Volume 64, Issue 2, 2020
Journal of Scientific Research
Institute of Science,
Banaras Hindu University, Varanasi, India.

Phytochemical Analysis of *Leucas aspera*(Willd.)Link. from Dibrugarh

J. Chetia* and L. R. Saikia

Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam.
junali.chetia@yahoo.com*, lrsdueco@rediffmail.com

Abstract: **The main aim of the study was to determine the phytoconstituents present in different aerial parts of *Leucas aspera* (Willd.)Link and their antioxidant and antimicrobial activity collected from Dibrugarh, Assam. Acetone extract of young leaves recorded higher (6.00±0.12mgCE/g extract and 3.05±0.04mgQE/g extract respectively) phenol and flavonoid content. Methanol extract of young leaves recorded highest (83.84±0.68% and 88.09±0.04% respectively) antioxidant activity against DPPH and ABTS at 500µl of sample at 1mg/ml of concentration. Acetone extracts recorded higher antimicrobial activity than other extracts of the plant. The different solvent extract recorded antibacterial and antifungal activity. Using GC-MS analysis, hexane broad fraction of inflorescence was analysed for the phytoconstituent present in it. GC-MS analysis was done in Bombay, IIT, SAIF and the analysis revealed the presence of phytoconstituents like-7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (90.0%), Phenol,2,4-bis(1,1-dimethylethyl)-(53.6%), 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl)ester (23.4%), Stigmasta-5,22-dien-3-ol,acetate,(3β)-(23.2%), Spirost-8-en-11-one,3-hydroxy-,(3β,5α,14β,20β,22β,25R)-(22.7%), Neocurdione (17.6%), Isolongifolan-7-ol (15.7%).**

Index Terms: **antimicrobial, antioxidant, GC-MS, inflorescence, phytoconstituents.**

I. Introduction

Leucas aspera (Willd.)Link. is common is Assam and known as Durun bon and distributed all over India (Nadkarni, 1976). It is a perennial herb with woody root stock with hairs, erect and spreading. The young shoots are used as vegetables. The tender fried leaves are said to be good for cough. The plant has tremendous medicinal value as- analgesic, antipyretic, anti-rheumatic, anti-inflammatory, anti-bacterial, anti-fungal, psoriasis, chronic skin eruptions, chronic rheumatism, skin inflammation, painful swellings, stimulant, expectorant, aperients, diaphoretic, insecticide (Anonymous, 1962; Kirtikar *et al*., 1991; Reddy *et al*., 1993; Anonymous, 1994; Chopra *et al*., 1996; Gani, 1998; Srinivas *et al*., 2000; Srinivasan *et al*., 2001; Chopra *et al*., 2002; Gani, 2003; Goudgaon *et al*., 2003; Sadhi *et al*., 2003; Mangathayaru *et al.*, 2005; Kirtikar and Basu, 2005; Sadhu *et al*., 2006; Rahman *et al.*, 2007; Ilango *et al.*,2008; Unni *et al*., 2009; Prajapati *et al*., 2010; Morshed *et al.*, 2011; Shiney Ramya *et al*., 2012). During snake bites, tooth infection, wound and as insect and mosquito repellent, the leaves are used (Shirazi, 1947; Reddy *et al.*, 1993a; Reddy *et al*., 1993b; Reddy *et al*., 1993; Anonymous, 2001; Rai *et al*., 2005; Mangathayaru *et al*., 2006; Mangathayaru *et al*., 2006; Maheswaran *et al*., 2008; Rahmatullah *et al*., 2009; Unni *et al.*, 2009). Different phyto-chemicals have been isolated from the plant by various workers (Mangathayaru *et al*., 2006; Rahman *et al*., 2007; Ilango *et al.*, 2008; Prajapati *et al*., 2010; Srinivasan *et al*., 2011; Morshed *et al.*, 2011; Shiney Ramya *et al*., 2012). Some other experiments on the plant were carried out by, Prasad and Kumar (2012); Unni *et al.*, (2009); Mominul-Islam and Keto-Noguchi, (2012); Mohana *et al*., (2008).

The main aim of the study was to determine the phytochemicals present in different aerial parts of the plant and their antioxidant and antimicrobial activity.

II. Materials and Methods

Samples were collected from Dibrugarh district of Assam. Different aerial parts of flowering twig were separated and cleaned properly and washed under water. The materials were air dried at room temperature. Materials were grounded to fine powder using mortar and pestle and then in electric grinder. Extracts were prepared in five solvents viz- water, methanol, ethanol, acetone and petroleum ether by cold maceration methods. Hot petroleum ether extract was prepared using soxhlet extractor.

* Corresponding Author

The dried extracts were dissolved in DMSO (Dimethyl Sulfoxide) to obtain sample solution at 1mg/ml of concentration. Aqueous extracts were dissolved in distilled water at 1mg/ml of concentration.

