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Soxhlet extraction-obtained extracts of Cinnamomum verum bark: phytochemical profiling and antioxidant activity evaluation

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Abstract

This study focuses on the extraction and analysis of phytochemicals from Cinnamomum verum bark and their antioxidant activities. Utilizing the Soxhlet extraction method, the bark was subjected to sequential extraction with four solvents of varying polarities: hexane, petroleum ether, ethanol, and methanol. The aim was to compare the efficiency of each solvent in extracting different phytochemicals and to evaluate the antioxidant capacity of the resultant extracts.

The ground bark of *Cinnamomumverum* was processed in a Soxhlet apparatus, where each solvent was used to extract bioactive compounds over several cycles. Post-extraction, the solvents were evaporated under reduced pressure to yield concentrated extracts. These extracts were then subjected to qualitative and quantitative phytochemical analysis to identify and quantify various bioactive compounds, including alkaloids, flavonoids, tannins, and terpenoids.

Antioxidant activities of the extracts were assessed using three established assays: DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric Reducing Antioxidant Power), and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)). These assays provided insights into the scavenging activity, reducing power, and overall antioxidant potential of the extracts.

The study revealed significant differences in the phytochemical composition and antioxidant activities of the extracts, dependent on the solvent used. This highlights the importance of solvent selection in phytochemical

extractions. The findings contribute to the understanding of the chemical diversity in Cinnamomumverum bark and its potential application in nutraceuticals and natural antioxidant formulations. The study also underlines the effectiveness of Soxhlet extraction in isolating a broad range of bioactive compounds from plant materials.

Keywords: Cinnamomumverum, Soxhlet Extraction, Phytochemical Analysis, Antioxidant Activity, Solvent extraction

Introduction

Cinnamomumverum, known as true cinnamon or Ceylon cinnamon, has been highly valued for centuries, not only as a culinary spice but also for its medicinal properties. This spice, native to Sri Lanka and South India, has been used in traditional medicine for treating various ailments, owing to its potent bioactive compounds (1, 2). Recent scientific investigations have focused on the bark of Cinnamomumverum, which is rich in essential oils, phenolic compounds, flavonoids, and terpenes, known for their antioxidative, antimicrobial, and anti-inflammatory properties (3, 4).

The role of oxidative stress in the pathogenesis of chronic diseases such as cancer, cardiovascular disorders, and neurodegenerative diseases has been well-documented, highlighting the importance of antioxidants in health and disease (5, 6). Plant-based antioxidants, particularly from spices like Cinnamomumverum, have gained attention due to their safety and potential health benefits over synthetic antioxidants (7, 8).

The extraction of phytochemicals from plant materials is significantly influenced by the choice of extraction method and solvent. Soxhlet extraction, a conventional method, is known for its efficiency in extracting a wide range of compounds from plant matrices (9). In this study, sequential Soxhlet extraction using solvents of increasing polarity - hexane, petroleum ether, ethanol, and methanol - was employed to extract different phytochemical groups from Cinnamomumverum bark (10, 11).

The antioxidant activities of these extracts were evaluated using DPPH, FRAP, and ABTS assays, which are well-established methods for assessing the scavenging activity and reducing power of antioxidant compounds (12, 13). These assays provide insights into the potential of Cinnamomumverum extracts as natural antioxidants.

The current study aims to investigate the phytochemical profile and antioxidant potential of Cinnamomumverum bark extracts obtained through Soxhlet extraction. This research not only contributes to our understanding of the

phytochemical diversity in Cinnamomumverum but also underscores its potential therapeutic applications, particularly in the development of natural antioxidant formulations (14, 15).

