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CINNAMOMUM IMPRESSINERVIUM MEISN.: ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES

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ABSTRACT

Background: The *Cinnamomum impressinervium (CI)* Meisn. Tree, which belongs to the Lauraceae family and is endemic to India, is also called Assameses or Tejiya. Numerous pharmacological properties of this plant, including anti-tumor, anti-inflammatory, and antioxidant properties, have been demonstrated. **Materials and method:** This study examines the phytochemical profile of the leaf using qualitative and quantitative methods. Following the phytochemical analysis of the leaf, the antioxidant efficacy for scavenging free radicals (ABTS and DPPH) was measured. Using the agar well diffusion method, the antibacterial potential of the crude extract and its fractions (aqueous, methanolic, n-hexane, and chloroform) was investigated against six gram-negative, three gram-positive, and one fungal strain. **Result and discussion**: Antioxidant activities of various extracts viz: aqueous, hexane, chloroform, and ethanol were prepared and subjected to antioxidant and antimicrobial activities. Through qualitative analysis, several alkaloids, steroids, and flavones were identified. In the DPPH and ABTS assays, the aqueous extract had the most potent antioxidant activity, with IC50 values of 123.83±0.42 and 57.86±0.85 µg/mL, respectively. DNA nicking assay is a qualitative analysis that shows DNA protection from free radicals. All the extracts towards *B. atropheous* showed best inhibition activity but a maximum zone of inhibition was shown by aqueous extracts measuring 40 mm. Aqueous and methanolic extracts are completely inactive towards *S. typhi*. **Conclusion:** This study revealed the antioxidant and antimicrobial efficacy of various extracts of *Cinnamomum impressinervium* Meisn. Leaves In this paper, the antibacterial activity of leaf extracts from *Cinnamomum impressinervium* Meisn. were studied. The evaluated extracts showed varied levels of inhibitory zones against every tested bacterium.

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INTRODUCTION

Plants utilized for illness prevention, mitigation, or therapy are known as medicinal plants [1]. Herbal medications are made from various plant components, such as leaves, stems, bark, etc. [2]. Herbal medications may be inexpensively accessible. Plants are widely used in India's pharmaceutical industry and for traditional medical purposes. Folk medicine is used in traditional medical systems such as Ayurveda, Unani, and Siddha. Although the estimated number of plant species in India is 4.5 million, certain plants are still being investigated for photochemical and pharmacological uses [3]. Secondary metabolites, or extracts, are employed in the pharmaceutical industry to develop innovative medications. A WHO report indicates that almost 80% of Indians utilize herbs as their primary treatment since they are less expensive and have fewer adverse effects. Because of the benefits mentioned above, an extensive range of herbal medications are produced commercially [4].

Herbs and medicinal plants are essential components of traditional medicine. Natural goods and plants are essential for human health. The system of traditional medicines is gradually coming back into vogue due to its inherent therapeutic properties, simplicity of access, curative results, and low adverse effects. One of the many types of therapeutic plants found in nature is the Cinnamomum species. There were over 350 species of this genus (Cinnamomum) globally. These plants are indigenous to the Pacific Islands, Asia, and Australia, and their development at varied elevations depends on wet woods [1]. Included in the Lauraceae family, *C. Impressinervium* Meisn is also known as tejiya. The fragrant tree Cinnamomum *impressinervium* grows between 1500 and 2500 meters above sea level. It is a cultivable tree that grows to a height of around 8 meters. This plant has a peppery smell and a clove-like flavor. Usually used as a spice, certain studies have also shown that it has some pharmacological properties, including anti-inflammatory, anti-diabetic, gastro-protective, and free radical scavenging action. The plant extract reduces oxidative damage and is helpful in cases of renal toxicity. Treatments for several illnesses, including cancer, anal disorders, flatulence, liver and spleen disorders, and rectal disorders, can benefit from it [5].

The study's main objective is to explore the phytochemical profiling of this new species. It is hypothesized that this plant may have various activities like antioxidant, anti-inflammatory, antimicrobial activities, etc.