A. Qualitative phytochemical analysis

Qualitative analysis for detection of various phytochemical constituents were performed using standard methods.

B. Quantitative phytochemical analysis:

Total phenol content (TPC) of the sample extract was estimated following the method described by Malik and Singh, (1980). The Aluminium chloride method was used for determination of total flavonoid content of the sample extracts as described by Mervat and Hanan, (2009).

C. Antioxidant activity study:

DPPH radical scavenging activity was determined by the method described by Anti-Stanojevic *et al.,* (2009). The ABTS assay was carried out following the method of Re *et al*., (1999). Ascorbic acid was used as standard for antioxidant activity assay.

D. Antimicrobial activity study:

Antimicrobial activity of the bacterial strains was carried out by agar well diffusion method as described by Nair *et al.* (2005) using 6mm borer.

Gram positive and gram negative bacterial strains and fungal strains are used in this experiment to know the antimicrobial activity of the sample extracts.

a) Gram positive bacterial strains- *Bacillus subtilis* (MTCC 441), *Bacillus cereus* (MTCC 8750), *Staphylococcus aureus* (MTCC 3160), *Staphylococcus epidermis* (MTCC 3615) and *Proteus vulgaris* (MTCC 744).

b) Gram negative bacterial strains- *Escherichia coli* (MTCC 443), *Enterococcus faecalis* (MTCC 439).

c) Fungal strains- *Candida albicans* (MTCC 3017) and *Penicillium chrysogenum* (MTCC 947).

Strains were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The reference of bacterial strains were maintained on nutrient agar slants and fungal strains on PDA slants and stored in freeze. Strains were regularly sub-cultured using nutrient broth for bacterial strains and Potato Dextrose Broth for fungal strains.

E. GC-MS analysis:

50 gm of methanol extract was taken in a conical flask and broad fractionation was done using magnetic stir with the simultaneous use of solvents- hexane, chloroform, acetone, ethyl acetate and methanol. These fractions were kept separately for further work.

The hexane broad fraction showing clear bands in TLC is selected for characterization by GC-MS analysis. The analysis was done in Bombay, IIT, SAIF. A Shimadzu 17A gas chromatograph coupled with a Shimadzu QP-5000 quadrupole mass spectrophotometer and Varian 3800 gas chromatograph coupled with detector was used. Compounds were recognised using comparison of their mass spectra with the Wiley GC-MS library and Adams Library (65, 66). Using Fisons GC 8000 series chromatograph with flame ionization detector equipped with non-polar ZB-5 column (30m×0.25mm×0.25µm).

III. Results and Discussion

Table 1 presents the results of qualitative phytochemical test of different parts of *L. aspera*. Tannins, flavonoids, glycosides, cardiac glycosides, saponins, alkaloids, reducing sugar, phenols are recorded in different parts of the plant while terpenoids are not recorded in flower. In some earlier studies, Ilango *et al.,* (2008); Morshed *et al.,* (2011); Shiney ramya *et al.,* (2012); Rahman and Islam, (2013); Latha *et al*., (2013); Saravanasingh *et al.,* (2016) recorded the presence of these phytochemicals in the plant. Those studies were conducted from different places of our country like- Kattankulathur, Kerala, Chennai, Tamil Nadu. The variation in presence and absence of these phytochemicals might be due to the climatic and soil condition of the study area.

The quantitative analysis was performed for total phenol and flavonoid content of different parts of *L. aspera* and the results are presented in Table 2. Acetone extract of young leaves (6.00±0.12 mgCE/g extract) recorded highest phenol content than other extracts of the plant at 1mg/ml of concentration. Extracts recorded good amount of phenol content are, water extract of flower (4.28±0.00 mgCE/g extract); methanol extract of young leaves (4.94±0.00 mgCE/g extract) and inflorescence (5.68±0.97 mgCE/g extract); acetone extract of mature leaves (4.08±0.18 mgCE/g extract). Methanol extract of mature leaves (3.32±0.95 mgQE/g extract) and acetone extract of young leaves (3.05±0.04 mgQE/g extract) recorded higher flavonoid content than other extracts of the plant. Ethanol extract of stem showed lowest phenol and flavonoid content (1.03±0.00 mgCE/g extract and 0.70±0.00 mgQE/g extract respectively). Latha *et al*., (2013) recorded phenolic content of ethanol extract of the plant as 15.36 ±0.512 GAE/g of dry weight. The amount of phenol and flavonoid content present in plant may vary due to the extraction power of the solvent used for the study.

