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COMPREHENSIVE ANALYSIS OF ACTIVE AND TOXIC COMPOUNDS IN TWO CINNAMON SPECIES: IMPLICATIONS FOR PHARMACOLOGY AND TOXICOLOGY

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Abstract

Objective: This study investigated the potential toxicity of coumarin in cinnamon powder and cinnamon formulations. The focus was on the extraction, separation, and identification of coumarin and other essential oil components.

Method: Components from cinnamon samples were extracted using various solvents based on their polarities. Thin layer chromatography (TLC) was employed to screen the compounds. Essential oil components, including coumarin, were separated using column chromatography.

Result: The extraction process successfully isolated essential oils and water-soluble components from the cinnamon samples. TLC screening confirmed the presence of key compounds such as cinnamaldehyde, eugenol, and coumarin. Column chromatography effectively separated these compounds, allowing for the detailed analysis of their presence and concentration in the samples.

Conclusion: The study confirmed the presence of coumarin in cinnamon powder and formulations. Given the known toxicity of coumarin in animals and its potential risks to humans, these findings underscore the importance of monitoring and regulating coumarin levels in cinnamon-based products. Further research is recommended to fully understand coumarin's effects on human health and to develop guidelines for safe consumption.

1. Introduction

For centuries, plants have been used as spices, flavorants, and medicines across various societies. Examples include onions, pepper, vanilla, coriander, thyme, garlic, ginger, rosemary, and cinnamon. While these plants enhance culinary experiences, they also possess medicinal properties, treating diseases such as cancer, flu, and digestive issues, including type 2 diabetes. Medicinal approaches range from western pharmaceuticals to traditional and herbal remedies. Synthetic drugs are based on single chemical entities, whereas traditional and herbal remedies combine multiple plant extracts, enhancing efficacy. Examples include BuscopanTM, derived from the Corkwood tree, and GlycominTM, used for diabetes and hypertension. Traditional medicines, available from healers, use plant extracts, often prepared as aqueous solutions or oils, to treat various ailments. Aloe vera, for instance, is used for burns, while Artemisia afra treats colds. Unlike herbal formulations, traditional medicines use only water extracts. This study focuses on the cinnamon plant, particularly its water-soluble compound, methyl hydroxy chalcone polymer (MHCP), which has shown promise in treating type 2 diabetes by reducing blood sugar levels. Despite its medicinal benefits, cinnamon contains coumarin, a toxic compound that poses health risks. Individuals consume cinnamon in various forms, such as powder, tea, or tablets, to manage diabetes, inadvertently ingesting coumarin.

Medicinal plants and herbal remedies are not always regulated, raising concerns about toxicity. Plants like cinnamon produce toxins, such as coumarin, to protect against predators. The essential oil of cinnamon contains both beneficial and harmful components, including cinnamaldehyde, eugenol, and coumarin. Coumarin can cause liver and kidney damage if consumed excessively. Monitoring its levels in food and medicines is crucial, with the German Federal Institute for Risk Assessment recommending a tolerable daily intake of 2 mg/kg body weight. The study aims to identify, characterize, and quantify active and toxic compounds in cinnamon species and formulations. Objectives include isolating and identifying compounds in water-soluble and essential oil components, characterizing and quantifying coumarin, and comparing the composition of Cinnamomum cassia and Cinnamomum zeylanicum species.

The study uses solvent extraction and thin-layer chromatography to analyze cinnamon powder and formulations. Essential oil components are quantified using Thin Layer Chromatography (TLC) and Column Chromatography (CC) Diabetes, characterized by insulin deficiency, is a significant health issue. Type 1 diabetes requires insulin administration, while Type 2 diabetes, often linked to lifestyle and genetics, involves insulin resistance. Both types lead to glucose accumulation in the body. Research has increasingly focused on traditional and herbal medicines for Type 2 diabetes treatment, with cinnamon's MHCP gaining attention for its insulin-like properties. MHCP can activate adipocyte cells, enhancing insulin responsiveness. Studies in Pakistan and elsewhere have highlighted Cinnamomum cassia's role in managing insulin resistance. Beyond MHCP, cinnamon's essential oil components exhibit antioxidant, antimicrobial, antifungal, and antibacterial properties, except for coumarin's toxicity. Limited studies monitor coumarin levels in foods and remedies, emphasizing the need for this research. By identifying and quantifying coumarin and other compounds, the study aims to ensure cinnamon-based formulations are safe for diabetic individuals. The findings will contribute to understanding cinnamon's medicinal potential and risks, guiding safer use in managing diabetes and other ailments.

2. Literature Review

Here's a structured literature review on cinnamon covering various aspects such as botany, chemical composition, applications, and medicinal uses:

- **2.1. Introduction to Cinnamon:** Cinnamon, derived from various species of the Cinnamonum genus, is a widely used spice known for its distinctive aroma and flavor. It has historical roots in traditional medicine and culinary practices worldwide.
- **2.2. Botanical Overview:** Cinnamon plants are evergreen trees belonging to the Lauraceae family. They grow to heights of 10-15 meters, with ovate-oblong leaves and distinctive quill-like barks. The genus includes several species, with C. cassia and C. zeylanicum being the most commercially significant due to their aromatic compounds and medicinal properties (*Figure 1*).





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Figure1:a)Cinnamon plant.b)QuillsCinnamonzeylanicum (left)and Indonesian Cinnamomum cassia (right)

- **2.3. Chemical Composition:** Cinnamon contains diverse chemical compounds categorized into essential oils and water-soluble components. Essential oils are rich in phenylpropanoids (like cinnamaldehyde), terpenes (e.g., linalool, β -caryophyllene), and flavonoids. Water-soluble components include procyanidins, catechins, and methyl hydroxy chalcone polymers (MHCP).
- 2.4. Variation Among Species: Different cinnamon species vary in chemical composition and

physical characteristics of their bark, which influences their culinary and medicinal uses. For instance, C. cassia is known for its higher coumarin content compared to C. zeylanicum.

