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PHYTOCHEMICAL ANALYSIS OF *CINNAMOMUM ZEYLANICUM* AND DETERMINED ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

Introduction: Medicinal plants have been used traditionally in order to address or treat various disorders. Phytochemical investigation of these plants revealed the presence of primary along with secondary metabolites qualitatively and quantitatively. Due to its richness with respect to phytochemical content in medicinal plants and reported variousimmunopharmacological activities. In view of this, one of the medicinal plant i.e. *Cinnamomum zeylanicum*is still under investigation. **Methods**: In this regard, we screened bark (aqueous, methanolic and acetone) extractof *Cinnamomum Zeylanicum*for determining its metabolites qualitatively and estimated its antimicrobial activities using bacterial strains (i.e. *Pseudomonas aeruginosa, Bacillus subtilis* and *E.coli*) and fungal (*candida glabrata*) pathogen. In addition, extractedcrude oil was also isolated from bark extract for determining its antimicrobial activity. **Results:** The results showed that bark extract of *Cinnamomum Zeylanicum*showed moderate antimicrobial activity at higher concentration in case of aqueous, methanolic and acetone extracts. The results of these studies were compared with control values and also found that oil extracted from bark showed better antimicrobial activity. **Conclusion**- In short, oil (from bark) and aqueous extract of this medicinal plant should be used as a potential bioactive component in various disorders.

INTRODUCTION

Aromatic plants are the sources of some secondary metabolites e.g. flavonoids, polyphenols etc. Because of which they are being used as a source of condiments, spices, essential oils, food supplements and aromatic extracts [1, 2]. Aromatic plants have emerged with a boom in India including abroad and these are being commercially used because of the presence of some valuable essential oils in it [3-5]. The demand is therefore increasing day by day in Indian as well as international market and so its cultivation as well. *Cinnamonum* is a large genus, varieties of species are reported and some of which yields volatile oil on distillation. Therefore, its medicinal value, composition of oil and its use, it's totally much dependent on the species which is being distilled as well as on the part of the plant which is utilized [6, 7]. Natural products were obtained through medicinal plant products and these are available in the form of pure compounds or extracts and provide some unlimited opportunities especially for new drug candidate leads because of the unequalled availability of chemical diversity. Due to its chemical diversity of new drug candidate which may be identified through common phytochemical screening assays, chromatographic techniques such as HPLC (high performance liquid chromatography) [8]

and non-chromatographic techniques such as immunoassay and Fourier Transform Infra Red (FTIR). Historically, medicinal plants used as traditional medicine and considered them as one of the sources of natural antimicrobial substances for the treatment of various infectious diseases [9, 10]. Different classes of antimicrobial substances are reported and considered them as one of the major defense systems pertaining to protect them against various biotic (living) and abiotic (non-living) stresses or components. Much attention is needed especially for medicinal plants as a source of alternative antimicrobial strategies. However, there is a great demand for those extracts especially medicinal plants with antimicrobial activity have recently been reported [9, 10]. Cinnamomum, genus of evergreen aromatic trees (more than 300) and belonging to the family *Lauraceae*. Various species of Cinnamomum are reported i.e. *Cinnamomumtamala*(tejpatra; family *Lauraceae*); *Cinnamomum cassia* or *aromaticum* (Chinese cassia); *Cinnamomumverum* or *Zeylanicum* (true cinnamon); *Cinnamomumburmannii* (Indonesian cinnamon/*Padang cassia*/Batavia cassia) and *Cinnamomumloureiroi* (Vietnamese cinnamon/ Vietnamese cassia). All these species of Cinnamomum reported the existence of aromatic oils especially in leaves and bark [6, 7]. So, these oils are widely used as well as applicable in various industries i.e. cosmetics and pharmaceuticals [11-13]. Phytochemical investigation process was done regarding oil composition and revealed its richness of mono terpenoids and phenyl propanoids. In addition, major component of oil is also reported in bark (i.e. Cinnamaldehyde) and leaves (i.e. eugenol). One of the compounds were isolated and identified in *cinnamon zeylanicum* which belongs to two chemical classes i.e. polyphenols (cinnamon contains mainly vanillic, caffeic, gallic, protocatechuic, p-coumaric, and ferulic acids) and volatile phenols (hydrocarbons and oxygenated compounds i.e. β-caryophyllene, eugenylacetate, cinnamyl acetate etc.) [11-13]. *CinnamomumZeylanicum*, native species of Sri Lanka and southern parts of India. Three major components were reported i.e. trans-cinnamaldehyde, eugenol, and linalool, which represent 82.5% of the total composition. As per the literature, this species showed various biological activities i.e. lowering of blood glucose; serum cholesterol; anti-oxidant and free-radical scavenging properties; anti-nociceptive and anti-inflammatory activity; wound healing properties and hepato-protective effects [6, 7]. In addition, this species reported as less toxic and adverse effects but showing considerable economic importance as well e.g. bark used as a spice, perfumery and also in ayurvedic and traditional Chinese medicine for its hypoglycemic and antiseptic properties. As per the literature, bark of this species is used especially for treating bronchitis, asthma, nausea, vomiting, fever and for restoring normal skin, etc [11-13]. The major objective of our study is to evaluate the effect of *CinnamomumZeylanicum*using different solvent system and then determined its metabolites qualitatively and also extracted oil from bark of the medicinal plant and analyzing its antimicrobial studies.

