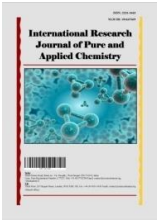

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Phytochemical Screening, Chemical Composition and Antimicrobial Activity of *Cinnamon verum* Bark

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Authors' contributions

This work was carried out in collaboration among all authors. Author IYE designed and administrated the study. Authors AMR and MBS conducted the experimental work. Author HMA managed the literature and wrote the first draft of the manuscript. Author OAOI managed the literature searches and revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

cinnamon dating from 1000 AD when it was firstly recorded in English due to its important as aroma and as herbs. The aim of this study was to investigate phytochemicals constitutes, chemical composition and antimicrobial activity of the essential oil of commercial samples of *Cinnamon verum* bark. The essential oil was extracted by hydrodistillation, while the crude extracts were prepared by three different solvents methanol (70%), acetone and aqueous. Phytochemical screening of crude extracts was performed using standard methods. The essential oil was subjected to GC-MS analysis and tested against *Staphylococcus aureus, Salmonella typhimurium, Pseudomonas aeruginosa, Eschericchia coli* and *Candida albicans*. The obtained results indicated the presence of alkaloids, flavonoids, coumarin, tannins, terpenoids, saponin, glycoside, anthrocyanin and phenolic compounds in the methanolic, aqoueous and acetone extracts of *C. verum* bark; while the major components of the extracted essential oil of *C. verum* bark were

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cinnamaldehyde (85.50%), stigmasterol (3.69%), Cadinene (1.37%), (E)-cinnamaldehyde (1.35%), alpha-amorphene (1.33%), hydrocinnamaldehyde (1.28%), alpha-cubebene (1.25) and ergosterol (1.09%) respectively. The antimicrobial activity result indicated the high activity of the extracted essential oil against all tested microorganisms at high concentration; except in *S. typhimurium* and *C. albicans* at concentrations of 25% and 12.5% no activity was noticed. Based in our obtained results the essential oil of *C. verum* bark had high potential as antimicrobial agent, therefore, recommended for more advanced studies to be conducted on this abundant plant as natural source of antibiotics.

Keywords: Cinnamon verum; bark; phytochemical screening; chemical composition; antimicrobial activity.

1. INTRODUCTION

Cinnamomum is a member of Lauraceae. It consists of approximately 250 species distributed in tropical and subtropical regions of Southeast Asia, Australia and North, Central and South America [1]. *Cinnamomum verum,* J Presl. (Syn. *Cinnamomum zeylanicum Nees*) (*Lauraceae*) is basically utilized as a culinary herb like different cinnamons in the conventional Eastern and Western medication [2]. Phytochemicals are plant-derived, bio-active chemicals. Due to the presence of phytochemical constituents, Therapeutic plants are helpful for healing as well as for treating human diseases [3]. Barks and leaves of cinnamomum are commonly used in foods as seasoning and flavoring agent [4]. *Cinnamomum verum J. Presl* (Lauraceae family), generally referred to cinnamon, develops for the most part in South and South-East Asia and its bark is rich in essential oil (EO) with strong antimicrobial activity Although the oil from various parts of the world has appeared extraordinary differences in chemical composition [5]. Previous reports revealed that an acetonic and ethylacetate extract of *C. verum* containing numerous bioactive constituents specifically alkaloids, tannins, saponins, terpenoids and remarkable amounts of polyphenols and flavonoids, was found in the Qualitative phytochemical estimation [6]. In the last few years *C. verum* bark and essential oils extracts demonstrated strong antimicrobial activity against a wide range of bacteria and fungi [7,8]. Yap et al. [7]; revealed that trans-cinnamaldehyde (72.81%), benzyl alcohol (12.5%), and eugenol (6.57%) were the major components of the essential oil of *Cinnamomum verum* in Gas-MS spectrometry. whereas Ainane et al. [9]; indicated in GC-MS that the main composition consists of three compounds: cinnamaldehyde (89.31%), cinnamyl acetate (2.44%), linalool (1.60%). The aim of this study was to investigate phytochemicals constitutes chemical composition and antimicrobial activity of the crude extract and essential oil of commercial sample of *cinnamon* bark.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The Cinnamon barks commercial sample used in this study was purchased from local market at Khartoum – Sudan; the purchased sample was cleaned, washed and shade dried for two days. Then the dried sample was grinded into proper size and kept in polyethylene bags for further study.