- **2.5. Harvesting and Processing:** Cinnamon bark is harvested after about two years of growth. The bark is then dried and rolled into quills or ground into powder. Variations in processing methods and geographical locations affect the flavor, aroma, and chemical composition of the final product.
- **2.6. Applications in Food:** Cinnamon is widely used as a spice in cooking and baking, adding flavor and aroma to various dishes and beverages. Despite concerns about coumarin content in some species, it remains a popular ingredient globally.
- **2.7. Industrial Uses:** Components of cinnamon essential oils, such as cinnamaldehyde and linalool, find applications in perfumery and hygiene products due to their aromatic properties and antimicrobial effects.
- **2.8. Medicinal Uses:** Cinnamon has been traditionally used in herbal medicine for its various health benefits. Compounds like cinnamaldehyde and MHCP exhibit antibacterial, antifungal, and antioxidant properties. Cinnamon is also studied for its potential role in managing Type II diabetes by improving insulin sensitivity.
- **2.9. Biological Activities:** The biological activities of cinnamon compounds include antimicrobial, antifungal, antiviral, and anti-inflammatory properties. Cinnamaldehyde, in particular, shows strong antimicrobial effects against various pathogens.
- **2.10. Cinnamon in Diabetes Management:** MHCP from cinnamon has been investigated for its ability to enhance insulin sensitivity in adipocyte cells, potentially aiding in glucose metabolism and blood sugar regulation in Type II diabetes.
- **2.11. Toxicological Considerations:** Coumarin, found in varying amounts in different cinnamon species, raises concerns due to its potential toxicity in high doses. Regulatory guidelines exist to limit its intake in food and medicinal products.
- **2.12. Isolation and Identification Techniques:** Various chromatographic and spectroscopic methods are employed for the isolation, identification, and quantification of cinnamon compounds. Thin-layer chromatography (TLC), gas chromatography-mass spectrometry (GC-MS), and UV-visible spectroscopy are commonly used techniques.
- **2.13. Structural Characterization:** The structures of cinnamon compounds, such as cinnamaldehyde, eugenol, and MHCP, are elucidated using advanced analytical methods like nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy, providing insights into their molecular properties.
- **2.14.** Commercial Implications: The commercial cultivation and trade of cinnamon involve multiple stakeholders across regions like Sri Lanka, Indonesia, and China, reflecting its economic significance in global markets.
- **2,15. Future Directions:** Future research could focus onexploring novel applications of cinnamon compounds in pharmaceuticals, nutraceuticals, and functional foods. Additionally, further studies are needed to understand the mechanisms underlying cinnamon's health-promoting effects, particularly in chronic disease management.

These literature reviews encapsulate the multifaceted aspects of cinnamon, ranging from its botanical origins to its diverse chemical composition, applications in food and industry, medicinal uses, and ongoing research trends. Each section provides a comprehensive overview based on current scholarly literature, highlighting both the traditional uses and modern scientific insights into this globally cherished spice.

3. Methodology

3.1 Chemical Reagents and Materials

This study employed a range of solvents and chemicals to ensure comprehensive extraction and analysis of compounds from cinnamon powder and its formulations. The analytical grade solvents—hexane, diethyl ether, toluene, acetone, ethanol, methanol, acetic acid, and chloroform. For Thin Layer Chromatography (TLC) and Column Chromatography. The cinnamon spice powder and its commercial formulations were acquired from local stores.

3.3 Solvent Extraction

The extraction process aimed to isolate non-polar, medium-polar, and polar compounds from

cinnamon powder and its formulations using a range of solvents. Two primary extraction procedures were followed: serial extraction and non-serial extraction.

3.3.1 Solvent Extraction of Cinnamon Powder (Serial Extraction)

In the serial extraction method, 100 g of cinnamon powder was first mixed with 1L of hexane and stirred for 24 hours. The mixture was then filtered using Whatman 540 hardened Ashless 18.5 cm filter paper. The residue was left to dry in a fume hood, while the supernatant was dried using a rotary evaporator at room temperature. This process was repeated with diethyl ether, dichloromethane, ethyl acetate, methanol, and deionized water. The residues from each solvent (hexane: 96.65 g, diethyl ether: 94.15 g, dichloromethane: 94.00 g, ethyl acetate: 91.90 g, methanol: 90.03 g) were dried, and the crude extracts were refrigerated for future use. The supernatants were analyzed using thin-layer chromatography (TLC) and concentrated using a rotary evaporator at 25°C.

3.3.2 Solvent Extraction of Cinnamon Formulation (Serial Extraction)

For the cinnamon formulation, a similar serial extraction process was followed. One gram of powdered formulation was extracted sequentially with 10 mL of hexane, methanol, and boiled deionized water. The residue from each solvent (hexane: 0.9969 g, methanol: 0.9884 g) was dried and weighed, and the supernatants were immediately analyzed using TLC.

3.3.3 Solvent Extraction of Cinnamon Powder and Formulation (Non-Serial Extraction)

For non-serial extraction, three separate 1 g samples of cinnamon powder and its formulation were extracted individually with 10 mL each of hexane, methanol, and boiled deionized water for 24 hours. The mixtures were filtered, and the filtrates were analyzed using TLC. The residues were dried and stored in sealed glass vials for future use.