Table 3 presents the antioxidant activity study of sample extracts of *L. aspera*. Methanol extract of mature leaves (84.30±0.24%) and young leaves (88.09±0.04%) recorded highest inhibition against DPPH and ABTS respectively. The extracts showed antioxidant inhibition at 500µl of sample at 1mg/ml of concentration. Higher inhibition against DPPH was also recorded by water extract of young and mature leaves (82.00±0.00% and 81.12±0.12% respectively); methanol extract of young leaves (83.84±0.68%). Against ABTS, water extract of young leaves and flower (87.65±0.80% and 83.47±0.40% respectively) and ethanol extract of stem (83.86±0.00%) recorded higher inhibition than

other extracts of the plant. Latha *et al*., (2013) recorded antioxidant content of ethanol extract of the plant as 190.00±7.95mg/g of dry weight extract. Chew *et al.,* (2012) from Malaysia showed that methanol extract of all the parts of the plant have antioxidant activity at a concentration of 2mg/ml. Flower, leaf and stem extracts have inhibition like- 26.39±0.07%, 17.04±0.82% and 13.42±0.56% respectively. According to Rahman and Islam (2013), ethanolic extract have a percentage of (86.62±0.49)% as compared to ascorbic acid (97.34±1.22)% at 800µg/ml of concentration and have IC_{50} of 99.58±1.62 µg/ml and 1.25±0.21 µg/ml respectively. Morshed *et al.,* (2011) showed that flower, leaf and stem extracts exhibited scavenging activity as 26.39±0.07%, 17.04±0.82% and 13.42±0.56% respectively as compared to BHT (65.67±0.58%) and vitamin E (41.67±0.58%). The difference in antioxidant activity against DPPH and ABTS, may be due to the methods and chemicals used for the study.

The results of antimicrobial activity study of the sample extracts of *L. aspera* are presented in Table 4. Acetone extract of young leaves against *S. aureus* (12±2mm), *E. coli* (14±0mm), *E. faecalis* (11±3mm), *P. vulgaris* (14±4mm) have higher inhibition than the other extracts of young leaves. Various extracts of mature leaves, methanol extract against *P. chrysogenum* (14±2mm), acetone extract against *E. coli* (12±0mm), *E. faecalis* (12±0mm), *P. vulgaris* (14±2mm); petroleum ether extract against *S. aureus* (16±3mm), *E. coli* (12±0mm) and hot petroleum ether extract against *P. vulgaris* (12±1mm) recorded good inhibition than the other extracts of mature leaves. Methanol extract against *E. faecalis* (15±3mm) and petroleum ether extract against *B. cereus* (12±2mm), *E. coli* (12±1mm), *E. faecalis* (18±4mm) of inflorescence recorded higher inhibition than other extracts of the plant. Water extract of flower against *S. epidermis* (12±4mm), acetone extract against *B. cereus* (12±1mm), *S. epidermis* (14±2mm), *E. coli* (14±2mm), *C. albicans* (17±3mm); petroleum ether extract against *B. cereus* (12±2mm), *S. aureus* (16±4mm), *S. epidermis* (18±2mm), *E. faecalis* (19±1mm) revealed good inhibition. Methanol extract of stem against *S. epidermis* (16±2mm), *E. coli* (12±1mm), *P. vulgaris* (12±2mm), acetone extract against *B. subtilis* (12±1mm), *E. coli* (14±0mm), *E. faecalis* (14±2mm), *P. vulgaris* (14±2mm) and *C. albicans* (14±0mm) recorded higher inhibition than other extracts of the plant. Extracts from mature leaves, flower and stem of the plant recorded some good inhibition against fungi. Chew *et al.,* (2012) showed that ethanolic extract of flower, stem and leaves have inhibition like- 7.0±0.7mm, 0.0±0.0 and 0.0±0.0 respectively against *E. coli* and 7.0±0.6mm, 7.0±0.4mm and 7.0±0.5mm respectively against *S. aureus* at a concentration of 100mg/ml which was lower than the standard antibiotic tetracycline. Rahman and Islam, (2013) revealed that the extract from the plant have inhibition against *B. subtilis* (12.00±1.32mm), *B. cereus* (7.00±1.01mm), *S. aureus* (8.00±0.50mm), *E. coli* (7.00±0.75mm). Methanol and ethanol extract of leaf exhibited antibacterial activity against *Shigella* sp., *Salmonella typhi and Escherichia coli* (Shiney ramya *et al*., 2012). Ethyl acetate extract and methanol extracts recorded antibacterial activity against the selected bacteria at different concentrations (Ilango *et al.,* 2008). Unni *et al.,* (2009) recorded antibacterial activity of water extract as 30mm of zone of inhibition against *E. coli*. Morshed *et al.,* (2011) recorded that n-hexane, ethyl acetate and ethanol extract did not show any antimicrobial activity against 14 strains of micro-organisms using Disc-diffusion method at 500µg/ml of concentration. Srinivasan *et al.,* (2001) recorded antibacterial activity against *S. aureus*, *E. coli* and *P. aeruginosa.* Babu *et al.,* (2016) carried out antifungal activities of methanol leaf extracts of *Leucas aspera* and recorded inhibition against *Candida albicans* and *Penicillium spp.* along with some other fungal strains. In the present study, flowers of *L. aspera* recorded antimicrobial activity. Acetone, cold and hot petroleum ether extracts of flowers of the plant recorded higher inhibition than water, methanol and ethanol extracts. Mangathayaru *et al.,* (2005) recorded antibacterial activity of methanol extract of flower of the plant. Sabri and Vimala, (2018) recorded antimicrobial activity of ethyl acetate extract of flowers of *L. aspera*. The antimicrobial activity of *L. aspera* flowers is due to the presence of phenolic content like tannins, which have antimicrobial inhibition against pathogens. According to Scalbert, (1991) tannins are responsible for the antimicrobial inhibition.

