RESEARCH ARTICLE

Assessment of Phytochemicals and Antioxidant activities of *Leucas indica* aerial parts- A comparative study

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Manuscript details:

Received: 05.02.2016
Revised: 29.02.2016
Accepted :15.03.2016
Published : 11.04.2016

Editor: Dr. Arvind Chavhan

Cite this article as:
Sowjanya M, Kiran Kumar M and Sandeep BV (2016) Assessment of Phytochemicals and Antioxidant activities of *Leucas indica* aerial parts- A comparative study. *International J. of Life Sciences*, 4(1): 29-43.

ABSTRACT

The exploration was to evaluate the phytochemical ingredients and antioxidant activities of annual herb *Leucas indica* methanolic leaves and flowers extracts. These were estimated by distinguished methods. Phytochemical constituents and non enzymatic antioxidants showed enhancement in increasing concentration from 25 mg/ml to 100 mg/ml. All the assays were carried out in triplicate and the results uttered as mean values ± standard deviations. The levels of phytochemical, explicitly phenolics, flavonoids, tannins, alkaloids, saponins, vitamin C, β-Carotene and lycopene, evidently exposed that methanolic leave extract had elevated phytochemical constituents than methanolic flower extract, except alkaloids. Same pattern was observed for antioxidant capacity assays, specifically DPPH, FRAP, reducing power assay, Hydroxyl radical scavenging activity and ABTS radical scavenging activity, this may be due the presence of high phytochemical ingredients. Even though, leave extract had superior activities than flowers extract, they both have profound therapeutic potential.

Keywords: Antioxidants, ABTS, butylated hydroxy toluene, β-Carotene, phytochemicals, rutin and lycopene.

INTRODUCTION

A complete storehouse of remedies has been provided by nature to cure ailment of mankind (Kumar and Chandrashekar, 2011) one of which includes medicinal plant. Medicinal plants contribute drugs in the form of phytochemcals, which are reported to have various biological activities (Samy *et al.,* 2008). Phytochemcals may minimize the production of reactive oxygen species ROS or it may protect itself from deleterious effect of ROS by efficiently scavenging of ROS. The scavenging system controlling reactive oxygen species (ROS) comprises of antioxidant components (Sharma, 2013). Free radicals act as a trigger to a number of degenerative diseases. Therefore, samples having free radical scavenging activity can be of potent medicinal importance (Chen and Ho, 1995).

Leucas indica (family- Lamiacae) is an annual herb found throughout India as a weed in cultivated fields, wastelands and roadsides. The flowers are given with honey to treat cough and cold in children. The leaves are applied to the bites of serpents, poisonous insects and scorpion sting. *L. indica* leaves are also used as insecticides and mosquito repellent in rural area. The plant extract with honey is a good remedy for stomach pain and indigestion (Madhava Chetty *et al.,* 2008). In the underway effort a comparative cram was conceded out in between, methanolic leave and flower extracts of *Leucas indica* for their phytochemicals and antioxidant activities.

MATERIALS AND METHODS

Plant material:

The Arial parts, leaves and flowers of *Leucas indica* Linn were collected from Marlapudi village, Nellore district, Andhra Pradesh. India, in the month of November – March, 2015. Plant materials were recognized with the help of Gamble, "Flora of the Presidency of Madras" and later verified by comparison with the authentic specimens available in the herbariums of National Botanical Research Institute (NBRI), Lucknow and these plant materials were authenticated by Dr. M. Venkaiah, faculty of Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh. Leaves and flowers of *Leucas indica* were cleaned, shade dried, mechanically grinded. Finally, the coarse powders were separated by sieving and stored in an air tight container for further use.

Preparations of extracts:

Accurately weighed 10 gms of *Leucas indica* leave and flower powder were extracted with 250 ml methanol by stirring at 50^{oC} for 3hr. The extracts were then filtered through whatmann filter paper and the filtrate was concentrated with a vacuum rotary evaporator under low pressure and temperature and stored in desiccator.

Evaluation of Phytochemicals

Estimation of total phenolics

The amount of total phenolics in extracts was determined according to the Folin- Ciocalteu procedure (Javanmardi *et al.,* 2003). Samples (200 µl) were introduced into test tubes. One milliliter of Folin Ciocalteu reagent and 0.8 ml of sodium carbonate (7.5%) were added. The tubes were mixed and allowed to stand for 30 min. Absorption at 765 nm was measured. The total phenolic content was expressed as gallic acid equivalents (GAE) in micrograms per gram of extract as calculated from standard gallic acid graph.

Estimation of total flavonoids

Total flavonoid content of the extract was determined according to a modified colorimetric method (Bao *et al.,* 2005). Test extract (1.0 ml) was mixed with 1ml of distilled water and 75 µl of a 5% $NaNO_2$ solution. After 5 min, 75 µl of 10% $AlCl_3.H_2O$ solution was added. After 5 min, 0.5 ml of 1M Sodium hydroxide was added. The solution was mixed well and kept for 15 min. The increase in absorbance was measured at 510 nm using a UV-Visible spectrophotometer. The total flavonoid content was calculated using standard quercetin calibration curve. The results were expressed as micrograms of quercetin equivalents (QE) per gram of extract.

