HPTLC Analysis and Screening Antioxidant Activity of Cinnamomum zeylanicum Bark Hexane Extract

Riddhi M. Patel
Assistant Professor in Botany, H. & H. B. Kotak Institute of Science, Rajkot, Gujarat, India.

***Abstract*—During the usual metabolism in any living system, oxidation reactions does generate free radicals. Free radicals are an assembly of bioactive compounds that possess unpaired electrons which start off further chain reactions; which can react and harm other stable molecules. When the free radicals harm lasts for an extended time, it may lead to disease development. Whereas Antioxidants are compounds that provides protection to cells against such reactive oxygen or free radicals in the body. Antioxidants are free radical scavengers and they counteract these chain reactions by being oxidizing themselves and acting as reducing agents. More concisely, antioxidants can effectively restrict oxidation process by reacting with free radicals, chelating metals and also by acting as oxygen scavengers, triplet as well as singlet form and transferring hydrogen atoms to the free radical structure.**

The plants are similarly vulnerable to impairment produced by active oxygen and thus develop frequent antioxidant defence systems resulting in establishment of numerous potent antioxidant components, also referred as plant secondary metabolites (PSMs). Fruits, vegetables, herbs, medicinal plants, spices etc. are frequently known to comprise huge variety of antioxidants. With the development in techniques and recent researches, it has been shown that certain non-nutritive chemicals in plants such as PSMs like Flavonoids, Isoflavones, Flavones, Anthocyanins, Coumarins, Lignans, Catechins, Isocatechins, β-Carotene, etc. which were formerly considered to be of no importance to human diet, found to possess antioxidant properties. are reported to possess antioxidative property. The aromatic nature of spices, herbs and other plants/ plant parts is due to presence of Aromatic components, Volatile or Essential oils. The aromatic principles or essential oils are composed of a complex mixture of organic substances with diverse functional groups like Phenolic compounds comprising hydroxyl groups (-OH) and the low molecular volatile Terpenoids mainly Mono- and Sesquiterpenes. Therefore, several Spices and Aromatic plants are medicinally important as well due to presence of rich diversity of secondary metabolite content and therefore their usage in regular diet, not only serve as source of fragrance and flavouring agent, but likewise delivers dietary antioxidants.

In this context: *Cinnamomum zeylanicum* plant Hexane extract was screened for the presence of DPPH Radicle Scavenging Activity, Total Phenol Content and Ferrous Ion Chelating Activity representing Antioxidative property. The selected plant exhibited potential antioxidant activity in all above mentioned three assays and showed a rich Phyto-chemical profile in HPTLC screening.

***Index Terms*—Free radicals, Antioxidant, Plant Secondary Metabolites (PSMs), chain reactions**

I. INTRODUCTION

Biochemically Plant-based antioxidants own wide-ranging physiological roles within body and are a key for good health. Antioxidants are expected to avert many chronic diseases caused by free radicals such as Atheroscelerosis, Cancer, Diabetes, Arthritis, Inflammation, Cardiovascular, Ageing related problems etc.. From the food preservation perspective, antioxidants can evade lipid peroxidation and microbial spoilage of food and works as a natural preserving agent. Thus currently, herbs and spices have great potential in a growing Nutrition industry and becoming an essential part amongst packaged food category (Markowicz *et al,* 2007). Consequently today, there has been growing interest in screening plant extracts and essential oils to cater novel sources of natural antioxidants including broad-spectrum actions. Nevertheless, contempt of enormous medicinal value of Indian medicinal plants, a rich diversity of plants is left to be scientifically assessed for such properties and to appreciate their mechanism of action.

A. Area of Concern and Therapeutic Importance of Antioxidants:

Free radicals such as hydroxyl and reactive oxygen radicals produced in our body during the normal metabolic process; are highly reactive in nature and harm almost every molecule found within living cells, distress human health and lead to numerous degenerative diseases including Diabetes, Arthritis, Atherosclerosis, Hypertension, Heart Attack, Immunosuppression, Neurodegenerative, Parkinson and Alzheimer diseases, Cancer and premature body aging (Porwal *et al*, 2010). At a molecular level; these free radicals can cause rupture of DNA strand and thereby contribute to Carcinogenesis, Mutagenesis and Cytotoxicity. In recent years, effective free radical scavengers have intricated a great interest as 'Therapeutic agents' against oxidative stress and the free radical arbitrated diseases by the exogenous consumption of antioxidants to benefit the body to scavenge harmful free radicals. Scientific studies have confirmed that antioxidant activity shown by Phyto-chemicals or PSMs found to play roles in various pharmacological activities by inhibition of free radical induced injury for example Antibacterial, Antiviral, Anti-Inflammatory, Anti-Atherosclerosis, Anti-Aging, Anti-Cancer activities etc. Also stimulate Immune system, control Gene expression in Cell proliferation & Apoptosis, Hormone metabolism, as well reduce coronary heart disease and Cancer mortality (Ghafar *et al*, 2010). Consequently, subjoining of antioxidants has become an attractive healing approach for reducing the risk of diseases.