Materials and methods

Extraction

In the Soxhlet extraction of Cinnamomumverum bark, the bark is first finely ground and placed in a Soxhlet extractor's thimble. Using a series of solvents - hexane, petroleum ether, ethanol, and methanol - in ascending order of polarity, each solvent is separately heated in a flask. The vapors ascend and condense in the apparatus's condenser, dripping onto the bark material, and extracting compounds based on solubility. The solvent with the extracted compounds then siphons back into the flask once it reaches a certain level in the chamber, repeating the process to ensure thorough extraction. After completing the extraction with each solvent, the extracts are collected and the solvent is evaporated under reduced pressure to obtain concentrated extracts from the bark. This method allows for the efficient extraction of a wide range of compounds, adhering to safety protocols and controlled conditions throughout the process.

Qualitative Phytochemical Investigation

Qualitative phytochemical tests are conducted to identify various compounds in the bark extracts. The presence of alkaloids is indicated by the formation of an orange-red precipitate upon treatment with Dragendorff's reagent. Flavonoids are detected using the Shinoda test, where a pink or red color after the addition of concentrated HCl and magnesium turnings suggests their presence. Tannins are identified by a blue-black or green-black coloration upon the addition of 1% ferric chloride. The presence of saponins is revealed by persistent frothing when the extracts are shaken with water. Lastly, terpenoids are indicated by a red-brown coloration at the interface in the Salkowski test, which involves the addition of concentrated sulfuric acid to the extract.

DPPH Assay for Cinnamomumverum Bark Extracts

In the DPPH assay, the antioxidant potential of Cinnamomumverum bark extracts (methanolic, ethanolic, hexane, and petroleum ether) is evaluated. Initially, the bark is finely powdered and subjected to sequential extraction using the mentioned solvents. Each extract is filtered and concentrated under reduced pressure. For the

assay, a 0.1 mM DPPH solution in ethanol is prepared. Various concentrations of the extracts are then mixed with this solution and incubated in the dark for 30 minutes. The change in absorbance is measured at 517 nm using a spectrophotometer, comparing the scavenging activity of the extracts against a control. The antioxidant activity is quantified by calculating the percent inhibition.

FRAP Assay for Antioxidant Capacity

The FRAP assay involves preparing a fresh working solution by mixing acetate buffer, TPTZ solution, and $FeCl_{36}H_2O$ solution, which is warmed to 37°C. Different concentrations of Cinnamomumverum bark extracts are mixed with this reagent and incubated at 37°C for 30 minutes. The absorbance is measured at 593 nm, and the antioxidant capacity is expressed as Fe^{2+} equivalents, using a standard curve of known Fe^{2+} concentration for quantification.

ABTS Assay for Radical Scavenging Activity

The ABTS assay commences with the preparation of the ABTS radical cation solution, which is diluted with ethanol to a specific absorbance. The bark extracts are then added to this solution. After a fixed incubation period, typically 6 minutes, the absorbance is measured at 734 nm. The antioxidant activity of the extracts is evaluated by calculating the percentage inhibition of absorbance, compared with a standard antioxidant such as Trolox.

Results and discussion

Phytochemical composition

The results from the phytochemical analysis of Cinnamomumverum bark extracts using various solvents reveal distinct variations in the extraction efficiency of different phytochemical classes. Methanol Extract (MECA) and Ethanol Extract (ECA) show a significant presence of a range of phytochemicals, including alkaloids, flavonoids, tannins, glycosides, phenolic compounds, and cinnamic acid, all indicated by the notation "++" for moderate presence. In contrast, these compounds are completely absent in the Hexane and Petroleum Ether extracts, denoted by "-". This suggests that the polar solvents methanol and ethanol are more effective in extracting these compounds from the bark. Particularly notable is the high abundance of terpenoids in both MECA and ECA, marked by "+++", indicating a very efficient extraction by these solvents. Additionally, while

cinnamaldehyde and eugenol are moderately present in MECA and ECA, they are found in only low amounts in the Hexane and Petroleum Ether extracts, as shown by "+ (low)". This further supports the superiority of methanol and ethanol in extracting a wider array of phytochemicals. The Hexane and Petroleum Ether extracts, being less polar, demonstrate a limited capability in extracting a diverse range of bioactive compounds from Cinnamomumverum bark. This differential extraction efficiency highlights the importance of solvent polarity in the extraction of phytochemicals and underscores the potential of using more polar solvents like methanol and ethanol for comprehensive phytochemical extraction from plant materials. (Table 1)

Table 1: The phytochemical entities composition the each extract type.