The antimicrobial activity of cold and hot petroleum ether extract recorded difference in their inhibition against bacterial strains. Sharma *et al.,* (2013) recorded that ethanol extract have more activity than the water extracts, which may be due to the hot extraction of ethanol using soxhlet apparatus. Zalazare *et al.,* (2018) recorded that ethanol and hot water extracts contained higher bioactive substances than cold water extracts of the tested mushrooms. Variable antimicrobial activity was observed in cold and hot water extracts against tested bacterial pathogens (Nwachukwa and Ozoeto, 2010; Khan *et al*., 2013; Nagananda and Satishchandra, 2013; Samie *et al*., 2017). According to some other workers, activity of samples also may vary with temperature (Wang, 1985; Su and Qiao, 1989; Zhu 1998; Chan *et al*., 2008). Traub and Leonhard, (1995) showed that out of 62 types of antimicrobial material, 25 types were found stable after the heat treatment which is very essential for antimicrobial agents of foods. This kind of differences occurs may be due to the age of the plant, the time of harvest of the material, method of extraction or may be the thermo-sensitivity of the active compounds.

Table 1. Phytochemical analysis of *Leucas aspera* (Willd.)Link.

Sample	**Tannins**	**Phlobatannins**	**Flavonoids**	**Terpenoids**	**Steroids**	**Glycosides**	**Cardiac Glycosides**	**Saponins**	**Anthraquinones**	**Free Anthraquinones**	**Carotenoids**	**Alkaloids**	**Reducing Sugar**	**Phenols**
Young Leaf	+	-	+	+	-	+	+	+	-	-	-	+	+	+
Mature Leaf	+	-	+	+	-	+	+	+	-	-	-	+	+	+
Inflorescence	+	-	+	+	-	+	+	+	-	-	-	+	+	+
Flower	+	-	+	-	-	+	+	+	-	-	-	+	+	+
Stem	+	-	+	+	-	+	+	+	-	-	-	+	+	+

Table 2. Quantitative estimation for total phenol and total flavonoid content of sample extracts of different parts of *Leucas aspera* (Willd.) Link.

Sample (mg/ml) ↓	**Total phenol content (mg catechol equivalent/gm dry extract)**					**Total flavonoid content (mg quercetin equivalent/gm dry extract)**				
	Water Extract	**Methanol extract**	**Ethanol extract**	**Acetone extract**	**Petroleum ether extract**	**Water Extract**	**Methanol extract**	**Ethanol extract**	**Acetone extract**	**Petroleum ether extract**
Young leaf	3.62 ±0.16	4.94 ±0.00	3.76 ±0.00	6.00 ±0.12	2.01 ±0.63	1.78 ±0.14	2.44 ±0.00	2.56 ±0.00	3.05 ±0.04	2.31 ±0.99
Mature leaf	1.62 ±0.00	3.54 ±0.06	2.88 ±0.00	4.08 ±0.18	1.34 ±0.00	1.67 ±0.00	3.32 ±0.95	2.73 ±0.00	2.54 ±0.00	2.36 ±0.00
Inflorescence	2.14 ±1.14	5.68 ±0.97	1.77 ±0.07	2.10 ±0.05	2.17 ±0.00	1.54 ±0.00	1.77 ±0.00	1.19 ±0.00	2.25 ±0.06	1.40 ±0.00
Flower	4.28 ±0.00	2.85 ±0.00	2.10 ±0.00	1.89 ±0.00	1.64 ±0.00	1.28 ±0.00	2.08 ±0.00	1.39 ±0.00	1.35 ±0.04	1.68 ±0.00
Stem	2.33 ±0.52	3.87 ±0.00	1.03 ±0.00	1.34 ±0.02	1.86 ±0.00	1.19 ±0.06	2.82 ±0.00	0.70 ±0.00	1.23 ±0.02	2.29 ±0.00

Table 3. Anti-oxidant activity study of sample extracts of different parts of *Leucas aspera* (Willd.) Link.