3.4 Thin Layer Chromatography (TLC)

- **3.4.1 Solvent Preparation (TLC):** Various solvent systems were prepared for TLC analysis: hexane/dichloromethane (9:1), toluene/ethyl acetate (93:7), benzene/acetone (19:1), and methanol/water/acetic acid/ethyl acetate/chloroform (10:8:30:40:12). Toluene/ethyl acetate (93:7) was also prepared for column chromatography.
- **3.4.2 Chemical Reagent Preparation:** The vanillin-sulphuric acid reagent for TLC was prepared by dissolving 1.32 g of vanillin in 5 mL of concentrated sulphuric acid, followed by the addition of 200 mL ethanol. The mixture was stored in a refrigerator.
- **3.4.3 Screening and Isolation of Cinnamon Compounds:** TLC plates with 0.20 mm silica gel 60 and a UV254 fluorescence indicator were used for screening the crude extracts and reference standards. The solvent systems used were toluene/ethyl acetate (93:7) for non-polar and medium-polar compounds, and methanol/water/acetic acid/ethyl acetate/chloroform (10:8:30:40:12) for polar and very-polar compounds. Compounds were visualized under UV light at 254 and 366 nm, and through vanillin-sulphuric acid spraying, followed by heating at 110°C.
- **3.4.3.2 Isolation of Cinnamon Powder Components PTLC:** Preparative thin-layer chromatography (PTLC) was used to isolate cinnamon powder hexane crude extracts on glass silica gel plates. Isolated compounds were washed with toluene/ethyl acetate (93:7) solvent, filtered, dried in a fume hood, and stored in a refrigerator for further characterization.

3.5 Column Chromatography (CC)

Crude hexane extracts were further purified using a normal-phase silica gel column packed with a slurry of silica gel in toluene/ethyl acetate (93:7). The extracts were eluted, and fractions were collected in 2 mL portions. Approximately 750 fractions were combined, and the solvent was evaporated. The purified compounds were stored in a refrigerator for subsequent analysis.

3.5.1.1 Sample Preparation

The crude extracts were prepared as described in sections 3.3.1 and 3.3.2. Standard materials included cinnamaldehyde, eugenol, linalool, coumarin, and thymol, with stock solutions prepared in methanol. Working solutions were obtained through serial dilutions for analysis (Table 1).

Table1:Summary of standard reference materials concentrations.

C:	T	T :11	C	T11
Cinnamaldehyde	Eugenol	Linalool	Coumarin	Thymol

(mg/L)	(mg/L)	(mg/L)	(mg/L)	(mg/L)
0.0423	0.0427	0.0359	0.0039	0.0041
0.0846	0.0760	0.0720	0.0779	0.0082
0.1260	0.1140	0.1080	0.0117	0.0123
0.1680	0.1520	0.1441	0.0126	0.0165
0.2100	0.1900	0.1800	0.0195	0.0206

4. Result and Discussion

The screening of their components was done with mobile phases of different polarities to suit the polar nature of the compounds of interest. Components of essential oil were eluted with a non-polar solvent toluene / ethyl acetate (93:7) and those of water soluble with a polarsolvent (methanol / water / acetic acid / ethyl acetate / chloroform (10:8:30:40:12) by using thin layer chromatography (TLC). The numbers and types of compounds (phenyl propanoids, flavonoids and terpenes) extracted with different solvents for cinnamon powder are shown in Figure 2. Table2 below represent cinnamon extracts with their respective symbols as shown in Figure 2.

Table2:Cinnamonpowderandformulationextractsandsymbols

Symbol	Cinnamon extract
A1	Cinnamonpowderdiethylethercrudeextract
A2	Cinnamonpowderdichloromethane crudeextract
A3	Cinnamonpowderethylacetatecrude extract
A4	Cinnamonpowdermethanolcrude extract
A5	Cinnamonpowderwatercrude extract
A6	Cinnamonpowderwatercrude extract
A7	Cinnamonpowdertoluenecrudeextract
A8	Cinnamonpowderhexanecrude extract
B1	Cinnamonformulationmethanolcrude extract
B2	Cinnamonformulationwatercrude extract

Figure 2: Screening of cinnamon powder crude extracts from serial extraction

- a) essentialoilcomponentselutedwithtoluene/ethylacetate(93:7)
- b) watersolublecomponentselutedwithmethanol/water/aceticacid / ethyl acetate / chloroform (10:8:30:40:12). c) methanol and water extracts of cinnamon formulation eluted with methanol / chloroform (2:1)

From Figure 2 above, a wide range of solvents were used for extraction in order to determine the types of compounds which could be extracted with different solvents. A TLC plate in Figure 2a shows most compounds (non-polar) A8 and a fewwithA1,A2and(medium-polar)A3,onetracecompoundA4andnoneatA5 and A6. Also Figure 2a showed the presence of a common compound (light blue spot circled in red) from A8, A1, A2 and A3 and a trace in A4. Whereas, only A8 and A1 shared a common compound (blue glowspot circled in yellow). A4, A5 and A6 components were not resolved on this TLC plate due to their polarity natureand a non-polar mobile phase been used. Hence a more suitable mobile phase (polarmedium)toresolvecomponentsofA4,A5andA6asillustratedinFigure 2b. A7 in Figure 2a was also tested to investigate the compounds which could be extracted with toluene. It was shown that A7 had similar compounds as those extracted in A8, and hence only A8 was considered instead of A7. From TLC plates shown in Figure 2, it was evident that A8 and A4 underdifferent elution conditions (polar and non-polar) could extract most compounds and

hence the focus in these solvents with the exception of water, since the main objective of this study was to investigate the water soluble components of both cinnamon powder and cinnamon formulation. Furthermore, no components of A8, A1, A2 and A3 were observed in A4, A5 and A6 when a polar elution solvent was used.

