in diameter and crowded with white bell shaped flowers. Calyx is variable, with an upper lip and short, triangular teeth. Fruit nut lets, brown and smooth.

Leucas aspera-Habit

The whole plant is more popular in the treatment of many diseases, may be due to the presence of rich biologically active compounds. Selected plant drug is used as an antifungal, antiinflammatory, antimicrobial, antioxidant, cytotoxic, hepatoprotective, antibacterial and antihyperglycemic agents[1]. Its paste as an ointment is applied topically to inflamed areas and in India the decoction of dried aerial parts of plant is used orally for diarrhea[2,3]. The decoction of entire plant is used orally to reduce fever[4]. The aqueous extract of entire plant is used orally as an appetizer[5]. The flowers and leaves are applied externally as poultice to treat headache[6]. The decoction of flower is used orally to treat jaundice[7]. Hot water extract of dried flowers is used orally for cough and colds[8,9]. The juice of unripe fruits is used externally to treat scabies[10]. The juice of leaves is used externally in treating snake bites and the dried leaves are used as a blood purifier[11,12].

Leucas aspera possess enormous therapeutic potential, besides being safe and efficacious. This prompted us to investigate the cytotoxic and antioxidant efficacy of aqueous extract of *Leucas aspera* employing *in vitro* methods and attempts were also made to identify the nature of compounds present in the extracts by GC-MS.

EXPERIMENTAL

The whole plant was collected from in and around Thanjavur, authenticated by comparing the specimens deposited at Raphinet Herbarium, St. Joseph College, Trichirapalli, India. The various parts of the plant were washed, dried in shade, powdered and processed for aqueous extraction.

†Presented at PHYTOCONGRESS-2013, held on 7-8 March 2013, SASTRA University, Thanjavur, India

Preparation of aqueous extract: 10 g of the sample was taken and soaked in distilled water over night, filtered through Whatmann filter paper No. 1 and boiled, concentrated to 1/8th part and dried to get crude extract[13,14].

Phyto-chemical screening: The preliminary phytochemical analysis of leaves, flower, stem, root and aerial parts were performed according to Harborne[15] and the results were tabulated (Table-1).

TABLE-1
PHYTOCHEMICAL SCREENING OF DIFFERENT PARTS OF *Leucas aspera*

Phyto constituents	Leaf	Flower	Stem	Root	Aerial part
Alkaloids	+	+	+	+	+
Carbohydrates	+	–	+	–	+
Glycosides	+	+	+	+	+
Saponins	+	–	–	+	+
Sterols	+	+	+	+	+
Phenols	+	+	+	+	+
Tannins	+	+	+	+	+
Flavonoids	+	+	–	–	+
Amino acids	+	–	–	+	+

Antioxidant activity

DPPH radical scavenging activity: The hydrogen atom or electron donating ability of the *Leucas aspera* aqueous extracts was measured from the bleaching of methanol solution of DPPH. The DPPH assay was performed as per Arvind *et al.*[16]. The extracts of different parts of the plant dissolved in methanol. Then 0.5 mM DPPH solution in methanol was prepared and 0.5 mL of this DPPH solution was mixed with 0.1 mL of various amounts of the extract and vortexed thoroughly. Then, 4 mL of methanol was added to the solution and allowed to stand for 1 h in a dark room. The absorbance was measured at 516 nm using UV-spectrophotometer (Perkin Elmer). Decreasing absorbance of the DPPH solution indicates an increase in DPPH radical scavenging activity. The percentage inhibition was calculated and tabulated.

Reducing capacity: The reducing power was measured according to Oyaizu[17]. The reaction mixture (1 mL) containing the samples in phosphate buffer (0.2 M, pH 6.6) was incubated with potassium ferricyanide (1 g/100 mL water) at 50 ºC for 20 min. The reaction was terminated by adding TCA (10 g/100 mL water), the mixture was centrifuged at 3000 rpm for 10 min and the supernatant was mixed with ferric chloride (0.1 g/100 mL of water); the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

***Ehrlich ascites carcinoma* MTT assay:** The MTT assay is a test of metabolic competence based upon assessment of mitochondrial performance, relying on the conversion of the yellow dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) to the purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Cells were incubated with RPMI-1640 medium, supplemented with 10 % fetal calf serum, 2 mM glutamine, 100 U/mL streptomycin and 100 U/mL penicillin at 37 ºC with 5 % CO_2 before plating in 96-well plates (10^6 cells/well in 100 µL of medium). After 24 h, the extracts were added, dissolved in DMSO to obtain the final concentrations (500-7.8 µg/mL). At the end of 72 h incubation, the medium in each well was replaced by fresh medium (200 µL) containing 0.5 mg/mL of MTT. After 3 h, the formazan product of MTT reduction was dissolved in DMSO and absorbance was measured using a micro-plate reader. The cytotoxic effect of extracts against *Ehrlich ascites carcinoma* was determined as the percentage of reduced dye[18].