MATERIAL AND METHODS

Reagents and chemicals

HCl, ethanol, methanol, n-hexane, gallic acid, acetic acid, sodium nitrite, sodium hydroxide, and quercitin. DPPH, sodium carbonate, mercuric chloride, ferric chloride hexahydrate, ascorbic acid, pot. Iodide, trichloride, Folin-ciocalteu phenol reagent, pot. Acetate, aluminum trichloride, and Dragendorff's reagent were purchased from Delhi, CDH. The solvent was purchased from Merck. All chemicals were of analytical grade.

Collection and identification of plants

Cinnamomum impressinervium meisn. leaves were collected from the Sikkim region. Identification was done by the Department of Botany, Punjabi University, Patiala with letter no. SPL-114/BOT, dated 30/11/2016.

Preparation of extracts

Leaves of *Cinnamomum impressinervium meisn*. were cut into small pieces and dried in sunlight. Soxhlet apparatus was used for extraction, and plant material was extracted sequentially using aqueous, n-hexane, chloroform, and methanol as solvents with increasing polarity. Plant material (50°C) was boiled for two hours using a hot plate. Extraction was collected and kept in a desiccator under vacuum [6].

Oil isolation

Leaves of *Cinnamomum impressinervium meisn*. were broken into smaller pieces, and isolation was done using the Clevenger apparatus [7].

Phytochemical screening

The crude extract's active ingredients like flavones, terpenoids, flavonoids, carbohydrates, and polyphenols, were further characterized. For the assessment, the approved methods were applied [8].

Alkaloid's detection

15mg of extract was mixed in 5% HCl (2ml). After proper mixing, screening of the material was done. 4 separate tubes were taken with different reagents: Dragendorff's reagent, Mayer's reagent, Wganer's reagent, and Bouchardat's reagent. The creation of yellowish white (Mayer's), red-orange (Dragendorff's), brown (Bouchardat's), and red-brown ppt (Wagner's) was the evidence against the presence of alkaloids [8].

Saponin detection

To a small amount (10 mg) of extract, 2ml of water, 20 drops of isopropyl alcohol, and 1ml of olive oil were added. Saponin was confirmed by the presence of foamy layers [9].

Flavonoid detection

A test tube containing a small amount (10 mg) of extracts was taken, and three drops of 10% NaOH were put into it. The emergence of purple-red and yellow-orange ppt. revealed the presence of flavonoids[10].

Tannin verification

1ml of the extract (dissolved) + 2ml of water (distilled) and around 2ml of ferric chloride were taken in test tube. The appearance of a green-blue color confirmed the presence of tannins [11].

Glycoside detection (Salkowaski test)

A trace quantity of crude drug was dissolved in a 2ml solution of sulphuric acid and distilled water. The reddish brown color appeared to confirm the presence of the aglycon part of glycoside [12].

Keller killani test

10ml of extract in dissolved form was taken in a test tube, and to this extract, 1.5ml of conc. sulphuric acid, and a few drops of 2%Fe_2 Cl_3 with 4ml glacial acetic acid were added. Brown rings between layers confirmed the formation of glycosides [12].

Anti-oxidant assay

Evaluation of free radical scavenging potential

DPPH radical scavenging assay and $ABTS^+$ radical cation test were used to reveal the free radical scavenging efficacy of the extracts [13].

DPPH radical scavenging potential

Brand-Williams et al. (1995) described methods for free radical scavenging using DPPH. Samples were assessed for their ability to scavenge the free radicals. A 0.02mM concentration was prepared in 70% methanol, and stirring was done at room temperature for 24 hours. Samples (10g/ml-200g/ml) and ascorbic acid standards (5g/ml-50g/ml) were combined to create a final volume of 1 ml with the above solution. It was determined how well they could scavenge free radicals. The samples were shaken and allowed to come to room temperature in the dark. Absorbance reduction for test materials and ascorbic acid was measured at 517 nm after 30 min; a plot was created to calculate ascorbic acid concentration using scavenging capacity and concentration [14].

$ABTS^+$ radical scavenging ability

The technique outlined by Re et al. was used to calculate the ABTS+ radical scavenging potential. 2.45 mM potassium persulfate was added to a 7 mM ABTS aqueous solution to produce the free radical cation. These radical cations were put in the dark and let to remain at room temperature for 12 to 16 hours. To create the working solution, the stock solution was diluted with ethanol; at 745 nm, this solution had an initial absorbance of 0.70. Samples (5g/ml-100g/ml) and ascorbic acid standards (5g/ml-50g/ml) were combined to create a final volume of 1 ml with solution. It was determined how well they could scavenge free radicals [15].

