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Preliminary Phytochemical Screening and *In vitro* Antioxidant Activity of Ethiopian Indigenous Medicinal Plants, *Ocimum lamiifolium* Hochst. ex Benth and *Ocimum basilicum* L.

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ABSTRACT

Antioxidants are the chemical substances which prevent the free radicals damage in the body. Numerous researches are going globally focussed on investigating natural antioxidants of plants origins. The aims of the present study were to evaluate preliminary phytochemical investigation and *in vitro* antioxidant activities of Ethiopian indigenous medicinal plants, *Ocimum lamiifolium* Hochst. ex Benth and *Ocimum basilicum* L. Aqueous, benzene and hexane crude leaves extracts of *O. lamiifolium* and *O. basilicum* were subjected to qualitative phytochemical screening using standard procedures. In addition, we investigated the antioxidant potential of crude aqueous leaves extract of *O. lamiifolium* and *O. basilicum* using tests involving inhibition of superoxide anions, DPPH, H_2O_2, NO and ABTS. Preliminary phytochemical investigation for benzene, hexane and aqueous extracts found alkaloids, sterols, carbohydrate and glycosides, tannins and flavonoids. The fraction inhibition of lipid peroxide at the first stage of oxidation illustrated antioxidant activity of *O. lamiifolium* and *O. basilicum* as 90% and 88% compared to those of gallic acid (97%) and BHT (84%) respectively. Also, the aqueous leaves extract of *O. lamiifolium* and *O. basilicum* exhibited significant DPPH free radical scavenging activity, nitric acid free radical scavenging activity assay, superoxide anion scavenging activity, ABTS scavenging activity and hydrogen peroxide free radical scavenging assay. Our findings provide confirmation that the aqueous leaves extract of *O. lamiifolium* and *O. basilicum* are potential source of natural antioxidants, and this warranted its uses in traditional medicine systems.

Keywords: *O. lamiifolium* Hochst. ex Benth, *O. basilicum* L, preliminary phytochemical investigation, *in vitro* antioxidants, traditional medicine.

INTRODUCTION

Oxidative stress caused by reactive oxygen species (ROS) is associated with the pathogenesis of a numerous dreaded chronic diseases such as diabetes, cancer, atherosclerosis, coronary artery diseases and other degenerative diseases. [1-2] ROS causes tissue damage includes intracellular protein, lipids, and DNA damages and oxidation of membrane bound, and mitochondrial enzymes. [3] The use of antioxidants derived from plants such as flavonoids and

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polyphenols has been most valuable in the anticipation of these dreaded diseases. [4] Phenolic compounds are recognized as radical scavengers to slake oxygen-derived radicals by donating its hydrogen atom [5] and they have revealed to be nullifying free radicals. [6] Many researchers have exposed that these antioxidant agents have antihyperglycemic, anti-inflammatory, antitumor, anticarcinogenic, antibacterial and antiviral activities. [7] Eating of natural antioxidants has been connected with diminished risks of diabetes, cancer, cardiovascular disease and ageing. [8] Nowadays, it has been a global trend towards the consumption of the natural phytochemical present in herbs, oilseeds, fruits and vegetables. [9]

The genus Ocimum (Lamiaceae) is an aromatic annual and perineal medicinal herbs cultivated in temperate regions especially in Africa and Asia. *Ocimum lamiifolium* Hochst. ex Benth (local name Dama Kesse, Amharic; Anchabi, Oromifa) and *Ocimum basilicum* L. (local name Besobila, Amharic; Kefosa, Oromifa), are mostly found in mountain forests, grasslands, deserted fields at an elevation between 1000 and 3000 m. Traditionally, these fresh leaves are clutched and the juice is snuffled to treat cough, malaria, headache, febrile illness and cold. The juices are also used as eye infections and nose bleeding.

Based upon ethanobotanical survey of Ethiopian indigenous medicinal plants, the plant of *O. lamiifolium* and *O. basilicum* have been selected to prove scientifically having phytoactive compounds and antioxidant activity on *in vitro* studies. The phytochemicals generated data from the three different extracts of these plants may be used as tools for quality control of drugs in the future, for the healing of a diversity of disease conditions.