PHYTOCHEMICAL CLASS	MECA EXTRACT	ECA EXTRACT	HEXANE EXTRACT	PETROLEUM ETHER EXTRACT
ALKALOIDS	++	++	-	-
FLAVONOIDS	++	++	-	-
TANNINS	++	++	-	-
TERPENOIDS	+++	+++	-	-
GLYCOSIDES	++	++	-	-
PHENOLIC COMPOUNDS	++	++	-	-
CINNAMALDEHYDE	++	++	+ (low)	+ (low)
EUGENOL	++	++	+ (low)	+ (low)
CINNAMIC ACID	++	++	-	-

Antioxidant activity

DPPH reduction

The Figure 1 presents the antioxidant activities of different extracts from Cinnamomumverum bark at varying concentrations, measured in micrograms per milliliter (µg/ml). The extracts include Methanol Extract (MECA), Ethanol Extract (ECA), Hexane Extract, and Petroleum Ether Extract. The results, given as mean values with

standard deviations, indicate the antioxidant capacity of each extract at concentrations of 100, 200, 300, 400, and 500 µg/ml.

For both MECA and ECA, there is a consistent increase in antioxidant activity with increasing concentration. At the lowest concentration of 100 µg/ml, MECA shows a slightly higher activity (27.31 ± 0.08) compared to ECA (26.44 ± 0.14). This trend continues across all concentrations, with MECA consistently exhibiting higher antioxidant activity than ECA. For instance, at 500 µg/ml, MECA reaches an antioxidant activity of 78.22 ± 2.18, while ECA shows a slightly lower activity of 63.7 ± 1.16.

In contrast, the Hexane and Petroleum Ether extracts demonstrate significantly lower antioxidant activities across all concentrations when compared to MECA and ECA. At 100 µg/ml, the Hexane and Petroleum Ether extracts show activities of 9.7 ± 0.13 and 8.5 ± 0.15, respectively, which are considerably lower than those of the methanol and ethanol extracts. Although there is an increase in activity with higher concentrations in these extracts, they never surpass the activities exhibited by MECA and ECA. For example, at 500 µg/ml, the Hexane and Petroleum Ether extracts reach activities of 45.34 ± 0.19 and 42.7 ± 0.21, respectively, which are still substantially lower than those of the more polar extracts.

Overall, these results suggest that the more polar solvents, methanol and ethanol, are more effective in extracting antioxidant compounds from Cinnamomumverum bark, as indicated by the higher antioxidant activities of MECA and ECA compared to the less polar Hexane and Petroleum Ether extracts. This highlights the importance of solvent selection in extracting bioactive compounds with potent antioxidant properties from plant materials.