Estimation of total tannins

The total tannins were determined using the Folins-Ciocalteau method (1927), briefly, 0.1 ml of test extract, 6.5 ml of water and 0.5 ml of Folins-Ciocalteau reagent and 1.5 ml of 20% sodium carbonate at overnight standard solution were added and incubated at 1 h the absorbance of sample was measured in spectrophotometer at 725 nm. The total tannin content was calculated using standard tannic acid calibration curve and the results were expressed as micrograms of tannic acid equivalents per gram of extract.

Estimation of total alkaloids

Total Alkaloid content was estimated by the method of Sreevidya and Mehrotra (2003). A standard solution was prepared by dissolving 5 mg of boldine and test extracts separately in 5 ml of warm distilled water each. Five ml of boldine solution/extract was adjusted to PH 2-2.5 (with 0.01 M HCl), and 2 m of DR (Dragendorff's reagent) was added to form an orange precipitate that was centrifuged at 5000 rpm for 15 min. Afterward, DR was added to the supernatant to check for complete precipitation. 2 ml amount of 1% sodium sulfide was added to the residue to form a brownish black precipitate which was centrifuged at 5000 rpm for 15 min. Complete precipitation was checked by further adding 1% sodium sulfide. The resulting residue was dissolved in 2 ml of nitric acid with warming and sonication and then made up to 10

ml with distilled water.5 ml of 3% thiourea was added to 1 ml of the resulting solution to form a yellow bismuth complex, of which the absorbance was measured at 435 nm. The amount of bismuth present in the boldine solution/extract was achieved from the calibration curve of bismuth nitrate. The results were expressed as boldine, considering that is a monobasic alkaloid, and therefore the complex formed with bismuth follows a 1:1 stoichiometry.

Estimation of Saponins

The method used was that of Obadoni and Ochuko (2001). 25 g, 50g and 100g of samples powder were put into conical flasks separately and 100 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated as percentage.

Estimation of Vitamin C:

Ascorbic acid content was determined by the procedure described previously (Sadasivam and Theymoli, 1987). Briefly, to 5.0 ml of ascorbate solution (10µg per ml), 10 ml of 4% oxalic acid was added and titrated against 0.026% dichlorophenol indophenol. The amount of the dye consumed was equivalent to the amount of ascorbic acid present in the plant extracts. Similar titration was carried out with 5.0 ml of leave and flower extracts alone.

Estimation of β-Carotene and lycopene:

β-Carotene and lycopene were determined according to the method of Nagata and Yamashita (1992). The dried methanolic extract (100 mg) was vigorously shaken with 10 ml of acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm. Contents of β-carotene and lycopene were calculated according to the following equations: lycopene (mg/ 100 ml) = 0.0458 A_{663} + 0.372 A_{505} 0.0806 A_{453}; β-carotene (mg/100 ml) = 0.216 A_{663} 0.304 A_{505} + 0.452 A_{453}. Results were expressed as µg /gram of extract.

Antioxidant capacity assays

Diphenyl picryl hydrazyl radical scavenging Assay (DPPH)

3 ml of test extract was added to 1ml of 0.1 mM solution of DPPH in methanol. After 30 min incubation at 37°C absorbance was measured at 517 nm against control using a spectrophotometer (Cuendet *et al.,* 1997). Rutin and BHT were used as the reference materials. The percentage of inhibition was calculated by comparing the absorbance values of the test samples with those of the controls. The inhibition percentage (I) was calculated as radical scavenging activity as follows

Percentage of inhibition (I) = (Absorbance of Control-Absorbance of Test)/ Absorbance of control × 100

Ferric reducing or antioxidant power assay (FRAP)

The total antioxidant power of the sample was assayed by the method of Benzie and Strain (1996). 3.0 ml of FRAP working reagent was taken in a test tube then 100 µl of test extract was added, this is vortex mixed, and the absorbance was read at 593 nm against a reagent blank at a predetermined time after sample-reagent mixture. The results are expressed as Ascorbic acid equivalents (µ moles/ml) or FRAP units.

Determination of Iron (III) to Iron (II) Reducing Activity (or) reducing power assay

The ability of the extracts to reduce iron (III) was assessed by the method of Oyaizu (1986). One ml of seed extract was mixed with 2.5 ml of 0.2 M phosphate buffer, PH 6.6, and 2.5 ml of 1% aqueous potassium hexacyanoferrate [$K_3Fe(CN_6)$] solution. After 30 min of incubation at 50 °C, 2.5 ml of 10% trichloroacetic acid was added, and the mixture was centrifuged for 10 min. Finally, 2.5 ml of the upper layer was mixed with 2.5 ml of water and 0.5 ml of 0.1% aqueous $FeCl_3$, and the absorbance was recorded at 700 nm using a UV-Visible spectrophotometer. Increasing absorbance at 700 nm was interpreted as increasing reducing activity. The results were expressed as micrograms of ascorbic acid equivalents (AscAE) per gm of extract. Butylated hydroxy toluene (BHT) and ascorbic acid were used as positive controls. Ascorbic acid was used