MATERIALS AND METHODS

Chemicals

Trichloroacetic acid, Ferric chloride, HCl, Dragendorff 's reagent, benzene, hexane, methanol, gallic acid, chloroform, H_2SO_4, Folin-Ciocalteu reagent, aluminium chloride, potassium acetate, phosphate buffer, $K_3Fe(CN)_6$, 2-thiobarbituric acid, ferric thiocyanate, butylated hydroxyl toluene, 2, 2-diphenyl-1-picrylhydrazyl, 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid, potassium persulphate, hydrogen peroxide, sulfanilic acid, glacial acetic acid, potassium metabisulphite, NADH were all purchased from Chemico Glass & Scientific Company, Erode, Tamilnadu, India. All the chemicals used in this experiment were of analytical grade.

Collection and authentication of plant material

The plant of *O. lamiifolium* and *O. basilicum* were collected from Jimma University Garden, Jimma, South West Ethiopia in the month of October-November-2014. The plant has been taxonomically identified and authenticated by the Jimma University Botanist Dr. Ramesh Moochikkal and kept in Jimma University Botanical Science and Herbarium for future references.

Preparation of the extract

O. lamiifolium and *O. basilicum* were collected and air dried under shade and then roughly powdered with the help of mechanical blender. The powder was conceded through filter and stored in an airtight container for the solvent extraction.

Preparation of Extracts

Benzene extract of leaves of *O. lamiifolium* and *O. basilicum*

The shade dried coarsely powdered *O. lamiifolium* L. and *O. basilicum* L. were immersed and extracted with benzene for 72 hours. After completion of extraction, the defatted extracts were sieved by Whatman filter paper No.1 to eliminate any contamination. The extract was intensed by vaccum distillation to decrease the volume; the intensed extract was transferred to 100 ml beaker and the remaining solvent was volatalised. Dark greenish yellow coloured extract was acquired. The intensed extract was then kept in a dessicator to eliminate the unnecessary moisture. The dried extract was packed in air tight glass container for further studies.

Hexane extract of leaves of *O. lamiifolium* and *O. basilicum*

The marc left after benzene extraction was dried and then immersed and extracted with hexane, up to 72 hours. After completion of extraction, the solvent was removed by distillation. Dark greenish yellow colour residue was obtained. The residue was then stored in a dessicator.

Aqueous extract of leaves of *O. lamiifolium* and *O. basilicum*

The marc left after hexane extraction was again dried and then macerated with distilled water in a 2 litre round bottom flask, for 72 hours. 10 ml of chloroform was added daily to avoid fungal growth. After completion of extraction, it was filtered and the solvent was removed by evaporation to dryness on a water bath. Green coloured extract was obtained and it was stored in a dessicator to remove the excessive moisture.

Identification of phytochemical active constituents

Preliminary phytochemical studies [10-11]

The extracts obtained (benzene, hexane and aqueous) was subjected to the following preliminary phytochemical studies.

Test for Alkaloids

Dragendorff's test: To 1 ml of the extract, 2 ml of distilled water was added; 2 M hydrochloric acid was added until an acid reaction occurs. To this 1 ml of Dragendorff's reagent was added. Formation of orange or red precipitates indicates the presence of alkaloids.

Hagger's Test: To 1 ml of the extract was taken in test tube, a few drops of Hager's reagent was added. Formation of yellow precipitate confirms the presence of alkaloids.

Wagners Test: 1 ml of extracts was acidified with 1.5% v/v of hydrochloric acid and a few drops of Wagners reagent were added. A yellow or brown precipitate indicates the presence of alkaloids.

Mayers Test: To a few drops of the mayers reagent, 1 ml of extract wase added. Formation of white or pale yellow precipitate indicates the presence of alkaloids.

Test for Carbohydrates

Anthrone Test: 1 ml of extract was shaken with 10 ml of water, filtered and the filtrate was concentrated. To this 2 ml of anthrone reagent solution was added. Formation of green or blue colour indicates the presence of reducing sugars.

Benedict's Test: 1 ml of extract was shaken with 10 ml of water, filtered and the filtrate was concentrated. To this 5 ml of Benedict's solution was added and boiled for 5 min. Formation of brick red coloured precipitate indicates the presence of reducing sugars.

Fehling's Test: 1 ml of extract was shaken with 10 ml of water, filtered and the filtrate was concentrated. To this 1ml mixture of equal parts of Fehlings solution A and B were added and boiled for few minutes. Formation of red or brick red coloured precipitate indicates the presence of reducing sugar.

