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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL STUDIES OF *CINNAMOMUM TAMALA*

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ABSTRACT

Introduction- Medicinal plants are able to produce a wide range of primary and secondary metabolites and also claimed them its properties as defence compounds against various microbes. In general, existence of these secondary metabolites in medicinal plants exhibits a wide range of immunobiological and pharmacological properties. In view of this, phytochemical analysis and antimicrobial potential of leaves extract of *Cinnamomum tamala* leaves including oil were explored. **Materials and methods**- For phytochemical and antimicrobial studies of leaves extract of *Cinnamomum tamala* using different solvent system (i.e. aqueous, methanolic and acetone). In addition, extraction of oil from leaves and determined its antimicrobial action against various bacterial (*Pseudomonas aeruginosa*, *Bacillus subtilis* and *E.coli*) pathogens and also determined its hemolytic activity as well. **Results**- The results showed its existence of secondary metabolites in *Cinnamomum tamala* leaves and showing better antimicrobial activity at higher concentration in case of aqueous extract. In contrast, oil extracted from leaves also showed better antimicrobial activity as compared to control and methanolic/acetone extracts. In addition, all these test materials does not showed any hemolytic activity as compared to distilled water control. **Conclusion**- In short, aqueous leaves extract and oil squeeze out from leaves should be used as a potential bioactive component in various disorders.

INTRODUCTION

Medicinal plants reported various immunobiologically active compounds in the form of primary and secondary metabolites [1]. These metabolites are considered them as more valuable for human health care and it belongs to the category of traditional healthcare system [2]. According to Ayurveda, researchers worked on medication (natural remedies) and that can be exploited as sources for new and effective therapeutic agents. As per the literature, more than 5000 medicinal plants are reported and exploited them for the treatment of various diseases (intracellular or extracellular) [1, 2]. In other words, these medicinal plant products may play a key role in drug discovery and development process as well. So, these medicinal plants are exploited for various purposes (e.g. timber, fuel etc.) and now become threatened through man's activities. Lot of efforts was taken related to its sustainability of medicinal plant species because of its potential source of new drugs and also relies on human healthcare [1-3]. So, conservation of these medicinal plant products especially endangered ones which is totally depending largely on its conservation of the ecosystem in which they occur. Due to enhancement in over exploitation of these natural resources, government should be realized that in order to have sustainable utilization of natural resources so local people must be involved in the management of their natural resources. This study is aimed to generate necessary data and assessed the

knowledge associated with utilization of medicinal plants for health care needs and factors associated in using medicinal plants in the study area [4, 5]. The study aimed to assess the knowledge associated with utilization of medicinal plants for health care needs and factors associated in using medicinal plants in the study area. In this regard, one of the medicinal plants i.e. *Cinnamomum tamala* [6, 7] is still under investigation.