DNA nicking assay

Antioxidant assay of extracts (rich in bioactive polyphenols) was evaluated by studying DNA damage protection. Zhao et al. described the method in which supercoiled pBSK plasmid DNA was used. The same method was followed with minor adjustments [16]. Extracts and plasmid DNA were mixed in 50µg/ml and 0.5 µg/ml, respectively. The incubation was done at 30 minutes at normal body temperature. An equivalent amount of Fenton's reagent was added. Incubation of the reaction mixture was done for 30 min at room temperature. Ethidium bromide staining was done for DNA analysis. Curcumin served as a positive control [17].

Antimicrobial activity

Susceptibility studies were conducted utilizing the modified Agar Well Diffusion method [18] to investigate whether the leaves of *Cinnamomum tamala* have antimicrobial properties. The MHA served as the medium. The fixed temperature for incubation was 37°C, and the culture was stored in triplicate for 24 to 72 hours. The test organism's broth culture (0.6 mL) was taken in a Petri plate. To this, the sterilized melted MHA (20mL) was added. Wells were bored, and then 2 mL of an extract made from *Cinnamon impressinervium* leaves was added to the medium. An hour was spent on inoculation to guarantee the strong diffusion of antimicrobial agents to the medium [10,18,19].

Anti-fungal Assay: Agar well diffusion assay was used for antifungal activity. The plant extracts were dissolved in different test tubes containing DMSO. Dextrose agar medium (5mL) was placed in a test tube, and inoculation was done. A slanting position was selected for keeping the test tube overnight at room temperature. The fungal culture was inoculated on a slant. The incubation period was for 7 days at a temperature of 29°C. The zone of inhibition was calculated [20,21].

RESULTS

Qualitative screening

The presence of various biomolecules was confirmed by qualitative screening of all extracts. Biomolecules exist in tannins, alkaloids, carbohydrates, tannins, steroids, phenol, and saponins. Screening results are shown in Table 1.

Free radical scavenging potential

The nature of phytochemicals present in various extracts might affect the methods that are used to analyze the anti-oxidant potential of extracts. Accordingly, two methods, namely DPPH and ABTS+, were selected to understand the anti-oxidant potential of various extracts. In the DPPH and ABTS assays, the aqueous extract had the most potent antioxidant activity, with IC50 values of 123.83±0.42 & 57.86±0.85µg/mL, respectively. The experiment was done in triplicates, and SD was calculated. The CI oil has IC50 values of 12.38 ± 3.45 and 12.92 ± 0.98 µg/mL, respectively. The results of DPPH and $ABTS^{+}$ are summarized in Table 2.

Table 1: Phytochemical screening of CI leaves

Phytochemical	Screening test	n-hexane extract	Chloroform extract	Methanolic extract	Aqueous extract
Alkaloids	a. Mayer's test	++++	+	++	++
	b. Dragondorff's test	++	+	+	+
Steroids	a. Salkowaski's test	+	- -	-	--
Tannins	a. Ferric chloride test	++	-	++	+
Flavones	a. Aqueous test	++	-	+	++
Glycosides	a. Sulphuric acid test	+++	-	+	+
	b. Keller Killani's test	++++	+++	++	+
Saponins	a. Aqueous test	+++	+	+	++
Phenols	a. Phenol test	+++	-	+	++

++++ sign indicates the Excellent, +++ indicates the Very Good quantity, ++ Indicates Good quantity, + indicates significant, - Poor, -- Negative

Antioxidant potential by DNA nicking assay

Finally, to determine the protective effect of a particular extract against the damaging effects of hydroxyl radicals on DNA, a DNA nicking test was employed. Fenton's reaction mixture produced hydroxyl radicals that broke down the supercoiled form of plasmid DNA (Lane 1, Type 1) into linear, single-stranded, nicked, and double-stranded forms (Lane 7 Type II and III) outcomes demonstrated that the supercoiled DNA is protected by curcumin at 25 µg/mL concentrations (Lane 3). In a similar vein, the reaction mixture including 50µg/mL quantities of cinnamon oil, hexane extract, MeOH extract, and chloroform extract reduced the amount of DNA damage.