In recent few years increased interests in nutrition, fitness and beauty have improved concerns over health and attended more emphasis on the positive aspects of diet. Accordingly, functional foods are recognized all over, able of providing supplementary physiological benefits such as preventing or declining chronic diseases together with the completion of elementary nutritional supplies. Bioactive compounds commonly found in medicinal plants are studied in many *in vitro* and *in vivo* systems and have exhibited to own several valuable pharmacological properties like antioxidative, anticarcinogenic, atherosclerosis, anti-inflammatory and angiogenesis inhibitory actions etc.

B. Spice: Rich Source of Beneficial Antioxidants:

Nowadays people are conscious and became more health sensible. Owing to possible toxicological side effects produced due to artificial food additives on human health; the over-all trend is shifted to reducing the usage of synthetic food seasonings. This has resulted in a growing of the hunt for the natural materials owning antimicrobial and antioxidant belongings and henceforth they are appreciated in averting cellular damage, the cause of ageing, and chronic human diseases. For this purpose, herbal extracts and essential oils are widely used due to their complex composition of various constituents of mostly Hydrocarbons and Oxygenated compounds. On the foundation of this background, many studies are in development (Singh *et al.,* 2004).

Utmost Medicinal plants, Aromatic plants, Herbs and Spices are an important source of Plant Secondary Metabolites (PSMs) which frequently exhibits free radical scavenging antioxidant activity. Amount of these chemicals and their physical property varies from plant to plant. Owing to the difference in their chemical composition and mechanism of action; finding the antioxidant capability is a complex task. In order to find a precise type of antioxidant effect; various *in vitro* and *in vivo* assays can be implemented (Mirghani *et al.*, 2012).

Aromatic plants exhibit an antioxidant outcome due to the presence of Hydroxyl groups within their Phenolic compounds. Other structurally alike compounds to Plant Phenolics are the Terpenoids containing a complex composition of Hydrocarbons and Oxygenated compounds. They are frequently present in Aromatic plant parts and stated as Essential oils (Souri *et al.*, 2008).

In this context; Spice Cinnamon was exploited to find out presence of Antioxidant activity apart from its culinary, and therapeutic applications implementing various Antioxidant Screening Assay. The beneficial health benefits claimed for the mentioned spice is included below (Fig. 1, Table 1).

Fig. 1 Spice- *Cinnamomum zeylanicum* Bark

Table 1. Medicinal Properties of Cinnamon Spice

Common name: Cinnamon
Family: Lauraceae
Phyto-chemicals: Cinnamaldehyde, Cinnamyl alcohol, Eugenol, Acetyl-Eugenol, Methyl-Eugenol, Nerol, Ethyl-Cinnamate, Dihydro-Eugenol, 1, 8-Cineol, Geranial, Trans-Cinnamic acid, Cinnamyl alcohol, Hydroxy Cinnamaldehyde, o-Methoxy Cinnamaldehyde, α- Pinene, α-Caryphyllene, α-and β-Phellandrene, α-Terpineol, p-Cymene, Catechins, Oligomeric Proanthocyanidins, Limonene, and Linalool.
Uses: The bark is acrid, bitter, sweet, aromatic, deodorant, tonic, antiseptic, antifungal, astringent, appetite stimulant, expectorant, febrifuge, diuretic emenagogue, aphrodisiac, and alexeteric. It treats nausea & vomiting, diarrhea, flatulence, halitosis, asthma, bronchitis, fever, cephalalgia, odontalgia, cardiac diseases, haemorrhage, uropathy, and reinstate normal skin colour on face. Cinnamon essential oil is recognized as stomachic, carminative, emenagogue and astringent.

C. Plant Phenolics as Potential Phyto-Antioxidants and its Importance:

Phenolic compounds present in many Spices that displayed natural antioxidant properties have been studied for replacement of synthetic antioxidants, owing to their likely side effects which may in several situations act deleterious (Kitazurua *et al.,* 2004). Phenolic derivatives signify the major group known as 'Secondary Plant Products' or 'Plant Secondary Metabolites-PSMs' produced by higher plants. Numerous of these Phenolic compounds are vital to plant life, e.g., by providing defence against microbial attacks and makes food unpalatable to herbivorous predators. Although a detailed chemical description may be given for Plant Phenolics, it would unavoidably comprise other structurally similar compounds such as the terpenoid sex hormones.

Substantial antioxidant, antiviral, antibiotic and antitumoral activities are frequently reported in various studies for plant Phenols. They have frequently been recognized as active principles of many folk herbal medicines. In recent years, the regular intake of fruits and vegetables has been highly recommended, since the plant Phenols and Polyphenols they encompass are supposed to play significant role in long-term health and reducing the risk of chronic and degenerative diseases. Acknowledgement of the aids taken by these natural products to human health has encouraged the presence in everyday diets of some characteristic plant-derived food and beverages (Apak *et al.,* 2007). Phenolic antioxidants in plants are mainly composed of Phenolic acids, Polyphenols, Phenylpropanoids, Flavonoids, Flavones, Isoflavones, Anthocyanins, Alkaloids Coumarins, Lignans, Catechins, Isocatechins, Tannins, etc (Patel and Jasrai, 2009).

