

**Research Article****Identification, isolation and elucidation of compounds from fraction of methyl hydroxyl chalcone polymer from aqueous extract of *Cinnamomum zeylanicum*****T. Sivapriya<sup>1\*</sup>, Sheila John<sup>2</sup>**<sup>1</sup>Asst Professor, Department of Clinical Nutrition and Dietetics, SDNB Vaishnav College for Women, Chrompet, Chennai, TamilNadu, India<sup>2</sup>Head and Associate Professor, Department of Home Science, Women's Christian College, Chennai, India

Received: 5 December 2018

Revised: 18 January 2019

Accepted: 27 January 2019

**Abstract**

**Objective:** Cinnamon has been touted as an alternative medicine for type 2 diabetes in recent times. There has been multitude of discrepancies about the bioactive compound responsible for insulin sensitising action. The objective of the study is to purify, identify, isolate and elucidate the molecular structure of bio active compounds present in enriched fraction of methyl hydroxyl chalcone polymer (MHCP) in *Cinnamomum zeylanicum* (CZ). **Materials and methods:** Phytochemical screening was carried out to detect the presence of phytochemicals present. Aqueous extract of CZ was subjected to purification and isolation of the enriched fraction of (MHCP). Then the chemical structure of the enriched fraction of (MHCP) was elucidated using spectroscopic techniques like infrared spectroscopy, nuclear magnetic resonance and mass spectroscopy. **Results and Conclusion:** MHCP enriched fraction was subjected FT infrared analysis, nuclear magnetic resonance spectroscopy and mass spectrometry. The bioactive compounds identified were Ellagic acid 3-O-pentoside, Afzelechin 3-O-glucopyranoside, galocatechin 3-O-pentoside.

**Keywords:** Cinnamon, phytochemical, type 2 Diabetes, insulin, sensitivity

**Introduction**

As per the ancient adage “spice chest is your pharmacy”, the concept of spices being our medicine is acknowledged from historical times, their flavour and properties make them important for culinary and medicinal uses (Parthasarathy et al., 2008). Spices are used for diverse medicinal therapies, such as stimulants, diuretics, carminatives, anti-inflammatory, stomachic, antibiotics, digestives, astringents, antihelminitics, expectorants and tonics (Chattopadhyay et al., 2004; Platel and Srinivasan, 2004). They are also used in numerous forms such as infusions, decoctions, macerations, tinctures, fluid extracts, teas, juices, syrups, poultices, oils, ointments and powders. Essential oils of spices have been used as aromatherapy for depression, stress and anxiety (Peter and Shylaja, 2012).

Among spices, the inner bark of cinnamon is most accepted,

used universally for cooking and as medicines. The medicinal use of this plant has been authenticated in Ayurveda for over 6000 years (Sangal, 2011). Health benefiting property associated with the consumption of cinnamon could be attributed to its polyphenolic constituent. The main polyphenolic compound in cinnamon is a complex polymer proanthocyanidins and is related to the bioactive components of grape and tea polyphenols (Martin and Ernst, 2003).

Proanthocyanidins, after lignans, are the second most common class of natural phenolic substances found in nature. Since they occur in many foods like apple, tea, cinnamon and cocoa, there is a strong and ever increasing interest in determining their biological properties and significance as dietary antioxidants. Proanthocyanidins have demonstrated beneficial actions including anti-inflammatory, hypoglycemic, insulin activation, antioxidant, hypocholesterolemic and anti-allergic properties (Blade et al., 2010).

Research by Anderson's (2004) group established that the active component in cinnamon responsible for its insulin-like

**\*Address for Corresponding Author:**

Dr. T. Sivapriya

Department of Clinical Nutrition and Dietetics, SDNB Vaishnav College for Women, Chrompet, Chennai, Tamil Nadu, India

Email: sivaamanick@gmail.com

DOI: <https://doi.org/10.31024/ajpp.2019.5.3.22>2455-2674/Copyright © 2019, N.S. Memorial Scientific Research and Education Society. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

activity is a water-soluble chemical compound called methyl hydroxyl chalcone polymer (MHCP). They found that methyl hydroxyl chalcone polymer was highly effective, providing essentially the same biological activity as insulin itself. It was effective not only in increasing the uptake of glucose by cells, but also of stimulating the synthesis of glycogen.

