Original Article

Antioxidant Activities and Thin Layer Chromatographic Analysis of Aqueous Extract of Barks of *Cinnamomum zeylanicum* Blume.

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ABSTRACT

Objective: The objective of the present study was to investigate phytochemical constituents, free radical scavenging activities and TLC of the aqueous extract of barks of *Cinnamomum zeylanicum* Blume.

Methodology: The qualitative and quantitative analyses were carried out for the aqueous extract of barks of *C. zeylanicum* using standard procedures. The antioxidant activities were carried out by DPPH, $ABTS^{\bullet+}$, $OH^{\bullet}$ and $NO^{\bullet}$ radical scavenging assay as well as Fe^{3+} reducing power and phosphomolybdenum reduction assay methods. The aqueous extract of barks of *C. zeylanicum* showed good radical scavenging as well as reducing power activities.

Results: The Phytochemical estimation revealed that the concentration of phenols and flavonoids in the aqueous extract of barks of *C. zeylanicum* were 42.3 ± 2.96 mg/g and 5.81 ± 0.40 mg/g respectively. The maximum percentage of $DPPH^{\bullet}$, $OH^{\bullet}$, $ABTS^{\bullet+}$ and $NO^{\bullet}$ radicals inhibition were 84.39 ± 5.90, 54.84 ± 3.83, 67.06 ± 4.69, and 55.61 ± 3.89 respectively.

Conclusion: The results revealed that the aqueous extract of barks of *C. zeylanicum* significantly inhibits the radicals and the antioxidant activity increases with increasing concentration.

Keywords: *Cinnamomum zeylanicum*, Phenols, Flavonoids, antioxidant, DPPH, $ABTS^{\bullet+}$ $OH^{\bullet}$, $NO^{\bullet}$, Reducing power, Phosphomolybdenum and TLC.

INTRODUCTION

The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment, Pharmaceutical industries during the 19th and 20th century mainly depended on the leads from medicinal plants and their derivatives. To discover new therapeutic molecules and also to identify the new sources of phytochemicals for the synthesis of targeted drug systems, information of chemical constituents found in form of secondary metabolites is utmost needed[1]. *Cinnamomum zeylanicum* Blume belongs to the family Lauraceae, which is popularly known as Cinnamon is biologically classified in the botanical class Magnoliopsida and division Magnoliophyta[2]. Generally in India, *Cinnamomum zeylanicum* is cultivated in South India[3]. But it originates from south east of India and the island of Srilanka. It is cultivated in India, Mauritius, Brazil and Jamaica. The production of cinnamon is mostly limited to the wettest low land area of the South East Asia and cultivated upto an altitude of 500 meters above mean sea level having the mean temperature 27ºC and annual rainfall 2000-2400 mm. It prefers sandy soil enriched with organic matter. The genus *Cinnamomum* has 250 species and many of them are aromatic and flavouring[4]. Bark of Cinnamon is being used for the flu-preventive medicines, mouth washes[5], potent antibacterial[6], anti-fungal and uterine stimulant. The diterpenes present in the cinnamon oil have shown antiallergenic activity. *Cinnamomum zeylanicum* is also used for the treatment of toothache, mild spastic conditions of the gastrointestinal tract and diabetes[7]. Antioxidants are the substances which inhibit oxidation, and have the ability to remove the potentially damaging oxidizing agents in a living organism. Phytochemicals present in many plants are able to prevent the oxidative damage to the human cells which can even cause cancer. Thus it is necessary to know the antioxidant activities of phytocompounds responsible for inhibiting radicals. In this study, the phenols, flavonoids, antioxidant, DPPH, $OH^{\bullet}$, $NO^{\bullet}$, reducing power, phosphomolybdenum free radical scavenging activity of the extracts of *C. zeylanicum is* analyzed.

MATERIALS AND METHODS

Collection of plant material

The barks of *C. zeylanicum* were collected from the market. The plant material was authenticated by Prof. N. Raaman, CAS in Botany, University of Madras, Chennai. The bark was cleaned and shade dried for 3 days. The tree grows to a height of 7-10 meters in its wild state. The plant has strong branches and thick scabrous bark and has deeply veined ovate leaves that are dark green underneath, both bark and leaves are aromatic. It has small yellowish-white flowers with disagreeable odour and bears dark purple berries.

