**E-ISSN:** 2583-9667
**Indexed Journal**
**Peer Reviewed Journal**
https://multiresearchjournal.theviews.in

**Received: 20-07-2024**
**Accepted: 25-08-2024**

**INTERNATIONAL JOURNAL OF ADVANCE RESEARCH IN MULTIDISCIPLINARY**

**Volume 2; Issue 3; 2024; Page No. 342-345**

# Study the therapeutical applications of *Cinnamomum verum* Buffer Extract [CVBE]

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**DOI:** https://doi.org/10.5281/zenodo.13369636

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**Abstract**

The purpose of the current study is to investigate the potential therapeutic uses of *Cinnamon verum* Buffer Extract [CVBE]. According to the results of the initial screening assay, CVBE has a wealth of macro and micronutrients, such as terpenoids, phytosterol, tannins, phenolic compounds, saponins, flavonoids, carbohydrates, alkaloids, and more. The presence of several phytochemicals was also confirmed by GC-MS and HPLC analysis, which produced chromatograms with 3 and 10 peaks, respectively, indicating their presence. Additionally, the extract of CVBE lacks a number of minerals, including zinc, manganese, cadmium, iron, boron, and aluminium. Aside from the aforementioned assays, we are lucky to have discovered that the non-toxic characteristic of CVBE prevents it from cleaving packed red blood cells during *in vitro* experiments. Remarkably, CVBE has antibacterial properties against gram positive (S. aureus) and gram negative (E. *coli*), respectively, producing 1.5 and 2.1 Minimum Inhibitory Concentration values against the positive control medication, amoxicillin.

**Keywords:** *Cinnamomum verum* Buffer Extract [CVBE], GC-MS, RP-HPLC, anti-bacterial and non-toxic property

## Introduction

One of the most significant and well-liked spices in the world, cinnamon bark comes from a variety of species and is utilized in both traditional and modern medicine in addition to cooking [1]. Studies have been conducted on the phytoconstituent, pharmacological characteristics, and traditional therapeutic use of Cinnamomum verum plants [2]. One of the essential elements that are used in many cuisine preparations is spice. These are typically aromatic dried plants, such as fruits, leaves, bark, roots, etc., [3]. The principal active ingredient in cinnamon, cinnamon aldehyde, is mostly responsible for giving food its favour, aroma, and taste [4]. Essential oils are significant pharmacological and commercial substances that are widely used in industry, medicine, aromatherapy, and other fields. They are also commonly used for cosmetic purposes [5]. Ginger promotes blood flow to the limbs (fingers, toes), and plants that contain cinnamon oil are a natural alternative remedy for easing the pain in muscles and other cold and flu-related symptoms [6]. Using an empirical approach, the use of medicinal plants to treat human diseases stretches back thousands of years, and scientific advancements have made it possible to identify the elements causing these health benefits [7]. The plant is found throughout Southeast Asia and is grown in some areas of Indonesia and the Philippines. It has glossy green, oblong-elliptical leaves that are placed in opposition and an ovoid, 1-cm-long fruit [8]. The plant's dried bark, which is used for flavoring and cooking, can be purchased in the market in rolls and quills. Vanillin acid, caffeic acid, gallic acid, p-coumaric acid, ferulic acid, proanthocyanidins A and B, keampferol, cinnamic acid, and cinnamon aldehyde are some of the most intriguing compounds found in bioactive Cinnamomum accessions [9]. These compounds have a variety of positive effects on humans, including neuroprotective, hepatoprotective, cardio protective, and gastro protective properties [10].

## Materials and Methods

All the chemicals used were of analytical grade. Microbial cultures were purchased from MTCC.

### Preparation of CVBE

*Cinnamon Verum* was purchased from local market and subjected for Soxhlet extraction method using PBS buffer. The finally obtained extract was termed as *Cinnamon Verum* Buffer Extract (CVBE) and it utilized for further assays.

### Preliminary phytochemical screening of CVBE

CVBE was screened for terpenoids, phytosterol, tannin, phenolic, glycoside, Saponin, flavonoid, carbohydrates, proteins, steroids and alkaloids [11].