4.1 Screeningandidentification of cinnamon components (TLC)

The essential oil and water soluble components were screened under different conditions as represented in **paragraph 4.2** and therefore screening and identification of these components were done separately. This led to a simultaneous running and screening of compounds alongside with standard materials (cinnamaldehyde, eugenol, linalool, thymol and coumarin) for identification purposes.

4.1.1 Screeningofessential oilcomponents

On a TLC plate, Figure 3 below developed with toluene / ethyl acetate (93:7), A8 showed 8 components resolved and none for C2, with A4, 4 compounds were observed and 1 compound was observed in B1. Both A5 and A6 showed no presence of any compounds, due to the differences in polarities of crude extracts and the mobile phase. The screening of A4, A5, B1, B2, A8, C2, C3, C4, C5, C6 and C7 is illustrated in Figure 3 below. Table 4.2 represents again cinnamon extracts and their symbols. The colours observed on the TLC plate are reactions of specific compounds with a specialized chemical spray (vanillin sulphuric acid in ethanol). Where it was clear noted in a TLC plate shown in Figure 3 below that standardmaterials; thymol, coumarin, eugenol, linaloolandcinnamaldehydewould reflect; pink, faint blue, yellow-brown, violet-blue and powdered blue colours respectively. Likewise, similar colours observed on the same plate for cinnamon components would then indicate the presence of possible compounds as those of standard materials.

Table3:CinnamonextractsscreenedonTLCplateand eluted with toluene / ethyl acetate (93:7)⁶

Symbols	Cinnamon extracts
A8	Cinnamonpowderhexanecrudeextract
C2	Cinnamonformulationhexanecrudeextract A4
	Cinnamon powder methanol crude extract
B1	Cinnamonformulationmethanolcrudeextract A5
	Cinnamon powder water crude extract
B2	Cinnamonformulationwatercrudeextract C3
	Thymol standard material
C4	Coumarinstandardmaterial C5
	Eugenol standard material C6
	Linalool standard material
C7	Cinnamaldehydestandardmaterial

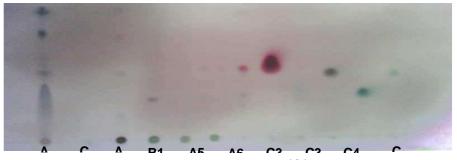


Figure 3:TLCscreeningof essentialoilcomponents elutedwithtoluene/ethyl acetate (93:7) The screening of polar components of methanol and water (A4, A5, B1, C2 andB2) of both cinnamon powder and cinnamon formulation are shown in Figure 4.3a, where none of the standard materials (C3 to C7) could be used to identify cinnamon components due to the polarity (medium to very-polar) of the mobile phase used and them being non-polar. Figure 4.3b B1 and A4 showed the presence of most polar components, followed by A5 and B2 and in C2 no components were expected to befound since this would be the reverse of the non-polar components. Table 4.3 below represents cinnamon crude extracts and their symbols respectively.

Table3:Cinnamonpowder watersolublecomponentselutedwithmethanol/ water / acetic acid / ethyl acetate / chloroform (10:8:30:40:12).

Symbols	Cinnamon extracts
C7	Cinnamaldehydestandardmaterial C5
	Eugenol standard material
C6	Linalool standard material C3
	Thymol standard material C4
	Coumarinstandardmaterial
B1	Cinnamonformulationmethanolcrudeextract A4
	Cinnamon powder methanol crude extract
C2	Cinnamonformulationhexanecrudeextract A5
	Cinnamon powder water crude extract
A5	Cinnamonpowderwatercrudeextract B2
	Cinnamonformulationwatercrude extract

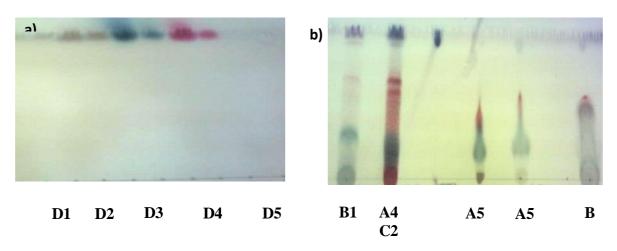


Figure4:TLCscreeningof watersolublecomponentselutedwith methanol/ water / acetic acid / ethyl acetate / chloroform (10:8:30:40:12).

Identificationofcinnamon components

A way of identifying these compounds was by comparing R_f values (crudeextracts) with those of standard materials on the same TLC plates run under the same conditions. From the observations, the following compounds were identified: cinnamaldehyde, coumarin, eugenol and linalool. was also used toverify the identities of cinnamon components by matching R_f-values and characteristic colours of these compounds viewed after chemical treatment with vanillin sulphuric acid in ethanol spraying.¹ Table 4.4 illustrates data obtained from literature and the one obtained from this project.

Identification by comparison of R_f-values of cinnamon compounds

The experimental R_f -values of individual compounds and were calculated and compared with those from literature to verify their identities. Since it has been shown and discussed in paragraph 4.3.1 that A8 extracted most non-polar compounds and hence only its crude extract was considered in this regard. From the deductions based on the R_f -values of cinnamon components compiled from literature, the following compounds (β -caryophyllene; cinnamaldehyde; eugenol; coumarin; linalool; and methyl chavicol) were identified and they matched thesame compounds found in A8. A summary of comparison of the experimental R_f - values of cinnamon powder components to those from literature is tabulated in Table 4.5 below.