Methyl hydroxyl chalcone polymer has the ability to promote phosphorylation process which in turn activates beta cells to improve insulin receptivity of the cells that will then convert glucose into glycogen. Methyl hydroxyl chalcone polymer induces changes in insulin signalling cascade and regulate glucose uptake by exerting insulin mimicking effects (Khunoana, 2011).

There are discrepancies in the results of supplementation studies involving cinnamon in type 2 diabetes, which may be due to the difference in the species of cinnamon and the compounds present in polymeric polyphenol MHCP. With these background the objective of the study has been framed as to purify, identify, isolate and elucidate the molecular structure of bio active compounds present in enriched fraction of methyl hydroxyl chalcone polymer (MHCP) in *Cinnamomum zelaynicum* (CZ).

## Materials and methods

### Chemical reagents

Most of the chemicals used for phytochemical screening were purchased from Biosar, India. For purification and identification study, analytical grade chemicals were purchased from Loba Chem. The IR spectra were obtained using JASCO 4100 by KBr Pellet method. NMR spectra were recorded on JEOL spectrometer at 500 and 125 MHz, respectively with DMSO-d<sub>6</sub> as a solvent. Mass spectra were obtained using JEOL-Accu TOF JMS- T 100 LC mass spectrometer.

### Preparation of test samples

The aqueous, methanolic and ethanolic extracts were subjected to preliminary phytochemical screening to detect the presence of phytoconstituents by the following methods:

Test samples (5.0 g each) were transferred to conical flasks and 50 ml of methanol, ethanol and distilled water were added as a single solvent in each flask. The mixtures were stored for 24 hours at room temperature. Finally, the samples were filtered with Whatmann filter paper No. 1 and used for qualitative analysis. Phytochemical screening was done by the methods suggested by Trease and Evans, (1989).

### Detection of alkaloids

To 5 ml of extract, dilute hydrochloric acid was added and filtered. The filtrate was used to perform the following test for the detection of alkaloids.

Mayer's test: Filtrates were treated with Mayer's reagent

consisting of potassium mercuric iodide. Formation of a yellow coloured precipitate confirms the presence of alkaloids.

Dragendorff's test: About 1 ml of the filtrate was treated with Dragendorff's reagent. The appearance of reddish brown precipitate indicates the presence of alkaloid.

### Detection of glycosides

Legal's test: Test sample extracts (1 ml each) were added to individual test tubes and 1 ml pyridine and 1 ml sodium nitroprusside were added to each test tube. Development of pink to red colour confirms the presence of glycosides.

### Detection of saponin

Froth Test: Extracts were diluted with distilled water to 20 ml and vigorously shaken in a conical flask for 15 minutes. Formation of foam indicates the presence of saponin.

Foam Test: 0.5g of the extract was shaken with 2 ml of water. If the produced foam continues for ten minutes, it indicated the presence of saponin.

### Detection of phenols

Ferric Chloride Test: Extracts were treated with 3 to 4 drops of 1% ferric chloride solution. Formation of bluish black colour confirms the presence of phenols.

### Detection of flavonoids

Lead Acetate test: To the test sample, 12 - 15 drops of lead acetate solution were added. Formation of yellow colour precipitate confirms the presence of flavonoids.

Shinoda's test: The bark extracts were dissolved in alcohol. To this one piece of magnesium, and concentrated hydrochloric acid were added drop wise and heated. The appearance of magenta colour shows the presence of flavonoids.

### Detection of steroids

Liebermann Burchard test: Samples were treated with concentrated sulphuric acid and a few drops of glacial acetic acid was followed by the addition of acetic anhydride. The appearance of green colour indicates the presence of steroids.

### Detection of tannins

Lead Acetate test: Extracts were treated with 10% lead acetate solution; the appearance of white precipitate confirms the presence of tannins.

Ferric Chloride Test: Extracts were treated with 3 to 4 drops of 1% ferric chloride solution. The appearance of bluish black colour confirms the presence of tannins.