Extraction

The dried barks were cut into small pieces and the process of extraction was carried out with distilled water using a hot plate apparatus at 80 °C. The hot aqueous extract was cooled and completely filtered using filter paper. Water was completely removed in a hot plate apparatus at 50° C and the aqueous extract was used for antioxidant activities as well as thin layer chromatographic (TLC) analysis.

Phytochemical estimations

Determination of total phenol

Total phenolic content was determined by the Folin-Ciocalteu method[8]

with slight modifications. The aqueous extract of barks of *C. zeylanicum* 1 mL (0.1 mg/mL) was mixed with 1mL of Folin-Ciocalteu's reagent (1:10 diluted with distilled water). After 5 min, 1 mL of aqueous Na_2CO_3 (20%) was added. The mixture was then allowed to stand for 30 min incubation in dark. The quantification of phenolic compounds was performed spectrophotometrically by measuring the absorbance in UV-VIS spectrophotometer at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

Determination of total flavonoid

Aluminium chloride colorimetric method was used to determine the total flavonoids[9] with slight modifications. One mL of aqueous extract of barks of *C. zeylanicum* (0.1 mg/mL) was mixed with 0.5 mL of 10 % aluminium chloride, 0.5 mL of 1 M potassium acetate and 0.5 mL of distilled water. It was incubated at room temperature for 30 min. The absorbance of the reaction mixture was measured by spectrophotometer at 415 nm. The total flavonoid content was expressed in terms of quercetin equivalent, which is a common reference standard.

Antioxidant activities

Screening of antioxidant activity by dot-blot DPPH staining method

Drops of DPPH (0.4 mM) were loaded onto a 5 cm x 5 cm TLC plate (silica gel 60 F254; Merck) in each column and allowed to dry for 3 min. The first row of TLC plate was considered as control, containing only DPPH. In second row, various concentrations of aqueous extract of barks of *C. zeylanicum* was carefully loaded onto the DPPH spot. The third row of TLC plate was considered as standard reference, where ascorbic acid was carefully loaded onto the DPPH spot. The staining of the silica plate was based on the procedure of Soler- Rivas[10]. Stained silica gel layer revealed a purple background with yellow to white spots at the location where radical scavenging capacity observed. The intensity of disappearance of purple colour depends upon the amount and nature of radical scavenger[11] present in the aqueous extract of barks of *C. zeylanicum*.

DPPH radical scavenging activity

The antioxidant activity of aqueous extract of barks of *C. zeylanicum* was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhyorazyl (DPPH) free radical according to the method described by Brand-Williams et al[12] with slight modifications. In the Experiment, 1 mL of 0.1 mM DPPH solution in methanol was mixed with 1mL of various concentrations of aqueous extract (2-12 µg/mL) of barks *C. zeylanicum*. Mixer of 1mL methanol and 1mL DPPH solutions were used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517 nm after 30 minutes in dark using UV-Vis spectrophotometer. Ascorbic acid was used as reference standard. The inhibition % was calculated using the following formula.

$$\% \text{ of DPPH radical inhibition} = \frac{\text{Control-Sample}}{\text{Control}} \times 100$$

$ABTS^{\bullet+}$ radical cation scavenging activity

The antioxidant capacity was estimated in terms of the $ABTS^{\bullet+}$ radical cation scavenging activity following the procedure described by Delgado-Andrade et al[13]. Briefly, 7 mM ABTS stock solution by reacting with 2.45 mM potassium persulfate and the mixture was left to stand in the dark at room temperature for 12-16 h before use. The ABTS solution (stable for 2 days) was

diluted with 5 mM phosphate-buffered saline (pH 7.4) to an absorbance at 730 nm of 0.70±0.02. To the various concentrations (2-12 µg/mL) of aqueous extract of barks of *C. zeylanicum*, 1 mL of diluted $ABTS^{\bullet+}$ solution was added and after 10 min, the absorbance was measured at 730 nm. Ascorbic acid was used as reference standard. The $ABTS^{\bullet+}$ radical cation scavenging activity was expressed as

$$\% \text{ of } ABTS^{\bullet+} \text{ radical inhibition} = \frac{\text{Control-Sample}}{\text{Control}} \times 100$$