as the standard control with concentrations 10, 20, 40, 60, 80 and 100 µg/mL.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging was carried out by measuring the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe^{3+}/Ascorbate/EDTA/H_2O_2 system, a method carried out by Gulhan *et al.,* (2003). 0.1 ml of the test extract was added to the reaction mixture containing 0.1ml of 3.0 mM deoxyribose, 0.5 ml of 0.1 mM $FeCl_3$, 0.5 ml of 0.l mM EDTA, 0.5 ml of 0.1mM ascorbic acid, 0.5 ml of 1mM H_2O_2 and 0.8 ml of 20 mM phosphate buffer, PH 7.4, in a final volume of 3.0 ml. The reaction mixture was incubated at 37 ^{o} C for 1 h. The formed thiobarbituric acid reactive substances (TBARS) were measured by adding 1.0 ml of thiobarbituric acid (TBA) and 1.0 m of trichloroacetic acid (TCA) to the test tubes and incubated at 100 ^{o} C for 20 min. After the mixtures were cooled, absorbance was measured at 532 nm against a control containing deoxyribose and buffer. A blank was carried out similar way as the test except test compound. Inhibition (I) of deoxyribose degradation in percent was calculated in the following way.

I= (Absorbance of control –Absorbance of test / Absorbance of control) x100.

ABTS radical scavenging activity

ABTS radical scavenging measurements were performed according to Re *et al.,* (1999) with modifications described previously (Barton´, Fołta and Zachwieja 2005). ABTS radical cation was generated by the interaction of ABTS and $Na_2S_2O_8$. For measurement of sample scavenging activity, 2 ml of ABTS were added to the cuvettes containing the pre-diluted samples (0.15, 0.3, 0.45, 0.6, 1.0 ml extracts with addition of 0.85, 0.7, 0.55, 0.4, and 0 ml (phosphate buffered saline) PBS respectively. The absorbance was measured after 6 minutes at the wavelength of 734 nm. The total antioxidant capacities (TAC) were estimated as (6-hydroxy-2, 5, 7, 8-tetramethyl-2-carboxylic acid) trolox equivalents (TEAC) interpolation to 50% inhibition (TEAC50).

RESULTS AND DISCUSSION

Evaluation of phytochemicals and Antioxidant components

The lofty phytochemicals and antioxidant components in the *Leucas indica* methanolic leave and flower extracts indicate that these bioactive agents might partly be responsible for the folkloric use of the fractions in traditional medicine. All the above assays were carried out in triplicate and the results expressed as mean values ± standard deviations. The levels of phytochemical constituents of analysis mentioned below were at 100 mg/ml. The total phenolic content of leaves was 4.42 ± 0.03µg of gallic acid equivalents g-1 of extract whereas for flowers, it was 2.37 ± 0.05µg of gallic acid equivalents g-1 of extract. Our findings showed in figure: 1(a).

Table: 1 (a): Total phenolic content in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves µg of gallic acid equivalents/gram of extract	Flowers µg of gallic acid equivalents/gram of extract
25	1.59 ± 0.03	0.68 ± 0.02
50	3.70 ± 0.02	1.83 ± 0.04
100	4.42 ± 0.03	2.37 ± 0.05

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	12.970	2	6.485	8.338E3	.000
	Within Groups	.005	6	.001		
	Total	12.975	8			
Flowers	Between Groups	3.867	2	1.934	1.513E3	.000
	Within Groups	.008	6	.001		
	Total	3.875	8			

Each value represents the mean±SD of three replicates

P<0.05 was considered as significant difference

Table: 1 (b): Total flavonoid content in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves µg of quercetin equivalents/gram of extract	Flowers µg of quercetin equivalents/gram of extract
25	7.48 ± 0.20	7.35 ±0.45
50	15.06 ± 0.35	14.53 ± 0.30
100	22.82 ± 0.32	22.40 ± 0.20

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	353.142	2	176.571	1.986E5	.000
	Within Groups	.005	6	.001		
	Total	353.148	8			
Flowers	Between Groups	340.140	2	170.070	1.531E5	.000
	Within Groups	.007	6	.001		
	Total	340.147	8			

Each value represents the mean±SD of three replicates
P<0.05 was considered as significant difference

Table: 1 (c): Total tannin content in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves µg tannic acid equivalents/gram of extract	Flowers µg tannic acid equivalents/gram of extract
25	0.56 ±0.03	0.42 ±0.02
50	0.94 ± 0.03	0.84 ± 0.02
100	1.56 ± 0.02	1.15 ± 0.02

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	1.538	2	.769	1.473E3	.000
	Within Groups	.003	6	.001		
	Total	1.541	8			
Flowers	Between Groups	.833	2	.416	1.171E3	.000
	Within Groups	.002	6	.000		
	Total	.835	8			

Each value represents the mean±SD of three replicates
P<0.05 was considered as significant difference