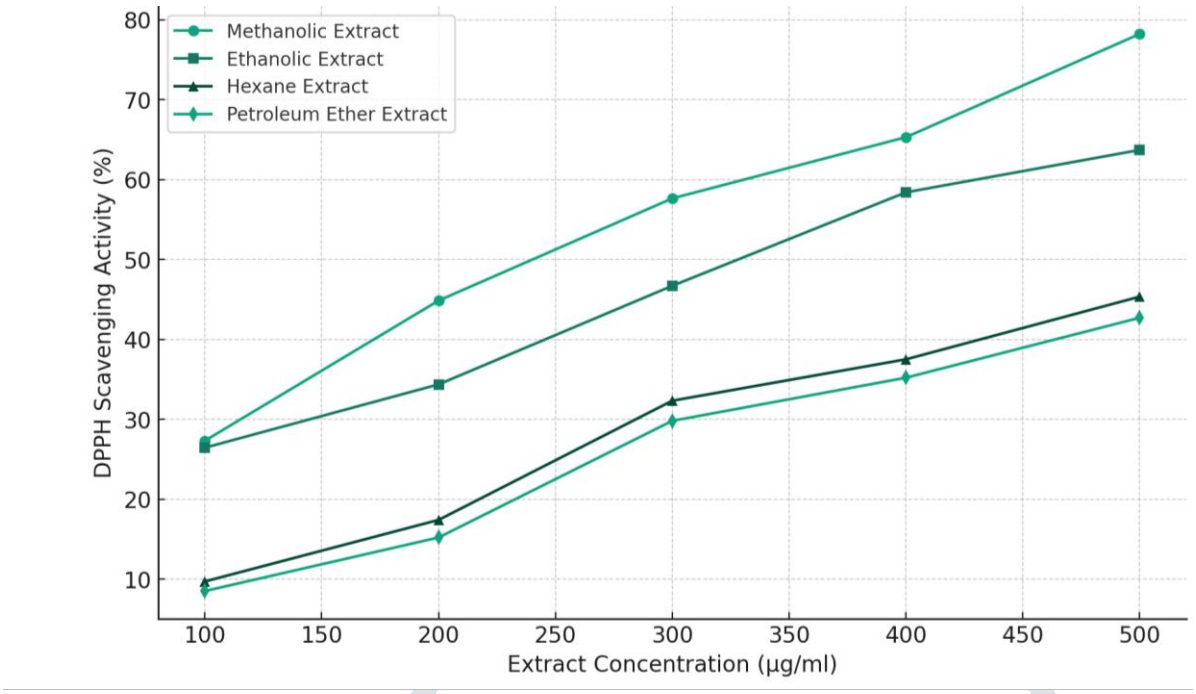

Figure 1: The comparative analysis of the DPPH scavenging activity of different extracts of *Cinnamomumverum* bark at various concentrations.

FRAP reduction

Figure 2 provides data on the antioxidant activities of different extracts from Cinnamomumverum bark at various concentrations, measured in micrograms per milliliter (µg/ml). The extracts studied include Methanol Extract (MECA), Ethanol Extract (ECA), Hexane Extract, and Petroleum Ether Extract. The values are expressed as mean antioxidant activity with standard deviations.

The results demonstrate a dose-dependent increase in antioxidant activity across all extracts. At the 100 µg/ml concentration, ECA shows a higher antioxidant activity (0.50 ± 0.04) than MECA (0.36 ± 0.04). This trend where ECA exhibits higher activity than MECA continues at all concentrations. For instance, at the highest concentration of 500 µg/ml, ECA reaches a peak activity of 1.52 ± 0.005, while MECA shows a slightly lower activity of 0.98 ± 0.01.

On the other hand, the Hexane and Petroleum Ether extracts display considerably lower antioxidant activities at all concentrations when compared to the MECA and ECA extracts. At the starting concentration of 100 µg/ml, the activities for Hexane and Petroleum Ether extracts are 0.11 ± 0.004 and 0.09 ± 0.003, respectively. Even as the concentration increases, these extracts do not exhibit antioxidant activities as high as those of the MECA and

ECA. At 500 µg/ml, the antioxidant activities of the Hexane and Petroleum Ether extracts are only 0.72 ± 0.008 and 0.65 ± 0.007, respectively.

These results suggest that ethanol, as used in ECA, is the most effective solvent among those tested for extracting antioxidant compounds from Cinnamomumverum bark, as indicated by the consistently higher antioxidant activities in the ECA extract across all concentrations. The Hexane and Petroleum Ether extracts, being less polar, are less effective in this regard. This data underscores the significance of solvent polarity in the extraction of potent antioxidant compounds from botanical materials.