MATERIALS AND METHODS

Preparation of extract

The bark of *Cinnamomumzeylanicum* was procured from local market of Dehradun, Uttarakhand. The dried bark were pulverized or crushed into fine powder and then extracted with different solvent system (i.e. acetone, methanol and aqueous). Each plant powder (5 g) was separately extracted in three different solvents i.e. phosphate buffered saline, PBS for 4 h; methanol; MeOH for 4 h and acetone for 3 h using Soxhlet apparatus. Extracts were filtered using Whatman filter paper 1 and determined its antimicrobial activity.

Qualitative analysis of metabolites: Various tests were performed in order to determine the metabolite qualitatively using various tests i.e.

- **Flavonoids test-** 2 ml of extract is treated with 2 ml of 10% lead acetate, brownish green/yellow color indicates the presence of flavonoids.
- **Terpenoid (Salkovski test)-** 1 ml of extract solution was treated with 5 drops of concentrated H_2SO_4 and 1 ml of chloroform, color will change i.e. yellow color into reddish brown which indicates the presence of terpenoids.
- **Glycosides-** Extract was treated with 2 ml of H_2SO_4, appearance of reddish

brown color which indicated the presence of glycosides.

- **Saponins test (Foam test) –** Extract (1 ml) was taken and diluted with water (10 ml) and shaken in a test tube for 10-15 minutes. If there is formation of foam which indicates the presence of saponins.
- **Alkaloids (Wagner's test) -** Extract 2 ml was treated with 2 ml of Wagner's reagent (1.27gm iodine and 2 gm of potassium iodide in 100 ml distilled water). Appearance or formation of brownish red precipitate which indicates the presence of alkaloids.

Isolation of essential oils: Samples of dried bark (400 g each) were separately hydro-distilled for 4 hours using Clevenger apparatus. Oil isolation of samples was carried out in triplicates. The oil samples obtained were dehydrated over anhydrous Na_2SO_4 and stored in sealed vials at 4 °C until analyzed.

Gas-chromatography (GC): Gas chromatography (GC) of essential oil samples was carried out by Agilent (model 6890 N) gas chromatograph, equipped with flame ionization Detector (FID) using N2 as carrier gas. The column was HP-5 fused silica capillary column (30 m x 0.32 mm, 0.25µm film thickness) and temperature program was used as follows: Initial temperature i.e. 60°C (hold: 2 min; programmed at a rate of 3°C/min) to a final temperature i.e. 220° C (hold 5 min). So, injector and FID temperature were maintained at 210°C and 250°C, respectively and its injection volume was 0.2 µl.