D. Free Radical Scavenging Activity of Phenolics:

Phenolic compounds produced by plants alike Phenolic acids, Favonoids and Catechins are very vital for plant growth and play a significant role in their defense mechanisms by providing resistance against microbial attacks and by making food unpalatable to herbivorous predators. When consumed; Phenolic antioxidants present in herbs exhibits beneficial effects on our body cellular mechanism such as reduction in lipid peroxidation, prevention in DNA oxidative damage and scavenging reactive oxygen species (ROS) like superoxide, hydrogen peroxide, and hydroxyl radicals (Yoo *et al.*, 2008).

Phenolic compounds are ubiquitous in the Plant Kingdom but their distribution at cellular, intracellular and tissue levels vary from plant Family and Genus. Generally hydrophobic Phenolics are found inside cell-wall and they deliver the mechanical strength to cell, however; hydrophilic Phenolics found in vacuoles and support the cell in reducing stress and pathogenic outbreaks. In plants; they achieve varied physiological functions alike they contribute in the process of growth and development, pigment formation, pollination, resistance from pathogens and herbivores, etc (Saeed *et al.*, 2012).

E. Mechanism of action:

i) Total Phenolic Content (TPC) or Total Antioxidant Capacity Assay: The Phosphomolybdenum method or Folin-Ciocalteu method usually perceives antioxidants such as Ascorbic acid, some Phenolics, Polyphenols, Aromatic Amines, Glutathione, Cysteine, α-Tocopherol and Carotenoids by their hydrogen and electrons donating capability. These compounds undertake a complex redox reaction with the

Phosphotungstic and Phosphomolybdic acids present in the Folin-Ciocalteu reagent (Ghafar *et al,* 2010).

ii) DPPH Radical Scavenging Activity: DPPH is a nitrogen-centered free radical that receives an electron or hydrogen radical to become a stable diamagnetic molecule. The DPPH scavenging ability and reducing power assays provides preliminary information on the reactivity of the test compound with a free radical and its hydrogen-donating tendency and the reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm. During this free radical scavenging assay, extract containing solution of DPPH changes its colour from deep violet to a pale yellow by antioxidants. The colour of DPPH solution changes due to formation of colourless α-Diphenyl-β-Picryl Hydrazine a stable diamagnetic molecule, via either transfer of an electron or hydrogen atom to DPPH, therefore neutralizing its free radical character. In the other words, odd electron of the radical becomes paired off with hydrogen donated by the extract, resulting in the reduction of absorption strength. The resulting decolorization from purple to yellow is associated with respect to the number of electrons captured by the extract. DPPH, has characteristic absorbance maxima at 517 nm, which decreases with the scavenging of the radical. The reduction of DPPH radicals can be observed by the decrease in absorbance at 517 nm and on the degree of discoloration due to the radical scavenging ability of antioxidant (Sreelatha and Padma, 2009). Many researchers have reported positive correlation and detected that high reduction of DPPH is related to the high scavenging activity and higher amount of antioxidants present in the sample. EC_{50} (Effective Concentration of Extract needed for 50% Free radical Inhibition) is the amount of antioxidant present in the sample essential for 50% DPPH inhibition. Thus lower the EC_{50} value, higher the antioxidant activity (Ghafar *et al,* 2010).

iii) Metal Ion Chelating Activity: Transition elements like iron and copper are the free radicals having one or more unpaired electrons and act as powerful catalyst of oxidation responses since they contain electron that can enable to participate in electron transfer reactions. Metal ion chelating activity of an antioxidant molecule can inactivate, catalyse and inhibit the harmful transition metal ions accountable for the generation of Oxygen free radicals in living organisms. Chelating agents contribute in the conversion of H_2O_2 (Hydrogen Peroxide) in to -OH also called Fenton and Haber-Weiss reaction, form low redox potential complexes, decompose Alkyl Peroxides to the heavy reactive Alkoxyl and Hydroxyl radicals and averts Oxyradical generation and the resulting oxidative damage (Ghimiry *et al,* 2009). In metal ion chelating assay, the extract and standard compounds repressed the formation of ferrous and Ferrozine complex and are able to capture Ferrous ion before the formation of Ferrozine by their chelating activity. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disturbed, resulting in decrease of the red coloured complex. Thus, reduction of the colour is equivalent to the metal chelating activity (Singh *et al,* 2009).

F. HPTLC Phyto-Chemical Profiling:

The importance of conducing Phyto-chemical studies, is to not solitary to be used for the chemical characterization but also significant for linking the chemical contents with precise functional properties. In the present study, also an effort was directed to develop HPTLC based Phyto-chemical profile from selected Spice plant *Cinnamomum zeylanicum*. HPTLC study of extracts aids to recognize active metabolites intricate and as an analytical tool in quality control as well as quantitative aspects of the medicinal product. Various Components of the sample separated on TLC plate over designated solvent system since the Polar, Non-polar or the intermediate nature/ composition of the Secondary metabolites. This is then followed by Chromatogram scanning at diverse wavelengths, for detection of R_f value (Retention value) and λ_{max} (Wavelength of maximum absorption) for each separated compound. Also, the HPTLC technique is helpful to compare profiles of the crude plant extracts prepared by means of various solvents, for the maximum extraction of the desired metabolites in selected solvents. The relative analysis with different polarity solvent extracts likewise assists the value addition feature of the invention.