Molischs Test: 1 ml of extract was shaken with 10 ml of water, filtered and the filtrate was concentrated. To these 2 drops of freshly prepared 20% alcoholic solution of α-naphthol was added. 2ml of conc. sulphuric acid was added so as to form a layer below the mixture. Red-Violet ring appear, indicating the presence of carbohydrates which disappear on the addition of excess of alkali.

Test for flavonoids

Shinods test: 1 ml of extract was dissolved in 5 ml of ethanol and to this 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish or brown colour indicates the presence of flavonoids.

With Con. Sulphuric acid test: Yellow colour (anthocyanins), yellow to orange colour (flavones) and orange to crimson (flavonones).

Test for Glycosides

Molisch Test: 1ml of extract was shaken with 10 ml of water, filtered and the filtrate was concentrated. To this 2-3 drops of Molisch reagent was added, mixed and 2ml of conc. sulfuric acid was added carefully through the side of the test tube. Reddish violet ring appears, indicating the presence of glycosides.

Test for proteins and free amino acids

Millions reagent-Appearance of red colour shows the presence of protein and free amino acid.

Ninhydrin reagent-Appearance of purple colour shows the presence of protein and free amino acids.

Biuret test: Equal volumes of 5% sodium hydroxide solution and 1% copper sulphate solution was added. Appearance of pink or purple shows the presence of proteins and free amino acids.

Test for gums and mucilage

Precipitation with 95% alcohol: Small quantities of the extracts were added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitate was dried in air and examined for its swelling properties for the presence of carbohydrates.

Test for anthraquinones

About five ml of the extract solution was hydrolysed with diluted Conc. H_2SO_4 extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones.

Test for Saponins

Foam test: In a test tube containing about 5 ml of extracts, a drop of sodium bicarbonates solution was added. The test tube was shaken vigorously and left for 3 min. Formation of honeycomb like froth indicates the presence of saponins.

Test for Sterols

Liebermann-Buchards test: 1 ml of extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green color indicates the presence of steroids.

Salkowski reaction: 1 ml of extract was shaken with chloroform, to the chloroform layer sulphuric acid was added slowly by the sides of test tube. Formation of red colour indicated the presence of steroids.

Test for fixed oils

Spot test: Small quantities of various extracts were separately pressed between the two filter papers. Appearance of oil stains on the paper indicates the presence of fixed oil. Few drops of 0.5N alcoholic potassium hydroxide were added to small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

Test for triterpenoids

About two ml of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of Conc.H_2SO_4. Formation of reddish violet colour indicates the presence of triterpenoids.

Test for phenolic compounds and tannins

Small quantities of the extracts were taken separately in water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.

Ferric chloride solutions (5%)-Violet color

1% solution of gelatin containing 10% sodium chloride-white precipitate

10% lead acetate solution-white precipitate

Antioxidant assay

The antioxidant activity of the aqueous plant extract was determined using ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods. The FTC method was used to measure the amount of peroxide at the beginning of peroxidation while TBA method was used to measures free radicals present after peroxide oxidation.

Ferric thiocyanate (FTC) method

The standard method described by Kikuzaki *et al*. [12] was used for FTC determination. A mixture of 2 ml of sample in 4 ml of 99.5% ethanol, 4.1 ml of 2.51% linoleic

acid in 99.5% ethanol, 8.0 ml of 0.02 M phosphate buffer (pH 7.0) and 3.9 ml of distilled water contained in screw cap vial (Ø38 × 75 mm) was placed in an oven at 40°C in the dark. To measure the extent of antioxidant activity, 0.1 ml of the reaction mixture was transferred to a test tube (Ø38 × 150 mm) and, to it; 9.7 ml of 75% (v/v) aqueous ethanol, followed by 0.1 ml of 30% aqueous ammonium thiocyanate and 0.1 ml of 0.02 M ferrous chloride in 3.5% hydrochloric acid were added. Three minutes after the addition of ferrous chloride to the reaction mixture, the absorbance of the resulting mixture (red colour) was measured at 500 nm every 24 h until the absorbance of the control reached its maximum. Butylated hydroxyl toluene (BHT) (final concentration of 0.02% w/v) was used as positive control, while the mixture without the plant extract was used as the negative control.