The word *Cinnamomum* is derived from Greek word 'kinnamomon' (meaning spice). One of the medicinal plant species i.e. *Cinnamomum tamala* belongs to *Lauraceae* family and is commonly called as Tejpatta and its native place is India (leaves used as a popular spice) [7-10]. In addition, leaves yield an essential oil (eugenol and phellandrene) and also used in the perfume industry for their fragrance whereas essential oil from bark contains aldehyde. According to the literature, leaves of this medicinal plant are generally used by traditional peoples especially for the treatment of hyperlipidemia, diabetes and associated wound healing problems [8-11]. In contrast, major constituents of the leaf contained various essential oils i.e. furanosesquiterpenoids, principal constituents. Apart from this, Furanogermenone (59.5%) reported another one major compound in the leaf contained essential oil [9] (i.e. β-caryophyllene, 6.6%; sabinene, 4.8%; germacrene D, 4.6% and curcumenol, 2.3%. In general, Cinnamon leaf oil contains a variety of constituents including eugenol and cinnamaldehyde, which is a local mucous and dermal membrane irritant [9]. According to the Unani medicine, leaves of *Cinnamomum tamala* has a sharp taste and always use as a tonic for the brain and also used as anthelmintic, diuretic, sore eyes, stops salivation and good for liver and spleen condition [7-10]. On the other hand, bark of *Cinnamomum tamala* is also given especially for disease like gonorrhoea. Apart from these functions, these leaves are also used for flavouring food and widely used in pharmaceutical preparation because of its hypoglycaemic, stimulant and carminative, antidiabetic, antioxidant and anti-ulcer properties [12-14]. Now a day, synthetic based drugs are commonly used in medicines and played an important role in the successful treatment of various microbial diseases. In view of this, various biotechnological and pharmaceutical based industries worked on various antibiotics but these microorganisms are highly resistant against these synthetic based drugs and utilized as therapeutic agents [15]. In this regard, enhancement in multiple drug resistance may slow down its research along with development of new synthetic antimicrobial drugs [15, 16]. Due to this reason, researchers focused on natural plant sources and tried to develop or isolate or synthesize series of antimicrobial agents and found them as effective and potent molecule against various diseases. In this paper, we focused on leaves using three different solvent systems and quantified its secondary metabolites simultaneously and also quantified oil through GC-MS analysis and also tested against various bacterial pathogens including determination its hemolytic activity.

EXPERIMENTAL

Plant material/oil extraction

The leaves of *Cinnamomum tamala* were collected from the local market of Dehradun, Uttarakhand, India. It was duly authenticated by Centre for Aromatic Plant, Institute of Govt. of Uttarakhand, selaqui. Leaves of this medicinal plant part were shed-dried, made coarse powder and stored in an air-tight container for determined its secondary metabolites and also extracted oil with the help of Clevenger apparatus by hydro distillation method. Overall, yield percentage of oil (light yellow colour) was 0.3 -0.4 % respectively and its solubility was also checked and found that oil was soluble in acetone. In oil, major components were reported i.e. methyl eugenol (47 %), eugenol (27 %), trans-cinnamyl acetate (13%) and Beta-Caryophyllene (6 %) through GC-MS analysis [Agilent GC system equipped with MS detector]. In addition, dried leaves powdered plant material was further macerated in acetone, methanol and water, respectively, and crude extract of this plant was obtained.

Phytochemical analysis

Preliminary qualitative phytochemical screening was carried out with the following methods.

- **Terpenoids-** 1 ml of extract solution was treated with 5 drops of concentrated sulphuric acid and 1 ml of acetic anhydride, colour will change and appearance of blue (green rings)

which indicates the presence of terpenoids.

- **Glycosides-** Extract was treated with 2 ml of sulphuric acid, appearance of reddish brown colour which indicated the presence of glycosides.
- **Saponins test (Foam test)** - 5 ml of distilled water was added to 2 ml of the extract and shaken it, appearance of froth will occur which confirms the presence of saponins.
- **Alkaloids** - Extract (2 ml) was treated with diluted hydrochloric acid (0.2 ml) and then adds 2 ml of Wagner's reagent. (1.27 g iodine and 2 g of potassium iodide in 100 ml distilled water). Following reaction takes place and appearance or formation of yellowish coloration which indicates the presence of alkaloids.
- **Flavonoids test-** 2 ml of extract is treated with 2 ml of 10% lead acetate, brownish green/yellow colour indicates the presence of flavonoids.

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 N_2 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^o C (hold: 2-3 min) programmed at a rate of 3 degree celsius/min to a final temperature of 220^o C (hold 5 min). So, temperature of the injector along with FID was maintained at 210^o C and 250^o C, respectively. The injection volume was 0.2μL.