Table 2: Antioxidant potential of various extracts of CI leaves.

Sample	DPPH IC_{50}µg/mL	ABTS IC_{50}µg/mL
Ascorbic Acid	25.20 ± 0.23	8.02 ± 0.13
CI Hexane extract	67.81 ± 2.45	35.67 ± 0.80
CI chloroform extract	49.95 ± 6.22	20.31 ±0.35
CI MeOH extract	42.75 ± 6.64	18.16 ± 0.54
CI aqueous extract	123.83 ± 0.42	57.86 ± 0.85
CI oil	12.38 ± 3.45	12.92 ± 0.98

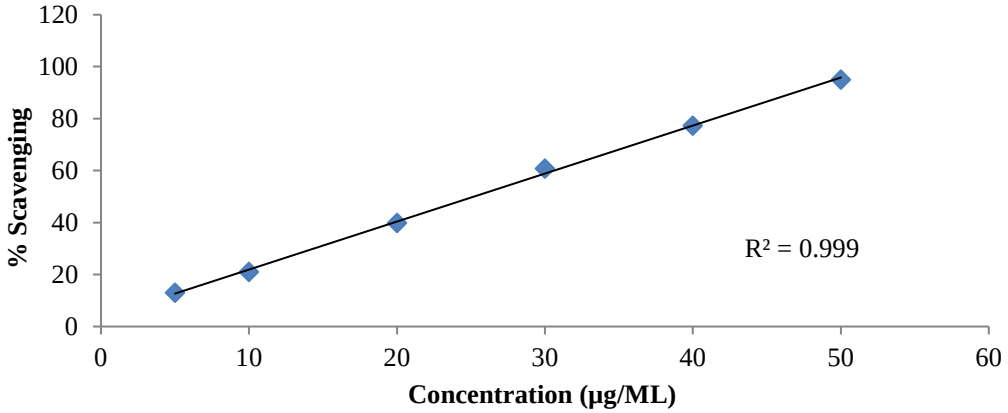

Figure 1: % scavenging versus concentration graph (DPPH assay)

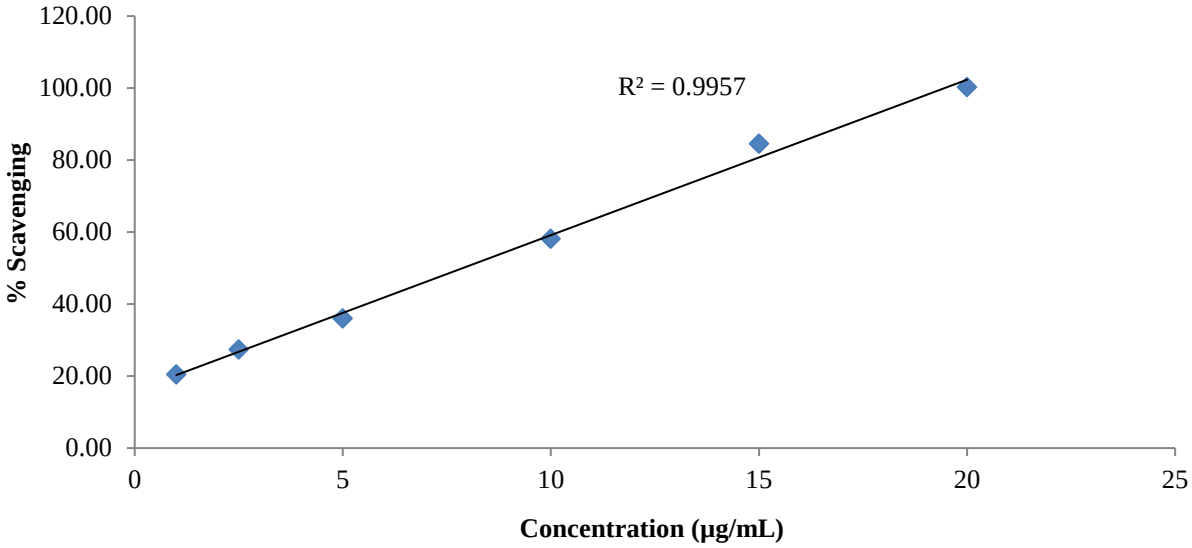

Figure 2: % scavenging versus concentration graph (ABTS$^+$ assay)