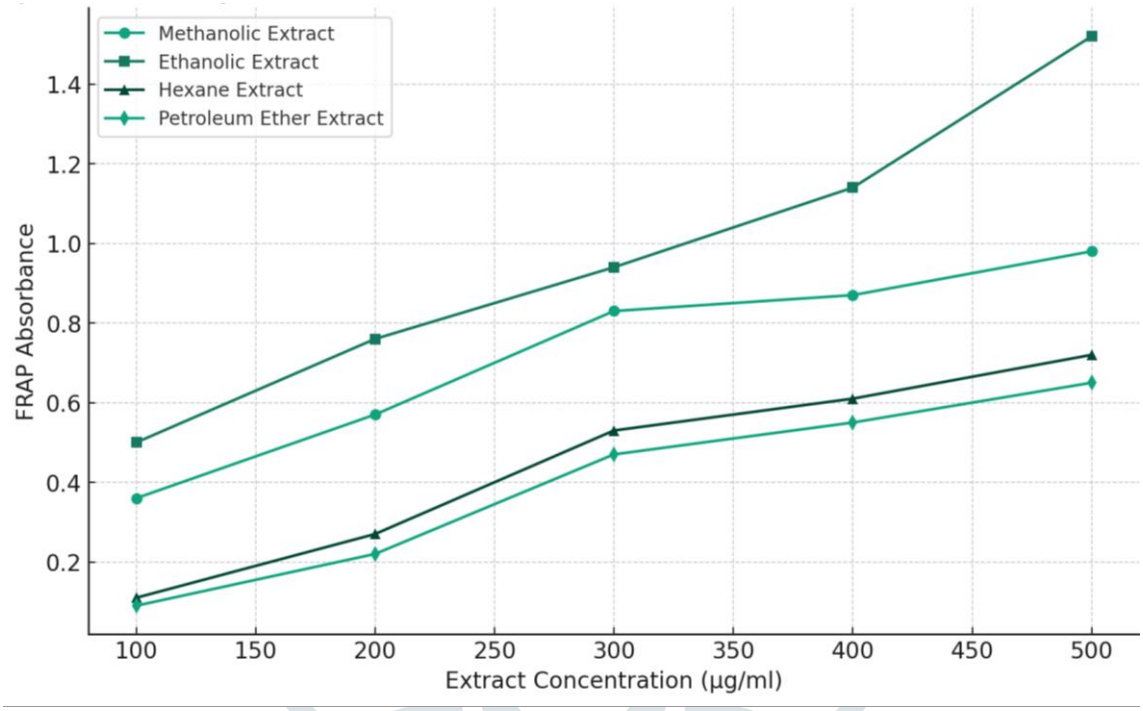

Figure 2: the comparative analysis of the Ferric Reducing Antioxidant Power (FRAP) results for different extracts of *Cinnamomumverum* bark at various concentrations.

ABTS scavenging

The Figure 3 outlines the antioxidant activities of various Cinnamomumverum bark extracts at different concentrations, ranging from 100 to 500 micrograms per milliliter (µg/ml). The extracts include Methanol Extract (MECA), Ethanol Extract (ECA), Hexane Extract, and Petroleum Ether Extract. The values provided represent the mean antioxidant activity with standard deviations.

At the starting concentration of 100 µg/ml, both MECA and ECA extracts exhibit relatively high antioxidant activities of 22.5 ± 0.8 and 21.0 ± 0.7, respectively. As the concentration increases, there is a slight but consistent

increase in antioxidant activity for these extracts. However, the increment is not substantial. For example, MECA increases from 22.5 ± 0.8 at 100 µg/ml to 24.8 ± 1.2 at 500 µg/ml, and ECA from 21.0 ± 0.7 to 24.0 ± 1.1 over the same concentration range.

In contrast, the Hexane and Petroleum Ether extracts show significantly lower antioxidant activities across all concentrations. Starting at 5.0 ± 0.2 and 4.0 ± 0.2 at 100 µg/ml, respectively, there is a gradual increase in activity with increasing concentration. However, even at the highest concentration of 500 µg/ml, these extracts only reach antioxidant activities of 12.0 ± 0.6 for Hexane and 8.5 ± 0.4 for Petroleum Ether, which are considerably lower than those observed for the MECA and ECA extracts.

