Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2018, 10(4): 88-92

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Anti-Oxidant Potential and HPLC Analysis of Phytochemical Components in *Leucas Aspera*

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ABSTRACT

Leucas aspera is commonly known as pandharphedha. It is mostly found in all parts of south India and has many medicinal values. Drug from the plants are simply available less pricey, safe and efficiency. The main reason to conduct qualitative and quantitative analysis is to reveal the amount of bioactive components present in the leaves of the plant and the components has the properties to put off a numerous chronic diseases by physiological action on the human body. The chloroform extracts of L. aspera was extracted and subjected to various experiments. Anti-oxidant activity of this plant was performed by DPPH - radical scavenging assay. These results confirm that this plant consists of anti-oxidant property. The final confirmation was done by HPLC. From these in vitro studies it can be concluded that the chloroform extract of L. aspera showed good antioxidant activity.

Keywords: *L aspera*; Chloroform; Anti-oxidant; DPPH; HPLC

INTRODUCTION

Plant materials keep on as a significant part of health care. They also remain as source of many chemical drugs. Studies reported that almost 49% of small molecule chemical entities discovered between 1981-2002 were natural products or synthetic analogues of natural products **[**1**]**. Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value [2]. Medicinal herb is considered to be a chemical factory as it contains multitude of chemical sesquiterpene, lactones and oils (essential and fixed) [3]. Many pharmacognostical and pharmacological investigations are going on to identify new drugs for the development of novel therapeutic agent to treat various human diseases such as cancer and tuberculosis [4]. *Leucas aspera* is widely used to cure many diseased conditions, which connote that *L. aspera* has an infinite capacity for the discovery of new drugs. *Leucas* genus embraces 80 species. The family lamiaceae family is an aromatic herb widely distributed in tropical Asia, Africa, and India and it grows in lands along with the weeds, homesteads, fallow lands and road side. The leaves of *leucas aspera* are yellowish green in color 3-9 cm long, 1-2.5 cm wide ovate or ovate-lamellate, sub-acute more or less pubescent, crenate and serrate, taste pungent.

Leucas aspera an annual, branched herb erecting to a height of 15-60 cm with short and hispid acutely quadrangular stem and branches [5]. In ancient days the plants are the only source for medicine. These plants are recognized for their potency to cure various ailments. *L. aspera* is one among the herbs found momentous due to its overriding medicinal outcomes. Researchers are trying many attempts for producing phytochemicals and also to discover new compounds from this plant to use it in pharmaceutical industry. Phytochemicals are also available in supplementary forms, but the evidence is lacking that they provide the same health benefits as dietary phytochemicals [6]. This plant has been used for antipyretic and insecticide. Parts of the plant are also used for many disorders like rheumatism, psoriasis, and chronic skin eruptions. The leaves of this plant contain sterols, alkaloids, galactose, glycosides, saponins, phytosterols, flavanoids, proteins and amino acids, phenols.

EXPERIMENAL SECTION

Collection and Processing of Plant Samples

Leucas aspera were collected from vellapottal area, Virudhunagar. They were properly washed in distilled water and dried in shade at room temperature of 37°C- 40°C for one week and it was pulverized using mixer grinder to obtain in powdered form. The powdered form is kept in air tight containers, protected from sunlight until required for analysis.

Preparation of Chloroform Extract

The extraction process was done by cold percolation method using chloroform for 24 hours. The sample extracts were filtered by using muslin cloth and Whattman No 1 filter paper. Then the sample was stored at 4°C temperature.

Phytochemical Screening Methods

Phytochemical screening was carried out using chloroform extract for quantitative analysis as per the standard methods.

Detection of Saponins

Froth test:

Extracts were taken in a test tube and it was diluted with 20 ml distilled water then, this was shaken for about 15mins. Formation foam for about 1cm layer inside the test tube indicates the presence of saponins.

Detection of Flavanoids

Leads acetate test:

Few drops of lead acetate solution was added into the sample. Presence of lead acetate was confirmed by formation of yellow color.

Detection of Tannins

Gelatin test:

Extracts were treated with 1% gelatin solution. Presence of tannins was indicated by white color.