Phenolics or phenolic acids, intermediates in phenylpropanoid metabolism, play many important roles in plant cells, tissue and organs (Dixon and Paiva 1995). Earlier discoveries have revealed that phenolic compounds are involved in plant development during seed germination and in plant-microbe recognition and signal transduction (Lynn and Chang 1990). Phenolic acids and flavonoids are the most persistent group of plant phenolics that play significant role in plants, for human health and function as reducing agents and as free radicle scavengers (Mamta *et al.,* 2012). Flavonoids were remarkable reactive oxygen

species scavengers and fight continuously against polluted atmosphere. These metabolites were effective in temperature stress, drought situation, freezing injuries of cell membranes and unusual salinity. Flavonoids act as signal molecules to take preventive measures in order to save them from pathogenic microbial attacks (Shirley, 1998).Recent studies have reaffirmed the link between flavonoids and plant architecture by showing that flavonoid-defective mutants display a wide range of alterations to root and shoot development (Buer *et al.,* 2009). Flavonoids have recently been implicated in the anti-venom protease activity of some Nigerian tropical plants (Ibrahim *et al.,* 2011). These large groups of compounds serve as UV protectants (Schmelzer *et al.,* 1988), signal molecules in plant microbe interactions (Long, 1989) and antibiotics in plant defence responses (Lamb *et al.,* 1989). Flavonoids carry out antioxidant action through scavenging or chelating process and are reported to play a preventive role in cancer and heart disease (Middleton *et al.,* 2000). Flavonoids and its related compounds also exhibit inhibition of arachidonic acid peroxidation, which results in reduction of prostaglandin levels thus reducing fever and pain (Baumann *et al.,* 1980). Flavonoids have a protective role during drought stress (Tattini *et al.,* 2004) and help plants to live on soils rich in toxic metals such as aluminium (Barcel´o and Poschenrieder 2002). Epicatechin, quercetin and luteolin types of flavonoids inhibit the development of fluids that result in diarrhoea by targeting intestinal cystic fibrosis membrane conductance regulators (Schuier *et al.,* 2005). Flavonoid content in leaves and flowers assessed as 22.82 ± 0.32 and 22.40 ± 0.20 µg of quercetin equivalents g-1 of extract correspondingly. The outcomes acquired were presented in fig. 1 (b).

Tannins are a group of polymeric phenolic substances capable of tanning leather or precipitating gelatic from solution (Scalbert 1991), causing local tumours (Kapadia *et al.,* 1978), inactivating and killing microorganisms. Some tannins are also known to have strong anticarcinogenic, antioxidant activities (Hausteen, 2005) and anti -HIV agent (Sayeed, 2007). Tannins are reported to exhibit antiviral, antibacterial and antitumor activities and also used as diuretic (Aiyelaagbe and Osamudiamen 2009). Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues and also used for treating intestinal disorders such as diarrhoea and dysentery (Dharmananda, 2003). Tannins complexes proteins, divalents metals, cellulose, hemicellulose, pectin and other carbohydrates (Mahanato *et al.,* 1982). Tannin content of *Leucas indica* leaves and flowers was 1.56 ± 0.02 and 1.15 ± 0.02 µg of tannic acid equivalents g -1 of extract correspondingly, results were put on show in figure: 1(c).

Table: 1 (d): Total alkaloid content in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves µg of boldine equivalents/gram of extract	Flowers µg of boldine equivalents/gram of extract
25	124 ± 4	159 ± 5
50	255 ± 5	291 ± 9
100	392 ± 5	495 ±8

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	107488.222	2	53744.111	2.325E3	.000
	Within Groups	138.667	6	23.111		
	Total	107626.889	8			
Flowers	Between Groups	171624.222	2	85812.111	1.560E3	.000
	Within Groups	330.000	6	55.000		
	Total	171954.222	8			

Each value represents the mean±SD of three replicates

P<0.05 was considered as significant difference

Table: 1(e): Total saponin content in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration Mg	Leaves µg/gram of extract	Flowers µg/gram of extract
25	5.15 ± 0.57	4.63 ± 0.55
50	9.66 ± 0.41	7.87 ± 0.21
100	13.07 ± 0.53	11.27 ± 0.26

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Leaves	Between Groups	94.619	2	47.310	181.975	.000
	Within Groups	1.560	6	.260		
	Total	96.179	8			
Flowers	Between Groups	66.147	2	33.074	240.419	.000
	Within Groups	.825	6	.138		
	Total	66.973	8			

Each value represents the mean±SD of three replicates
P<0.05 was considered as significant difference

Table: 1 (f): Ascorbate content in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves µg/gram of extract	Flowers µg/gram of extract
25	0.158 ± 0.004	0.152 ± 0.007
50	0.295 ± 0.015	0.292 ± 0.012
100	0.481 ± 0.022	0.454 ± 0.015

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Leaves	Between Groups	.157	2	.079	330.269	.000
	Within Groups	.001	6	.000		
	Total	.159	8			
Flowers	Between Groups	.137	2	.068	525.021	.000
	Within Groups	.001	6	.000		
	Total	.138	8			

Each value represents the mean±SD of three replicates
P<0.05 was considered as significant difference

Alkaloid is one of the most diverse groups of secondary metabolites found in living organisms with a wide array of biosynthetic pathways, structural types and even pharmacological activities (Roberts and wink 1998). Recently, alkaloids obtained from plants have been reported to have antiangiogenic activity and these alkaloids may act through different mechanisms to inhibit angiogenesis (Flavia *et al.,* 2013). The biological function of alkaloids is very important and is used in analgesic, antispasmodic and bactericidal activities. Morphine, quinine, ephedrine, nicotine and strychnine are the major types of alkaloids some of these are nacrotic analgesics as well as are anti-tissue agent. Our outcomes specifically alkaloid content of leaves and flowers was 392 ± 5 and 495 ±8 of boldine equivalents g-1of extract respectively. Tabular and graphical representations were in figure: 1(d).

