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Identification of Insulin like Polyphenol from The Inner Bark of CinnamomumZeylanicum by IR, NMR Spectroscopy

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ABSTRACT

Cinnamomumzeylanicum (CZ) 's inner bark has been a product of much interest to scientists owing to its multifaceted medicinal properties. Indian and Chinese traditional medical system have been using cinnamon from antique times due to its antimicrobial, anti-inflammatory and antidiabetic properties. The study aimed to identify, isolate and elucidate the molecular structure of compounds found in methyl hydroxychalcone polymer, which has insulin-like biological activity. The qualitative and quantitative phytochemical assay was carried out to detect various phytochemicals present in Cinnamomumzeylanicum inner bark. Aqueous CZ extract was subjected to purification, and the enriched fraction of methyl hydroxychalcone was isolated.Spectroscopic methods elucidated the chemical structure of the enriched fraction of (MHCP). The results reveal that the bioactive compounds in enriched MHCP fraction were ellagic acid 3-O-pentoside, afzelechin 3-O-glucopyranoside, and gallocatechin 3-O-pentoside. It was found that these compounds possessed biological activity similar to grapes and tea polyphenols.

KEYWORDS

Cinnamon, polyphenols, mass spectrometry, methyl hydroxychalcone polymer.

INTRODUCTION

The cinnamon's inner bark has been used as a culinary product in various cultures to enhance flavour and taste. The traditional medical systems worldwide also tout cinnamon as a miracle spice due to its medicinal properties. Recently, cinnamon has been found to alleviate blood glucose levels in type 2 diabetes subjects at an encouraging level. Scientific evidence also suggests that cinnamon possesses antioxidant, antimicrobial, anticancer, and hypotensive effects (Huda et al., 2019).

Aqueous cinnamon extracts have been shown to potentiate insulin activity >20 fold higher than any other compound in the in-vitro assay of type 2 diabetes. The positive health effects of cinnamon consumption could be attributed to its polyphenolic composition, mainly the methyl hydroxychalcone polymer (MHCP) responsible for its insulin-likeactivity (Qureshi et al., 2019). This polyphenolic polymer enhances enzyme lipase activity that hydrolyses dietary fat molecules, increases glycogen

synthesis in the liver, enhances glucose uptake and phosphorylation of insulin receptor in skeletal muscles and adipocytes (Jain et al., 2017).

Methyl hydroxyl chalcone polymer can promote the phosphorylation process, which activates beta cells to improve the cells' insulin receptivity, converting glucose into glycogen. Methyl hydroxyl chalcone polymer induces insulin signalling changes and regulates glucose uptake by exerting insulin mimicking effects(Khunoana, 2011).

Traditional medicines are preferred by many people due to the fewer side effects and unaffordability of allopathic medicines. Cinnamon is also widely consumed as concoctions and tea to cure diabetes. MHCP has been used in many herbal formulations to treat type II, diabetes individuals. It contains a phenolic compound coumarin that produces hepatotoxicity when consumed in excess (Muhammad D and Koen D., 2017).

Controversial results were found in the supplementation studies involving cinnamon in type 2 diabetes, which may be due to the difference in cinnamon species and the compounds present in polymeric polyphenol MHCP. The work aimed to purify, identify, isolate and elucidate the molecular structure of phenolic components present in an enriched fraction of methyl hydroxyl chalcone polymer (MHCP) of Cinnamomumzelaynicum species.

MATERIALS AND METHODS

REAGENTS AND MATERIALS

Chemicals used for phytochemical screening and characterisation study were procured from Biosar and Lobachem, India Pvt Ltd. JASCO 4100 by KBr Pellet method, JEOL spectrometer at 500 and 125 MHz,werewith DMSO-d6 as a solvent.

PREPARATION OF TEST SAMPLES

The aqueous, methanolic and ethanolic extracts of CinnamomumZeylanicum were subjected to preliminary phytochemical screening to detect phytoconstituents' presence by the procedure of Trease and Evans., 1989.

PURIFICATION OF ENRICHED FRACTION OF MHCP

Since the aqueous extract of CZ had an abundance of polyphenols,the aliquot part was used as a sample throughout the analysis. The enriched fractions were isolated from aqueous cinnamon, as Krishnan et al. (2006) described. Isolation and purification were carried out by successive extraction with chloroform, ethyl acetate and n-butanol, followed by acidification and n-butanol. The chloroform extract was discarded while the other extracts' residues 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) (Sivapriya T and John S., 2019).