Gas-chromatograph and Mass-spectrometry (GC-MS)

GC-MS analyses of the oils was performed with a Perkin Elmer Claurs 500 gas chromatography 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), carrier gas with 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. Similarly, temperature program was used in GC analysis. The temperature of the injector, transfer line and ion source as maintained at 210^o C. 210^o C and 200^o 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 (C_8-C_{24} 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. The results of GC-MS analysis as shown in **Fig.1**

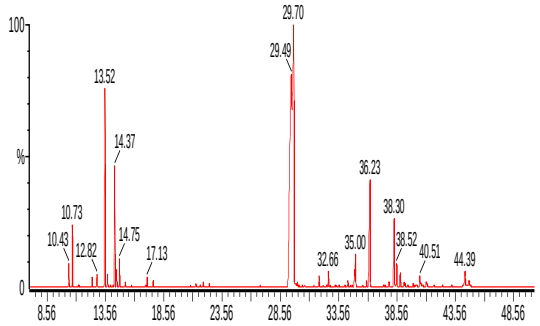

Fig.1. GC-MS data analysis of oil content (*Cinnamomum tamala)*

Collection of microorganism: The bacterial strains such as *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and fungal strain *Candida glabrata* were collected from Graphic era Deemed to be University, Department of Life Sciences (Microbiology), Dehradun. Bacterial strain were grown in LB medium contains in water at pH 7.2 and incubated at 37^o C for overnight. Whereas fungi strain *Candida glabrata* were grown in YPD (yeast potato dextrose agar. Medium containing potato infusion and dextrose at pH 5.6 incubated at 25^{o}C for three days.

Antimicrobial assay: In this study, we determined its antimicrobial activity of leaves extract using three different solvent systems (i.e. acetone, methanol and water) along with oil squeeze out from leaves against three bacterial pathogens. This study was designed in such a way in order to estimate its exact concentration of extract/oil that can kill bacterial pathogen i.e. approx. 10^8CFU/ml. So,

bacterial cultures were harvested after 18-24 h in presence or absence of test material through high speed centrifugation and collect the supernatant for measuring its bacterial population. In contrast, bacterial was added to each test tubes used as control and culture without the plant extract was taken as negative 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. 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. Finally, spectrophotometric method was performed.

Hemolytic activity: Oil and aqueous/methanolic/acetone leaves extracts of *Cinnamomum tamala* 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 hemolytic study. In this study, crude extract of variable concentrations (100–1000 µg) 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 haemoglobin at 540 nm.

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

RESULTS AND 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 [17, 18]. 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, flavanoids, alkaloids, tannins and glycosides and showed various activities such as antimicrobial and some have been reported to exhibit hemolytic activity. In view of this, we worked on leaves extract using different solvent system for determining its antimicrobial activity against bacterial and fungal strain. The results of phytochemical screening of leaves extract using solvent system i.e. acetone (carbohydrate, phenol and tannins); methanol (carbohydrate, phenol, tannin, alkaloid, flavonoid, terpenoid and glycosides) and aqueous (carbohydrate, phenol, tannins, terpenoids and glycosides) revealed its presence of primary and secondary metabolites. In addition, protein is totally absent in all the cases as shown in **Table 1**.

Table 1. Qualitative phytochemical screening of leaves extracts of *Cinnamomum tamala*

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

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) 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 *subtilis*. In other words, effect of leaves extract using three solvent systems along with oil for determining its antimicrobial activity against bacterial strain as shown in **Fig.2.** 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. Similarly, oil extracted from the leaves of *Cinnamomum tamala* exhibited anti-microbial activity against some pathogenic bacteria. In other words, aqueous leaves 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. In short, our results assessed antimicrobial activity of leaves extract using different solvent system that are used in folk medicine for the treatment of skin diseases, respiratory problems and nervous disorders against nine bacterial strains (*Bacillus subtilis,* that *Cinnamomum tamala* (aqueous extract) 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 *Cinnamomum tamala showed* less antimicrobial activity against *E. coli, P. aeruginosa* and *B.*

Escherichia coli, *Pseudomonas aeruginosa*). So, its major reason for its antimicrobial activity is due to the presence of phytochemicals and also showing synergistic effects in medicinal plants against pathogenic bacteria. This study has revealed that the medicinal plants especially leaves aqueous extract and oil from *Cinnamomum tamala* possess potentially antimicrobial properties against *Bacillus subtilis, Escherichia coli and Pseudomonas aeruginosa*. In contrast, effect of leaves extract using three solvent systems along with oil for determining its hemolytic activity against bacterial strain as shown in **Fig.3.** The results showed that leaves aqueous/acetone/methanolic extract does not show any hemolytic activity as compared to control whereas distilled water showed hemolytic activity. Further work is needed to isolate the active compounds and could be used in the development of novel antibacterial agents.