Saponins in *Leucas indica* leaves and flowers are ascertained, as they are glycoside components often referred to as "natural detergent" because of their foamy nature (Seigler, 1998). Saponins have been known to posses both beneficial and deleterious properties depending on its concentration in the

sample (Seigler, 1998). Seigler (1998) reported that saponins have anticarcinogenic properties, immune modulation activities and regulation of cell proliferation as well as health benefits such as inhibition of growth of cancer cells and cholesterol lowering activity. Saponins have been found to be potentially useful for the treatment of hyperglycaemia (Olaleye, 2007). Saponins inhibit Na^+ efflux by the lockage of the entrance of the Na^+ out of the cell. This leads to higher Na^+ concentration in the cells, activating a Na^+-Ca^{2+} anti porter in cardiac muscle. The increase in Ca^{2+} influx through this anti porter strengthens the contractions of heart muscle (Schneider and Woliling, 2004). Antifungal activity of some saponins has been reported (Khan and Srivastava 2009). The estimated saponins in *Leucas indica* leaves and flowers were 13.07 ±0.53 and 11.27 ± 0.26 µg/gram of extract dry matter respectively. Results were put on show in figure: 1(e).

Table: 1 (g): β-Carotene content in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves µg/gram of extract	Flowers µg/gram of extract
25	13.42 ± 0.41	4.57 ± 0.33
50	25.86 ± 0.17	10.41 ± 0.40
100	41.22 ± 0.23	14.89 ± 0.17

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Leaves	Between Groups	1163.523	2	581.762	7.072E3	.000
	Within Groups	.494	6	.082		
	Total	1164.017	8			
Flowers	Between Groups	160.472	2	80.236	813.020	.000
	Within Groups	.592	6	.099		
	Total	161.064	8			

Each value represents the mean±SD of three replicates

P<0.05 was considered as significant difference

Table: 1 (h): Lycopene content in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves µg/gram of extract	Flowers µg/gram of extract
25	9.07 ± 0.04	1.79 ± 0.19
50	17.74 ± 0.22	3.47 ± 0.25
100	28.59 ± 0.51	5.54 ± 0.25

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	573.936	2	286.968	2.763E3	.000
	Within Groups	.623	6	.104		
	Total	574.560	8			
Flowers	Between Groups	21.201	2	10.600	202.988	.000
	Within Groups	.313	6	.052		
	Total	21.514	8			