Gas-chromatograph and Mass-spectrometry (GC-MS): GC-MS analyses of oils was performed using Perkin Elmer Clarus 500 gas chromatography which is equipped with a split/split less injector (split ratio 50:1) data handling system. The column was Rtx-5 capillary column (60 m x 0.32 mm, 0.25 µm film thickness). Helium (He) considered them as carrier gas at a flow rate 1.0 ml/min. The GC was interfaced with (Perkin Elmer Clarus 500) mass detector operating in the El positive mode. The mass spectra were generally recorded over m/z 40-500 am that revealed the total ion current (TIC) chromatograms. Temperature program is almost same as already mentioned above in GC analysis. The temperature of the injector, transfer line and ion source was maintained at and 200°-210 °C respectively. Identification of the components was done on the basis of retention time, retention indices, determined with reference to homologous series of n-alkenes (C8-C24 Sigma-Aldrich) under identical experimental conditions, co-injection with authentic standard compounds, mass spectra with library provided by instrument software (NIST/Wiley) and by comparing their mass spectra with those reported in literature. Qualification of each compound was performed on the basis of their GC peak area, using the normalization procedure without using correction factors.

Preliminary *In vitro* antimicrobial assay – Spectrophotometric method: A preliminary *in vitro* antimicrobial assay of crude bark extract using three different solvent system (i.e. acetone, methanol and water) along with oil extracted from bark against three bacterial pathogens and one fungal strain. This experiment was designed as well as carried out in order to investigate its concentration of the extracts or oil that can kill the bacterial cells. Therefore, decrease in the bacterial population was monitored by the decrease in the optical density (OD) value and the survivor (time-kill) curve was plotted. A spectrophotometric method was performed [14]. Culture (18-24 h) was harvested through centrifugation and supernatant was collected and adjusted its population i.e. approx. 10^8CFU/ml. For these studies, different concentration of extracts or oil was added using three different bacterial and one fungal strain. Then 1ml of the bacterial or fungal strain was added to each test tubes used as control. So, these samples were added in test tube pertaining to monitor its bacterial growth by measuring the absorbance at 600nm and incubated at 37°C. In this study, culture without plant extract considered them as negative control.

Hemolytic activity: Aqueous, methanolic and acetone extracts of *Cinnamomumzeylanicum* was assayed on human erythrocytes (blood samples collected from healthy volunteers). Firstly, blood (2-3 ml) was diluted in PBS and then centrifuged at 2500 rpm for five minutes). Finally, prepared 2 % erythrocyte suspension in sterile PBS for measuring its hemolytic study. In this study, crude extract of variable concentrations (50–500 µg/ml) were added to 0.85% NaCl solution and then received a 2% suspension of human erythrocytes. After 30-

min incubation at room temperature, cells were centrifuged and supernatant was used to measure the absorbance of the liberated hemoglobin at 540 nm [15].

Statistical analysis: The difference between control versus standard versus variable concentration of bark oil and its extract using different solvent system is determined through one way ANOVA test (Boniferroni multiple comparison test).

RESULTS

Phytochemical investigation: The result of the phytochemical screening of the acetone, methanol and aqueous extracts of *cinnamomumzeylanicum* bark revealed the presence of metabolites in acetone (phenol, tannins saponins, alkaloids, terpenoids, flavonoids, glycosides); methanol (carbohydrate, phenol, tannin, alkaloid, terpenoid and glycosides) and aqueous (carbohydrate, phenol, tannins, saponinsterpenoids and glycosides) extract. In addition, protein is totally absent in all the cases as shown in **Table 1.**

Plant constituents	Acetone	Methanol	Aqueous
Carbohydrate	-	+	+
Protein	-	-	-
Phenol	+	+	+
Tannin	+	+	+
Oxalate	-	-	-
Saponins	+	-	+
Alkaloids	+	+	-
Terpenoids	+	+	+
Flavanoids	+	-	-
Glycosides	+	+	+

Antimicrobial activity: The effect of bark extract using three solvent systems along with oil for determining its antimicrobial activity against bacterial strain as shown in **Fig 1**.The results showed that leaves aqueous extract showed antimicrobial activity at higher concentration as compared to control whereas acetone and methanolic leaves extract showed less activity as compared to control. In addition, antimicrobial activity is also checked by agar well diffusion method using bark extract (acetone , methanol and aqueous) and bark oil in 20 µl concentration on three bacteria *Pseudomonas aeruginosa, Bacillus subtilis*, and *E. coli* and one fungal strain *Candida glabrata*. Only bark essential oil showed inhibition in bacterial population and fungal as compared to bark extract as Shown in **Fig 2**.