Sample (500µl) ↓	**DPPH radical scavenging activity (% inhibition in mg/ml)**					**ABTS radical scavenging activity (% inhibition in mg/ml)**				
	Water Extract	**Methanol extract**	**Ethanol extract**	**Acetone extract**	**Petroleum ether extract**	**Water Extract**	**Methanol extract**	**Ethanol extract**	**Acetone extract**	**Petroleum ether extract**
Young leaf	82.00 ±0.00	83.84 ±0.68	65.34 ±0.00	73.56 ±0.00	65.10 ±0.00	87.65 ±0.80	88.09 ±0.04	68.26 ±0.55	76.21 ±2.16	68.37 ±0.00

Mature leaf	81.12 ±0.12	84.30 ±0.24	69.99 ±1.75	78.81 ±0.94	69.42 ±0.00	75.38 ±3.28	74.04 0.00	78.63 ±0.00	77.75 ±2.65	63.70 ±1.08
Inflorescence	60.14 ±0.00	68.58 ±0.78	70.13 ±0.00	78.99 ±1.34	67.14 ±0.00	79.57 ±0.34	75.12 ±0.01	75.26 ±0.01	78.54 ±0.00	58.97 ±1.57
Flower	40.14 ±0.00	43.41 ±0.00	62.16 ±0.00	72.78 ±0.00	61.13 ±0.00	83.47 ±0.40	71.19 ±4.98	71.88 ±0.07	76.66 ±0.00	57.10 ±0.62
Stem	36.67 ±0.00	43.00 ±0.00	50.62 ±1.50	52.98 ±1.15	49.00 ±0.00	58.11 ±1.10	76.79 ±5.56	83.86 ±0.00	70.65 ±0.44	51.16 ±2.97
Ascorbic acid	90.28 ±0.02					89.00 ±0.00				

Table 4 Antimicrobial activity study of the sample extracts of different parts of *Leucas aspera* (Willd.) Link.

Sample	Extracts (mg/ml)	**Diameter of Zone of Inhibition (mm)**								
		Bacterial strains							**Fungal strains**	
		B. subtilis	***B. cereus***	***S. aureus***	***S. epidermis***	***E. coli***	***E. faecalis***	***P. vulgaris***	***C. albicans***	***P. chrysogenum***
Young Leaves	Water Extracts	-	8±0	-	8±2	-	-	-	-	-
	Methanol Extract	-	8±1	-	8±0	-	-	9±1	-	-
	Ethanol Extract	-	8±0	8±1	-	-	-	8±2	-	-
	Acetone Extract	10±1	-	12±2	10±2	14±0	11±3	14±4	-	-
	Petroleum Ether Extract	-	8±0	8±1	8±0	-	8±2	8±0	-	-
	Hot Petroleum Ether extract	8±0	10±2	-	8±1	-	8±1	10±2	-	-
Mature Leaves	Water Extracts	-	-	-	-	-	-	-	-	-
	Methanol Extract	-	-	10±2	8±1	-	-	8±0	-	14±2
	Ethanol Extract	-	8±1	-	-	-	-	8±0	-	10±1
	Acetone Extract	8±0	-	8±0	8±0	12±0	12±0	14±2	8±0	10±1
	Petroleum Ether Extract	-	8±2	16±3	-	12±0	-	-	-	-
	Hot Petroleum Ether Extract	-	8±1	10±2	-	9±1	-	12±1	-	-
Inflorescence	Water Extracts	10±2	-	-	-	-	-	-	-	-
	Methanol Extract	-	10±2	10±2	10±0	10±2	15±3	8±0	-	-
	Ethanol Extract	-	-	8±1	8±0	-	8±1	10±1	-	-
	Acetone Extract	8±0	8±0	8±0	8±0	-	-	10±2	8±0	-
	Petroleum Ether Extract	-	12±2	9±2	8±0	12±1	18±4	-	-	-
	Hot Petroleum Ether Extract	-	8±0	8±1	10±2	9±1	10±2	8±0	-	-
Flower	Water Extracts	-	8±0	-	12±4	-	-	8±0	-	-
	Methanol Extract	-	8±1	-	10±1	-	-	-	-	-
	Ethanol Extract	-	8±0	-	8±0	-	-	-	-	-
	Acetone Extract	8±1	12±1	8±2	14±2	14±2	-	10±2	17±3	-

	Petroleum Ether Extract	-	12±2	16±4	18±2	10±1	19±1	8±0	-	-
	Hot Petroleum Ether Extract	10±1	8±0	10±2	8±1	-	-	8±0	-	-
Stem	Water Extracts	10±1	8±0	-	-	10±2	-	-	-	-
	Methanol Extract	10±1	10±1	8±0	16±2	12±1	8±1	12±2	-	-
	Ethanol Extract	-	-	8±0	-	-	-	8±1	-	-
	Acetone Extract	12±1	8±0	8±1	10±1	14±0	14±2	14±2	14±0	-
	Petroleum Ether Extract	10±2	8±0	-	-	-	-	-	-	-
	Hot Petroleum Ether Extract	8±0	-	-	-	-	-	-	-	-
Ampicillin (10mcg)		-	-	-	-	12±2	10±1	10±0	-	46±0