### Detection of terpenoids

Salkowski test: Test sample extract (5 ml) were transferred to separate test tubes. 2 ml of chloroform and 3 ml of concentrated sulphuric acid were added to each to form a layer. Formation of a reddish brown colouration at the interface shows positive results for the presence of terpenoids.

#### Detection of coumarin

About 0.5 g moistened dry powder of each test sample was taken in separate test tubes. The test tubes were covered with filter paper, pre-soaked in dilute NaOH. The tubes were kept in a water bath. The filter papers were then exposed to UV light and observed for colour development. Appearance of yellowish green fluorescence indicated the presence of coumarin.

#### Detection of Anthocyanin

Ammonia and 2 ml of 2N HCL were added to 2 ml of aqueous extract. The appearance of pinkish red colour, that later turns bluish-violet confirms the presence of anthocyanins.

#### Purification, identification, isolation and elucidation of compounds in enriched fraction of methyl hydroxyl chalcone polymer (MHCP)

After the preliminary phytochemical screening, the aqueous extract of CZ was used to carry out the purification process as the aqueous sample had abundance of polyphenols. The polymeric MHCP enriched fractions were isolated from aqueous extract of cinnamon as described by Krishnan et al. (2006). Isolation and purification were carried out by successive extraction of an aqueous extract of cinnamon with chloroform, ethyl acetate and n-butanol, followed by acidification and further extraction with n-butanol. The chloroform extract was discarded while the residues from the other extracts were purified by fractional precipitation, employing mixtures of acetone/ether and methanol/ether. The resulting solid was dried in a vacuum oven overnight and subjected for Infrared Spectroscopy (IR), Nuclear Magnetic Resonance Spectroscopy (NMR) and Mass Spectral analysis (MS).

#### Results and discussion

There is limited research about the bioactive components of different species of cinnamon that are ingested. The conflicting results of cinnamon supplementation trials might be due to the consumption of different cinnamon species with undefined compounds. Thus, investigation about the active constituents in *Cinnamomum zeylanicum* regarding its therapeutic potential in the cure for diabetes is crucial.

*Cinnamomum zeylanicum* species contain both water and oil soluble compounds and are made of phenyl propanoids, terpenes, flavonoids and saponins. All these compounds polymerize to form methyl hydroxy chalcone polymer which is purported to be the core polymer in lowering blood glucose levels in type 2 diabetes individuals (Khuanona, 2011).

#### Qualitative estimation of phytochemicals in *Cinnamomum zeylanicum*

A thorough understanding about the phytochemicals present in *Cinnamomum zeylanicum* and their properties is vital for finding novel functional foods. For any intervention study, to have a positive impact upon human health, it is necessary to evaluate its efficacy and safety, so that it does provide the anticipated benefits and is harmless.

Phytochemical screening of cinnamon extracts (table 1) revealed the presence of various phytochemicals which have pharma and industrial values including aromas, dyes, gums, resins, pulp, fibre etc. with high bearing on health and commercial sectors. Saleem, (2009) in his research has identified bioactive compounds in *Cinnamomum zeylanicum* bark as polyphenols, flavonoids, saponins, tannins and coumarins.

Preliminary phytochemical assay on the extract of *Cinnamomum zeylanicum* bark established the presence of

**Table 1.** Phytochemical assay of *Cinnamomum zeylanicum*

Phytochemicals	Aqueous extract	Ethanolic Extract	Methanolic extract
Alkaloids	+	+	-
Flavonoids	++	+	+
Terpenoids	+	+	+
Steroids	-	-	-
Anthocyanin	+	+	+
Coumarin	+	-	+
Tannin	+	+	+
Glycosides	+	-	+
Polyphenol	++	+	+
Saponins	+	+	-

+presence of phytochemicals, ++ abundant presence of phytochemicals, - absence of phytochemicals

alkaloids, flavonoids, terpenoids, anthocyanins, coumarins, tannins, glycosides, polyphenols, and saponins in judicious amounts and indicated the absence of steroids. Results of the present study showed that aqueous extract of *Cinnamomum zeylanicum* indicates an abundance of phytochemicals such as flavonoids and polyphenols. After carrying out the preliminary assay for the presence of phenols, flavonoids, tannins, coumarins, and saponins the dried aqueous extract powder of cinnamon zeylanicum bark was considered for purification, isolation and elucidation of molecular structure of compounds.