GC-MS analysis: GC analysis was carried out on a Clarus 500 Gas Chromatograph using a non-polar, Elite-5 ms column (30 m × 0.25 mm × 0.25 µm film thickness, coated with 5 % diphenyl-95 % dimethyl polysiloxane) interfaced with mass detector. Helium was used as carrier gas with a flow rate of 1 mL/min. Temperature programme was 50-150 ºC hold for 2 min at the rate of 3 ºC/min and increased to 290 ºC (10 min) at the rate of 8 ºC/min. The plant extract was dissolved in methanol and 1 µL of the filtered methanol extract was injected with split ratio as 1:10. Mass spectra were recorded in the EI mode at 70 eV in a scan range of 40-600. Injector and ion source temperature were maintained at 280 and 200 ºC, respectively. The resulted spectra were compared with NIST library database.

Statistical analysis: All the experiments were carried out in triplicates and represented as mean ± SD. The IC_{50} (µg/mL) values were calculated. The statistical analyses were performed using the computer software Graph Pad Prism 6 for Windows (GraphPad Software, USA).

RESULTS AND DISCUSSION

The preliminary phytochemical screening revealed the presence of tannins, proteins, steroids, glycosides, carbohydrates, saponins, flavonoids and alkaloids in different extracts of *L. aspera.* These results indicate that *L. aspera* contain number of phytochemicals, which may be responsible for the various pharmacological actions although their specific roles remain to be investigated. It has been observed that most active principles present in the plant are alkaloids, flavonoids, phytosterols, tannins and glycosides (Table-1).

Data obtained in the DPPH radical scavenging efficacy of extracts of different parts of *Leucas aspera* were given in (Table-2).

Several studies revealed that various phytoconstituents were present in *Leucas aspera* like triterpenoids, oleanolic acid, urosilic acid and β-sitosterol, nicotine, sterols, glucoside, diterpenes and phenolic compounds. Thus it is a rich source of medicinal compounds and possess various pharmacological activities[19,20].

All the extracts showed moderate to potent activity. Aerial part extract demonstrated the strongest antioxidant activity with IC_{50} value of 125 µg/mL. Aqueous extract of the aerial part must be possessing rich source of natural antioxidants than the individual parts. Total reducing capacity of extracts of different parts of *Leucas aspera* were presented in (Table-3).

Aqueous extracts of various parts of *Leucas aspera* showed potent inhibitory activity against *Ehrlich ascites carcinoma* cancer cell lines in which flower extract showed an IC_{50} value of 15.62 µg/mL and all other extracts possessed an IC_{50} value of 31.25 µg/mL. Data of the results obtained in the present study depicted that the aqueous extract of different parts of *Leucas aspera* possess good cytotoxic effect against *Ehrlich ascites carcinoma* cell lines. The data obtained were present in Table-4. Fragmentation pattern of few compounds identified

TABLE-2
DPPH RADICAL SCAVENGING EFFICACY OF EXTRACTS OF DIFFERENT PARTS OF *Leucas aspera*

Concentration (µg/mL)	Inhibition (%)				
	Leaf	Root	Stem	Aerial	Flower
31.25	0.17 ± 2.91	2.26 ± 2.91	6.89 ± 2.18	15.12 ± 5.09	–
62.5	0.84 ± 0.73	8.44 ± 2.91	16.15 ± 2.18	20.78 ± 1.45	–
125	1.23 ± 1.45	16.67 ± 4.36	19.24 ± 2.18	51.13 ± 2.18	1.23 ± 2.91
250	11.52 ± 2.91	31.07 ± 2.91	66.05 ± 1.45	76.34 ± 2.91	15.12 ± 9.46
500	27.47 ± 12.37	62.45 ± 2.18	73.77 ± 0.73	80.97 ± 0.73	56.28 ± 3.64
1000	55.76 ± 2.91	75.31 ± 1.45	81.48 ± 1.45	83.02 ± 2.18	77.88 ± 2.18