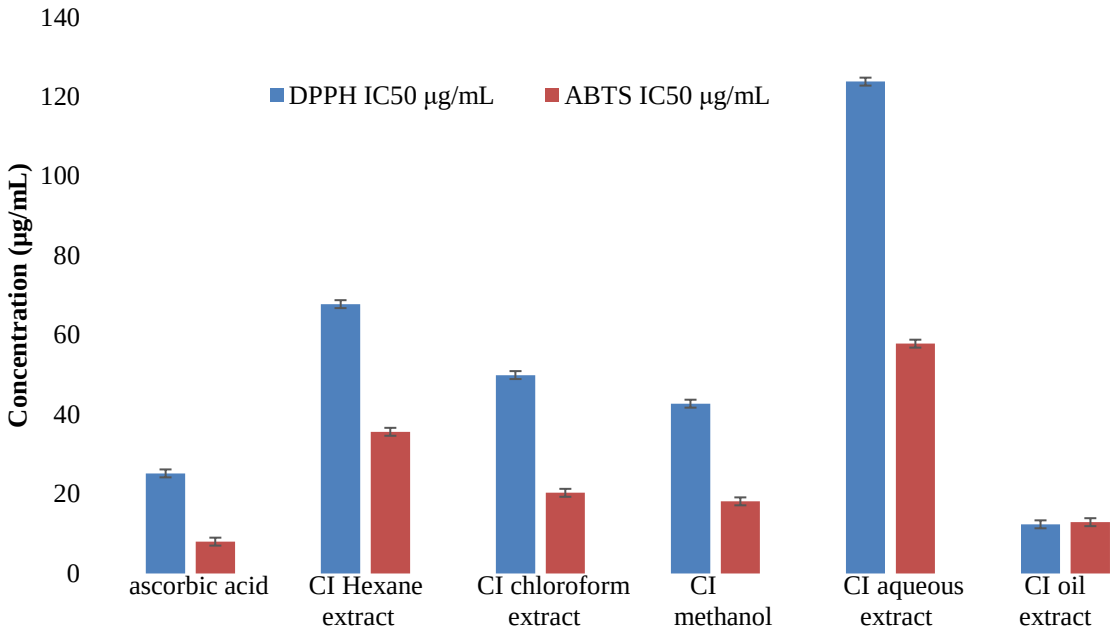

Fig 3: Antioxidant values of various extracts

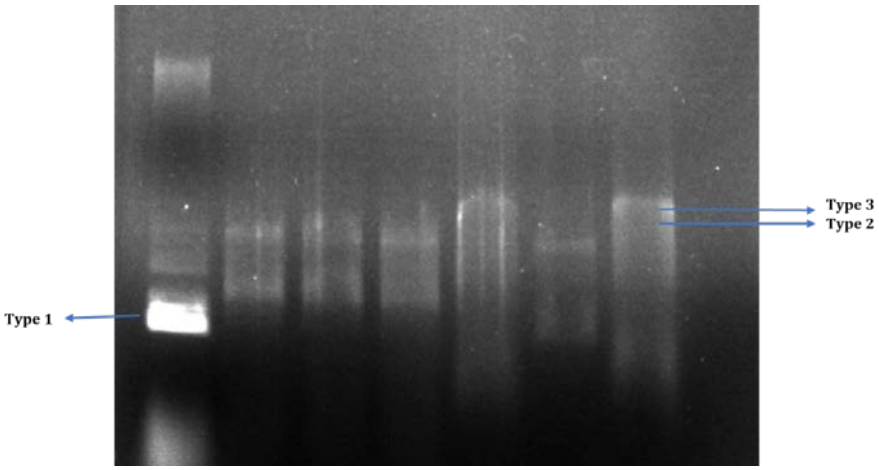

Figure 4: DNA Nicking assay showing a protective effect of cinnamon extracts against hydroxyl radical generated by Fenton's reagent. Lane 1: Native plasmid DNA pBSK without treated with Fenton's reagent; Lane 2: DNA treated with Fenton's reactant and 50 µg/mL Curcumin; Lane 3-6: DNA treated with Fenton's reactant and cinnamon oil, MeOH extract, hexane extract, and chloroform extract (25 µg/mL,) Lane 7: DNA treated with Fenton's reagent