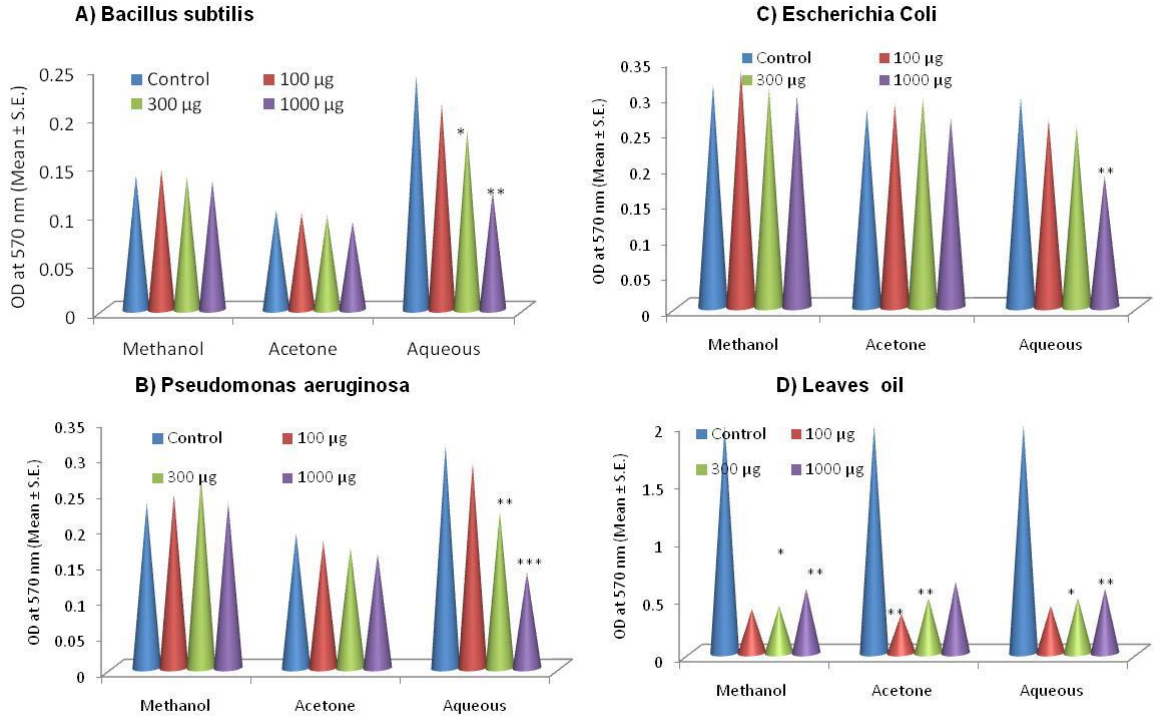

Fig.2. Effect of variable concentration of leaves extract (using different solvent system i.e. acetone, methanol and aqueous) and oil for determining its antimicrobial activity. The difference

between controls versus standard versus variable concentration of leaves extract along with oil using is determined through one way ANOVA test (Boniferroni multiple comparison test).