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was determined using standard protocol[14]. Various concentrations (10-60 µg/mL) of the aqueous extract of barks of *C. zeylanicum* were taken in different test tubes and evaporated to dryness. One mL of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 mL of EDTA (0.018%), and 1mL of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added to these tubes, and the reaction was initiated by adding 0.5 mL of 0.22% ascorbic acid. The test tubes were capped tightly and heated on a water bath at 80- 90°C for about 15mins. The reaction was completed by the addition of 1mL of ice-cold TCA solution (17.5% w/v). One mL of Nash reagent (18.75 g of ammonium acetate, 0.75 mL of glacial acetic acid and 0.5 mL of acetyl acetone were mixed and raised to 250 mL with distilled water) was added to all the tubes and left at room temperature for about 15 min for color development. The intensity of the yellow color was measured at 412 nm against reagent blank. Ascorbic acid was

$$\% \text{ of } OH^{\bullet} \text{ radical inhibition} = \frac{\text{Control-Sample}}{\text{Control}} \times 100$$

used as reference standard. The percentage of hydroxyl radical scavenging was calculated by the following formula

Nitric oxide radical scavenging activity

Nitric oxide generated from aqueous sodium nitroprusside (SNP) solution interacts with oxygen to produce nitrite ions at physiological pH, which may be quantified and determined according to Griess Illosvoy reaction[15]. The reaction mixture contained 1 mL of 10 mM SNP in 0.5 M phosphate buffer (pH 7.4) and 1 mL of various concentrations (20–120 µg/mL) of the aqueous extract of barks of *C. zeylanicum*. After incubation for 60 min at 37°C, Griess reagent (0.1% napthyl ethylenediamine dihydrochloride in water and 1% sulphanilic acid in 5% H_3PO_4) was added. The pink chromophore generated during diazotization of nitrite ions with sulfanilamide and subsequent coupling with napthyl ethylenediamine dihydrochloride were measured spectrophotometrically at 540 nm. Ascorbic acid was used as a positive control. The percentage of nitric oxide radical scavenging activity was calculated by the following formula

$$\% \text{ of } NO^{\bullet} \text{ radical inhibition} = \frac{\text{Control-Sample}}{\text{Control}} \times 100$$

Reducing power assay

The reducing power of the aqueous extract of barks of *C. zeylanicum* was evaluated according to the method of Ravisankar et al[16]. Different concentrations of aqueous extract of barks of *C. zeylanicum* (20–120 µg/mL) were mixed with 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1% $K_3Fe(CN)_6$. This mixture was incubated at 50°C for 20 min. After, 1 mL of 10% TCA was added and centrifuged at 3000 rpm for 10 min. The upper layer of the solution was mixed with 0.5 mL of $FeCl_3$ (0.1%) solution and the absorbance was measured at 700 nm using a spectrophotometer. Ascorbic acid was used as the standard reference.

Phosphomolybdenum reduction assay

The total antioxidant capacity was measured by spectrophotometeric method of Prieto et al[17]. At different concentration, aqueous extract of barks of *C. zeylanicum* (20–120 µg/mL) was combined with 1mL of reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate, 4 mM ammonium molybdate). The tubes were incubated at 95ºC for 90 min in a water bath. After, the mixture was cooled to room temperature and the absorbance was read at 695 nm. Ascorbic acid was used as the standard reference.

RESULTS AND DISCUSSION

Medicinal plants are an important source of practical and inexpensive new drugs[18]. Oxidative stress has been implicated in the pathology of many diseases such as inflammatory conditions, cancer, diabetes and the aging. This study was made to evaluate polyphenolic content and antioxidant activity. Phenolic compounds are composed of one or more aromatic rings bearing one or more hydroxyl groups and are therefore potentially able to quench free radicals by forming stabilized phenoxyl radicals[19].

Total phenol and flavonoid

The phenolic and flavonoid compounds quantified in the extracts seemed to be responsible for the antioxidant activity. Phenolic acids, flavonoids are the most commonly found polyphenolic compounds in plant extracts[20]. The antioxidant activity of phenolics plays an important role in absorption or neutralization of free radicals[21]. Polyphenols also enhance the level of cellular antioxidative system and induce the cytochrome P-450 resulting in detoxifying the activity of carcinogens intracellularly[22]. Total phenol content was 42.3 ± 2.96 mg/g of GAE and flavonoid content was 5.81 ± 0.40 mg/g of QE in the aqueous extract of barks of *C. zeylanicum*. These investigations provide a comprehensive profile of the antioxidant activity of extracts of plants with respect to their phenols and flavonoids content.