### Purification process

Polymeric MHCP enriched fractions were isolated from aqueous extract of *Cinnamomum zeylanicum* as described by Krishnan et al. (2006). Isolation and purification was carried out by successive extraction of an aqueous extract of cinnamon zeylanicum with chloroform, ethyl acetate and n-butanol, followed by acidification and further extraction with n-butanol. The chloroform extract was discarded while the residues from the other extracts were purified by fractional precipitation, employing mixtures of acetone/ether, methanol/ether. The final form of solid was dried out under a vacuum overnight and submitted for IR, NMR and Mass spectral analysis.

### Spectroscopic techniques

Using infrared spectroscopy, nuclear magnetic resonance and mass spectroscopy techniques, scientists confirmed that a proanthocyanidin compound in *Cinnamomum zeylanicum* affects insulin signalling in fat cells (Gallessich and Magruder, 2004). Hence one of the main aim of the present investigation is to identify, isolate and elucidate the structure of the polyphenolic polymers found in the aqueous extract of *Cinnamomum zeylanicum*. The following identification techniques were used to identify the compounds present in AECZ, their structures and their molecular weights.

### Fourier transform infrared spectroscopy (FT-IR analysis)

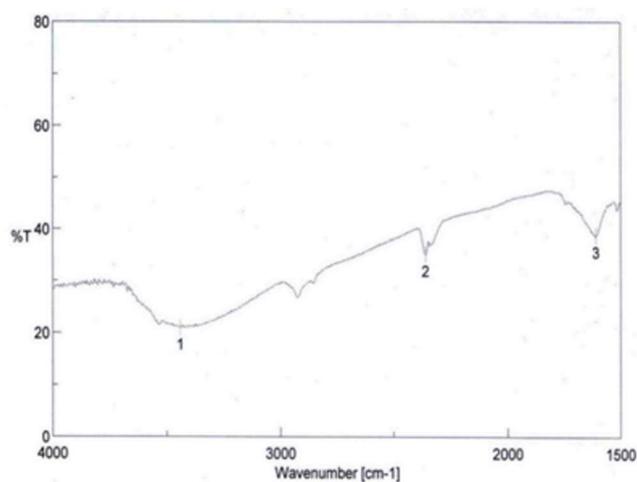
FTIR analysis was used to detect the characteristic peak value

**Table 2.** Functional groups detected in MHCP enriched fraction by FTIR analysis

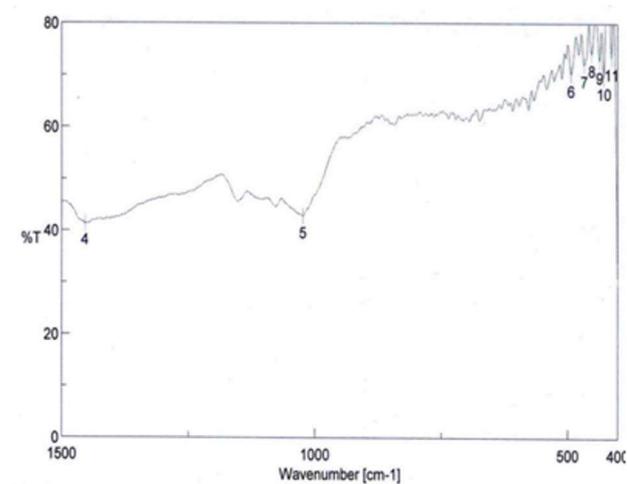
Peak No.	Wave number $\text{cm}^{-1}$	Functional groups
1	3440.39	(OH) aromatic
2	2360	(=CH) aromatic
3	1610	(-C=O) aromatic
4	1454	(-CH <sub>2</sub> ) aromatic
5	1024	(-CH) aromatic
6	456	(-COOH) aromatic

and their functional groups of the active components present in MHCP enriched fraction of *Cinnamomum zeylanicum*. When the aqueous extract was passed into the FTIR, the spectral data showed the broader peaks in the 3000-3500  $\text{cm}^{-1}$  region due to the presence of polyphenols containing hydroxyl groups (3440.39  $\text{cm}^{-1}$ ) in MHCPs enriched fraction. The peak at 1610  $\text{cm}^{-1}$  indicates the presence of (-C=O) group in the polyphenolic structure. The peak at 2360  $\text{cm}^{-1}$  was attributed to the presence of (=CH) stretch for the aromatic nature. The absorption showed at 1454  $\text{cm}^{-1}$  is due to the presence of (-CH<sub>2</sub>) groups in the molecule. The peak at 1024  $\text{cm}^{-1}$  exhibited the presence of (-CH) groups.