Each value represents the mean±SD of three replicates

P<0.05 was considered as significant difference

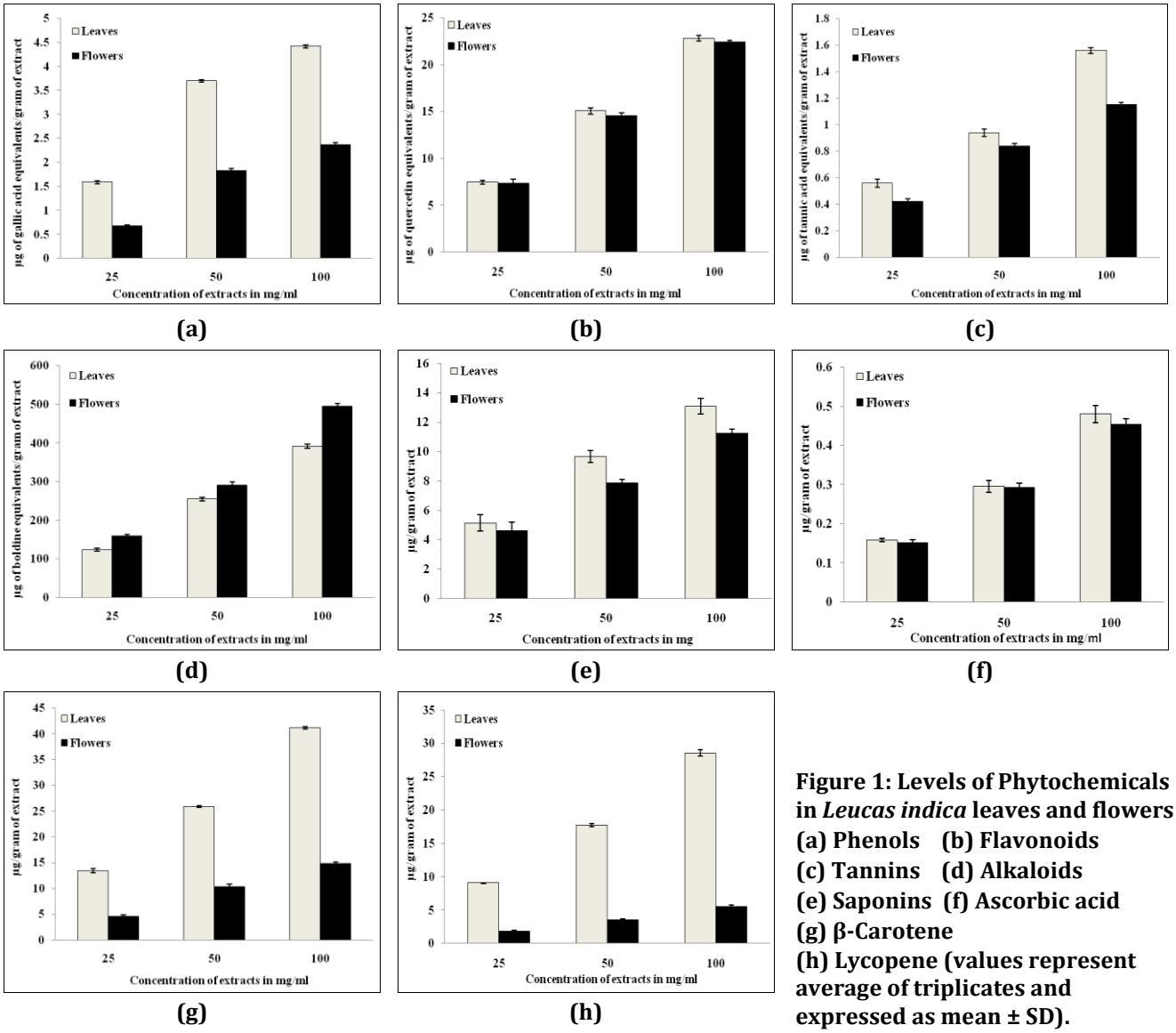

Figure 1: Levels of Phytochemicals in *Leucas indica* leaves and flowers (a) Phenols (b) Flavonoids (c) Tannins (d) Alkaloids (e) Saponins (f) Ascorbic acid (g) β-Carotene (h) Lycopene (values represent average of triplicates and expressed as mean ± SD).

Natural ascorbic acid is vital for the body performance (Aiyelaagbe and Osamudiamen, 2009). Vitamin C is the most important vitamin for human nutrition that is supplied by fruits and vegetables. L-Ascorbic acid (AA) is the main biologically active form of vitamin C. AA is reversibly oxidized to form L-dehydroascorbic acid (DHA), which also exhibits biological activity. Ascorbic acid provides first line of defence against oxidative stress (Nicolas Smirnoff 1996). The concentration of ascorbic acid in *Leucas indica* leaves and flowers was 0.481 ± 0.022 and 0.454 ± 0.015 µg/gram of extract respectively. Results were put on show in figure: 1(f).

The dietary carotenoids serve as precursor for vitamin A and prevent several chronic-degenerative diseases. Carotenoids have been extensively studied in different matrices to analyze their distribution and levels, as diet rich in carotenoids imparts health benefit properties. They are the most widely distributed pigments in nature (Gupta *et al.,* 2015). Lycopene is a vibrant red carotenoid that serves as an intermediate for the biosynthesis of other carotenoids and is found in moderate to high concentrations in foods such as tomato, watermelon, red grapefruit, and Brazilian guava (Stahl and Sies, 1996). Like its biosynthetic derivatives such as β-carotene, lycopene is an efficacious free radical scavenger (DiMascio *et al.,* 1989) and its presence in the diet positively correlates with reduced cancer incidence (Rao and Agarwal, 1998). Carotenoids and Lycopene contents of *Leucas indica* leaves and flowers were 41.22 ± 0.23, 14.89 ± 0.17 µg/gram of extract and 28.59 ± 0.51, 5.54 ± 0.25 µg/gram of extract correspondingly; results were put on show in figures: 1(g) and (h). From the above consequences, it evidently exposed that methanolic leave extract had elevated phytochemical constituents than methanolic flower extract, apart from alkaloids.

Antioxidant capacity assays

Antioxidants are radical scavengers which protects the human body from the pathological effects of free radicals. These chemical compounds are found in various processed foods or medicines as preservatives. But the increasing concern about chemical toxicity of the synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) has triggered public interest in naturally derived antioxidants from diet, supplements and medicinal plants. The reducing ability of plant extracts may be indicators of their antioxidant potentials (Oyaizu 1986). The presence of phenolic antioxidants causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form which indicates higher reducing ability. Our results suggest that *Leucas indica* methanolic leave and flower extracts had high electron donating capacity as well as strong redox potential and can act as a reducing agent in quenching free radicals.

Table: 2 (a): DPPH activities in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves % of inhibition/50µl of extract	Flowers % of inhibition/50µl of extract	RUTIN % of inhibition/50µl of Rutin	BHT % of inhibition/50µl of BHT
25	22.92±0.12	18.18 ±0.42		
50	40.36±0.23	31.45±0.41		
100	61.17±0.16	50.22±0.25		
1			46	75

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	2201.037	2	1100.519	3.517E4	.000
	Within Groups	.188	6	.031		
	Total	2201.225	8			
Flowers	Between Groups	1554.967	2	777.484	5.643E3	.000
	Within Groups	.827	6	.138		
	Total	1555.794	8			

Each value represents the mean±SD of three replicates

P<0.05 was considered as significant difference

Table:2 (b): FRAP activities in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves FRAP Units	Flowers FRAP Units
25	32.52±0.44	25.35±0.31
50	64.34±0.41	49.20±0.23
100	98.11±0.30	78.21±0.31