II. MATERIALS AND METHODS

A. Plant Material Collection:

Plant used in the present work for screening was *Cinnamomum zeylanicum* Blume bark (Table 1). The plant material for the study were purchased from the

local market of Gujarat State, India. Major cultivation of above spice plant is South India and it supplies to other areas of country.

B. Plant Material Extraction:
Bark Material was finely grinded in to a fine powder using domestic mixture grinder machine and subsequently subjected for the solvent extraction in a non-polar solvent Hexane to extract the bioactive terpenoid and other soluble compounds. Plant powder extracted in the ratio of 10 gm powder vs. 100 ml solvent with occasional shaking and overnight drenching in the air tight erlenmeyer flask (Harborne, 1984). The soaked content then filtered through the Whatman filter paper no.1 and concentrated in indoor open air until all the solvent gets evaporated. The concentrated extract was afterward collected in the glass vial and dry weight of extract residue was recorded (Table 1).

C. Screening Antioxidant Activity: In the present study, commonly used Cinnamon spice- *Cinnamomum zeylanicum* Blume belonging to Lauraceae family was used to find the potential antioxidant activity in terms of presence of %TPC, DPPH Radicle Scavenging Assay and Ferrous Ion Chelating Assay following standard protocols.

1) Phenol Estimation by Folin-Ciocalteau Method:
Plant extract was subjected for the screening antioxidant activity by instigating standardized protocols of % Total Phenol Content assay. The chemicals utilized in the study were of pure and analytical grade. Antioxidant assay readings were taken in six replicates for sample and calculated for their standard errors. The readings were calculated with reference to Standard Gallic acid. IC_{50} value was calculated for standard compound, representing the concentration of the compounds that caused 50% inhibition of free radicles or antioxidant activity. The detailed procedure of the *in vitro* assay is cited underneath.
For the assay, to 6 ml of double distilled water; added 2 mg of extract sample, 0.5 ml Folin-Ciocalteau reagent and 1.5 ml 20% Na_2CO_3 (Sodium Carbonate) solution. Then the total volume made up to 10 ml by addition of double distilled water in a test tube. The mixture was then incubated for 30 min. at 25ºC and afterwards OD taken at 760 nm using UV-VIS Spectrophotometer. The existence of antioxidant activity was indicated by a change in a color from light yellow to blue. The intensity of blue color of the mixture can be directly correlated with the existent of the antioxidant activity or the amount of Total Phenol present. % Total Phenol Content was calculated using % extract yield and Standard compound Gallic acid equivalents (GAE) with reference to standard curve.

To 6 ml of double dist. water added 2 mg extract sample, 0.5 ml Folin-Ciocalteau reagent and 1.5 ml 20% Na_2CO_3 solution
↓
The total volume made up to 10 ml by addition of distilled Water
↓
The mixture incubated for 30 min. at 25ºC and formerly OD taken at 760 nm
↓
The occurrence of antioxidant activity designated by a color alteration from light yellow to blue. The blue color intensity of the mixture is directly interrelated with the existent of the antioxidant activity or the quantity of Phenol present
↓
% Phenol calculated by means of % Extract yield Gallic acid Equivalents (GAE) with reference to standard curve where IC_{50} obtained 0.50 mg/10ml

Fig 2. Antioxidant Activity Analysis by Total Phenol Content Estimation by Folin-Ciocalteau method*:*

2) DPPH Radical Scavenging Assay: For the assay, 2 ml 0.5 mM Methanolic solution of DPPH is mixed with the 2 ml methanolic solution comprising 3 mg extract. The mixture was shaken vigorously then allowed to incubate in dark for 30 min. Existence of antioxidant activity was specified by turning of dark violet to brownish color or light-yellow color of mixture. More the antioxidant activity, paler the color of mixture after incubation. Subsequently OD was taken at 517 nm by UV-VIS Spectrophotometer instrument. Ascorbic acid (Vitamin C) was used as a reference compound with IC_{50} at 0.35 mg/ 4 ml. The calculation was executed using following formula (Ghasemi *et al.,* 2009).

$$\% \text{ Radical scavenging activity} = \frac{\text{A control -A sample}}{\text{A control}} \times 100$$

A control = OD of DPPH solution without Extract or Standard
A sample = OD of DPPH solution with Extract or Standard

3) Ferrous Ion Chelating Activity:
For the assay, 3 mg extract was mixed with the 2 ml of 0.04 mM $FeCl_2$ and 2 ml of 0.5 mM aqueous Ferrozine solution. The mixture was then shaken vigorously and left standing at room temperature for 10 min. Amount of Ferrous ion chelating activity was designated by turning of mixture color from dark purple to light purple or pink. Here correlation occurs for example higher the chelating activity; lighter the color of the solution. OD was taken at 562 nm by UV-VIS Spectrophotometer instrument. BHT (Butylated Hydroxy Toluene) was used as a reference compound with and displayed superlative chelating activity at IC_{50} at 6 mg/4 ml. The calculation was accomplished using following formula (Dinis *et al.,* 1994).