The functional groups present in MHCP enriched fraction detected using FTIR analysis is presented in table 2 and figure 1 and 2. Results of the present study were similar to the results of Rajadurai et al. (2016), who characterized the bioactive constituents present in the bark extract of cinnamon zeylanicum using FTIR analysis. FTIR analysis results proved the presence of alcohols, phenols, alkynes,



**Figure 1.** FT-IR spectrum indicating peaks 1-3



**Figure 2.** FT-IR spectrum indicating peaks 4 - 11

alkanes, aromatic amines, alkyl halides, aliphatic amines in their samples.

### H- Nuclear Magnetic Resonance analysis

Nuclear magnetic resonance analysis serves as an important tool for detection of polyphenolic compounds in *Cinnamomum zeylanicum*. Hydrogen-1 NMR, or  $^1\text{H}$  NMR analysis was performed to confirm the presence of aromatic compounds present in MHCP enriched fraction of *Cinnamomum zeylanicum* (figure 3). In H-NMR spectrum, the chemical shift obtained at 3.21-3.83 indicates the presence of (-OH) hydroxyl groups. The peaks in the region of 6.35-6.99 showed that the structure of the compounds was aromatic in nature.

Results of the present study was similar to result obtained by Maria et al. (2012). A proanthocyanidin trimer, two tetramers, and a pentamer have been isolated in their free phenolic forms from the bark of *Cinnamomum zeylanicum*.  $^1\text{H}$ - and C-NMR analysis, has unequivocally established their structures as catechins and epicatechins by comparing the peaks obtained using NMR spectral data base.

Catechins and epicatechins are flavonoid compound possessing strong antioxidant activity. Consumption of cinnamon with these phytochemicals help in scavenging the free radicals. Free radicals are the main causative factor for degenerative disease like type 2 diabetes mellitus.

### Mass spectral analysis

Mass Spectrometry is an analytic technique that finds the relative masses of molecular ions. It is a powerful method as it provides a great deal of information like determination of molecular mass of a compound, finding out the structure of an unknown substance.

Infusion of the MHCP fraction into mass spectrometry showed that they consisted of oligomers ranging in masses from 376 to 1728 Da. The molecular masses of trimers and tetramer indicated that they were A-type doubly linked procyanidin oligomers of the catechins/epicatechins. The presence of an ion at  $m/z$  287 appears to be indicative of the doubly linked A-type catechin/epicatechin oligomers (Anderson et al., 2004).

In figure 4, the mass spectrum analysis showed the molecular ion peaks at  $m/z$ , 435.2, 437.2, 438.3 that corresponds to Ellagic acid 3-O-pentoside, Afzelechin 3-O-galloylpyranoside, galocatechin 3-O-pentoside respectively.

### Proposed structure of the compounds identified from MHCP enriched fraction

The mass spectrum displayed the molecular ion peaks at  $m/z$ , 435.2, 437.2 and 438.3. By comparing the above stated results with published data and by interpreting the above results, it was concluded that the compounds present in