Diameter of the cork borer=6mm, '-' indicates no inhibition

Table 5: Compounds identified in GC-MS analysis

Sl. No.	**Name of the compounds**	**% Propability**	**Chemical formula**
1	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	90.0%	$C_{17}H_{24}O$
2	Phenol,2,4-bis(1,1-dimethylethyl)-	53.6%	$C_{14}H_{22}O$
3	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl)ester	23.4%	$C_{16}H_{22}O_4$
4	Stigmasta-5,22-dien-3-ol,acetate,(3β)-	23.2%	$C_{31}H_{50}O_2$
5	Spirost-8-en-11-one,3-hydroxy-,(3β,5α,14β,20β,22β,25R)-	22.7%	$C_{27}H_{40}O_4$
6	Neocurdione	17.6%	$C_{15}H_{24}O_2$
7	Isolongifolan-7-ol	15.7%	$C_{15}H_{26}O$
8	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	15.0%	$C_{20}H_{40}O$

IV. STATISTICAL ANALYSIS

All the experiments were done in triplicate and mean and SD was calculated and are presented in ± form.

CONCLUSION

The phytochemicals present in the plant are responsible for its antioxidant and antimicrobial activity. Different parts of the plant having antimicrobial activity can be used in medicinal practices instead of using the whole plant at a time.

ACKNOWLEDGEMENT

I like to acknowledge Department of Life Sciences, Dibrugarh University for providing me the necessary laboratory facilities. I also like to acknowledge DST, for helping financially in the form of INSPIRE fellowship.

REFERENCES

Aja, P.M., Okaka, A.N.A., Onu, P.N., Ibiam, U. & Urako, A.J. (2010). Phytochemical composition of *Talinum Triangulare* (water leaf) Leaves. Pakistan Journal of Nutrition, 9(6), 527-530.

Ajayi, I.A., Ajibade, O. & Oderinde, R.A. (2011). Preliminary Phytochemical analysis of some plant seeds. Research Journal of Chemical Sciences, 1(3), 58-62.

Ajiboye, B.O., Ibukun, E.O., Edobog, G., Ojo, A.O. & Onikanni, S.A. (2013). Qualitative and quantitative analysis of phytochemicals in *Senecio biafrae* leaf. International Journal of inventions in Pharmaceutical Sciences, 1(5), 428-432.

Anonymous, (1962). The Wealth of India- Raw Materials, Vol-VI, Publication and Information Directorate, CSIR, New Delhi. 79.

Anonymous, (1994). The Useful Plants of India, Publication and Information Directorate, CSIR, New Delhi. 326.

Anonymous, (2001). In: Wealth of India (Raw Materials), Vol.4, publications and information Directorate, CSIR, New Delhi. 36.

Anti-Stanojevic, L., Stanojevic, M., Nikolic, V., Nikolic, L., Ristic, J., Canadanovic, & Brunet, V. (2009). Antioxidant activity and total phenolic and Flavonoid contents of *Hieracium Pilosella* L extracts. Sensors, 9, 5702-5714.

Chew, A.L., Jessica, J.J.A. & Sasidharan, S. (2012). Antioxidant and antimicrobial activity of different parts of *Leucas aspera*. Asian pacific journal of Tropical Biomedicine, 2(3), 176-180.

Chitravadivu, C., Manian, S. & Kalaichelvi, K. (2009). Qualitative Analysis of Selected Medicinal Plants, Tamilnadu, India. Middle-East Journal of Scientific Research, 4 (3), 144-146.

Chopra, R.N., Nayar, S.L. & Chopra, I.C. (2002). In: Glossary of Indian Medicinal Plants, NISCAIR, CSIR, New Delhi, 153.

Chopra, R.N, Nayar, S.L. & Chopra, I.C. (1996). Glossary of Indian Medicinal Plants. NISC, New Delhi, pp. 153.

Chopra, R.N., Nayar, S.L. & Chopra, I.C. (2002). In: Glossary of Indian Medicinal Plants, NISCAIR, CSIR, New Delhi.

De, C., M.G.I., Rehecho, S., Calvo, M.I., Cavero, R.Y., Navarro, I., Astiasaran, I. & Ansorena, D. (2010**)**. Effect of lyophilized water extracts of *Melissa officinalis* on the stability of algae and linseed oil-in-water emulsion to be used as a functional ingredient in meat products. Meat Science, 85, 373–377.

Edeoga, H.O., Okwn, O.E. & Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. African journal of biotechnology, 4 (7), 685-688.