1.1.1.1 Identification of cinnamon compounds by colour visualisation.

Figure 4.4 and Table 4.4 below illustrate identities of cinnamon components (both polarand non-polar) from cinnamon powder and formulation. In B1Figure 4. no components were identified, whereas A8, and A4 and a number of components were identified. From Figure 4.4-b B1 and A4 showed the presence of similar compounds, C2 had none and A5 and B2 also showed similar compounds.

Table4:Cinnamonpowderextracts		
Symbol	Extracts	
B1	Cinnamonformulation	
	methanolcrude extract	
A4	Cinnamonpowder methanol	
	crudeextract	

Figure 4: Identification of classes of cinnamon components a) TLC plate eluted withtoluene/ ethylacetate (93:7)b)TLCplateelutedwithmethanol/ water / acetic acid / ethyl acetate / chloroform (10:8:30:40:12) and viewed after spraying with VS.

The colours observed in A8 in Figure 4.4a and B1, A4, A5 and B2 in Figure 4.4b, when sprayed with vanillin sulphuric acid reagent (VS), were also compared with those from literature as tabulated in Table 4.5.¹ From the experimental results, the following cinnamon powder and formulation compounds' colours were matched (after spraying with VS) with those from literature and identified as; essential oil for cinnamon powder cinnamaldehyde (brownish), eugenol (yellow), linalool (blue), simple coumarin (purplish) methyl chavicol (red-violet), β-caryophyllene (blue- violet) and the water soluble for cinnamon powder and the formulation phenyl propanoids (purplish), saponnins (greenish) and flavonoids (pink-red).¹ The results obtained from this study and literature ones are summarised in Table 4.5 below.

Table 4.5: Summarized table of visualized cinnamon compounds from literature review

Compounds	Visual withVS (Lit.)	Visual withVS	Visual UV-light (366 nm)	R _f -values (Lit.)	R _f - values
β-caryophyllene	Red- violet ²	Red- violet	lime	Nearthe solvent line ¹	0.90
Cinnamaldehyde	Grey-	Grey-blue	Red-	0.50^{1}	0.50
Eugenol	brown ¹ Yellow-	yellow	brown Lime	0.46^{1}	0.47

Coumarin	Brown ¹ Purple ¹	Purple	Bright	0.40^{1}	0.40
Linalool	Blue ¹	blue-	light-blue blue	0.30^{1}	0.30
Methylchavicol	Red-	purple Red-	Light	0.20^{1}	0.20
Carvone	violet ¹ Red ¹	violet Red-pink	green Red	0.46^{1}	0.50
*Phenyl	Purplish ¹	Purplish			
propanoids **Flavonoids	Pink-red ¹	Pink-red			

^{*}Theelution solventsystemusedtoluene/ethylacetate(93:7) paragraph 3.4.1

4.4 Isolation of cinnamon compounds

Only cinnamon powder hexane crude extracts were isolated on column chromatography for the purpose of their characterizations with infrared and nuclear magnetic resonance.

4.4.1 Isolationwithcolumnchromatography(CC)

All hexane crude extracts (cinnamon powder) were isolated with CC using to luene

/ ethyl acetate (93:7) mobile phase and from the screening of these components, 8 compounds were identified. Upon isolation with CC a few compounds were recovered and the rest were lost or stuck in the column due to poor selection of solvent system and CC material as stationary phase, but nonetheless, from these findings an alternative isolation method preparative thin layer chromatography (PTLC) was used to isolate components of hexane crude extract.

4.4.2 Isolationwithpreparativethinlayerchromatography(PTLC)

From this isolation, 8 components screened from TLC according to the description in **paragraph 4.3.1.** Only 6 compounds were successfully isolated with the exception of F2 and F3 where similar compounds seen in both individual compounds and F3 showing a trace of another unresolved compound. Also compound F6 showed traces of 2 unresolved compounds. From the 6 isolated compounds the following cinnamon compounds were identified: F1 (carvone), F2 (2-methoxy cinnamaldehyde), F4 (coumarin), F5 (β -caryophyllene), F7 (cinnamaldehyde) and F8 (methyl chavicol). Figure 4.5 shows a developed TLC eluted with toluene / ethyl acetate (93:7) plate of compounds screened after PTLC isolation. Table 4.6 represents cinnamon powder hexane compounds and their symbols.

^{**}Theelutionsolventsystem used 1%vanillininethanol(solution1)and5% ethanol solution in sulphuric acid (Solution 2)

Table 4.6: Cinnamon powder hexane compounds and symbols represent

Symbol	Compound	
A8	Cinnamonpowderhex	anecrudeextract
F1	Compound1 ofA8	carvone
F2	Compound2 of A8	2-methoxycinnamaldehyde
F3	Compound3ofA8	(unresolvedcompounds)
F4	Compound4ofA8	coumarin
F5	Compound5ofA8	β-caryophyllene
F6	Compound6ofA8	(unresolvedcompounds)
F7	Compound7ofA8	cinnamaldehyde
F8	Compound8ofA8	methylchavicol

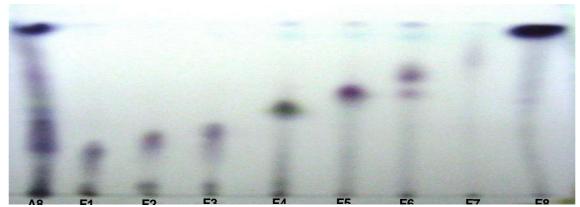


Figure5:TLCscreeningandidentification of cinnamon components eluted with toluene / ethyl acetate (93:7); viewed after spraying with VS

Conclusion

This study has found that cinnamon contains a number of compounds such as cinnamaldehyde, coumarin, eugenol, linalool, 2-methoxycinnamaldehyde,catechin, tannins, gallic acid etc. The major aim was to isolate and quantify coumarin which is toxic. This compound (coumarin) was found mainly in the non polar (hexane) and mid polar (ethyl acetate) fractions of the extracts though asmall fraction was found in methanol extracts. Only the major compounds found in both the non polar and mid polar fractions of the extracts (coumarin and cinnamaldehyde) were quantified. Coumarin the toxic compound in cinnamon was found to be 0.02445 mg/Kg implying that, this concentration falls far below the threshold limits set by The German Federal Institution for Risk Assessment (which is 2.0 mg/Kg/body weight). This means that unless this compound (coumarin) has a tendency to bio-concentrate in the body then the cinnamon spices are safe for human consumption.