$$\% \text{ Inhibition of Ferrozine - } Fe^{2+} \text{ complex} = \frac{\text{1-A}_1 \text{ sample}}{\text{A}_0 \text{ control}} \times 100$$

A_0control = OD of $Fecl_2$ and Ferrozine solution without Extract or Standard
A_1 sample= OD of $Fecl_2$ and Ferrozine solution with Extract or Standard

D. HPTLC instrumentation for HPTLC Phyto-Chemical Fingerprinting:
Quantitative and qualitative analysis was accomplished with the help of HPTLC instrument. The HPTLC system (Camag, Muttenz, Switzerland) consists of (1) TLC Scanner connected to a PC running WinCATS software under MS Windows NT; (2) Linomat V Sample applicator, (3) Photo documentation system Camag, Reprostar III. The HPTLC analysis requires sample and solvent preparation.

- Step 1: Sample Preparation: All reagents used in this study were of analytical grade. Each extract was redissolved at the concentration of 50 mg/ml in respective solvent (used for extraction) and taken in narrow glass vial and used for plate application.
- Step 2: Plate Activation: Aluminum sheet back coated with Silica gel $60F_{254}$ plates were used in the study. The plates were activated in an oven at 50 °C (10 min) before use. This process helps to remove the moisture and activates the active sites of Silica gel for better separation.
- Step 3: Sample Application: Camag Linomat V (Camag, Muttenz, Switzerland) was utilized for nitrogen gas-assisted and controlled application of samples on to TLC plate. The sample extract was streaked in form of narrow bands at requisite concentration, on the precoated Silica gel $60F_{254}$ Aluminum TLC plate, at a constant application rate of 250 µl/s and gas flow 10 s/µl employed with help of Camag 100 µl syringe connected to a nitrogen tank; using a Camag Linomat V (Camag, Muttenz, Switzerland). Samples were applied at the height of 10 mm from the base, having specific band width and space between two bands.
- Step 4: Plate Development and Chromatographic Conditions: After sample application, the plates were subjected to linear ascending development, in selected solvent system Toluene: Ethyl acetate (93: 7, v/v), up to a distance of about 70 mm. Twin trough glass chamber (with 10 min prior saturation with the solvent system) was used at room temperature.
- Step 5: Scanning of Chromatogram: Subsequent to the development, the TLC plates were dried in a current of air. Densitometric scanning was carried out using Camag TLC Scanner III (Camag, Muttenz, Switzerland) in the absorbance mode at 200 - 450 nm wavelength with a scanning speed of 20 mm/s, data resolution 100 µm/step and a precise slit dimension. The source of radiation utilized was Deuterium and Tungsten lamp. All remaining measurement parameters were at default settings. The chromatograms were integrated and regression analysis and statistical data were generated using WinCATS evaluation software (Version 1.4.2.8121).
- Step 6: Photo Documentation of Plate: After scanning, images captured at three different wavelengths i.e. 254 nm by UV lamp, 366 nm by Mercuric lamp, 400- 800 nm with Photo documentation system Camag, Reprostar III.
- Step 7: Post Chromatographic Derivatization of TLC plate: Post-chromatographic derivatization of developed TLC plates was also performed

wherever necessary. Various spray reagents used to mark/visualize invisible spots in visible range of light. This step is optional and used for better resolution and spotting of separated compounds on the plate.

III. RESULTS AND DISCUSSION

A. Extract yield:

In the present study, Hexane solvent was used for the extraction of powdered Spice material due to presence of good quantity of Essential oil and other

Aromatic principles. Hexane extract yield was obtained from *Cinnamomum zeylanicum* bark powder was 2.90 gm out of 100 gm representing a good extract yield. (Table 2).

Table 2. Spices and Extract yield in Hexane

Plants	Plant part used	% Extract yield*
Cinnamomum zeylanicum	Bark	2.90

[Note: * represents g extract/100g dry powder]

B. %TPC (Total Phenol Content) Estimation by Folin-Ciocalteu Assay:

In the present study, Cinnamon spice Hexane extract was selected (Fig. 1, Table 2) for the presence of % TPC Antioxidant screening using Folin-Ciocalteu assay (Figure 2). In this context, Cinnamon extract showed presence of significant amount of Phenol content representing an Antioxidant activity in the Folin-Ciocalteau assay for % Total Phenol Content (TPC) estimation. In the present study, the Hexane extract of selected Spice plant exhibited different quantity of %TPC as designated in Figure 3.

The results of the screening revealed a presence of possible antioxidant activity as indicated in Table 3. The assay shows colour change based upon the relative concentration of TPC in the test extract. Dark colour of the test solution designates good amount of Phenols 0.0157 ± 0.018 GAE (Figure 3). The OD (Optical Density) was detected using UV-VIS Spectrophotometer.

Phenolic compounds undergo a multifaceted redox reaction with the Phosphotungstic and Phosphomolybdic acids comprised in the Folin-Ciocalteu reagent (Ghafar *et al*, 2010). This way a blue colour develops in the reaction mixture. The intensity of blue colour is relative to quantitative occurrence of antioxidant reducing agent. By captivating the OD of the mixture solution, the quantity of Total Phenols can be calculated.