Egwaikhide, P.A. & Gimba, C.E. (2007). Analysis of phytochemical content and anti-microbial activity of *Plectranthus glandulosis* whole plant. Middle –East. Journal of Scientific Research, 2(3-4), 135-138.

Gani, A. (1998). Medicinal plants of Bangladesh: Chemical constituents and uses. Pp.467.

Gani, A. (2003). Medicinal Plants of Bangladesh, Chemical Constituents and Uses. Asiatic Society of Bangladesh, Dhaka. 215—216.

Goudgaon, N.M., Basavaraj, N.R. & Vijayalaxmi, A. (2003). Anti-inflammatory activity of different fractions of *Leucas aspera* Spreng. Indian Journal of Pharmacology, 35, 397-398

Ilango, K., Ramya, S. & Gopinath, G. (2008). Antibacterial activity of *Leucas aspera* spreng. International Journal of Chemical Science, 6(2),526-530.

Iyenger, M.A. (1995). Study of Crude Drugs. 8th edn. Manipal Power Press. Manipal, India. 2.

Kirtikar, K.R. & Basu, B.D. (1991). Indian medicinal plants, Singh B and Singh M.P. Publishers, Vol. 3.

Kirtikar, K.R. & Basu, B.D. (2005). Indian Medicinal Plants, Vol III, International Book Distributor, Dehradun, India: 2019.

Latha, B., Rumaisa, Y., Soumya, C.K. & Shahul, S. (2013). Phytochemical studies on *Leucas aspera*. 5(4), 222-228.

Majaw, S. & Moirangthem, J. (2009). Qualitative and Quantative analysis of some *Clerodendron colebrookianum* Walp. leaves and *Zingiber cassumunar* Ronb. Rhizomes. Ethno botanical leaflets, 13, 578 – 89.

Mangathayaru, K., Lakshmikant, J., Shyam, S.N., Swapna, R., Grace, X.F. & Vasantha, J. (2005). Antimicrobial activity of *Leucas aspera* flowers. Fitoterapia, 76, 752–754.

Mangathayaru, K., Thirumurugan, D., Patel, P.S., Pratap, D.V., David, D.J. & Kanthikeyan, J. (2006). Isolation and identification of nicotine from Leucas aspera (Willd.) Link. Indian Journal of Pharmaceutical sciences, 68,88-90.

Maheswaran, R., Sathish, S. & Ignachimuthu, (2008). Larvicidal activity of *Leuca aspera* (Willd.) against the larvae of *Culex quinquefasciatus* Say. and *Aedes aegypti* L. International journal of integrative Biology, 2, 214-217.

Mervat, M.M.E.I.F. & Hanan, A.A.T. (2009). Antioxidant activities total anthrocyanine, phenolics and flavonoids content of some sweet potato genotypes under stress of different concentrations of sucrose and sorbitol. Australian Journal of Basic Applied Science, 3, 3609-3616.

Mohana, D.C., Satish, S. & Raveesha, K.A. (2008). Antibacterial Evaluation of Some Plant Extracts against Some Human Pathogenic Bacteria. Advances in Biological Research, 2(3-4), 49-55.

Mominul-Islam, A.K.M. & Kato-Noguchi, H. (2012). Allelopathic potentiality of Medicinal plant *Leucas aspera*. International journal of Sustainable agriculture, 4(1),01-07.

Morshed, M.A., Uddin, A., Saifur, R., Barua, A. & Haque, A. (2011). Evaluation of antimicrobial and cytotoxic properties of *Leucas aspera* and *Spilenthes paniculata*. International Journal of Biosciences, 1(2), 7-16.

Malik, E.P. & Singh, M.P. (1980). Plant Enzymology and Hittoenzymology. Kalyani publishers, New Delhi, pp. 286.

Nadkarni, K.M. (1976). Nimbah. In Nadkarni (ed.), Indian Materia Medica Vol. I. 3rd edn. Bombay and Popular Prakashan Pvt. Ltd., Bombay, India, pp. 323–325.

Nair, R., Kalariya, T. & Chanda, S. (2005). Antibacterial activity of some selected Indian medicinal flora. Turkish Journal of Biology, 29, 41-47.

National Committee for Clinical Laboratory Standards (NCCLS) (1993). Performance standards for antimicrobial disk susceptibility tests. Approved standard. NCCLS document M2-A5. Wayne, Pa: National Committee for Clinical Laboratory Standards.

Prajapati, M.S., Patel, J.B., Modi, K. & Shah, M.B. (2010). *Leucas aspera:* A review. Pharmacognosy Review, 4, 85-7.

Prasad, E.R. & Kumar, P.S. (2012). Co-relation between Hymenopterans and *Leucas spp*. (Lamiaceae) of South India. International journal of plant, animal and environmental sciences, 2(4), 253-256.