### RP-HPLC analysis of CVBE

CVBE was subjected to RP-HPLC using $C_{18}$ column (150mm×3mm, particle size 2.7µm) with VWD detector in Agilent 1260-infinity II. The column was pre-equilibrated with HPLC water and Acetonitrile and sample was eluted at the flow rate of 1ml/min in linear gradient mode [12].

### GC-MS analysis of CVBE

CVBE was analyzed in GC-MSD, model number 5977B, Agilent Make on single quadrupole mass spectrometers in the Electron Capture Negative Ion Chemical Ionization (ECNICI) mode with capillary column (30m length X0.25mm ID, 0.25µm film thickness, composed of 5% Phenyl methyl poly siloxane). Helium (99.9%) gas was used as carrier gas at the flow rate of 6ml/min and the injection volume of 2µl. Split ratio of 10:1, temperature program was set as follows, injector analyzed in GC-MSD, model number 5977B, Agilent Make on single quadrupole mass spectrometers in the Electron Impact Ionization Total Ion Chromatography (EITIC) mode with capillary column (30m length X0.25mm ID, 0.25µm film thickness, composed of 5% Phenyl methyl poly siloxane). Helium (99.999%) gas was used as carrier gas at the flow rate of 1ml/min and the injection volume of 2µl. Split ratio of 10:1, temperature program was set as follows, injector temperature 350 °C; Auxiliary temperature 250 °C, oven temperature initially 50 °C (4min hold) with an increase in temperature of 10 °C/min to 150 °C (4min hold), thereafter 20 °C/min to 200 °C (4min hold), 25 °C/min ramp to 250 °C (4 min hold), 30 °C/min ramp to 280 °C (4 min hold). Total run time 35.5 min. Sample was analyzed in GC-MSD, model 5977B Agilent Make. Mass spectrum was taken at 70ev; a scan interval of 2.92s [13].

### Direct hemolytic activity of CVBE

Direct hemolytic activity was determined by using washed human erythrocytes. Briefly, packed human erythrocytes and Phosphate Buffer Saline (PBS) (1:9v/v) were mixed; 1mL of this suspension was incubated independently with the various concentrations of CVBE (100µL & 200µL) for 1hr at 37 °C. The reaction was terminated by adding 9mL of ice cold PBS and centrifuged at 1000g for 10min at 37 °C [14]. The amount of hemoglobin released in the supernatant was measured at 540nm. Activity was expressed as percent of hemolysis against 100% lysis of cells due to the addition of water (positive control), whereas PBS served as negative control.

### Antimicrobial assay of CVBE

The bacterial cultures (E. coli and S. aureus) were grown in Muller Hinton nutrient agar medium that contain peptone (1%), beef extract (1%) and NaCl (1%) at pH 6.8. Sterile nutrient broth swabs were prepared and 0.1mL of the overnight grown bacterial culture was spread on the solidified agar plates (Muller Hinton Agar) evenly with the help of a swab. Wells were made on the solidified agar using a cork borer. The test solution was made by dissolving 50mg of CVBE in 1.0mL of water to get 50mg/mL concentration followed by sonication for 2min. The 100µL of this test solution containing 5mg of CVBE was added into the respective wells. The standard antibiotic drug Amoxycillin was kept as positive control and tested against both the pathogens. These plates were incubated at 37 °C for 24hr. The diameter of 'zone of inhibition' at each well was measured and recorded [15]. The minimum inhibitory concentration (MIC) assay was carried out in triplicate and the average values were reported.

### ICP-OES analysis of CVBE

CVBE was analyzed in Agilent Make ICP-OES instrument, model number 5110. To evaluate the content of minerals in the extract, the samples were aspirated at 12 RPM pump speed, 25 seconds sample uptake time, 30 seconds of rinse time, 5 seconds, read time, 1.2 KW RF power, 15 seconds stabilization time, Axial viewing mode, 8mm viewing height, 0.7 L/Min nebulizer flow, 12 L/Min plasma flow, 0.75 L/Min Aux flow [16].

## Results and Discussion

### Chemical Characterization of CVBE

CVBE found to presence of phenolic compounds, flavonoids, alkaloids, terpenoids, phenolic compounds, saponins, phytosterol and etc., (Table 01). CVBE shows the presence of several minerals such as aluminium, boron, copper, iron, manganese, lead, zinc and etc., (Table 02).