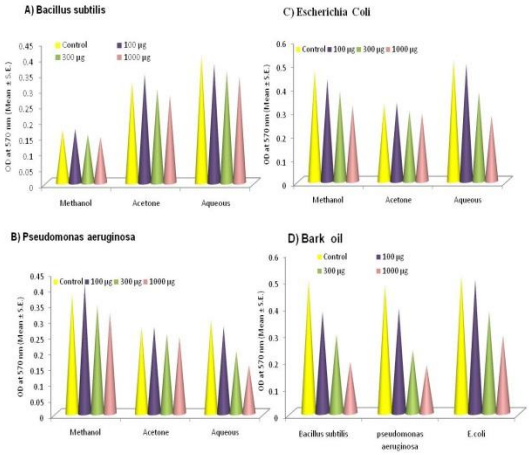

Fig.1. Effect of variable concentration of bark extract (using different solvent system i.e. acetone, methanol and aqueous) and oil for determining its antimicrobial activity. The difference between controls versus standard versus variable concentration of bark extract along with oil using is determined through one way ANOVA test (Boniferroni multiple comparison test).

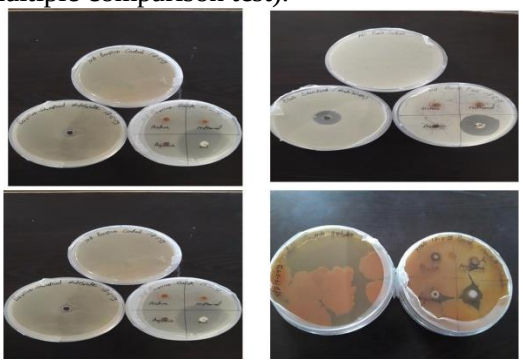

Fig 2: Effect of variable concentration of bark extract (using different solvent system i.e. acetone, methanol and aqueous) and oil for determining its antimicrobial activity using agar well diffusion method

Hemolytic activity: The effect of bark extracts using three solvent systems along with oil for determining its hemolytic activity against bacterial strain as shown in **Fig.3**. The results showed that bark aqueous/acetone/methanolic extract does not show any hemolytic activity as compared to control whereas distilled water showed hemolytic activity. The difference between control versus standard versus variable concentration of bark extract along with oil using is determined through one way ANOVA test (Boniferroni multiple comparison test).

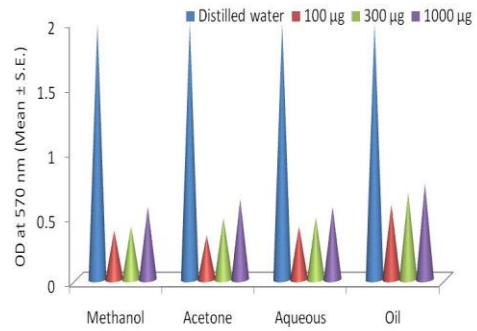

Fig.3. Effect of variable concentration of bark extract (using different solvent system i.e. acetone, methanol and aqueous) and oil for determining its hemolytic activity.

GC-MS DATA

GC-MS data of bark essential oil as shown in **Fig.4** and confirmed the presence of active metabolites

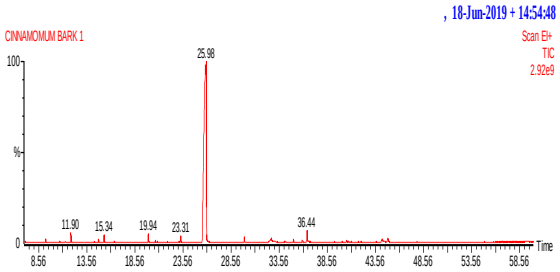

Fig.4. GC-MS data of *Cinnamomumzeylanicum* (bark)