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RESULTS AND DISCUSSION

There is fewer scientific evidence about the bioactive components present in different cinnamon species as there exist multiple cinnamon species, and their composition varies according to the geographical location. Discrepancies in the randomised control study results of CZ supplementation trials might be due to the ingestion of different cinnamon species with unidentified phytochemicals. Thus, an investigation about Cinnamomumzeylanicum species' active constituents regarding its therapeutic potential in treating diabetes is crucial. Cinnamomumzeylanicum species contain both water and oil-soluble compounds and are made of phenylpropanoids, terpenes, flavonoids and saponins. Compounds catechin, epicatechin, epigallocatechin, ellagic acid pentoside polymerise to methyl hydroxychalcone polymer, purported to be the core component lowering blood glucose levels in type 2 diabetes individuals (Hariri, M. and Ghiasv and R., 2016).

SCREENING FOR PHYTOCHEMICALS IN CINNAMOMUM ZEYLANICUM

Seamless analysis of CZ and its characteristics is pivotal for the development of novel functional foods. For any intervention study, it is necessary to evaluate its efficacy and safety to impact human health and provide the anticipated benefits positively. (Sivapriya T and John S., 2020). Phytochemical screening disclosed various components that have nutraceutical values with high demand on health and commercial sectors. Rajadurai M, Kumaresan R., 2016.in their research has identified bioactive compounds in Cinnamomumzeylanicum bark as polyphenols, flavonoids, saponins, tannins and coumarins.

TABLE 1- PHYTOCHEMICAL ASSAY RESULTS

S.No	Phytoconstituents	Aqueous extract	Ethanolic Extract	Methanolic extract
1	Alkaloids	+	+	-
2	Flavonoids	++	+	+
3	Terpenoids	+	+	+
4	Steroids	-	-	-
5	Anthocyanin	+	+	+
6	Coumarin	+	-	+
7	Tannin	+	+	+

8	Glycosides	+	-	+
9	Polyphenol	++	+	+
10	Saponins	+	+	-

+ shows the presence of phytochemicals

++ shows the abundant presence of phytochemicals

- Shows the absence of phytochemicals

Preliminary phytochemical assay on the extract of Cinnamomumzeylanicum bark established alkaloids, flavonoids, terpenoids, anthocyanins, coumarins tannins, glycosides, polyphenols, and saponins in judicious amounts and indicated the absence of steroids. The present study showed that aqueous extract of Cinnamomumzeylanicum 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 the molecular structure of compounds (Sivapriya T and John S., 2019).

PURIFICATION PROCESS

Polymeric MHCP enriched fractions were isolated from aqueous cinnamon extract as described by Krishnan et al., 2006. The following identification techniques were used to identify the compounds present in AECZ, their structures and their molecular weights.

PLATE 1 - PURIFIED MHCP ENRICHED FRACTION

FOURIER TRANSFORM - INFRARED SPECTROSCOPY ANALYSIS (FT-IR)

The FT-IR spectral data showed broader peaks in the 3000-3500 cm^{-1} region, assigned to the presence of polyphenols containing hydroxyl groups (3440.39 cm^{-1}) in MHCPs enriched fraction. The signal at 1610 cm^{-1} indicates the presence of (-C=O) group in the

polyphenolicstructure. The frequency at 2360 cm^{-1} is due to (=CH) stretch for the aromatic nature. The absorption showed at 1454 cm^{-1} is due to ($-CH_2$) groups in the molecule. The peak at 1024 cm^{-1} exhibited characteristics of (-CH) groups (Sivapriya T and John S., 2019).

TABLE 2- RESULTS OF PEAK PICKING

Peak No	Wave No cm-1	Functional group
1	3440.39	(OH) aromatic
2	2360	(=CH) aromatic
3	1610	(-C=O) aromatic
4	1454	($-CH_2$) aromatic
5	1024	(-CH) aromatic
6	456	(-COOH) aromatic