2.2 Extraction of Sample Essential Oil

The essential oil of the cinnamon bark sample was extracted by hydro distillation using Clevenger apparatus as mentioned by Kasim et al. [29]; with slight modification. About 120 g of sample was loaded in the apparatus and then the extraction run for two hours; the process was repeated and in each time distillate was collected. The extracted oil was kept in refrigerator under 4°C for further study.

2.3 Preparation of Sample Extracts

The sample extracts was prepared according to methods described by Ishag et al. [10]; with slight modification. About 10 g of the prepared sample of cinnamon was accurately weighed into three different conical flasks. A 100 mL of distilled water, 70% methanol and 75% acetone were added to each flask separately, with vigorous shaking for 10 minutes; then left for 48 hours at room temperature. The crude extracts of the three flasks were filtered, the solvents were evaporated (45 -50°C) and then the extracts were kept for further study.

2.4 Phytochemicals Tests of Sample Extracts

The cinnamon bark sample extracts were screened for alkaloids, coumarins, flavonoids, tannins, saponosids, glycosides, anthocyanin's, terpenoids and phenols. The aqueous test solution of sample was prepared according to method descried by Torres et al. [11]; with slight modification. About 1 g of each crude extract was dissolved in 20 mL of distilled water and the results solution was used in tests.

2.4.1 Test of alkaloids (Mayer's and Wagner's tests)

About 2 mL of the test solutions were transferred into three test tubes; a few drops of Mayer's and Wagner's reagents were then added into the tubes. The presence of alkaloids was evidenced by the development of precipitates in the tubes that contained the tested solutions; as mention by Djeussi et al. [12] and Abakar et al. [13].

2.4.2 Test of coumarins (Alkaline reagent test)

In a test tube, 2 mL of NaOH was added to 2 mL of the test solution in each case. Development of a greenish yellow or blue fluorescence indicated a positive test for Coumarins; as in Alzoreky and Nakahara [14] and Dandjesso et al. [15].

2.4.3 Test of flavonoids

Flavonoids were tested by adding drops of Lead acetate solution (10%) to a 1 mL of each extract. Formation of a yellow precipitate showed the presence of flavonoids [14, 16].

2.4.4 Test of Tannins

About 2 mL of test solution was added to 2 mL of water followed by drops of dilute ferric chloride solution (0.1%). A green to blue-green (cathechic tannins) or a blue-black (gallic tannins) coloration were positive indicators [16].

2.4.5 Test of saponosids (frothing test)

About 2 mL of test solution were introduced in a test tube containing 2 mL of distilled water. The tube was stopped and shaken vigorously for about 15 seconds. Allowed to stand for 15 min, persistent frothing indicated the presence of saponosids; as shown by Sofowora [17] and Mazimba et al. [16].

2.4.6 Test of glycosides

About 2ml of test solution was dissolved in 4ml of glacial acetic acid containing one drop of 5% ferric chloride solution which was under laid with 1 ml of concentrated H_2SO_4; A brown ring obtained at the interface indicate the presence of glycosides.

2.4.7 Test of anthocyanin's

About 2 mL of mixture contained HCl (2M, 1 mL) and ammonia (4M, 1mL) were added to 1 mL of test solution. The color change from pink-red to blue-violet indicates the presence of anthocyanin's [16,17].

2.4.8 Test of terpenoids (Chloroform test)

About 2 mL of chloroform were mixed with 2 mL of the test solutions. To this mixture, 2 mL of concentrated H_2SO_4 were added and heated for 120 s in a water bath (≈ 65°C). A reddish brown color that developed at the interface was evidence of the presence of terpenoids [16,18].

2.4.9 Test of phenols

About 1 mL of test solution was treated with drops of ferric chloride (5%) and observed for the formation of deep blue or black color [18].

2.5 Antimicrobial activity test

Antimicrobial activity of essential oil extracted from the sample of cinnamon bark was tested according to method described by Ishag et al. [10]; with slight modification. Microorganisms used for the antimicrobial assay in this study were as follows: *Staphylococcus Aureus, Salmonella typhimurium, Pseudomonas aeruginosa, Eschericchia coli* and *Candida albicans*. The organisms were obtained from Microbiology Laboratory, Department of Microbiology, International University of Africa, Khartoum, Sudan. Antimicrobial activity was evaluated by measuring the diameter of inhibition zone (DIZ) of the tested microorganism. DIZ was expressed in millimeters. Tests were performed in triplicate.