References

1. Oiye SQ, Muroki NM. Uses of spices in foods. J Food Technol Afr. 2002;7(2):39-44.

- 2. Lee N. The medicinal association of South Africa: Guide to medicines & drugs. 1st ed. Cape Town: The Readers Digest Association South Africa (PTY) Limited; 1992:12-16.
- 3. Buscopan® &Buscapina®: Duboisia a special plant: Active ingredient in Buscopan. [date accessed 21/10/2010]. Available from: http://www.buscapina.com.mx/com/ennext/main/about_buscopan/Duboisia/index.jsp
- 4. Van Wyk B, Wink M. Medicinal plants of the world: An illustrated scientific guide to important medicinal plants and their uses. 1st ed. Pretoria: Briza Publications; 2004:41.
- 5. Diabe-cinnTM Original: Water-based cinnamon extract (ZN112). www.diabecinn.co.za.
- 6. Anderson RA, Polansky M. Polyphenols found in cinnamon mimic job of hormone. Agric Res. 2004;(19):1-19.
- 7. WHFoods. Cinnamon, ground. [date accessed 19/08/2011]. Available from: http://www.whfoods.com/genpage.php?tname=foodspice&dbid=68
- 8. Rater M, Matissek R. Analysis of coumarin in various foods using liquid chromatography with tandem mass spectrometric detection. Eur Food Res Technol. 2008;227:637-642.
- 9. Gallessich G, Magruder J. Cinnamon may help to alleviate diabetes says UCSB researcher. The University of California, Santa Barbara; 2004 Apr 13.
- 10. Jarvill-Taylor KJ, Anderson RA, Graves DJ. A hydroxychalcone derived from cinnamon functions as a mimetic for insulin in 3T3-L1 adipocytes. J Am Coll Nutr. 2001;20(4):327-336.
- 11. Cao H, Urban JF, Anderson RA. Cinnamon polyphenol extract affects immune responses by regulating anti and proinflammatory and glucose transporter gene expression in mouse macrophages. J Am Soc Nutr. 2008;138:833-840.
- 12. Herbal healing and natural with culinary herbs and spices. Herbalism. 2005. Available from: http://www.culinaryherbsandhealth.com/Article/Herbalism.htm
- 13. Lungarini S, Aureli F, Coni E. Coumarin and cinnamaldehyde in cinnamon marketed in Italy: A natural chemical hazard? Food Addit Contam Part A. 2008;25(11):1297-1305.
- 14. Ziegenfuss TN, Hofheins JE, Mendel RW, Ladis J, Anderson RA. Effects of a water-soluble cinnamon extract on body composition and features of the metabolic syndrome in pre-diabetic men and women. J Int Soc Sports Nutr. 2006;3(2):45-53.
- 15. Symptoms of diabetes. [date accessed 30/03/2011]. Available from: http://www.informationaboutdiabetes.com/symptoms-of-diabetes
- 16. Gbolade AA. Inventory of antidiabetic plants in selected districts of Lagos State, Nigeria. J Ethnopharmacol. 2009;121:135-139.
- 17. Moon J, Shibamoto T. Antioxidant assays for plant and food components. J Agric Food Chem. 2009;57:1655-1666.
- 18. Preuss HG, Echard B, Polansky MM, Anderson RA. Whole cinnamon and aqueous extracts

- ameliorate sucrose-induced blood pressure elevations in spontaneously hypertensive rats. 2006;25(2):144-150.
- 19. Khan A, Safdar M, Khan MMA, Khan NK, Anderson RA. Cinnamon improves glucose and lipids of people with type 2 diabetes. Diabetes Care. 2003;26(12):3215-3218.
- 20. Mateljan G. The world's healthiest foods: Essential guide for the healthiest way of eating: Cinnamon, ground the health benefits of cinnamon. www.whfoods.org. [date accessed 08/15/05].
- 21. Atta-ur-Rahman, Choudnary MI, Farooq A, Ahmed A, Iqbal M, Demirci B, et al. Antifungal activities and essential oil constituents of some spices from Pakistan. H.E.J. Research Institute of Chemistry, University of Karachi, Pakistan. [received 20/08/1999; uploaded 29/08/1999.
- 22. Cinnamon Cultivation and Processing. [date accessed 19/08/2011]. Available from: http://www.science2day.info/2007/09/cinnamon-cultivation-and-processing.html
- 23. Parthasarathy VA, Chempakam B, Zachariah TJ. Chemistry of spices: Cinnamon and cassia. King's Lynn: Biddles Ltd; 2008. Chapter 7, p. 124.
- 24. Wang R, Wang R, Yang B. Extraction of essential oils from 5 cinnamon leaves and identification of their volatile compounds. Innov Food Sci Emerg Technol. 2009;10:289-292.
- 25. Cinnamon plant. [date accessed 13-06-2011]. Available from: http://medicineplants.blogspot.com/2010/11/cinnamon-tree-karuva-pattai-for.html
- 26. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. 2nd ed. Chapter 2. 1984. p. 52.
- 27. Friedman M, Kozukue N, Harden LA. Cinnamaldehyde content in foods determined by gas chromatography-mass spectrometry. J Agric Food Chem. 2000;48:5702-5709.
- 28. Wagner H, Bladt S. Plant drug analysis: A thin layer chromatography atlas. 2nd ed. Springer; 1996. p. 162.
- 29. Ivanova AB, Batovska DI, Todorova IT, Stamboliyska BA, Serly J, Molnar J. Comparative study on the MDR reversal effect of selected chalcones. Int J Med Chem. 2011:1-7.
- 30. Lazarus SA, Adamson GE, Hammerstone JF, Schmitz HH. High-performance liquid chromatography/mass spectrometry analysis of proanthocyanidins in foods and beverages. J Agric Food Chem. 1999;47(9):3693-3701.
- 31. Ferrer J, Jez JM, Bowman ME, Dixon RA, Noel JP. Structure of chalcone synthase and the molecular basis of plant polyketide biosynthesis. Nat Struct Biol. 1999;6(8):775-784.
- 32. Types of cinnamon: The spice you know and love isn't real cinnamon but cassia. Available from: http://www.thenibble.com/reviews/main/salts/cinnamon.asp
- 33. Shan B, Cai Y, Brooks JD, Corke H. Antibacterial properties and major bioactive components of cinnamon sticks (Cinnamomum burmannii): Activity against foodborne pathogenic bacteria. J Agric Food Chem. 2007;55(14):5484-5490.