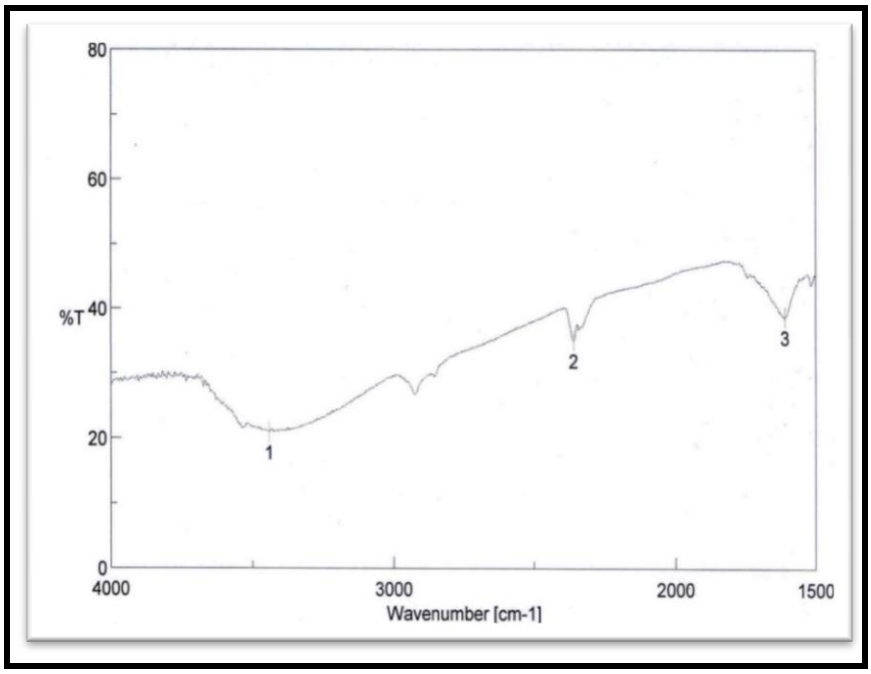

FIGURE 1 - FT-IR SPECTRUM INDICATING PEAKS 1-3

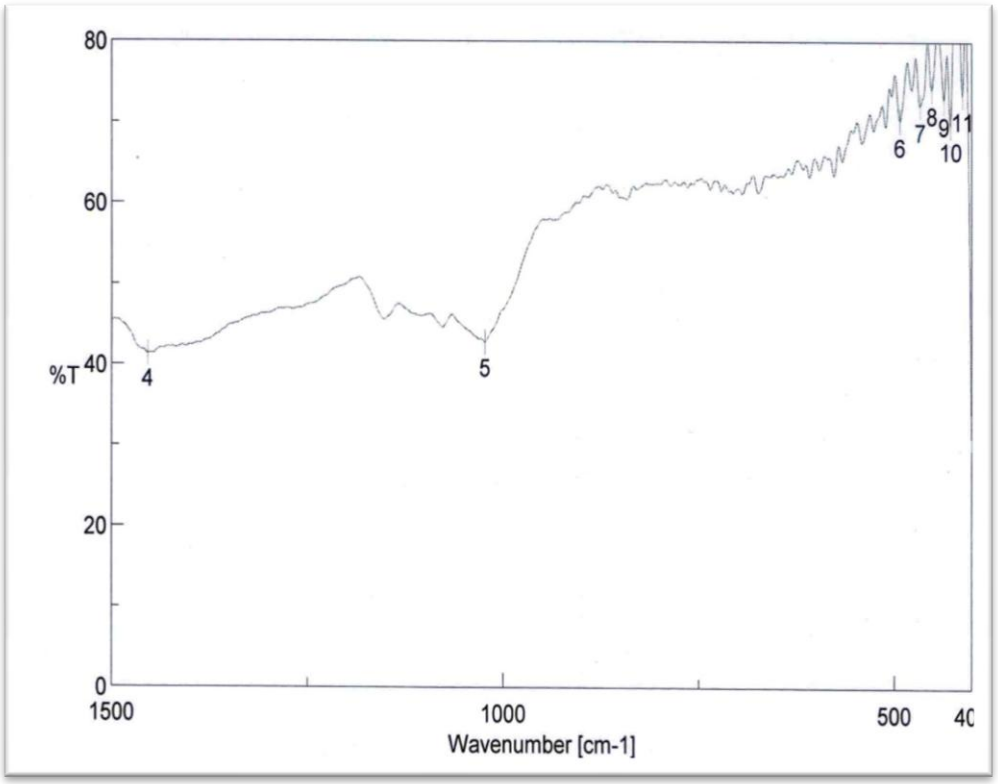

FIGURE 2 - FT-IR SPECTRUM INDICATING PEAKS 4-11

PROTON NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (H-NMR ANALYSIS)

In the 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 compounds' structure was aromatic.