2.6 GC-MS Analysis

The extracted essential oil of cinnamon barks sample was analyzed using Shimadzu Gas Chromatograph (Model GC.MS-QP2010 Ultra) coupled with a non-polar Rtx-MS capillary

column 30 meter in length, diameter (0.25 mm) and thickness (0.25 µL) using a mass spectrometer detector and helium was used as a mobile phase (carrier gas). The temperature range was from 50 – 300°C, with a temperature program rate of 10°C/min, starting at three minutes and finishing at thirty minutes. The pressure applied in this experiment was 100 kPa with a total flow of 50 mL/min and 1.69 mL/min of column flow. The injection, ion source and the interface temperatures were 300°C, 200°C and 250°C respectively.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening Results

The results obtained of phytochemical screening tests of the three different extracts of cinnamon bark sample were shown in Table 1.

The obtained results of phytochemicals screening tests showed the presence of alkaloids, flavonoids, coumarin, tannins, terpenoids, saponin, glycoside, anthrocyanin and phenolic compounds in all extracts. This result is in agreement to those of Mazimba et al. [16]; where they found presence alkaloids, tannins, phenol and saponins as strong positive results together with flavonoids in methanolic extracts of cinnamon steam bark; also Paliwal et al. [19]; showed the presence of alkaloids, phenolics, flavonoids, saponin, tannin and glucoside in the extract of *Cinnamomum zeylanicum* bark. While the results of Shreya et al. [20]; revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, and glycosides in methanolic bark extract while aqueous bark extract of cinnamon showed the absence of tannins and flavonoids where our obtained result showed the present of tannins and flavonoids in both extracts. Another previous study conducted by Adarsh et al. [21]; of methanol and aqueous cinnamon bark extract reported that alkaloids, saponins and terpenoids were present and exhibited strong agreement to our obtained results whereas the absence flavonoids, glycosides, tannins and phenolic compounds were disagreed our results in phytochemical screening as shown in Table 1. Gaurav Kumar and Sonu Garg [22]; evaluated the phytochemicals of aqueous extract of *C. verum*, their results exposed the presence of phenols, tannins and flavonoids, while saponins and alkaloids were found to be absent; which disagree our obtained results where saponins and alkaloids were present. Shreya et al. [20]; results showed the presence of alkaloids, flavonoids, tannins, terpenoids, saponin and glycoside in the methanolic extract of *C. verum* bark, although their results agreed our obtained results in this study except their aqueous extract of the bark where they obtained only alkaloids, terpenoids, saponin and glycoside; which disagreed our results; The variation in our obtained results and previous studies perhaps due to the environmental and soil issues.

3.2 Chemical Composition Result

The extracted essential oil of cinnamon bark sample was subjected to GC/MS analysis and the obtained result was shown in Table 2.

The obtained results of essential oil in this study showed the present of 21 chemical components; the major components were cinnamaldehyde (85.50%), stigmasterol (3.69%), cadinene (1.37%), (E)-cinnamaldehyde (1.35%), α-amorphene (1.33%), hydrocinnamaldehyde (1.28%), α-cubebene (1.25) and ergosterol (1.09%). This result are in agreement to those

Table 1. Phytochemical screening of Cinnamon crude extract

Chemical constitute	Acetone extract	Methanol extract	Aqueous extract
Alkaloid	++	++	++
Flavonoid	++	+	++
Tannins	++	++	++
Saponin	++	++	++
Glycoside	+	++	++
Anthrocyanin	++	++	+
Coumarins	++	++	+
Terpenoids	++	++	++
Phenol	++	++	**++**

++: high concentration
+: moderate concentration

found by Raeisi et al. [23]; where cinnamaldehyde was dominant major compound (79.74%) in hydro-distillated essential oil, other components were similar too. Also Huang et al. [24]; results of *C. cassia* the cinnamaldehyde (68.52%) was found to be the major compound was also agreed our result. Francisco et al. [25]; results of *C. zeylanicum* and *C. cassia* essential oil was also proved that cinnamaldehyde was the major component, where it's agreed our obtained results. Ainane et al. [9]; showed that in their result the major component of the essential oil of *C. verum* bark cinnamaldehyde (89.31%), which also in agreement with our result. Also Valizadeh et al. [26] and Al-fekaiki et al. [27]; their study result on the essential oil of the *Cinnamon* was shown the major component was cinnamaldehyde (69.15%), cinnamaldehyde (57.83%) respectively which similar to our result. Variation of cinnamon essential oil composition may be affected by geographical location and harvesting time.