Dot-blot assay for rapid radical scavenging activity

The results of dot-blot assay showed coloured spots where the aliquots of aqueous extract of barks of *C. zeylanicum* were placed in row. The purple zone on the plate indicates no (free radical scavenging) antioxidant activity and the yellow zone indicates antioxidant activity. The more intense the yellow colour, the greater the antioxidant activity (Figure 1). The result indicates that the aqueous extract of barks of *C. zeylanicum* have significant antioxidant activity when compared to standard ascorbic acid.

DPPH radical scavenging activity

To carry out DPPH assay, normally, methanol solution of DPPH is mixed with that of substances that can donate hydrogen atoms and then they give rise to the reduced form (Diphenylpicrylhydrazine; non radical) with the loss of violet colour[23]. To find out the aqueous extract of barks of *C. zeylanicum* to scavenge free radicals formed was assessed by using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). This was compared with a standard (ascorbic acid). As shown by the data aqueous extract of barks of *C. zeylanicum* revealed better scavenging of free radicals. The active phytochemicals present in the extract reduces the purple coloured stable (DPPH) 1,1-diphenyl-2-picrylhydrazyl radical to yellow coloured 1,1-diphenyl-2-picrylhydrazine and this capacity increases with increasing concentration as reported earlier by Raaman et al[24]. The maximum percentage of DPPH radical scavenging

activity was 84.39 ± 5.90 at 120 µg/mL concentration. It was compared with the standard ascorbic acid (70.95 ± 4.96) and the IC_{50} of DPPH radical scavenging activity was 6.28 µg/mL concentration. The scavenging ability of the aqueous extract of barks of *C. zeylanicum* may be due to its bio compositions such as phenolic acids and flavonoid. The radical scavenging activities of the extracts were determined by using DPPH a stable free radical at 517 nm. 1,1-diphenyl-2-picrylhydrazyl is a nitrogen-centred free radical, color of which changes from violet to yellow on reduction by donation of H or e^- by the aqueous extract of barks of *C. zeylanicum.*

$ABTS^{\bullet+}$ radical scavenging activity

ABTS•+ radical cation is a blue chromophore produced by the reaction between ABTS and potassium persulphate. The blue chromophore of ABTS•+ radical cation gets reduced in presence of the aqueous extract of barks of *C. zeylanicum* and was quantified spectrophotometrically[25]. The maximum percentage of ABTS•+ radical cation scavenging activity was 54.84 ± 3.83 at 12 µg/mL concentration. It was compared with standard (52.41 ± 3.66) ascorbic acid. The IC_{50} of ABTS•+ radical cation scavenging activity was 10.33 µg/mL concentration.

Hydroxyl (OH•) radical scavenging activity

Scavenging of hydroxyl radical is an important antioxidant activity because of very high reactivity of the OH radical which enables it to react with a wide range of molecules found in living cells, such as sugars, amino acids, lipids and nucleotides. Hydroxyl radicals are being the most reactive oxygen species that easily penetrate through the cell membrane and react with most of the biomolecules like proteins, polypeptides, nucleic acids and lipids causing tissue damage and cell death[26]. The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells[27]. The maximum percentage of $OH^{\bullet}$ radical scavenging activity was 67.06 ± 4.69 at 60 µg/mL concentration. It was compared with standard ascorbic acid. The IC_{50} of $OH^{\bullet}$ radical scavenging activity was 41.46 µg/mL concentration.

Nitric oxide (NO•) radical scavenging activity

The absorbance of chromophore formed during the diazotization of the nitrite ion with sulphanilamide and the subsequent coupling with naphthyethylenediamine dihydrochloride was measured by spectrophotometric method. NO, being a potent pleiotropic mediator in physiological processes and a diffusible free radical in the pathological conditions, reacts with superoxide anion and form a potentially cytotoxic molecule, the peroxynitrite (ONOO)[28]. Its protonated form, peroxynitrous acid (ONOOH), is a very strong oxidant. The main route of damage is the nitration or hydroxylation of aromatic compounds, particularly tyrosine. Under physiological conditions, peroxynitrite also forms an adduct with carbon dioxide dissolved in body fluid and responsible for oxidative damage of proteins in living systems. The maximum percentage of $NO^{\bullet}$ radical scavenging activity was 55.61 ± 3.89 at 120 µg/mL concentration. It was compared with standard ascorbic acid. The IC_{50} of $NO^{\bullet}$ radical scavenging activity was 110.11 µg/mL concentration.