Detection of Protein and Amino Acids

Ninhydrin test:

To the extract 0.25% ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

Detection of Carbohydrates

Iodine test: 2 drops of iodine solution added to extract and one or two drops of 0.1N HCl was added. Formation of colored complex indicates the presence of carbohydrates.

Estimating Invitro Anti-Oxidant Activity

The plant extracts were subjected to analyze the antioxidant activity of the plant *leucas aspera.*

Determination of Total Anti-Oxidant Activity

2ml of sample solution was mixed with 25 μg/ml of DPPH solution. The mixture was shaken vigorously and maintained at dark for 30 min. The spectrometer was kept at 517 nm and absorbance was measured. The lower the absorbance of the reaction mixture indicated higher free radical scavenging activity. DPPH free radical was measured using the following equation:

$$\text{Radical scavenging activity (\%)} = 100 - \frac{A_C - A_S}{Ac}$$

Where,

$A_{C=}$ control is the absorbance and

$A_{S\,=}$ sample is the absorbance of reaction mixture (in the presence of sample)

Method of Analysing HPLC

Phenolics Compounds were analyzed by using a HPLC method. The HPLC analysis of Extract was carried out with Chromatographic system (Shimadzu Class-VPV6.14SP2, Japan) consist of auto sampler with 20 µl fixed loop and an UV-Visible detector. The gradient elution of solvent A water-acetic acid (25:1 v/v)] and solvent B (chloroform) had a significant effect on the resolution of compounds. As a result, solvent gradients were formed, using dual pumping system, by varying the proportion of solvent A water-acetic acid (25:1, v/v)] to solvent B (chloroform). Solvent B was increased to 50% in 4 min and subsequently increased to 80% in 10 min at a flow rate of 1.0 mL/min. The samples were made to run for 25 mins and detection was done at 280 nm by UV detector (Lamp-D2). All chromatographic data were recorded and processed using autochro- software.

RESULTS

Preliminary phytochemical screening of chloroform extract of *l. aspera* leaves revealed the presence of various bio active components which include flavanoids, saponins, tannins, proteins and amino acids, phytosterols. The result of phytochemical test (qualitative) has been summarized in tables 1-3 below.

Table 1: Qualitative analysis of *Leucas aspera*

S.no	Phytochemical test	Reagent used(test performed)	Inference	Result
1	Carbohydrate test	Iodine test	Appearance of colored complex	Positive
2	Saponins test	Froth test	Formation of foam	Positive
3	Proteins and amino acid test	Ninhydrin test	Formation of blue color	Positive
4	Tannins test	Gelatin test	Appearance of white precipitate	Positive
5	Flavanoids test	Lead acetate Test	Yellow precipitate	Positive

Total Antioxidant Activity

The antioxidant activity of plant extract at 20, 40, 60 and 80 $\mu g/ml$ was chosen for invitro antioxidant activity and was measured by DPPH method. The total anti-oxidant capacity of extracts of *l. aspera* was calculated using the standard curve of ascorbic acid and is expressed as number of equivalents of ascorbic acid per gram of plant extract(AAE).

Table 2: DPPH radical scavenging activity of extract and Standard

S.NO	Concentrations (µg/ml)	*L.aspera* extract	Ascorbic acid (Standard)
1	20	20.46 ± 1.45	25.6 ± 2.04
2	40	43.19 ± 2.61	51.26 ± 4.90
3	60	61.82 ± 3.81	78.98 ± 7.11
4	80	85.46 ± 5.12	93.34 ± 7.94
	IC_{50}	47.43	39.37