**Table 1:** Phytochemical Analysis and CVBE Results

| SL NO | Phytochemical Analysis | CVBE Results |
|---|---|---|
| 1 | Teipenoid | Present |
| 2 | Phytosterol | Present |
| 3 | Tannin | Present |
| 4 | Phenolic | Present |
| 5 | Glycoside | Absent |
| 6 | Saponin | Present |
| 7 | Flavonoid | Present |
| 8 | Carbohydrates | Present |
| 9 | Proteins | Absent |
| 10 | Alkaloid | Present |
| 11 | Steroids | Present |
| 12 | Lipid Test | Absent |

**Table 2:** Name of the Metal in CVBE ppm

| Sl. No. | Name of the Metal | CVBE in ppm |
|---|---|---|
| 1 | Aluminium | 1.155 |
| 2 | Boron | 0.10 |
| 3 | Cadmium | 0.01 |
| 4 | Chromium | 0.02 |
| 5 | Copper | 0.11 |
| 6 | Iron | 1.28 |
| 7 | Manganese | 3.38 |
| 8 | Molybdenum | 0.00 |
| 9 | Nickel | 0.01 |
| 10 | Lead | 0.09 |
| 11 | Strontium | 0.68 |
| 12 | Zinc | 0.20 |

**RP-HPLC analysis of CVBE**

CVBE elutes 3 peaks at different retention time in reverse phase HPLC chromatogram which is attached to Variable Wavelength Detector. Sample was eluted at 216nm at room temperature (Fig.01).

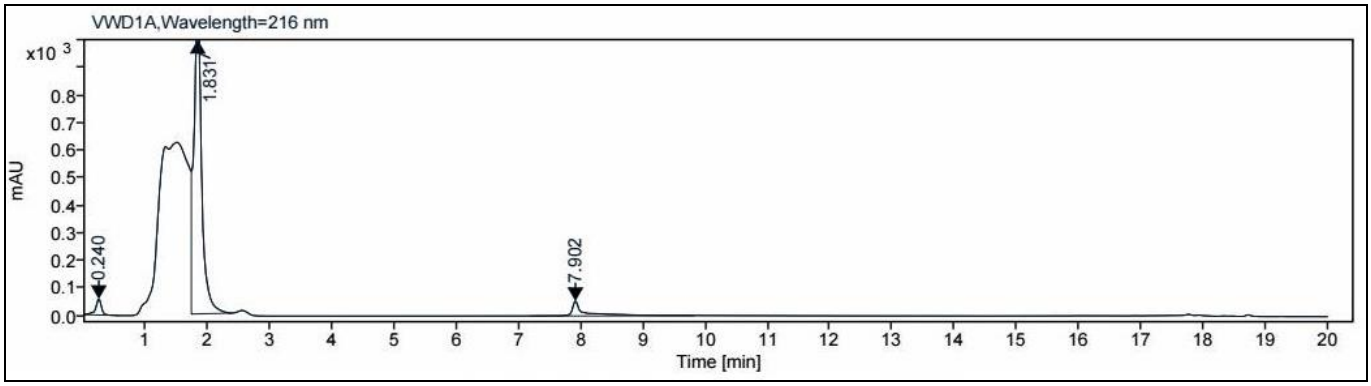


**Fig 1:** HPLC Chromatogram of CVBE

**GC–MS analysis of CVBE**

CVBE elutes 10 peaks in GC-MS chromatogram at the retention time of 2.2, 2.8, 3.9, 20.8, 23.5, 26.2, 26.6, 28.5, 31.1 and 32.5 respectively (Fig.02).