Thiobarbituric acid (TBA) method

The method of Ottolenghi [13] modified by Kikuzaki and Nakatani [14] was used for the determination of free radicals present in the aqueous leaf extract. The final sample concentration of 0.02% w/v from the same samples prepared for FTC assay was used. Two ml of 20% trichloroacetic acid and 2 ml of 0.67% of thiobarbituric acid were added to 1 ml of sample solution from the FTC method. The mixture was placed in a boiling water bath for 10 min and then centrifuged after cooling at 3000 rpm for 20 min. The absorbance activity of the supernatant was measured at 552 nm and recorded after it has reached its maximum.

2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) assay

The method of Liyana-Pathiana and Shahidi [15] was used for the determination of scavenging activity of DPPH free radical. One ml of 0.135 mM DPPH prepared in methanol was mixed with 1.0 ml of aqueous extract ranging from 0.2-0.8 mg/ml. The reaction mixture was vortexed thoroughly and left in dark at room temperature for 30 min. The absorbance was measured spectrophotometrically at 517 nm. The scavenging ability of the plant extract was calculated using this equation;

$$\text{DPPH Scavenging activity (\%)} = [(Abs_{control} - Abs_{sample})]/(Abs_{control})]\times 100$$

Where $Abs_{control}$ is the absorbance of DPPH + methanol; Abs_{sample} is the absorbance of DPPH radical + sample (i.e. extract or standard).

2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS) scavenging activity

The method of Re *et al*. [16] was adopted for the determination of ABTS activity of the plant extract. The working solution was prepared by mixing two stock solutions of 7 mM ABTS solution and 2.4 mM potassium persulphate solution in equal amount and allowed to react for 12 h at room temperature in the dark. The resulting solution was later diluted by mixing 1 ml of freshly prepared ABTS+ solution followed by the measurement of absorbance at 734 nm after 7 min. The percentage of scavenging inhibition capacity of ABTS+ of the extract was calculated and compared with Butylated hydroxyltoluene (BHT). The percent of scavenging inhibition capacity of ABTS+ of the extract was calculated from the following equation:

$$\text{\% inhibition} = [(Abs_{control} - Abs_{sample})]/(Abs_{control})]\times 100$$

Nitric oxide scavenging activity

The method of Garratt [17] was adopted to determine the nitric oxide radical scavenging activity of aqueous extract of *H. longifolium*. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generate nitric oxide which interacts with oxygen to produce nitrite ions determined by the use of Griess reagents. Two millilitre of 10 mM sodium nitroprusside dissolved in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of plant extract at various concentrations (0.2-0.8 mg/ml). The mixture was incubated at 25°C. After 150 min, 0.5 ml of incubation solution was withdrawn and mixed with 0.5 ml of Griess reagent [(1.0 ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthylethylenediamine dichloride (0.1% w/v)]. The mixture was incubated at room temperature for 30 min. The absorbance was measured at 540 nm. The amount of nitric oxide radical was calculated following this equation:

$$\text{\% inhibition of NO} = [A_0 - A_1]/A_0 \times 100$$

Where A_0 is the absorbance before reaction and A_1 is the absorbance after reaction has taken place.

Scavenging activity of superoxide anion

The scavenging activity of superoxide anion was determined by the method of Yen and Chen. [18] The reaction mixture consists of 1 ml of plant extract (1 mg/ml), 1 ml of PMS (60µM) prepared in phosphate buffer (0.1 M pH 7.4) and 1 ml of NADH (phosphate buffer) was incubated at 25°C for 5 min, the absorbance was read at 560 nm against blank samples.

Hydrogen peroxide scavenging activity

Scavenging activity of hydrogen peroxide by the plant extract was determined by the method of Ruch *et al*. [19] Plant extract (4 ml) prepared in distilled water at various concentration was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm against blank solution containing the plant extract without H_2O_2.

RESULTS

In the study, preliminary phytochemical investigation has been done in the three extracts (aqueous, benzene and hexane) of *O.lamiifolium* and *O.basilicum* leaves showed the presence of phytochemical constituents namely alkaloids, saponins, *anthraquinones*, flavonoids, tannins, total phenol and triterpenoids, and absence of steroids, aminoacids and glycosides described in Table 1.