Rahman, M.A. & Islam, M.S. (2013). Antioxidant, antibacterial and cytotoxic effects of the phytochemicals of whole *Leucas aspera* extract. Asian Pacific Journal of Tropical Biomedicine, 3(4), 273-279.

Rahman, M.S., Sadhu, S.K. & Hasan, C.M. (2007). Preliminary antinociceptive, antioxidant andcytotoxic activities of *Leucas aspera* root. Fitoterapia,78, 552-555.

Rahmatullah, M., Das, A.K., Mollik, M.A.H., Jahan, R., Khan, M., Rahman, T. & Chowdhury, M.H. (2009). An ethnomedicinal survey of Dhamrai sub district of Dhaka district, Bangladesh. American-Eurasian journal of sustainable agriculture, 3, 881-888.

Rai, V., Agarwal, M., Agnihotri, A.K., Khatoon, S., Rawat, A.K. & Mehrotra, S. (2005). Pharmacological evaluation of *Leucas aspera*. Natural Product sciences, 11, 109-114

Re, R., Pelleorini, N., Proteggente, A., Pannala, A., Yang, M. & Rice Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free radical Biology and Medicine, 26, 1231-1237.

Reddy, M.K., Viswananthan, S., Thirugnanasambatham, D., Santa, R. & Lalitha, K. (1993a). Analgesic activity of *Leucas aspera*. Fitoterapia, 64, 151-154.

Reddy, M.K., Viswananthan, S., Thirugnanasambatham, D., Santa, R. & Lalitha, K. (1993b). Effect of *Leucas aspera* on snake venom poisoning in mice and its possible mechanism of action. Fitoterapia, 64, 442-446

Sabri, G. &Vimala, Y. (2018). Antibacterial and antioxidant activity of *Leucas aspera* flowers from Bihar, India. Asian Journal of Pharmaceutical and Clinical Research,11(2),223-226.

Sadhu, S.K., Okuyama, E., Fujimoto, H. & Ishibashi, M. (2006). Diterpenes from *Leucas aspera* inhibiting prostaglandin-induced contractions. Journal of Natural Products, 69, 988–994.

Sadhu, S.K., Okuyama, E., Fujimoto, H. & Ishibashi, M. (2003). Separation of *Leucas aspera*, a medicinal plant of Bangladesh, guided by prostaglandin inhibitory and antioxidant activities. Chemical and Pharmaceutical Bulletin, 5, 595-598.

Saravanasingh, K. & Ramamuthy, M. (2016). Investigation of antibacterial activity of medicinal plants against wound pathogens. International Journal of Current Research in Chemistry and Pharmaceutical sciences, 3(1), 50-59.

Schlumbaum, A., Mauch, F., Vogeli, U. & Boller, T. (1986). Plant chitinases are potent inhibitors of fungal growth. Nature, 324, 365-367.

Shiney ramya, B., Ganesh, P. & Suresh Kumar, R. (2012). Phytochemical Screening of *Coleus aromaticus* and *Leucas aspera* and Their Antibacterial Activity against Enteric Pathogens. International Journal of Pharmaceutical and Biological Archives, 3(1), 162-166.

Shirazi, A.M. (1947). Studies on *Leucas aspera*. Indian Journal of Pharmaceutical Sciences, 9, 116-117.

Siddiqui, A.A. & Ali, M. (1997). *Practical Pharmaceutical chemistry*. Ist ed. CBS publishers and distributors, New Delhi, 126-131.

Srinivas, K., Rao, M.E.B. & Rao, S.S. (2000). Anti-inflammatory activity of *Heliotropium indicum* Linn and *Leucas aspera* Spreng in albino rats. Indian Journal of Pharmacology, 32, 37–38.

Srinivasan, D., Nathan, S., Suresh, T. & Perumalsamy, P.L. (2001). Antimicrocial activity of certain Indian medicinal plants used in folkloric medicine. Journal of Ethnopharmacology,74, 217-220.

Srinivasan, R., Ravali, B., Suvarchala, P., Honey, A., Tejaswini, A. & Neeraja, P. (2011). *Leucas aspera* -medicinal plant: A review. International Journal of Pharma and Bio Sciences, 2, 153-159.

Trease, G.E. & Evans, W.C. (2002). Pharmacognosy. 15th edition. London. Saunders Publisher.

Unni, B.G., Borah, A., Wann, S.B., Singh, H.R., Devi, B. & Bhattacharjee, M. (2009). Phytochemical and antibacterial study of traditional medicinal plants of North- East India on *Escherichia coli*. Asian Journal of Experimental Sciences, 23(1), 103-108.

Wagner, J., Pham, H.L. & Doepke, W. (1996). Alkaloids from *Hippeastrum equestre* Herb. D 5. Circular dichroism studies.Tetrahedron, 52, 6591-6600.

***