Ferric (Fe^{3+}) reducing power activity

Studies were made on total reduction ability of Fe^{3+} to Fe^{2+} transformation in the presence of the aqueous extract of barks of

C. zeylanicum and found increasing in showing reduction ability in a dose dependent manner, with increasing concentrations. Increase in absorbance of the reaction mixture indicated increased reducing power. Since the reducing capacity of the aqueous extract of barks of *C. zeylanicum* serve as a significant indicator of its potential antioxidant activity, the reducing ability was 0.219 ± 0.010 at 60 µg/mL concentration, which was compared with standard (0.359 ± 0.02/12 µg/mL) ascorbic acid. The antioxidant activity has been reported to be concomitant with development of reducing power[29].

Phosphomolybdenum reduction activity

The total antioxidant activity of aqueous extract of barks of *C. zeylanicum* was measured spectrophotometrically by the phosphomolybdenum method, which is based on the reduction of Mo (VI) by the petroleum ether fraction and the subsequent formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption 695 nm. It evaluates both water-soluble and fat-soluble antioxidants with a high absorbance value of the petroleum ether fraction indicated its strong antioxidant acitivity[30]. The maximum absorbance was 0.088 ± 0.006 at 60 µg/mL concentration, which was compared with standard (0.359 ± 0.02/12 µg/mL) ascorbic acid.

Thin layer chromatography

TLC analysis was carried out for aqueous extract of barks of *C. zeylanicum* by using chloroform: methanol (1.8:0.2) solvent system. The separated bands were visualized by UV light at 254 nm. The R_f values of the separated compounds were measured (Table 9, Figure 2).

CONCLUSION

The replacement of synthetic antioxidants with natural antioxidants may be advantageous. In the present study, aqueous extract of barks of *C. zeylanicum* tested with respect to their total phenolic and flavonoid contents, antioxidant capacity and oxidative stability. Extractions were performed using the conventional reflux method. The antioxidant capacity was measured by DPPH free radical scavenging method was proven to be significant. Finally, the results in this study indicate that the examined extract contains significant sources of antioxidants.

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Table 1. Quantitative phytochemical estimations of phenol and flavonoid of aqueous extract of barks of *C. zeylanicum*

S. No.	Phytochemicals	Value (mg/g)
1.	phenols	42.3 ± 2.96
2.	Flavonoids	5.81 ± 0.40

Table 2. DPPH radical scavenging activity of aqueous extract of barks of *C. zeylanicum*

S. No	Concentration (µg/mL)	% of inhibition
1.	2	24.75 ± 1.73
2.	4	35.34 ± 2.47
3.	6	47.75 ± 3.34
4.	8	59.87 ± 4.19
5.	10	72.13 ± 5.04
6.	12	84.39 ± 5.90

Table 3. DPPH, $OH^{\cdot}$ and $NO^{\cdot}$ radical scavenging activities of standard (Ascorbic acid)

S. No	Concentration (µg/mL)	DPPH	$ABTS^{\cdot+}$	$OH^{\cdot}$	$NO^{\cdot}$
1.	2	03.34 ± 0.23	25.00 ± 1.75	35.17 ± 3.02	37.14 ± 2.59
2.	4	13.88 ± 0.27	32.25 ± 2.25	52.42 ± 2.51	40.00 ± 2.81
3	6	21.59 ± 1.51	37.9 ± 2.65	69.35 ± 1.07	48.57 ± 3.39
4.	8	37.25 ± 2.60	43.54 ± 3.04	89.14 ± 6.23	54.28 ± 3.79
5.	10	70.62 ± 4.94	51.61 ± 3.61	92.24 ± 6.45	60.00 ± 4.23
6.	12	70.95 ± 4.96	52.41 ± 3.66	97.67 ± 6.83	62.85 ± 4.39

Table 4. $ABTS^{\cdot+}$ radical cation scavenging activity of aqueous extract of barks of *C. zeylanicum*

S. No	Concentration (µg/mL)	% of inhibition
1.	2	16.67 ± 1.16
2.	4	25.81 ± 1.80
3.	6	35.48 ± 2.48
4.	8	41.94 ± 2.93
5.	10	48.39 ± 3.38
6.	12	54.84 ± 3.83

Table 5. Hydroxyl radical scavenging activity of aqueous extract of barks of *C. zeylanicum*