Free radical scavenging activities

Total Antioxidant Capacity

Figure 1 illustrated *in vitro* antioxidant assay of the *O. lamiifolium* and *O. basilicum* extracts which significant

Table 1: Preliminary phytochemical investigation of *Ocimum lamiifolium* Hochst. ex Benth and *Ocimum basilicum L.*

Phytochemical Analysis	*Ocimum lamiifolium*				*Ocimum basilicum*			
	Leaves			Stem	Leaves			Stem
	Aqueous	Benzene	Hexane	Aqueous	Aqueous	Benzene	Hexane	Aqueous
Alkaloids	+++	+++	+++	+++	+++	+++	+++	++
Protein and aminoacids	-	-	-	-	-	-	-	-
Anthraquinones	-	++	-	-	-	-	-	-
Flavonoids	+++	+++	+++	+++	+++	+++	+++	+++
Glycosides	-	-	-	-	-	-	-	-
Saponins	-	-	-	-	++	-		++
Steroids	-	-	-	-	-	-	-	-
Total phenols and Tannins	-	-	-	-	+++	-	-	-
Triterpenoids	+++	++	++	++	++	++	-	+

+++ = appreciable amount (positive within 5 min); ++ = moderate amount (positive after 5 min but within 10 min); + = trace amount (positive after 10 min but within 15 min; - = completely absent.

Table 2: Radical scavenging activities of aqueous crude leaf extract of *Ocimum lamiifolium* and BHT as standard at different concentrations.

Percentage inhibition (% I) of radical scavenging of *Ocimum lamiifolium*					
Extract or BHT Conc (mg/ml)	Superoxide anion	Nitric oxide	DPPH	Hydrogen peroxide	ABTS
0.2	62.65(60.16)	31.71(40.27)	40.91(42.62)	58.99(68.61)	50.00(51.17)
0.4	67.55(73.49)	42.00(46.27)	47.63(53.00)	66.14(73.29)	59.31(63.39)
0.6	72.16(77.12)	62.28(61.87)	58.33(73.99)	70.85(76.22)	66.31(77.20)
0.8	75.01(79.96)	64.96(80.29)	75.91(82.32)	72.44(80.00)	75.10(77.95)

BHT values in parenthesis.

Table 3: Radical scavenging activities of aqueous crude leaf extract of *Ocimum basilicum* and BHT as standard at different concentrations.

Percentage inhibition (% I) of radical scavenging of *Ocimum basilicum*					
Extract or BHT Conc (mg/ml)	Superoxide anion	Nitric oxide	DPPH	Hydrogen peroxide	ABTS
0.2	60.14(58.38)	29.67(38.39)	42.44(45.53)	52.44(61.76)	52.34(53.55)
0.4	64.86(67.23)	39.45(42.67)	51.78(59.37)	62.89(69.57)	63.93(67.58)
0.6	69.16(72.56)	56.39(57.95)	64.53(79.54)	69.43(75.37)	69.53(80.34)
0.8	73.01(77.53)	66.45(82.76)	78.22(85.26)	74.75(83.24)	78.48(83.74)

BHT values in parenthesis.

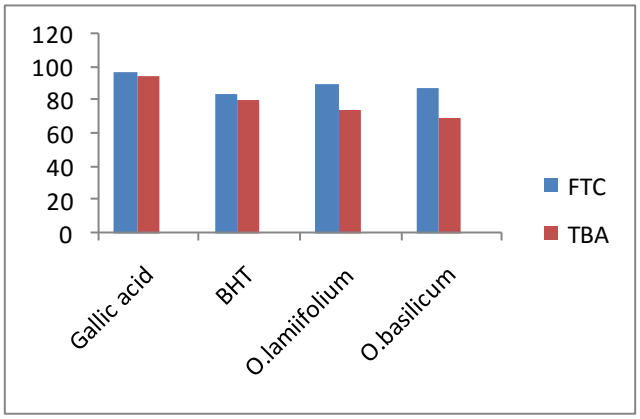

Fig. 1: Antioxidant properties of plant extract compared to the standards (Gallic acid and BHT) as determined with the FTC (500 nm) and TBA (552 nm) methods on the 7th day. TBA: Thiobarbituric acid. FTC: Ferric thiocyanate. BHT: Butylated hydroxyl toluene.

antioxidant potential has compared with standard Gallic acid and BHT. The proportion inhibition of lipid peroxide at the first phase of oxidation showed antioxidant activity of *O. lamiifolium* and *O. basilicum* as 90% and 88% compared to those of gallic acid (97%) and BHT (84%) respectively. The percentage inhibition of malondialdehyde by *Ocimum lamiifolium* and *Ocimum basilicum* showed percentage inhibition of 76% and 70% compared to both BHT (79.24%) and gallic (95.2%).