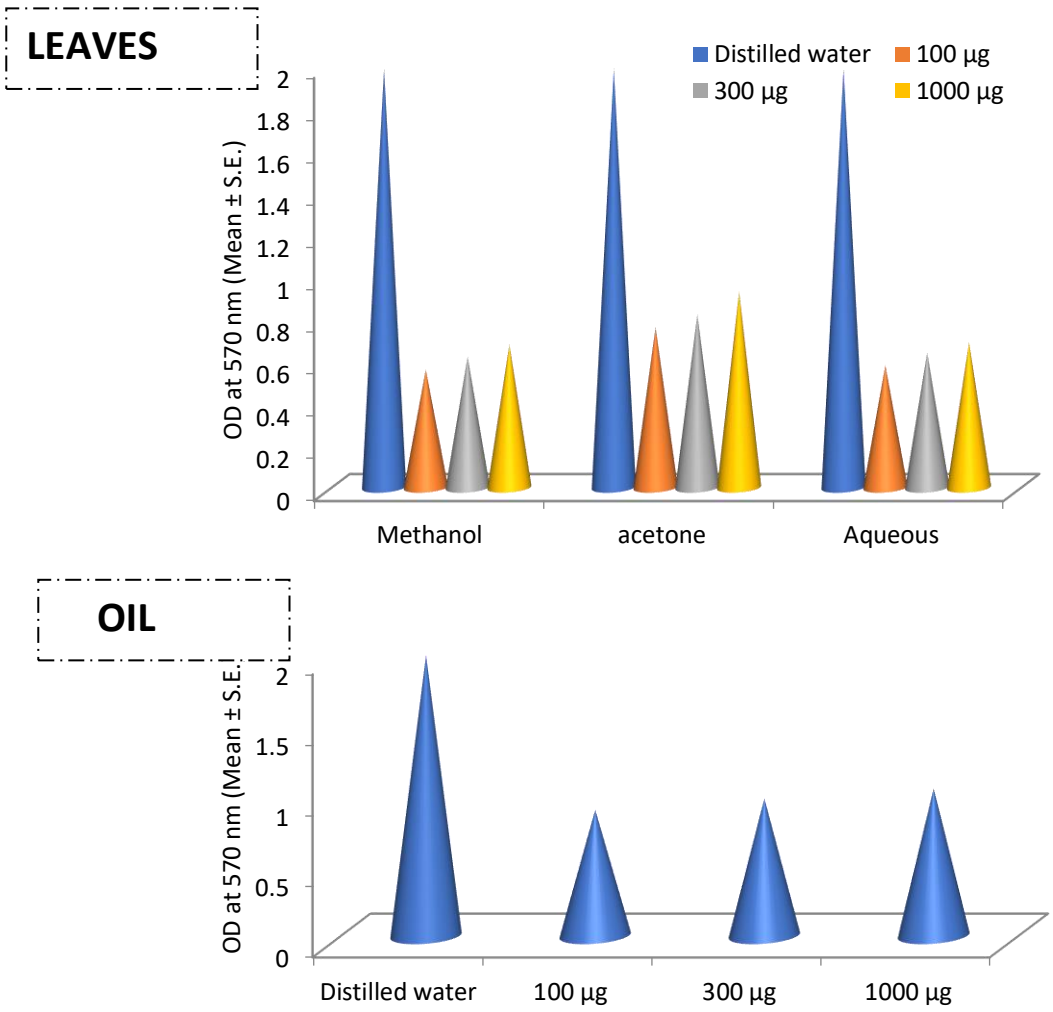

Fig.3. Effect of variable concentration of leaves extract (using different solvent system i.e. acetone, methanol and aqueous) and oil for determining its hemolytic activity. The difference between control versus standard versus variable concentration of leaves extract along with oil using is determined through one way ANOVA test (Boniferroni multiple comparison test).

CONCLUSION

The results obtained in the present study, medicinal plants possess antimicrobial properties. Therefore, there is need for further evaluation of the purified bioactive components of the plant extracts that can be exploited as new potent raw materials for the manufacture of herbal drugs and antimicrobial agent's productions. In short, these medicinal plants have provided so many things to humans including essential needs especially life-saving pharmaceutical agents.

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