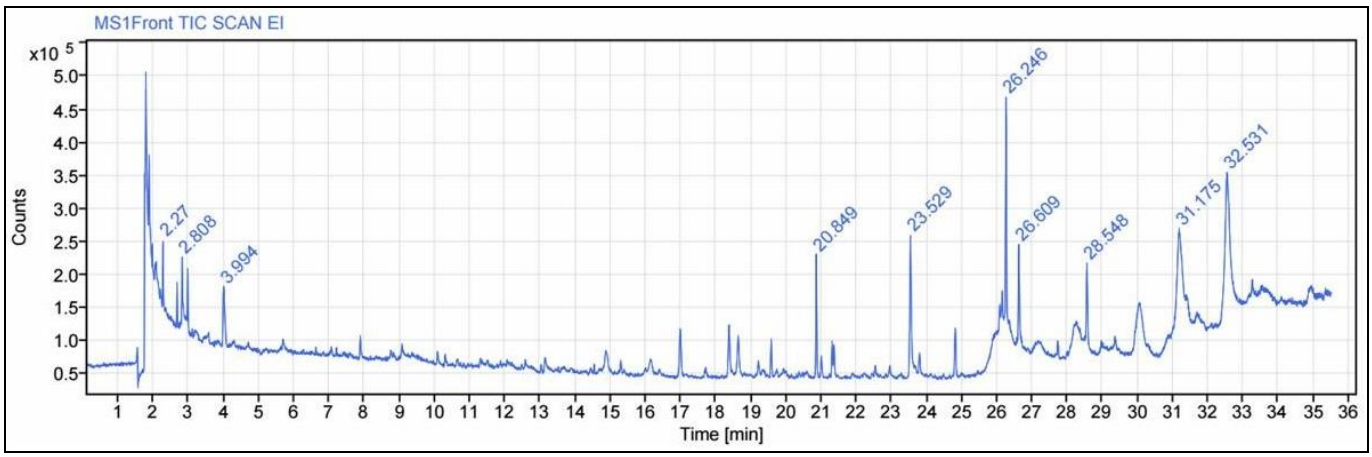


**Fig 2:** GC MS Chromatogram of CVBE

**Antimicrobial activity of CVBE**

CVBE antimicrobial property was performed with both gram negative bacteria (E.coli) and gram positive bacteria (S. aureus). Astonishingly, CVBE found to show zone of inhibition against both the bacteria (Fig.03).

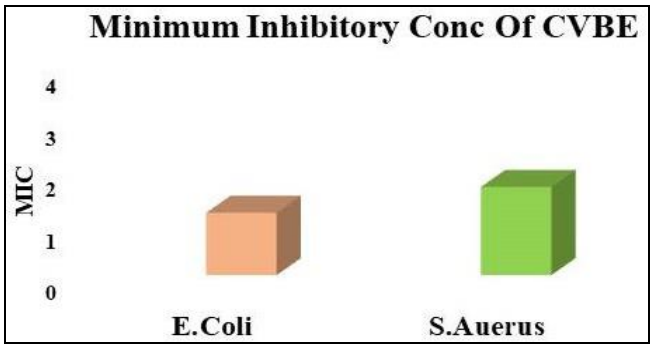


**Fig 3:** Antimicrobial Property of CVBE

Moreover, CVBE did not hydrolyze RBC suggested its nontoxic property (Fig.04).

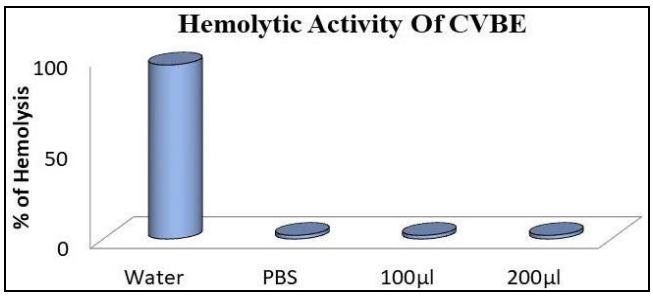


**Fig 4:** Haemolytic Activity of CVBE

## Conclusion

In conclusion, this study demonstrates the preliminary characterization of CVBE and its antimicrobial property against both gram negative and gram positive bacteria.

## Acknowledgments

Authors thank for Mr. Prakash [CEO of A TO Z ENVIRO TEST HOUSE LLP LAB] to given the opportunity for research work. We are very great full for all research associates of ENVIRO TEST HOUSE LAB namely Mahamad, Kavya, Vathsala, Shridevi, Mounika, Lavanya and Manjunath for their support during our research work.

## Declaration of Conflict of Interest

The authors declared no potential conflict of interest with respect to the authorship and publication.

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