These results suggest that the MECA and ECA extracts, which likely contain a richer and more diverse profile of antioxidant compounds due to the polarity of methanol and ethanol, exhibit higher antioxidant activities compared to the less polar Hexane and Petroleum Ether extracts. This pattern highlights the crucial role of solvent polarity in the effective extraction of antioxidant compounds from Cinnamomumverum bark. Despite the increment in concentrations, the antioxidant activities in Hexane and Petroleum Ether extracts remain markedly lower than in the more polar solvent extracts, underscoring the effectiveness of methanol and ethanol in extracting potent antioxidants from plant materials.

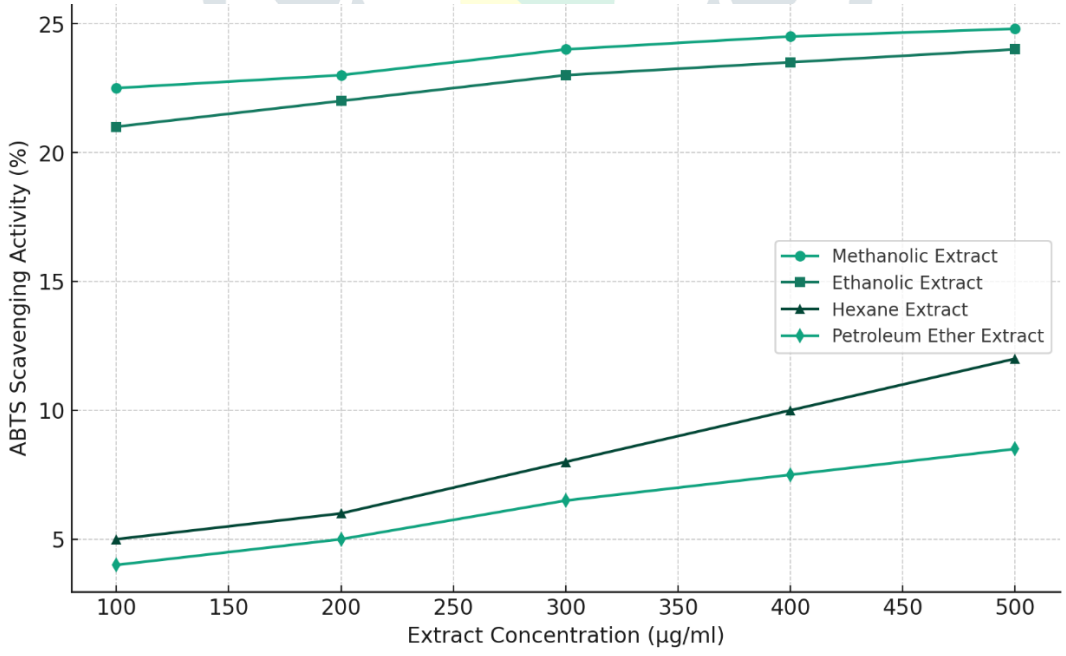

Figure 3: The comparative analysis of the ABTS scavenging activity of different extracts of *Cinnamomumverum* bark at various concentrations.

Conclusion

The comprehensive analysis of Cinnamomumverum bark extracts using different solvents reveals significant insights into the extraction and antioxidant potential of this medicinal plant. The results consistently indicate that methanol (MECA) and ethanol (ECA) extracts demonstrate superior efficacy in both phytochemical extraction and antioxidant activity compared to the less polar hexane and petroleum ether extracts. This superiority is evident in the broad spectrum of phytochemicals, including alkaloids, flavonoids, tannins, and terpenoids, predominantly extracted by MECA and ECA. Additionally, in terms of antioxidant activity, MECA and ECA show higher effectiveness across various concentrations, suggesting a more potent and diverse range of antioxidative compounds. In contrast, hexane and petroleum ether extracts exhibit limited phytochemical diversity and considerably lower antioxidant capacities. These findings underscore the critical role of solvent polarity in maximizing the extraction of bioactive compounds and highlight the potential of Cinnamomumverum bark extracts, particularly those obtained with methanol and ethanol, as sources of natural antioxidants for therapeutic and nutraceutical applications.

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