Scavenging activities of the extract

The proportion inhibition of scavenging activities of the aqueous extract of *O. lamiifolium* and *O. basilicum* for DPPH, ABTS, hydrogen peroxide, nitric oxide and superoxide anion radical were exhibited in Table 2. The nitric oxide and ABTS radical scavenging activity of the both extract at 0.8 mg/ml, which was the highest concentration of *O. lamiifolium* extract tested, was 77.95% and 80.29% and *Ocimum basilicum* extract tested, was 83.74% and 82.76% respectively. The leaves extracts of *O. lamiifolium* and *O. basilicum* exhibited a significant free radical scavenge activities at the maximum concentrations of 0.8 mg/ml on hydrogen peroxide, superoxide anion radical and DPPH (Table 2, 3).

DISCUSSION

The preliminary phytochemical analysis investigation on *O. lamiifolium* and *O. basilicum* extract revealed the presence of alkaloids, saponins, flavonoids, tannins, and triterpenoids. Tannins are recognized to be helpful in the treatment of chronic inflammation in tissues and they have notable activity on anticancer. [20] Thus, *O. lamiifolium* and *O. basilicum* containing these chemical compounds may provide as active principle in the treatment of various cancer.

Flavonoids are phenolic compounds that are acting as principal antioxidants or free radical scavengers and serve as health promoting compound as a results of its anion radicals. [20] Since these phenolic compounds were originated to be present in the extracts, it might be accountable for the potent antioxidant capacity of *O. lamiifolium* and *O. basilicum*. These phytochemicals of

medicinal plants have primarily reported for their medicinal value, which can be valuable folklore remedies in the treatment of cold, headache, acne, malaria and bacterial diseses. [21] The plant containing phenolic compounds contributed to their antioxidative properties and thus the value of the plants are in folklore medicine. Phenols have been practicing in the preparation of some antimicrobial agents such as dettol and cresol. Both plants are widely used regularly among many tribes in Africa for the treatment of various diseases. For instance, saponins proved as hypotensive and cardiodepressant properties [22], which are helpful for the management of heart failure and cardiac myopathy. [23] The occurrence of saponins in aqueous extracts leaves of *Ocimum basilicum* might play a role in the cardioprotective potential. Alkaloids have the potential of anti-hyperglycaemic and anti-inflammatory activities. [24]

The result of DPPH radical scavenging activity analysis indicates that both plants were potentially antioxidant properties. These results recommend that the plant extracts contain compounds that are potential to donate hydrogen atom to a free radical and makes them unstable. The capacity of these plant extracts to scavenge DPPH may possibly reproduce and prevent the generation of ABTS+. The radical scavenging activity of ABTS+ by these plant extracts were found to be significant; this shows that *O. lamiifolium* and *O. basilicum* may be useful for treating radical associated pathological tissue injury [25].

Superoxide anion radical is the strongest ROS among the free radicals [20], which was scavenged by both plant extracts compared positively with the standard compound such as gallic acid signifying that these plants are potent radical scavenger of superoxide anion. Hydrogen peroxide is another significant ROS, that ability to enter biological membranes. It may be cellular toxic if converted to hydroxyl radical [26]. The plant extracts may be attributed to their phenolics, which donate electron to H_2O_2, thus reducing it to water. The extracts were able of scavenging H_2O_2 in a dose dependent manner. Nitric oxide (NO) is another ROS created by phagocytes and endothelial cells, to yield more ROS such as peroxynitrite that decays to form OH radical. The composition of nitric oxide was significantly abridged in this study by the leaves extracts of *O. lamiifolium* and *O. basilicum*.

This study confirms the preliminary phytochemical compounds and *in vitro* antioxidant potential of leaves extract of the *O. lamiifolium* and *O. basilicum*, with results similar to individual standard such as gallic acid and BHT. Further studies are needed to elucidate the *in vivo* potential of these plants in the treatment of human diseases resulting from oxidative stress.

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