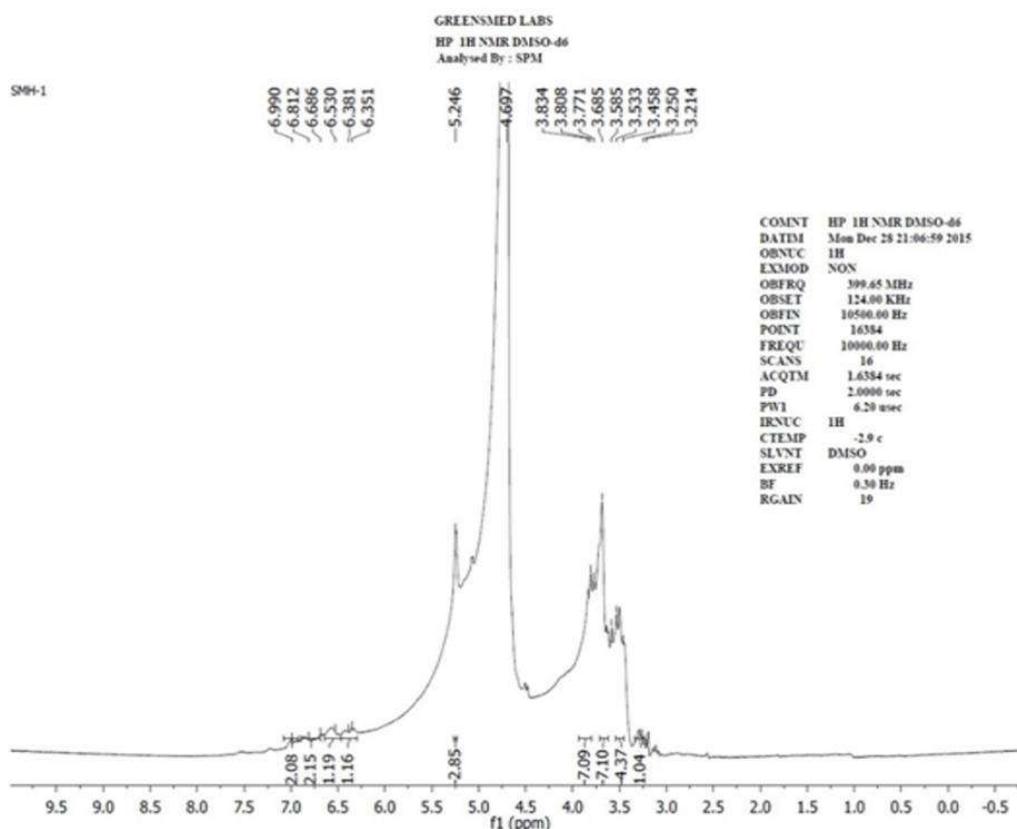


Figure 3. NMR spectra obtained from the MHCP enriched fraction of *Cinnamomum zeylanicum* extract