S. No	Concentration (µg/mL)	% of inhibition
1.	10	18.82 ± 1.31
2.	20	29.41 ± 2.05
3.	30	38.82 ± 2.71
4.	40	48.24 ± 3.37
5.	50	61.18 ± 4.28
6.	60	67.06 ± 4.69

Table 6. Nitric oxide radical scavenging activity of aqueous extract of barks of *C. zeylanicum*

S. No	Concentration (µg/mL)	% of inhibition
1.	20	12.24 ± 0.85
2.	40	20.49 ± 1.43
3.	60	34.18 ± 2.39
4.	80	41.84 ± 2.92
5.	100	45.41 ± 3.17
6.	120	55.61 ± 3.89

Table 7. Ferric (Fe^{3+}) reducing power and phosphomolybdenum reduction of aqueous extract of barks of *C. zeylanicum*

S. No	Concentration (µg/mL)	Fe^{3+} reducing power	Phosphomolybdenum reduction
1.	20	0.186 ± 0.009	0.034 ± 0.002
2.	40	0.191 ± 0.009	0.047 ± 0.003
3.	60	0.195 ± 0.009	0.054 ± 0.003
4.	80	0.202 ± 0.009	0.068 ± 0.004
5.	100	0.217 ± 0.010	0.083 ± 0.005
6.	120	0.219 ± 0.010	0.088 ± 0.006

Table 8. Ferric (Fe^{3+}) reducing power and phosphomolybdenum reduction of standard (Ascorbic acid)

S. No	Concentration (µg/mL)	Fe^{3+} reducing power	Phosphomolybdenum reduction
1	20	0.154 ± 0.01	0.015 ± 0.01
2	40	0.189 ± 0.01	0.093 ± 0.00
3	60	0.209 ± 0.01	0.172 ± 0.01
4	80	0.214 ± 0.01	0.205 ± 0.01
5	100	0.247 ± 0.01	0.317 ± 0.02
6	120	0.289 ± 0.02	0.359 ± 0.02

Table 9. Thin layer chromatography of aqueous extract of barks of *C. zeylanicum*

Test sample	Solvent system	R_f
Aqueous extract of *C.zeylanicum*	$CHCl_3$:CH_3OH (1.8 : 0.2)	1^{st} spot - 0.23 2^{nd} spot - 0.27 3^{rd} spot – 0.31 4^{th} spot – 0.44

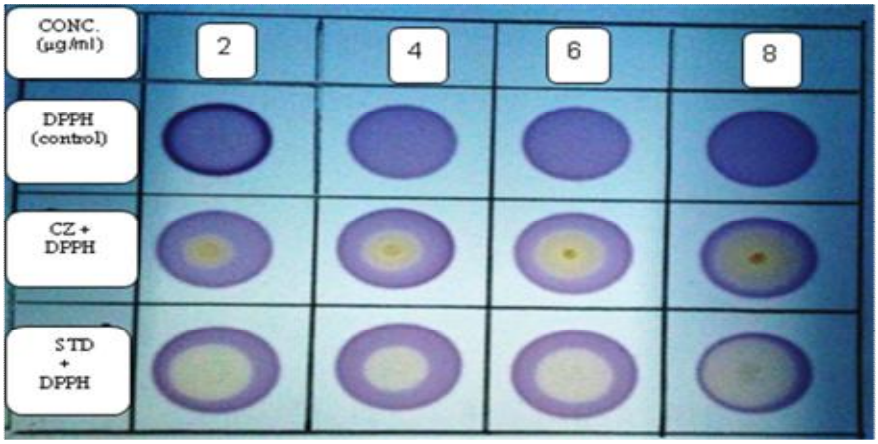

Fig. 1. Dot-blot assay of aqueous extract of *C. zeylanicum* in DPPH radical scavenging activity.

CONC- Concentration; DPPH - 1, 1-Diphenyl-2-picryl hydrazyl; CZ - *Cinnamomum zeylanicum*; STD- Standard (Ascorbic acid).

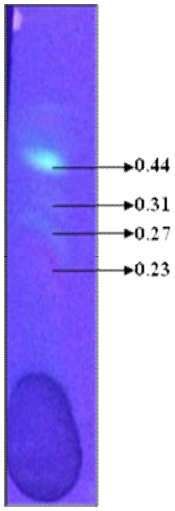

Fig. 2. Thin layer chromatography of aqueous extract of barks of *C. zeylanicum*.