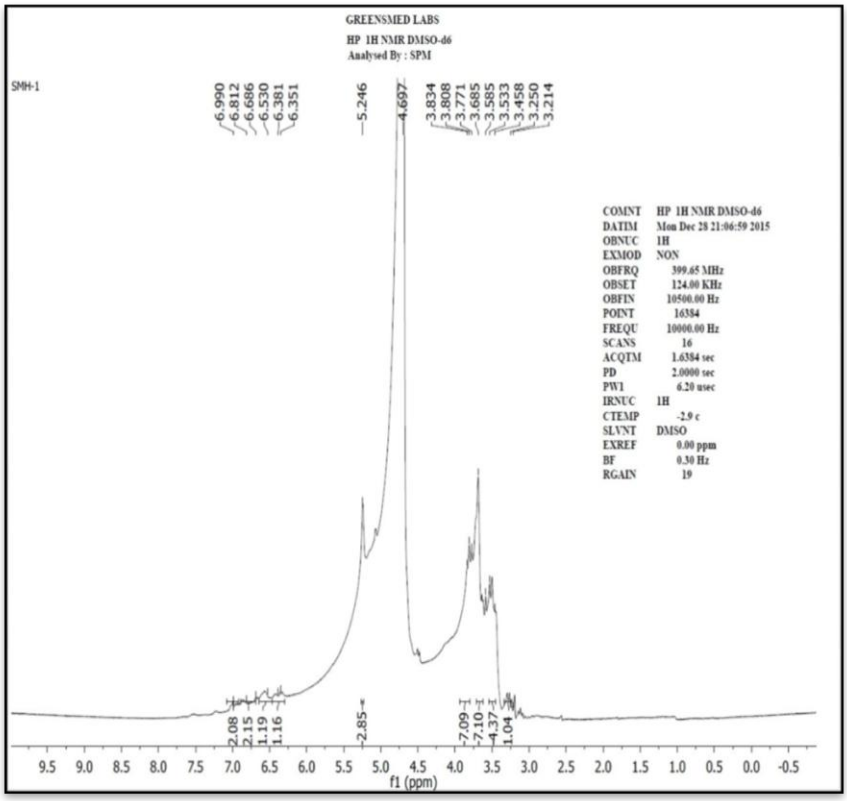

FIGURE 3 - NMR SPECTRUM INDICATING PEAKS AT 3.5 AND 6.5

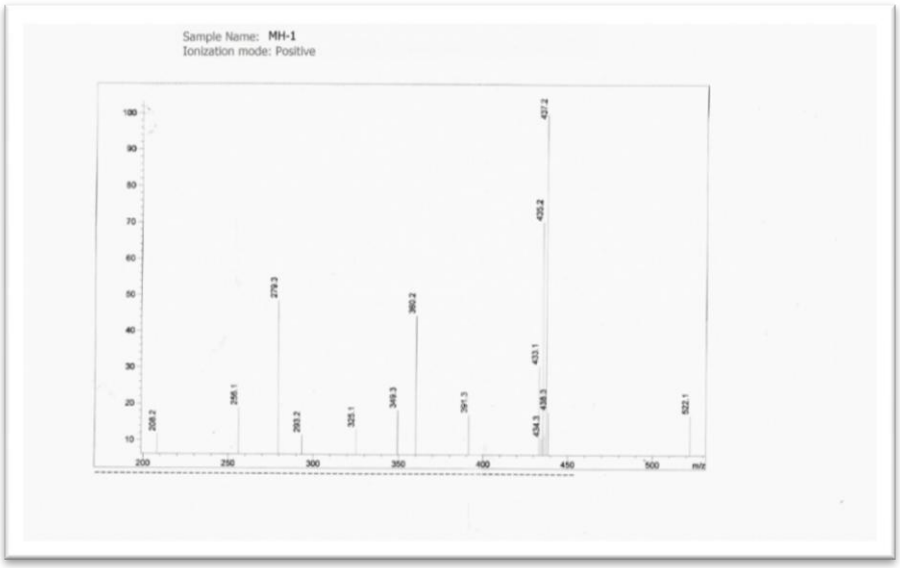

FIGURE 4 - MASS SPECTRAL ANALYSIS

The mass spectral 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-glucopyranoside, gallocatechin 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 were Ellagic acid 3-O-pentoside, Afzelechinglucopyranoside and gallocatechin 3-O-pentoside respectively.