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	6452.999	2	3226.500	2.121E4	.000
	Within Groups	.913	6	.152		
	Total	6453.912	8			
Flowers	Between Groups	4205.094	2	2102.547	2.608E4	.000
	Within Groups	.484	6	.081		
	Total	4205.577	8			

Each value represents the mean±SD of three replicates

P<0.05 was considered as significant difference

Table: 2 (c): Reducing power assay of methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves µg of Ascorbic acid equivalents/1ml of extract	Flowers µg of Ascorbic acid equivalents /1ml of extract
25	15.51±0.19	11.51±0.19
50	30.14±0.12	18.62±0.20
100	58.16±0.15	40.56±0.21

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	2818.091	2	1409.045	5.857E4	.000
	Within Groups	.144	6	.024		
	Total	2818.235	8			
Flowers	Between Groups	1375.138	2	687.569	1.710E4	.000
	Within Groups	.241	6	.040		
	Total	1375.380	8			

Each value represents the mean±SD of three replicates
P<0.05 was considered as significant difference

Table: 2 (d): Hydroxyl Radical Scavenging activities of methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves % of inhibition/ 0.1 ml of extract	Flowers % of inhibition/0.1 ml of extract	Ascorbic acid % of inhibition/0.1 ml of ascorbic acid	BHT % of inhibition / 0.1 ml of BHT
25	16.46±0.21	10.19±0.19		
50	27.67±0.29	20.47±0.24		
100	53.32±0.17	36.43±0.21		
1			73	73.6

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	2142.246	2	1071.123	2.095E4	.000
	Within Groups	.307	6	.051		
	Total	2142.553	8			
Flowers	Between Groups	1048.900	2	524.450	1.164E4	.000
	Within Groups	.270	6	.045		
	Total	1049.170	8			

Each value represents the mean±SD of three replicates
P<0.05 was considered as significant difference

The extracts under study demonstrated superb free radical scavenging activity that is similar to standard antioxidants at all concentration tested. High antioxidant activity of plant extracts has previously been attributed to the phenolic compounds (Odagbasoglu *et al.,* 2004) and flavonoids, saponins and tannins (Aliyu *et al.,* 2009). Free-radical scavengers are antioxidants which can provide protection to living organisms from damage caused by uncontrolled production of reactive oxygen species and subsequent lipid peroxidation, protein damage and DNA strand breaking (Palanisamy and Sellappa 2012).

Table: 2 (e): ABTS activities in methanolic extracts of leaves and flowers of *Leucas indica*

Concentration mg/ml	Leaves TEAC50	Flowers TEAC50
25	32.27±0.25	25.26 ±0.31
50	64.56±0.23	48.91 ±0.33
100	97.77±0.23	77.91 ±0.24

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Leaves	Between Groups	6436.456	2	3218.228	5.754E4	.000
	Within Groups	.336	6	.056		
	Total	6436.792	8			
Flowers	Between Groups	4172.345	2	2086.173	2.367E4	.000
	Within Groups	.529	6	.088		
	Total	4172.874	8			

Each value represents the mean±SD of three replicates
P<0.05 was considered as significant difference