Fig 3. Good amount of antioxidant activity exhibited by dark blue colour during the assay

Table 3. Plants extracted in Hexane and tested for % Total Phenol Content, Radicle Scavenging Activity, Ferrous Ion Chelating Activity

Plants	% Phenol	% RSA	% FICA
Cinnamomum zeylanicum	0.0157 ± 0.018	34.539 ± 0.432	91.62 ± 0.12

[Note: * represents g extract/100g dry powder]

C. DPPH Assay:

In the present study, % DPPH RSA activity IC_{50} value for the standard BHT (was observed at 0.08 mg/ml concentration. There are few reports available on antioxidant activity of essential oil and various solvent extracts of studied plants like *Cinnamomum zeylanicum* (El-Baroty *et al*., 2010). In the present study; 3 mg extract exhibited 34.539 ± 0.432 % RSA.

It is showed in the former studies that free radicals produced in the body during the usual metabolic procedures can unfavourably alter many crucial biological molecules leading to loss of form and function. Such unwanted variations in the body tissue can lead to onset of numerous diseased circumstances. The antioxidant action of Phenolic compounds is largely due to redox properties, which let them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, heavy metal chelators and hydroxyl radical quenchers. The key role of Phenolic compounds as scavengers of free radicals is emphasized in several previously published reports. A reasonable correlation

amongst antioxidant and free radical scavenging action and Phenolic content was observed in an Antioxidant Assay experiment by Aquil *et al*, 2006 and Singh *et al*, 2009.

D. Ferrous Ion Chelating Activity:

The reducing capacities of extract cannot be attributed solely to individual component of extract, but it is owing to synergistic effect. Therefore, crude extract is more effective than an individual phyto-chemical component. In the present study, % FICA (Ferrous Ion Chelating Activity) IC_{50} value for standard Ascorbic acid detected at 1.5 mg/ml concentration. In this respect, 3 mg Hexane extract test sample of *Cinnamomum zeylanicum*, showed an outstanding % FICA in the range 95 - 70%. Henceforth the test result supports the free radical scavenging ability of the non-polar extract of plants and consequently oil-based recipe can be prepared using the mentioned plant components, can provide the body with promising antioxidant and harmful free radical scavenging impact.

E. HPTLC Phyto-Chemical Fingerprinting:

Cinnamon Hexane extract was subjected for the HPTLC profiling using CAMAG Instrument using standardized protocols to develop its Phyto-Chemical profile for qualitative analysis and further research for therapeutic purposes (Table 4). Aluminum base silica gel coated TLC plates were developed in Toluene: Ethyl acetate (93: 7, v/v) after spotting shows separation of various Phyto-Chemicals in various concentration. The profile shows many Phyto-Chemical components as visible under Florescent, UV and visible light and confirmed in the TLC plate scanning at 200 - 450 nm wavelength range (Fig. 4, 5). R_f values were generated for the various component bands separated on the TLC plate (Table 5, 6). The profile can be used to verify the originality of the material or extract in the pharmaceutical product.

Table 4. HPTLC instrumentation details

Plant name:	*Cinnamomum zeylanicum*
Plant part used:	Bark
Spotting of Samples:	
Track 2	*C. zeylanicum*
Band width	6.0 mm
TLC plate size	10 cm ×10 cm
Two bands distance	23.3 mm
Sample conc.	50 mg/ml
Sample volume	2 µl
Solvent system	Toluene: Ethyl acetate (93:7) v/v.
Spray reagent	10% Ethanolic Sulphuric acid and 1% Ethanolic Vanillin reagent
Slit dimension	6.00 mm × 0.30 mm, Micro

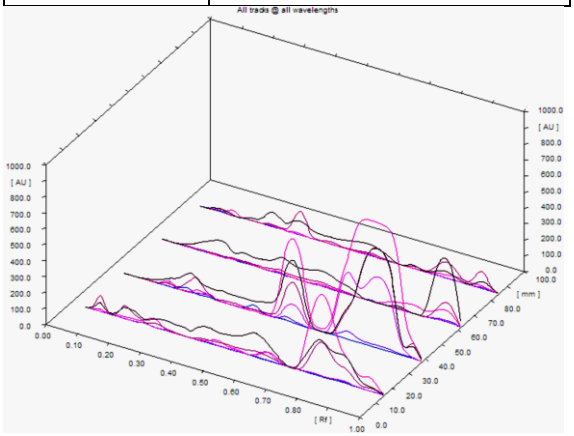
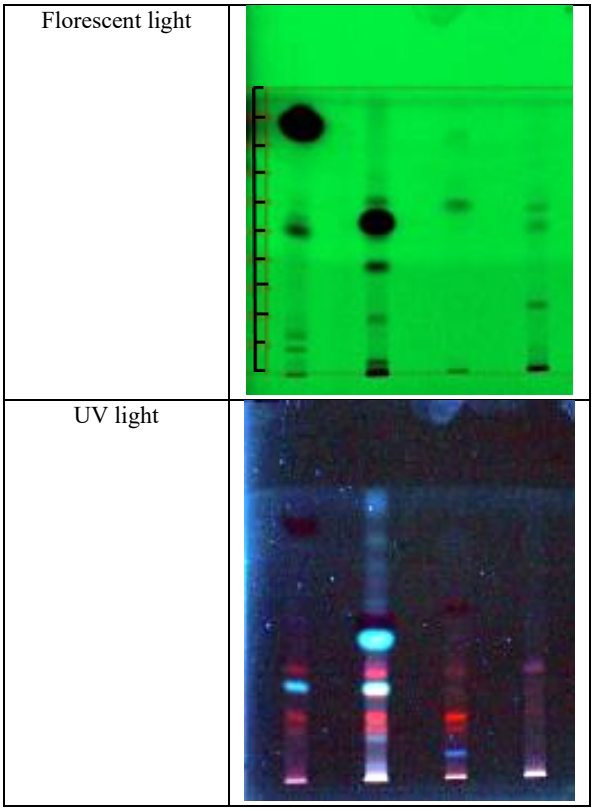