DISCUSSION

The subject of phytochemistry or pharmaceutical chemistry is well established and more advanced in coming years as a distinct discipline. This study is almost concerned with enormous production of organic substances (secondary metabolites) that are accumulated by medicinal plant products and deals with the chemical structures of these substances i.e. biosynthesis, turnover and its metabolism including natural distribution and their biological function. So, evaluation of all these drugs (natural/plant derived) is totally based on phytochemical and pharmacological approaches which leads to drug discovery and is commonly referred as natural product screening. According to the literature, plant (bark, leaves, flowers, roots, fruits and seeds) may contain bioactive components in the form of primary and secondary metabolites. So, these bioactive plant constituents are steroids, terpenoids, carotenoids, flavonoids, alkaloids, tannins and glycosides and showed various activities such as antimicrobial and some have been reported to exhibit hemolytic activity [16]. In view of this, we worked on bark extract using different solvent system for determining its antimicrobial activity against bacterial and fungal strain. India is a land of rich and diverse fauna and flora. According to the literature, various varieties of edible fruits along with spices are reported as traditional medicine against diseases/pathogens. Traditional type of medicines are well known for different remedies of common disease as they are rich in phytochemical constituents. One of the medicinal plants i.e. *Cinnamomumzeylanicum* were reported in the Uttarakhand region. This medicinal plant undergoes phytochemical screening in order to determine the presence of natural products (secondary metabolites) like alkaloids, steroids, flavonoids, saponins, proteins, tannins and polyphenols including glycosides. So, extraction of this medicinal plant using three different solvent systems and utilized various standards for screenings purpose related to its detection with respect to secondary metabolites. Overall, these phytochemicals are more essential for human health because they display different biological activities such as antifungal, antibacterial and antioxidant activities [17, 18]. Herbs along with spices have been used continuously because of its antimicrobial properties and also showed some enhancement in shelf life of food products by acting against food borne pathogens. Therefore, much attention has been needed related to medicinal plant, considered them as a source of alternative antimicrobial agents. Moreover, due to its huge demand related to preservative-free cosmetic products along with herbal extracts showing antimicrobial activity which is being used in the cosmetic industry pertaining to reduce the risk of allergies which is directly connected to the presence of methylparabens [19]. Most of the species belonging to the genus Cinnamomum, contained many antibacterial compounds that are reported. In this paper, we reported antimicrobial effects of bark using different solvent system and also extracted oil from bark portion for determining its antimicrobial properties. The prevalence of infectious (bacterial, viral and fungal) diseases are reported and considered them as one of the major global health problems. Most of the

bacterial (*Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis*) and fungal agents cause several human infections. In an effort to reduce the burden of various infectious diseases, researchers or doctor still relied on antibiotic treatment which is commonly and preferred choice for controlling these infections but still showed severe side effects. In this regard, researchers focused on various medicinal plant products against various infectious diseases and having no severe or signs of side effects. In view of this, we have investigated this plant for determining its antimicrobial activity against known human bacterial and fungal pathogens. This study revealed that *Cinnamomumzeylanicum* (aqueous extract) and bark oil showed inhibitory effects at higher doses for all the microbes and showing zone of inhibition was observed. On the other hand, acetone and methanol extract of *Cinnamomumzeylanicum* showed less antimicrobial activity against *E. coli, P. aeruginosa* and *B. subtilis*. Similarly, oil extracted from the leaves of *Cinnamomumzeylanicum* exhibited anti-microbial activity against some pathogenic bacteria. In other words, aqueous bark extract along with oil has more potential and showed inhibitory effects on all tested bacteria including fungi while acetone and methanol extracts of this plant show limited inhibitory effects on few tested bacteria.

CONCLUSION

In short,antimicrobial screening of *CinnamomumZeylanicum*was done against *Bacillus subtilis, Escherichia coli, Pseudomonasaeruginosa*. The results of these studies which indicated that aqueous extract and oil of bark extracted from *CinnamomumZeylanicum* inhibited the growth of pathogens whereas acetone and methanol extract does not show any antimicrobial effect. This activity is due to the presence of phytochemicals that are present in this medicinal plant. The major objective is to analyze these phytochemicals qualitatively in medicinal plants with its antimicrobial and hemolytic activity and understands them how effective these medicinal plants in terms of its properties so that it may lead to development of new medicines with lesser type of side effects.

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