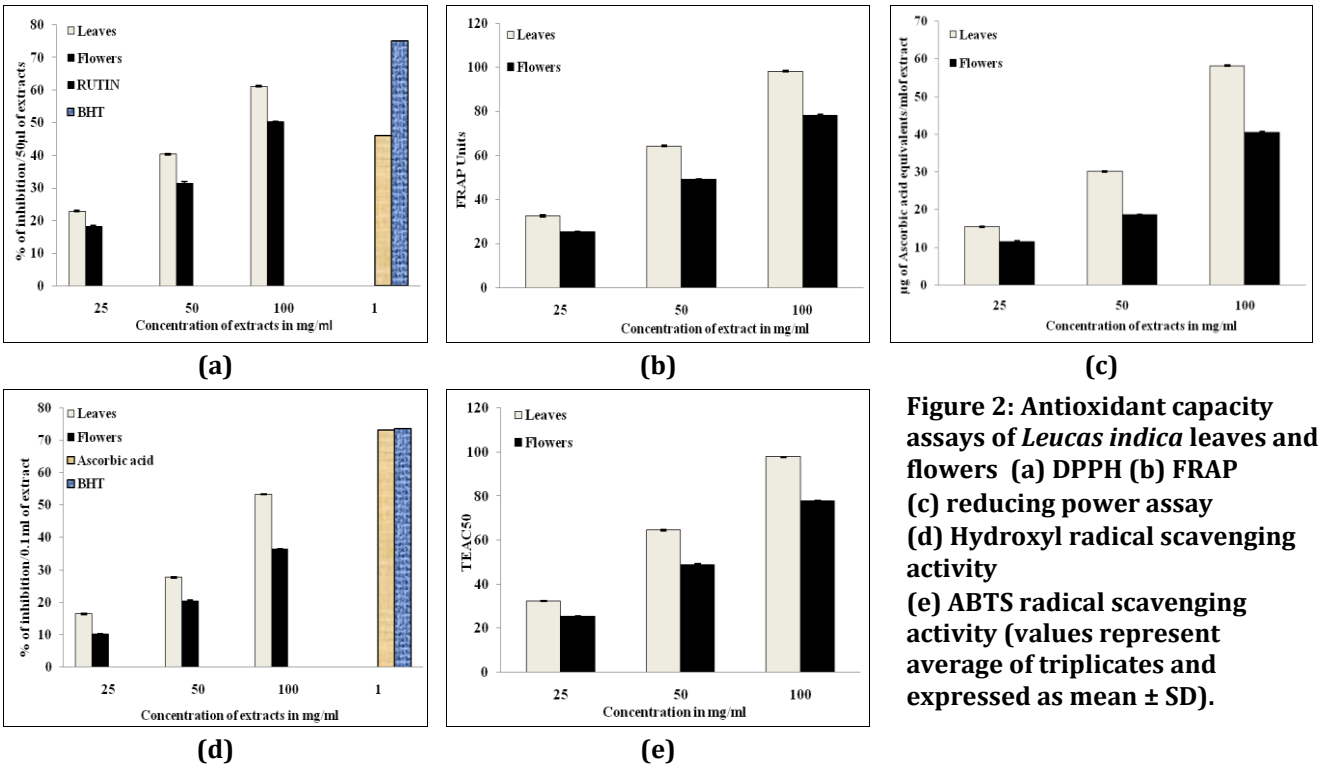

Figure 2: Antioxidant capacity assays of *Leucas indica* leaves and flowers (a) DPPH (b) FRAP (c) reducing power assay (d) Hydroxyl radical scavenging activity (e) ABTS radical scavenging activity (values represent average of triplicates and expressed as mean ± SD).

The over production of ROS has implicated in more than 100 degenerative diseases including heart diseases, atherosclerosis, diabetes, cancer etc (Nunomura *et al.,* 2006). Free radicals act as a trigger to a number of degenerative diseases. Therefore, samples having free radical scavenging activity can be of potent medicinal importance (Chen and Ho, 1995). DPPH is commonly used as a tool to evaluate the free radical scavenging activity of new compounds (Muchuweti *et al.,* 2007). Reducing power assay is

another convenient and rapid screening method for measuring the antioxidant potential (Chanda *et al.,* 2011). In addition, reducing power of a compound is related to electron transfer ability of the compound which could lead to the neutralization of free radicals (Zhu *et al.,* 2001). The FRAP assay is based on the ability of a sample to reduce Fe3+ in a Tripyridyltriazine (TPTZ) solution to Fe^{2+} and create the blue-coloured complex Fe^{2+}– TPTZ. Increased concentrations of the above complex means an increased FRAP value. Hydroxyl radicals are one of the quick initiators of the lipid peroxidation process by abstracting hydrogen atom from unsaturated fatty acids or simply auto oxidation of polyunsaturated fatty acids found primarily in membranes. Reduction capacity of a compound may serve as a significant indicator of its potential antioxidant activity (SerbetciTohma and Gulcin 2010)

Therefore, antioxidant capacities were ascertained in *leucas indica* leaves and flowers, they were increased in a dose dependent manner ranged from 25 – 100 mg/ml. The outcomes of antioxidant capacity assays mentioned below were at 100 mg/ml. DPPH radical scavenging activity of leaves and flowers was 61.17±0.16 and 50.22±0.25 % of inhibition/50µl of extract as compared to 46 and 75% of inhibition per 1 mg/ml of Rutin and BHT as positive controls. For ferric reducing ability power (FRAP), the outcome observed as 98.11 ±0.30 and 78.21±0.31 FRAP Units for leaves and flowers correspondingly. In favour of reducing power assay, results were observed as 58.16±0.15 and 40.56±0.21 µg of ascorbic acid equivalents per ml of extract for leaves and flowers in that order. Hydroxyl Radical Scavenging activity outcomes were 53.32±0.17 and 36.43±0.21 % of inhibition per 0.1 ml of extract for leaves and flowers of *leucas indica*, as compared to the results of Positive controls ascorbic acid and BHT at 1 mg/ml with percentage of inhibition 73 and 73.6 in that order. Finally, in ABTS method, the highest TAC value was observed in leaves (97.77±0.23 TEAC50) and the less value was observed in leaves (77.91 ±0.24 TEAC50), Previously, Paweł Pas´ko *et al.,* (2009) estimated ABTS in amaranth and quinoa seeds and sprouts during their growth. Penarrieta *et al.,* (2008) made a comparitive study of ABTS in between pseudocereals and cereals, There was a strong correlation between ABTS and DPPH, which was also observed previously (Awika *et al.,* 2003). All the above results agree with the preceding information and outcomes were portrayed in Figures: 2 (a), (b), (c), (d) and (e). correspondingly. Velioglu *et al.,* (1998) worked out on antioxidant activity and total phenolics in selected fruits vegetables and grain products. All the acquired results are in line with preceding information. From the above fallouts, it clearly reveeld that methanolic leave extract showed higher antioxidant capacity compared with methanolic flower extract, this may be due the presence of high phytochemical constituents.

CONCLUSION

In view of the consequences obtained from phytochemical screening and antioxidant activities of *Leucas indica* leave and flower methanol extracts contains promising antioxidant and phytochemical ingredients and that might be responsible for the therapeutic activities of the plant aerial part extracts in the treatment of various diseases. Further work to isolate and characterize the organic constituents as well as toxicological studies may enhance the understanding or the scientific basis of its uses in traditional medicine.

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