Fig. 4: 3-D graphical display of absorbance peaks (200-450 nm)



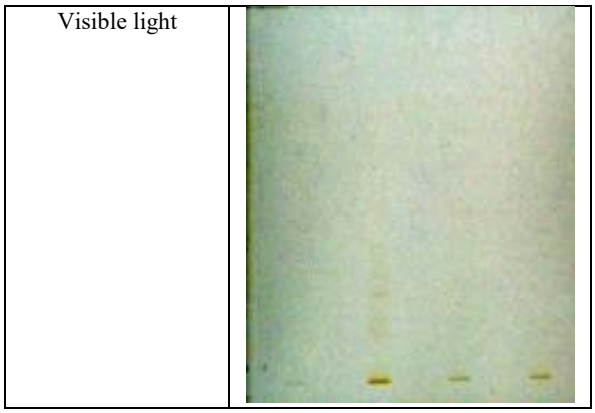
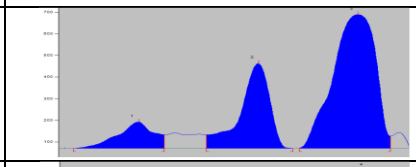
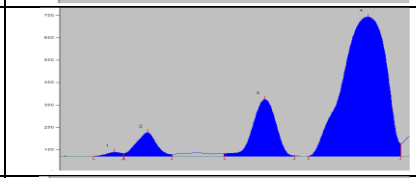
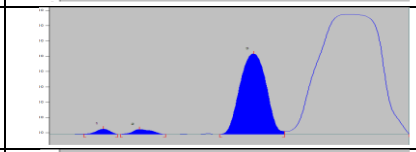
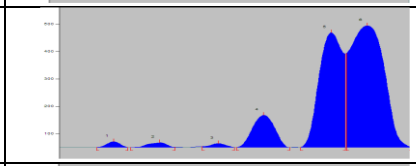
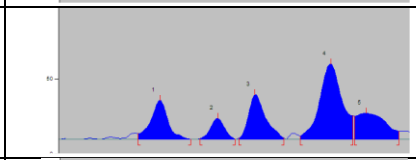
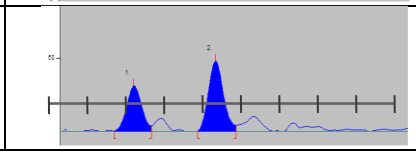


Fig. 5: TLC chromatogram visualized in various lights representing separated compounds (Vertical scale: Distance between two bars indicates 0.10 R_f value)

Table 5. HPTLC fingerprinting of *Cinnamomum zeylanicum*

λ (nm)	Density (AU) vs R_f value
	Track 2
200	
250	
300	
350	
400	
450	

Densitometric chromatogram showing peak display (Horizontal scale: Distance between two bars indicates 0.10 R_f value)

Table 6. Number of separated bands/ compounds and their Rf value documented during HPTLC densitometric scanning for *Cinnamomum zeylanicum* Hexane extract

No. of bands and Rf value	Wavelength (nm)					
	200	250	300	350	400	450
Bands	3	4	3	6	5	2
Rf	(0.09, 0.45, 0.70)	(0.14, 0.22, 0.48, 0.70)	(0.14, 0.24, 0.50)	(0.15, 0.24, 0.42, 0.51, 0.68, 0.80)	(0.26, 0.42, 0.52, 0.68, 0.83)	(0.19, 0.41)

Present work was to find out potential Antioxidant potency of common Spice plant Cinnamon. Its volatile components were isolated in Hexane solvent and the concentrated extract was screened for the amount of Antioxidant activity implementing three different Antioxidant Screening Assay- %TPC, DPPH RSC and FICA. Present finding indicates 0.0157 ± 0.018 % Phenol, 34.539 ± 0.432 % RSA, and 91.62 ± 0.12 % FICA. Also, the prepared extract was used to prepare Phyto-Chemical profile for further detailed work to be carried out to mark exact component having beneficial Antioxidant effect. The study revealed a good amount of Antioxidant activity present in the Spice and it imparts beneficial therapeutic effect on our body during its usage for culinary purposes and also separately used for therapeutic purposes.