TABLE-3
TOTAL REDUCING CAPACITY OF EXTRACTS OF DIFFERENT PARTS OF *Leucas aspera*

Concentration (µg/mL)	Absorbance				
	Leaf	Root	Stem	Aerial	Flower
31.25	0.0253 ± 0.007	0.025 ± 0.007	0.0233 ± 0.011	0.0152 ± 0.007	0.015 ± 0.007
62.5	0.0352 ± 0.007	0.054 ± 0.014	0.0251 ± 0.007	0.0251 ± 0.007	0.035 ± 0.007
125	0.0401 ± 0.006	0.056 ± 0.014	0.0456 ± 0.007	0.0560 ± 0.014	0.075 ± 0.007
250	0.0455 ± 0.007	0.065 ± 0.007	0.0655 ± 0.021	0.0652 ± 0.021	0.095 ± 0.021
500	0.0557 ± 0.007	0.085 ± 0.021	0.1424 ± 0.028	0.2353 ± 0.035	0.215 ± 0.092
1000	0.3102 ± 0.014	0.367 ± 0.014	0.492 ± 0.014	0.9351 ± 0.021	0.4823 ± 0.042

in the selected plant through GC-MS analysis are given in Table-5.

The presence of phenol group has been detected in the GC-MS analysis of aerial part of *L. aspera* (Figs. 1 and 2). This phenolic group act as hydrogen donors and can donate electrons to neutralize hydrogen peroxide as water[21]. This inter-relationship between total phenols content and antioxidant activity may be responsible for free radical scavenging activity. Hence, phenolic compounds present in the *Leucas aspera* aerial may be responsible for the antioxidant activity.

TABLE-4
CYTOTOXICITY EFFICACY OF EXTRACTS OF DIFFERENT PARTS OF *Leucas aspera* AGAINST EAC (MTT ASSAY METHOD)

Concentration (µg/mL)	Inhibition (%)				
	Leaf	Root	Stem	Aerial	Flower
7.812	37.49 ± 1.03	34.31 ± 7.58	39.71 ± 0.49	48.29 ± 4.60	39.21 ± 0.87
15.62	40.63 ± 1.25	44.99 ± 2.65	46.79 ± 1.08	53.11 ± 4.38	44.76 ± 1.89
31.25	48.17 ± 2.60	51.39 ± 5.52	56.36 ± 3.14	57.74 ± 3.03	56.25 ± 7.09
62.50	60.34 ± 8.12	57.09 ± 3.52	57.97 ± 2.92	59.57 ± 0.87	59.00 ± 5.14
125	62.37 ± 5.58	61.80 ± 2.92	60.80 ± 3.14	62.23 ± 2.44	60.88 ± 2.71
250	68.65 ± 5.68	62.60 ± 2.11	61.03 ± 3.03	66.47 ± 3.36	63.37 ± 0.38
500	73.51 ± 2.92	69.11 ± 1.46	67.62 ± 3.46	73.47 ± 4.60	83.96 ± 2.65

TABLE-5
TABLE SHOWING BIOACTIVE MOLECULES OF *Leucas aspera* AERIAL PART

Peak name	m.f.	m.w.	Retention time	Peak area	Peak area (%)
3-Allyl-6-methoxyphenol	$C_{10}H_{12}O_2$	164	18.89	948688	0.2614
4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180	24.21	5582139	1.5383
Hydroquinone	$C_6H_6O_2$	110	17.27	113073008	31.1604
Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$	154	16.97	2096691	0.5778
2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	16.27	11297228	3.1133
Phenol, 2-methoxy-	$C_7H_8O_2$	124	11.42	2191624	0.6040
Phenol	C_6H_6O	94	9.60	10921027	3.0096