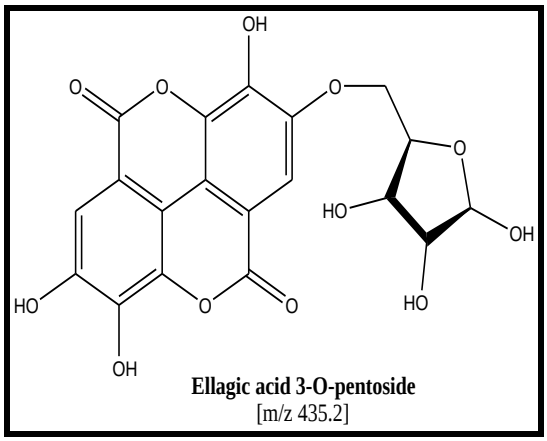

FIGURE 5 - STRUCTURE OF ELLAGIC ACID 3-O-PENTOSIDE [M/Z 435.2]

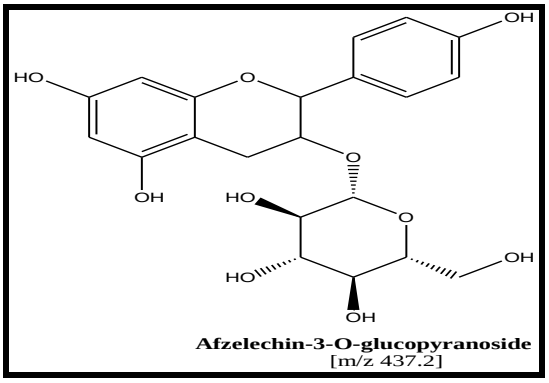

FIGURE 6 - STRUCTURE OF AFZELECHIN 3-O-GLUCOPYRANOSIDE [M/Z 437.2]

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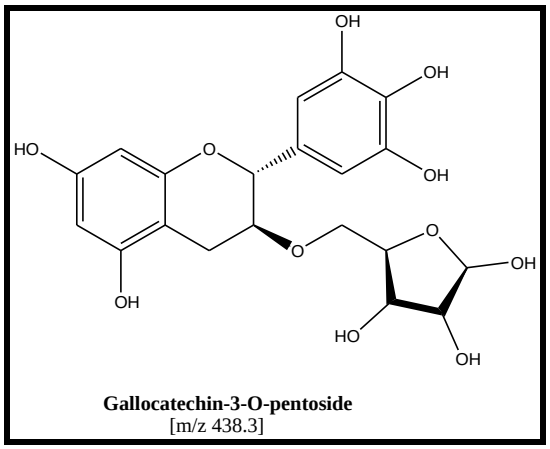

FIGURE 7 - STRUCTURE OF GALLOCATECHIN 3-O-PENTOSIDE [M/Z 438.3]

The mass spectral analysis, H-NMR and FT-IR spectral data of the sample indicated that the compounds present in the MHCP enriched fractions, was that of the polyphenolic compound tannins proanthocyanidin type of compounds. The peaks from spectral data have been assigned to be Ellagic acid 3-O-pentoside, Afzelechin 3-O-glucopyranoside, Gallocatechin 3-O-pentoside respectively(Sivapriya T and John S., 2019).

Polymeric polyphenols, mainly condensed tannins, i.e., proanthocyanidins and catechins, were observed. These Proanthocyanidins and (epi)catechins, were identified as procyanidin (b1, b2) dimers and trimers, and (+)catechin and (-)epicatechin when the peak values matched with values in the database indicating polyphenols. (Shan et al., 2017).

The procyanidin dimers, trimers further polymerise to form polyphenolic polymers, which are recognised as (MHCP), depending upon the orientation of functional groups attached to the general structure (Ferrer et al., 2006).

Scientists also identified that procyanidin (Type A) was methyl hydroxyl chalcone, which increases the uptake of insulin by the insulin receptors cells and can be effectively used to cure conditions of insulin resistance (Anderson et al., 2002; Landrault et al., 2003, Anderson et al., 2004).

CONCLUSION

Secondary metabolites detected in the aqueous extract will help scientist characterise the therapeutic property of CinnamomumZeylanicum. The spectral data indicated the compounds present in the MHCPs enrich fractions as polyphenolic compounds tannins and proanthocyanidin. The peaks attained in the CZ sample during spectral analysis were detected as Ellagic acid 3-O-pentoside, Afzelechin 3-O-glucopyranoside, gallocatechin 3-O-

pentoside respectively. Identification of ellagic acid, afzelchin and gallocatechin will help food professionals develop a novel nutraceutical that will keep diabetes and its associated insulin resistance disorders at bay. After getting a piece of thorough knowledge about the characteristics and concentration of the compounds present in Cinnamomumzeylanicum, undoubtedly cinnamon can be touted as a functional food with more benefits and fewer side effects. It will be of profound use to the community in treating and preventing the onset of various ailments. Simultaneously, people with good health can also consume cinnamon as an adjunct with their regular diet to remain healthy everlastingly.

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