- 34. Rahman A, Choudhary MI, Farooq A, Ahmed A, Iqbal MZ, Demirci B, et al. Antifungal activities and essential oil constituents of some spices from Pakistan. H.E.J. Research Institute of Chemistry, University of Karachi, Pakistan. 1999.
- 35. Levels of coumarin in cassia cinnamon vary greatly even in bark from the same tree. [date accessed 1999-2010]. Available from: http://www.sciencedaily.com/releases/2010/11/1011103135352.htm
- 36. Jham GN, Dhingra OD, Jardim CM, Valente VMM. Identification of the major fungitoxic components of cinnamon bark oil. Fitopatol Bras. 2005;30(4):404-408.
- 37. Kaul PN, Bhattacharya AK, Rajeswara Rao BR, Syamasundar KV, Ramesh S. Volatile constituents of essential oils isolated from different parts of cinnamon (Cinnamonum zeylanicum Blume). J Sci Food Agric. 2003;83(1):53-55.
- 38. High intake of coumarin in cassia cinnamon causes liver damage in sensitive people. [date accessed 2006-2010].
- 39. Sproll C, Ruge W, Andlauer C, Godelmann R, Lachenmeier DW. HPLC analysis and safety assessment of coumarin in foods. Food Chem. 2008;109(2):462-469.
- 40. Diogo CV, Felix V, Vilela S, Burgeiro A, Barbosa IA, Carvalho MJM, et al. Mitochondrial toxicity of the phytochemicals daphnotoxin and daphnoretin relevance for possible anticancer applications. Toxicol In Vitro. 2009;23:772-779.
- 41. Chaudhary SK, Ceska O, Warrington PJ, Ashwood-Smith MJ. Increased furanocoumarin content of celery during storage. J Agric Food Chem. 1985;33:1153-1157.
- 42. Seigler DS. Plant secondary metabolism Phenyl propanoids coumarin. Library of Congress Cataloging-in-Publication Data; 1998. p. 133-137.
- 43. Rodrigues A, Nerín C, Battle R. New cinnamon-based active paper packaging against Rhizopus stolonifer food spoilage. J Agric Food Chem. 2008;56(15):6364-6369.
- 44. Rater M, Matissek R. Analysis of coumarin in various foods using liquid chromatography with tandem mass spectrometric detection. Eur Food Res Technol. 2008;227:637-642.
- 45. Sugiyama H, Akazome Y, Shoji T, Yamaguchi A, Kanda T, Ohtake Y. Oligomeric procyanidins for apple polyphenol are main active components for inhibition of pancreatic lipase and triglyceride absorption. J Agric Food Chem. 2007;55:4604-4609.
- 46. Anderson RA, Broadhurst CL, Polansky MM, Schmidt WF, Khan A, Flanagan VP, et al. Isolation and characterization of polyphenol type-A polymers from cinnamon with insulinlike biological activity. J Agric Food Chem. 2004;52:65-70.
- 47. Cinnamon spice. Date accessed 31/03/2011. Available from: http://en.wikipedia.org/wiki/File:Canelle Cinnamomum verum Luc Viatour crop1.jpg
- 48. Lungarini S, Aureli F, Coni E. Coumarin and cinnamaldehyde in cinnamon marketed in Italy: A natural chemical hazard? Food Addit Contam Part A. 2008;25(11):1297-1305.
- 49. Linalool summary document registration review: Initial document (April 2007). Docket