Sample Name: MH-1  
Ionization mode: Positive

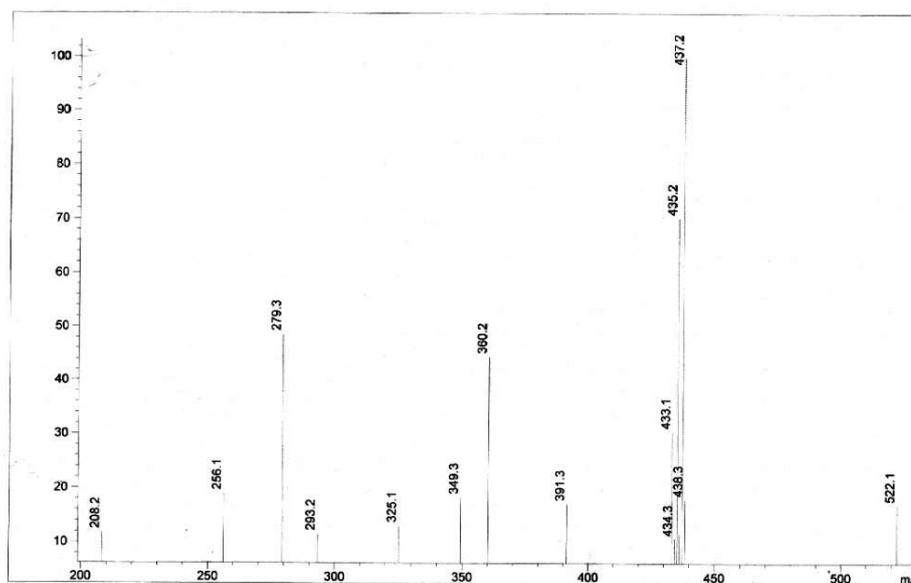


Figure 4. Mass spectral analysis of MHCP enriched fraction

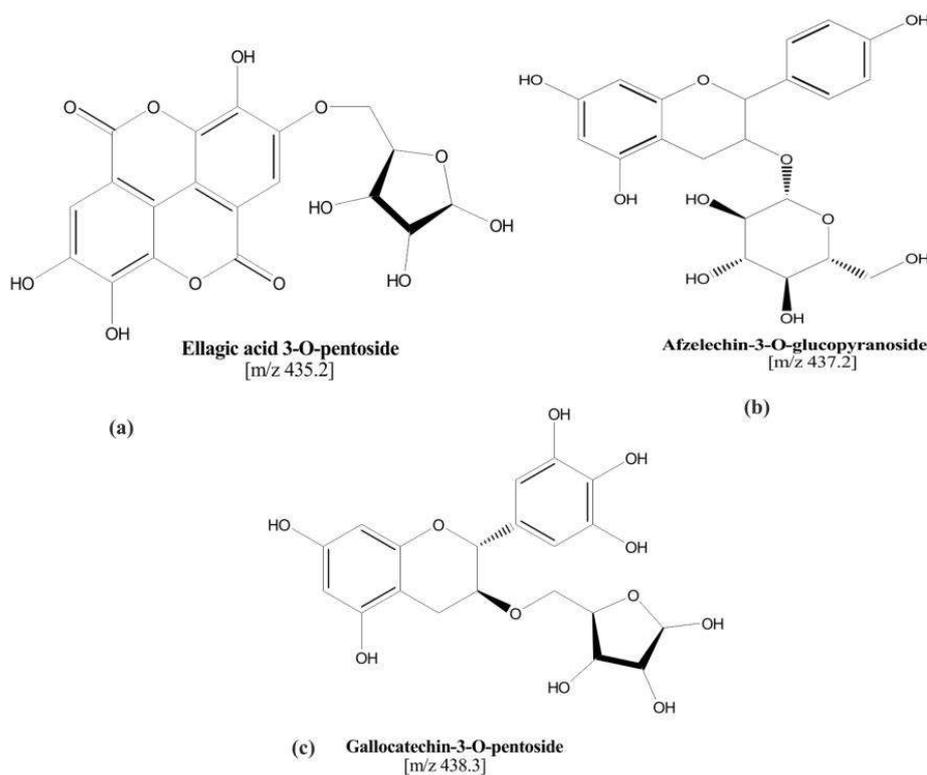


Figure 5. Structure of (a) Ellagic acid 3-O-pentoside, (b) Afzelechin glucopyranoside and (c) galocatechin 3-O-pentoside

enriched MHCP fraction were Ellagic acid 3-O-pentoside, Afzelechin glucopyranoside and galocatechin 3-O-pentoside respectively.

Structure of Ellagic acid 3-O-pentoside, Afzelechin glucopyranoside and galocatechin 3-O-pentoside is represented by figures.

The mass spectral analysis, H-NMR and FT-IR spectral data of the sample indicated that the compounds present in the MHCP enriched fractions, were that of the polyphenolic compound or proanthocyanidin type of compounds. The peaks from spectral data have been assigned to be Ellagic acid 3-O-pentoside, Afzelechin 3-O-glucopyranoside, Galocatechin 3-O-pentoside respectively.

Results of the present investigation matched with that of (Shan et al., 2007) who revealed the presence of polymeric polyphenols, mainly condensed tannins i.e., proanthocyanidins and catechins in cinnamon zeylanicum. Proanthocyanidins and (epi) catechins, were identified as procyanidin (b1, b2) dimers and trimers, and (+)-catechin and (-) epicatechin. These compounds further polymerize to form polyphenolic polymers, which are commonly known as Type A and Type B polymer or Methyl hydroxy chalcone polymer (MHCP), depending upon the orientation of functional groups attached to the general structure (Ferrer et al., 2006).

Scientists have also identified that procyanidin (Type A) was methyl hydroxyl chalcone, which improves the sensitivity of the insulin receptors cells and can be effectively used to cure cases of insulin resistance (Anderson et al., 2008; Landrault et al., 2003; Anderson et al., 2004).