Table 3: Results of antimicrobial activity

Name of organism	Zone of inhibition of various extracts in mm								Standard antibiotic inhibition zone (mm)
	n-Hexane		Aqueous		Methanolic		Chloroform		
	6µl	12µl	6µl	12µl	6µl	12µl	6µl	12µl	
E. coli	12	11	16	12	13	14	11	14	Ciprofloxacin 36
S. typhi	12	12	Nil	Nil	Nil	Nil	09	10	Ciprofloxacin 23
E. cartovora	16	14	12	14	12	15	09	11	Ciprofloxacin 17
A. tumifaciens	20	21	18	17	17	19	22	23	Azithromycin 25
S. aureus	14	12	14	12	12	13	12	11	Azithromycin 21
C. albican	15	14	16	13	15	17	14	13	Clotrimazole 32
B. subtilis	12	13	16	15	11	13	11	12	Azithromycin 24
B. atropheous	25	28	30	38	25	26	31	33	Azithromycin 28

Antimicrobial activity: The antimicrobial potential of CI leaves was assayed in vitro using the agar well diffusion method. The result is evaluated in Table No. 2. A Variable degree of inhibition was shown by various extracts of *C. impressinervium* against gram +ve bacterial stains, gram-ve bacterial stains, and fungus. The plant extracts showed a significant effectiveness against most bacterial and fungal species. All the extracts towards *B. atropheous* showed the best inhibition activity, but a maximum zone of inhibition was shown by aqueous extracts measuring 40 mm. Aqueous, methanolic extracts are completely inactive towards *S. typhi*.

In the case of fungal inhibition, all the extracts were effective and showed a significant zone of inhibition. There is no specific trend observed in antimicrobial activity. However, certain variations are observed as aqueous and methanolic extracts are completely inactive towards S. typhi and all other extracts show significant inhibition zones. Limitations: Susceptibility tests do not assess antimicrobial activity in patients; they only assess it in vitro. An antimicrobial agent's in vitro killing activity does not guarantee effective therapy [22].

CONCLUSION

Since ancient times, it has been recognized that Cinnamomum species and their essential oils have extraordinary medicinal potential. This investigation showed that phytochemicals like alkaloids, glycosides, tannins, etc., are present. Alkaloids already have antifungal and antispasmolytic properties, etc. It has been noted that leaf extract has the majority of phenolic compounds, and these can absorb free radicals and have neutralizing power. CI has a higher phenol concentration and may have some anticancer properties. In the DPPH and ABTS

assays, the aqueous extract had the most potent antioxidant activity, with IC50 values of 123.83± 0.42 and 57.86 ± 0.85 µg/mL, respectively. The CI oil has IC50 values of 12.38± 3.45 and 12.92 ± 0.98 µg/mL, respectively. The plant extracts showed a significant effectiveness against most bacterial and fungal species. All the extracts towards *B. atropheous* showed best inhibition activity but a maximum zone of inhibition was shown by aqueous extracts measuring 40 mm. Aqueous, methanolic extracts are completely inactive towards *S. typhi*. Like other plants and herbs, CI has long been valued for its spices and therapeutic qualities. The medicinal potential and phytochemical study of its extracts are the main topics of this paper. However, this species has to be investigated for further pharmacological potential. The observed results of the study show that plants have certain therapeutic activities, and, in the future, they may also be explored further for other activities.

FINANCIAL ASSISTANCE
Nil

CONFLICT OF INTEREST
The authors declare no conflict of interest

AUTHOR CONTRIBUTION
All authors contributed to the conception of study design by Amit Sharma. Materials and lab data results were prepared by Lovkesh Bhatia. Rishu Kalra helped in Anti- oxidant and antimicrobial activities. Varun Kumar wrote drafts of manuscript and all authors read and approved manuscript.

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