- number: EPA-HQ-EPA-2006-0356. Available from: www.regulations.gov.
- 50. Beta-caryophyllene; Fragrance Ingredient; Skin-Conditioning Agent- Miscellaneous; Masking; PERFUMING; Skin Conditions: Date Accessed 26/11/2010. Available from: http://www.cosmeticdatabase.com/ingredient.php?ingred06=716960.
- 51. Khan A, Safdar M. Role of diet, nutrients, spices and natural products in diabetes mellitus. Pak J Nutr. 2003;2(1):1-12.
- 52. Chang C, Chang W, Chang S, Cheng S. Antibacterial activities of plant essential oils against Legionella pneumophila. Water Res. 2008;42:278-286.
- 53. Shelef LA. Antimicrobial effects of spices. Division of Food Science & Nutrition, Wayne State University, Detroit, MI. Publication date 18 July 1983.
- 54. Herbal healing and natural with culinary herbs and spices. Herbalism. 2005. Available from: http://www.culinaryherbsandhealth.com/Article/Herbalism.htm.
- 55. Born SL, Candill D, Smith BJ, Lehman-McKeeman LD. In vitro kinetics of coumarin 3,3-epoxidation application to species differences in toxicity and carcinogenicity. Toxicol Sci. 2000;58:23-31.
- 56. Scientific Committee on Consumer Products: Opinion on coumarin. European Commission Health & Consumer Protection Directorate-General. Adapted by the SCCP during the 8th Plenary Meeting of 20 June 2006.
- 57. Nofal ZM, El-Zahar MI, Abd El-Karim SS. Novel coumarin derivatives with expected biological activity. Molecules. 2000;5:99-113.
- 58. Arnold GB, Bengele HH, Diehlk O, Grant JJ, Krupski SK, Sunal DW, et al. Health choosing wellness. Teacher's edition. Prentice Hall; 1989. p. 55, 209.
- 59. Zhang J, Liu L, He Y, Kong W, Huang S. Cytotoxic effect of trans-cinnamaldehyde on human leukemia K562 cells. Acta Pharmacol Sin. 2010;31(7):861-866.
- 60. Khan A, Safdar M, Khan MMA, Khan NK, Anderson RA. Cinnamon improves glucose and lipids of people with type 2 diabetes. Diabetes Care. 2003;26(12):3215-3218.
- 61. Schmidt E, Jirovetz L, Buchbauer G, Eller GA, Stoilova I, Krastanov A, et al. Composition and antioxidant activities of the essential oil of cinnamon (Cinnamomum zeylanicum Blume) leaves from Sri Lanka. Jeobp. 2006;9(2):170-182.
- 62. Ndlovu T. Isolation and characterization of some of the major compounds from PentanisiaPrunelloides. University of Johannesburg; 2007. Chapter 67.
- 63. Senanayake UM, Lee TH, Wills RBH. Volatile constituents of cinnamon (Cinnamomum zeylanicum) oils. J Agric Food Chem. 1978;26(4):822-824.
- 64. Archer AW. Determination of cinnamaldehyde, coumarin and cinnamyl alcohol in cinnamon and cassia by high-performance liquid chromatography. J Chromatogr. 1978;447:272-276.
- 65. Lopez P, Sanchez C, Battle R, Nerín C. Vapor-phase activities of cinnamon, thyme and oregano essential oils and key constituents against foodborne microorganisms. J Agric Food

- Chem. 2007;55(11):4348-4356.
- 66. Buttery RG, Ling LC, Bean MM. Coumarin off-odor in wheat flour. J Agric Food Chem. 1978;26(1):179.
- 67. He Z, Qiao C, Han Q, Cheng C, Xu H, Jiang R, et al. Authentication and quantitative analysis on the chemical profile of Cassia bark (Cortex Cinnamoni) by high-performance liquid chromatography. J Agric Food Chem. 2005;53:2424-2428.
- 68. Jang H, Chang K, Huang Y, Hsu C, Lee S, Su M. Principal phenolic phytochemicals and antioxidant activities of three Chinese medicinal plants. J Food Chem. 2007;103(3):749-756.
- 69. Goswami A, Rahman A. Antiviral activity of (E)-cinnamaldehyde revisited with nanoscience tools. Biological Science Division, India Statistical Institute; 2010. p. 1-4.
- 70. Dighe VV, Gursale AA, Sane TR, Menon S, Patel PH. Quantitative determination of eugenol from Cinnamomum tamala leaf powder and polyherbal formulation using reverse phase liquid chromatography. Chromatographia. 2005;61:443-446.
- 71. Abu-Eittah RH, EL-Tawil AH. The electronic absorption spectra of some coumarins. A molecular orbital treatment. Can J Chem. 1984;63:1173.
- 72. Villa C, Gambaro R, Dorato S, Mariani E. Metodo RP-HPLC per la determinazionesimultanea di 24 allergeni in prodotticosmeticiprofumati. Dipartmento di ScienzeFarmaceutiche, Universita Degli Studi di Genova; 2001. Viale Benedetto XV, (3-1-16132) Genova Italy.
- 73. Gatti R, Gioia MG, Di Pietra AM, Cavrini V. Analysis of phenols in pharmaceuticals by liquid chromatography after pre-column labelling and on-line post-column photochemical derivatization. Anal Chim Acta. 2001;447(1-2):89-99.
- 74. Tanaka T, Matsuo Y, Yamada Y, Kouno I. Structure of polymeric polyphenols of cinnamon bark deduced from condensation products of cinnamaldehyde with catechin and procyanidins. 2008;56(14):5864-5870.
- 75. de Carvalho CT, Siqueira AB, Ionashiro EY, Pivatto M, Ionashiro M. Synthesis and characterization of solid 2-methoxycinnamylidenepyruvis acid. Eclet Quim. 2008;33(4):1-11.
- 76. Gende LB, Floris I, Fritz R, Eguaras MJ. Antimicrobial activity of cinnamon (Cinnamomum zeylanicum) essential oil and its main components against Paenibacillus larvae from Argentine. Bull Insectol. 2008;61(1):1-4.
- 77. Currie DJ, Lough CE, McClusky FK, Holmes HL. Effect of functional group conformation on the infrared spectra of some gem difunctional phenylethylene derivatives. Can J Chem. 1969;47:3147.
- 78. Reddy CS, Reddy KRN, Mangala UN, Muralidharan K. Directorate of Rice Research, Rajendra Nagar, Hyderabad 500 030, India. Presentation at 3rd Advances Against Aspergillosis.