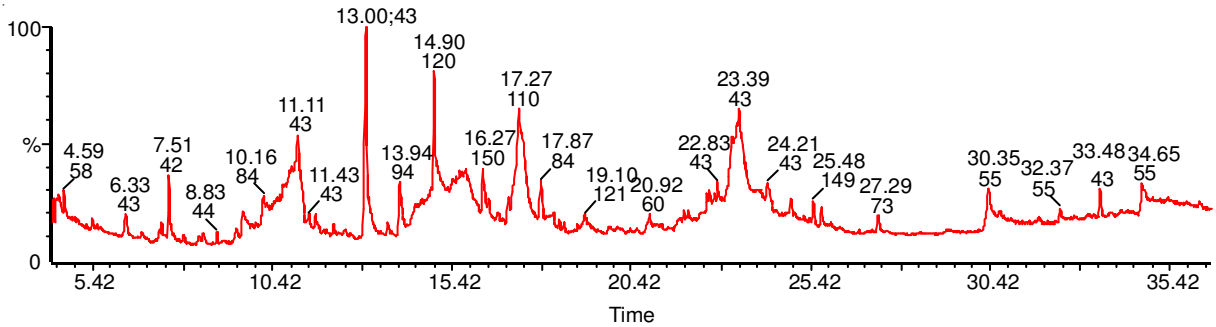

Fig. 1. GCMS total ion chromatogram of *Leucas aspera* Aerial part

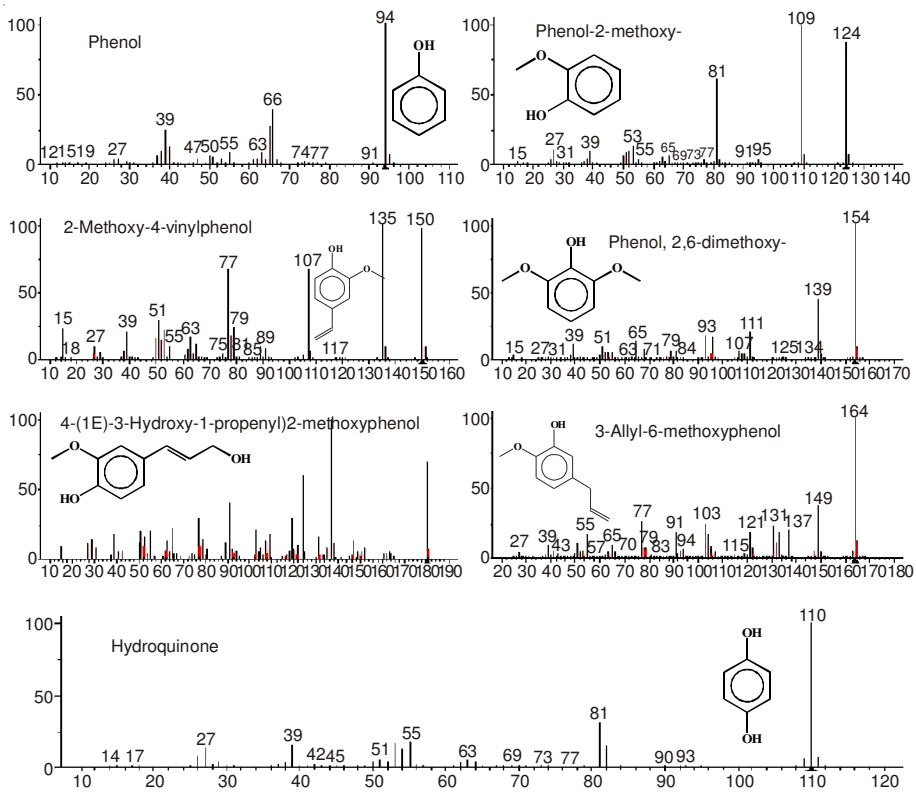

Fig. 2. GCMS spectrum of various compounds identified in *Leucas aspera*

In addition, polyphenolic contents present in extract act as good electron and hydrogen atom donors and thereby terminate the radical chain reaction involved in lipid peroxidation by converting free radicals and reactive oxygen species to become more stable and contribute in adsorbing and neutralizing super oxide ion, hydroxyl radical or peroxy radicals, quenching singlet and triplet oxygen or decomposing peroxides[22,23]. In the present study, the IC_{50} value of different parts of *Leucas aspera* was found to be 15-35 µg/mL. The cytotoxicity effect of *L. aspera* might be due to the presence of hydro-quinone, which is confirmed in GC-MS analysis (Fig. 2). GC-MS analysis of *L. aspera* showed the presence of 31.16 % of hydroquinone. This observation is in agreement with earlier reports by Terasaka *et al.*[24] which states that hydroquinone induces DNA fragmentation, suppression of MnSOD mRNA expression and cytochrome c release and activation of procaspase-3 and -9, but not procaspase-8 in hydroquinone-treated HL-60 cells.

Conclusion

The data obtained through *in vitro* studies depicted that *Leucas aspera* possess significant anti-cancer and anti-oxidant activities.

ACKNOWLEDGEMENTS

The authors are grateful to Honorable Vice-Chancellor, SASTRA University, Thanjvaur, Tamil Nadu for providing the necessary infrastructure and permission for carrying out this study.

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