Values are expressed as Mean ± SD for triplicates

DPPH radical scavenging activity of *L. aspera* extract and Standard

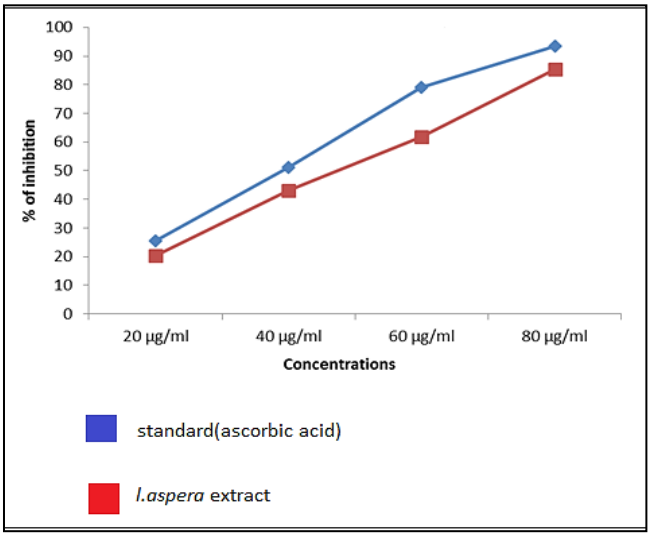

Figure 1: Calibration curve of DPPH

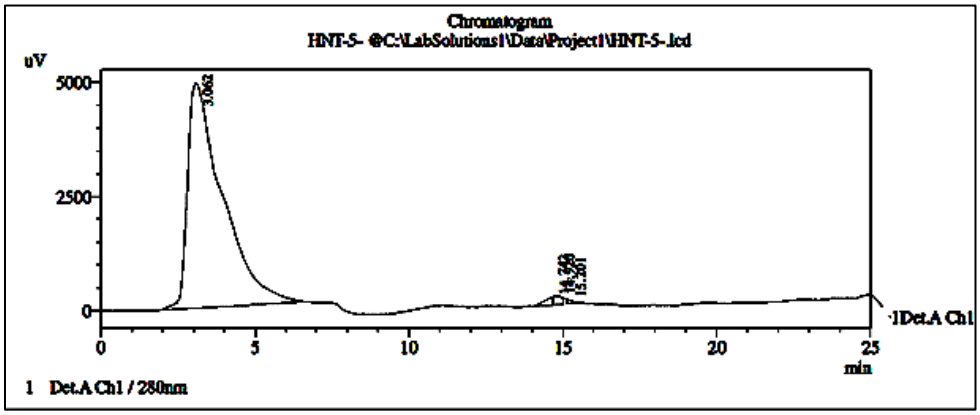

Figure 2: HPLC profile of *L. aspera* extract

Table 3: HPLC profile of *L. aspera* extract

Peak#	Ret. Time	Area	Height	Area %	Height	Compounds identified by literature **
	3.062	390848	4931	97.994	91.348	Quercetin
	14.742	3129	178	0.784	3.297	Delphinidin -3-O-glucoside
	14.92	3786	191	0.949	3.54	Delphinidin -3-O-retinoside
	15.201	1087	98	0.273	1.815	(+)-catechin
Total		398849	5398	100	100	

DISCUSSION

Previous Phytochemical screening revealed the presence of Carbohydrates, Saponins, Flavanoids, Tannins, Proteins and amino acids in chloroform extract of *L. aspera.* These results show that *L. aspera* contains a number of bionutrients which may be responsible for different pharmacological actions [7]. Flavanoids have potent antioxidant properties and it can modulate the various enzymatic activities due to their interaction with various biomolecules [8]. Flavanoids are natural product provide color to flower and fruits, which has major task in appeal of pollinating insects [9].Traditionally *L. aspera* was used as insecticide and antipyretic.. The *invitro* antioxidant activity of *L. aspera* extract was estimated by comparing it to the activity of known antioxidant Ascorbic acid by DPPH radical scavenging assay. DPPH radial scavenging activity was determined by previous study [10-12]. Where Ascorbic acid used as a positive control. According to this study the antioxidant activity was performed and the results were investigated by performing HPLC and we observed that the results were graphically represented which is shown in Figures 1,2 .Quercetin, Delphinidin -3-O-glucoside, Delphinidin -3-O-retinoside and (+)-catechin are the major components present in *L.aspera*.

CONCLUSION

The present investigation has been done to identify the bioactive components which are present in chloroform extract of *L. aspera*, which has the effective antioxidant activity and it was assured by HPLC analysis. Hence from the present experiments it was concluded that the bioactive components of *Leucas aspera*, which are therapeutically used.

ACKNOWLEDGEMENT

Authors are thankful to faculty members of biotechnology division and management of P.S.R. Engineering College. Sivakasi, for providing all the required facilities that helped us for successful completion of our project work.

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