### Conclusion

The Mass, IR, <sup>1</sup>H-NMR and FT-IR spectral data indicated the compounds present in the MHCPs enrich fractions were that of poly phenolic compounds viz Tannins, proanthocyanidin type of compounds. The peaks from spectral data have been assigned as Ellagic acid 3-O-pentoside, Afzelechin 3-O-glucopyranoside, galocatechin 3-O-pentoside respectively. By understanding the characteristics and concentration of the compounds present in Cinnamon zeylanicum, a functional food with more benefits and less side effects can be made from cinnamon which will be of great use to the community in preventing type 2 diabetes and delaying the onset of complications.

### Conflicts of interest

The author has no conflict of interest.

### References

- Anderson R. 2008. Chromium and poly phenols from cinnamon improve insulin sensitivity. *Proceedings of the Nutrition Society*, 67:48-53.
- Anderson RA, Broadhurst CL, Polansky MM, Schmidt WF, Khan A. 2004. Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. *Journal of Agricultural and Food Chemistry*, 52(1):65-70.
- Blade C, Arola L, Salvado MJ. 2010. Hypolipidemic effects of proanthocyanidins and their underlying biochemical and molecular mechanisms *Molecular Nutrition & Food Research*, 54:37-59.
- Chattopadhyay I, Biswas K, Bandyopadhyay U, Banerjee RK. 2004. Turmeric and curcumin: Biological actions and medicinal applications. *Current Science*, 87:1.
- Ferrer J, Jez JJ, Bowman ME, Dixon RA, Noel JP. 2006. Structure of chalcone synthase and the molecular basis of plant polypeptide biosynthesis. *Natural Structural Biology*, 8:775-784.
- Gallessich G, Magruder J. 2004. Cinnamon may help to alleviate diabetes says UCSB researcher, article from the University of California, Santa Barbara.
- Khunoana S. 2011. Identification, characterization and quantification of the active and toxic compounds of two cinnamon species, URL: <http://hdl.handle.net/10210/5042>.
- Krishnan R, Maru GB. 2006. Isolation and analysis of polymeric polyphenol from black tea. *Food Chemistry*, 96:331-340,
- Landrault N, Poucheret P, Azay J, Krosniak M, Gasc F, Jenin C, Cros G, Teissedre PL. 2003. Effect of a polyphenols-enriched chardonnay white wine in diabetic rats. *Journal of Agricultural and Food Chemistry*, 51(1):311-8,
- Maria M, Fuget E, Carmen Q. 2012. Identification of proanthocyanidin from cinnamon using MALDI-TOF mass spectrometry. *Analytical and Bio-analytical Chemistry*, 402:1327-1336.
- Martin W, Ernst E. 2003. Herbal medicines for treatment of bacterial infections: a review of controlled clinical trials. *Journal of Antimicrobial Chemotherapy*, 51:241-246.
- Parthasarathy VA, Chempakam Band Zachariah TJ. *Chemistry of spices*. 2008. CABI, 978-1-84593-405-7.
- Peter KV, Shylaja MR. 2012. Introduction to herbs and spices: definitions, trade and applications. In: *The Handbook of herbs and spices*. Second edition, 1:1-24. Woodhead Publishing.
- Patel K, Srinivasan K. 2004. Digestive stimulant action of spices: A myth or reality? *Indian Journal of Medical Research*, 119:167-179.
- Rajadurai M, Kumaresan R. 2016. Phytochemical Analysis of Bark Extract of *Cinnamomum verum*: A Medicinal Herb Used for the Treatment of Coronary Heart Disease in Malayali Tribes, Pachamalai Hills. *International Journal of Pharmacognosy and Phytochemical Research*, 8(7):1218-1222.
- Sangal A. 2011. Role of cinnamon as beneficial antidiabetic food adjunct: a review. *Advances in Applied Science Research*, 2:440-450.
- Shan B, Brooks J, Cai Y. 2007. The in vitro antibacterial activity of dietary spice and medicinal herb extract. *International Journal of Food Microbiology*, 117(1):112-9.
- Trease GE, Evans WC. *Pharmacognosy*. 11<sup>th</sup> edn., Bailliere Tindall, London; 1989 pp. 45-50.