Current work thus very well marks that designated Spice plant possess vigorous free radical scavenging mechanisms. Consequently, results of the current research work recommend that selected plant *C. zeylanicum* can be applied as a bio-safe source of latent antioxidants, as natural preservative agent in food industry, and as disease curing agent in pharmacological preparations besides from its culinary uses. The Spice in food also delivers medicinal benefits and cell protection from harmful free radicles. Clinical studies can be implemented to find out the dosage and its usage to treat the disease conditions.

REFERENCES

[1] Apak R., Güçlü K., Demirata B., Özyürek M., Çelik S. E., Bektaşoğlu B., Berker K. I. and Özyurt D. (2007) Comparative Evaluation of Various Total Antioxidant Capacity Assays Applied to Phenolic Compounds with the CUPRAC Assay. *Molecules*, 12: 1496-1547.

[2] Aqil F., Ahmad I., and Mehmood Z. (2006) Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. 30: 177-183.

[3] Dinis T. C., Madeira V. M. and Almeida L. M. (1994) Action of phenolic derivates (acetoaminophen, salycilate and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archive Biochemistry Biophysics, 315: 161-169.

[4] El-Baroty G. S., Abd El-Baky1 H. H., Farag R. S. and Saleh M. A. (2010) Characterization of antioxidant and antimicrobial compounds of cinnamon and ginger essential oils. *African Journal of Biochemistry Research*, 4(6): 167-174.

[5] Ghafar M. F., Prasad K. N., Weng K. K. and Ismail A. (2010) Flavonoid, hesperidine, total phenolic contents and antioxidant activities from *Citrus* species. *African Journal of Biotechnology*, 9 (3): 326-330.

[6] Ghasemi K., Ghasemi Y. and Ebrahimzadeh M. A. (2009) Antioxidant activity, phenol and flavanoid contents of 13 Citrus species peels and tissues. *Pak. J. Pharm. Sci.,* 22 (3):277-281.

[7] Ghimeray A. K., Jin C. W., Ghimire B. K. and Cho D. H. (2009) Antioxidant activity and quantitative estimation of azadirachtin and nimbin in *Azadirachta indica* A. Juss grown in foothills of Nepal. *African Journal of Biotechnology,* 8 (13): 3084-3091.

[8] Harborne J. B. (1984) A Guide to Modern Techniques of Plant Analysis. In: Phytochemical methods. 3rd edn, Chapman and Hall, Hong Kong.

[9] Kitazurua E. R., Moreirac A. V., Mancini-Filhoc J., Delince´ ed H., Villavicencio A. L. (2004) Effects of irradiation on natural antioxidants of cinnamon (*Cinnamomum zeylanicum* N.) *Radiation Physics and Chemistry* 71: 37-39.

[10] Markowicz B. D., Saldanha L. A., Catharino R. R., Sawaya A. C., Cunha I. B., Carvalho P. O. and Eberlin M. N. (2007) Phenolic antioxidants identified by ESI-MS from Yerba Maté (*Ilex paraguariensis*) and Green Tea *(Camelia sinensis*) extracts. *Molecules*, 12: 423-432.

[11] Mirghani M. E., Liyana Y. and Parveen J. (2012) Bioactivity analysis of lemongrass (*Cymbopogan citratus*) essential oil. *International Food Research Journal*, 19(2): 569-575.

[12] Patel R. M. and Jasrai Y. T. (2009) Plant secondary metabolites and their commercial production. *South Asian Journal of Social and Political Sciences,* 9 (2): 115-122.

[13] Porwal P., Shukla K., Mishra D. K., Mahajan S. C. and Tiwari A. (2010). comparative *in vitro* antioxidant activity of *Pongamia pinnata* Linn. leaves extracts and isolated compound. *International Journal of Pharmaceutical & Biological Archives*, 1(1) 69-75.

[14] Saeed N., Khan M. R. and Shabbir M. (2012) Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L. *BMC Complementary and Alternative Medicine*, 12:221.

[15] Singh G., Maurya S., Catalan C. and Lampasona M. P. D. (2004) Chemical Constituents, Antifungal and Antioxidative Effects of Ajwain Essential Oil and Its Acetone Extract. *J. Agric. Food Chem.*, 52: 3292-3296.

[16] Singh R., Singh B., Singh S., Kumar N., Kumar S. and Arora S. (2009) Investigation of ethyl acetate extract/fractions of *Acacia nilotica* willd. Ex Del as potent antioxidant. *Records of. Natural Products*, 3 (3): 131-138.

[17] Souri E., Amin G., Farsam H. and Barazandeh T. M. (2008) Screening of antioxidant activity and phenolic content of 24 medicinal plant extracts. *DARU*, 16 (2): 83-87.

[18] Sreelatha S. and Padma P. R. (2009) Antioxidant activity and total Phenolic content of *Moringa oleifera* leaves in two stages of maturity. *Plant Foods and Human Nutrition*, 64: 303-311.

[19] Yoo K. M., Lee C. H., Lee H., Moon B., and Lee C. Y. (2008) Relative antioxidant and cytoprotective activities of common herbs. *Food Chemistry* 106: 929-936.