3.3 Antimicrobial Activity Result

The activity of extracted essential oil of cinnamon bark was tested against some microorganism and the obtained result was shown in Table 3.

The obtained results in this study indicated that the extracted essential oil had high activity against *S. aureus* at all concentration; also showed high activity for *E. coli* at high concentration and low activity at low concentration (12.5%). For *P. aeruginosa* the extracted oil showed high activity at high concentration only; beside no activity was founded at low concentration (12.5%). In addition the extracted oil showed high activity against *S. typhimurium* and *C. albicans* only at high concentration (100 and 50%), while at concentration (25 and 12.5%) no activity was noticed. Ainane et al. [9]; their antimicrobial activity against*: E. coli, P. aeruginosa, S.aureus* and *C. albicans* demonstrated remarkable activity, which suggests the use of this oil in the treatment of mycoses; where their results were in agreement with our results. Vazirian et al. [5]; showed that the chemical constituents of the cinnamon oil determined by gas chromatography/mass spectrometry twenty five components were identified but cinnamaldehyde (79.73%) was the major constitute, where their result also agreed our result. Valizadeh et al. [26]; evaluated the antimicrobial activity of CEO against *S. thyphimurium, E. coli, B. cereus* and candida species using agar well diffusion and disc diffusion method with inhibition zone of 200, 100, 50 µL/mL of EO and reported that the CEO was most effective on *B. cereus* in both methods and diameter of inhibition zone of the highest concentration of CEO in disk and agar well diffusion methods was 30 mm. Mith et al. [28] showed that commercial essential oils from cinnamon exhibited antimicrobial effects against selected food-borne and food spoilage bacteria, which can be attributed to the presence of the principle bioactive components, particularly cinnamaldehyde.

Table 2. GC-MS result of cinnamon essential oil

No.	Name	Formula	Composition %
1	Cinnamaldehyde	C_9H_8O	85.50
2	Stigmasterol	$C_{29}H_{48}O$	3.69
3	Cadinene	$C_{15}H_{28}$	1.37
4	(E)-Cinnamaldehyde	C_9H_8O	1.35
5	Alpha- Amorphene	$C_{15}H_{24}$	1.33
6	Hydrocinnamaldehyde	$C_9H_{10}O$	1.28
7	Alpha-Cubebene	$C_{15}H_{24}$	1.25
8	Ergosterol	$C_{28}H_{44}O$	1.09

Table 3. Antimicrobial activity of EO of cinnamon bark

Concentration	Inhibition Zone (mm)				
(% v/v)	*E. coli*	*S. aureus*	*P. aeruginosa*	*S. typhimurium*	*C. albicans*
100	42	35	29	22	40
50	40	32	21	16	30
25	22	30	18	-	-
12.5	11	20	-	-	-

Weak inhibition zone for readings less than 14 mm (< 14 mm).
Strong inhibition zone for readings greater than 14 mm (> 14 mm).
No inhibition zone for readings less than 11 mm (< 11 mm)

4. CONCLUSION

The aim of this study was to investigate phytochemicals constitutes chemical composition and antimicrobial activity of the crude extract and essential oil of commercial sample of *cinnamon* bark. The obtained results indicated the presence of alkaloids, flavonoids, coumarin, tannins, terpenoids, saponin, glycoside, anthrocyanin and phenolic compounds in the methanolic, aqueous and acetone extracts of *Cinnamon* bark; while the major components of the extracted essential oil of *Cinnamon* bark were cinnamaldehyde, stigmasterol, cadinene, (E)-cinnamaldehyde, alpha-amorphene, hydrocinnamaldehyde, alpha-cubebene and ergosterol respectively. The antimicrobial activity result indicated the high activity of the extracted essential oil against all tested microorganisms at high concentration; except in *S. typhimurium* and *C. albicans* at concentration (25 and 12.5%) no activity was noticed.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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