SCREENING OF PHYTOCHEMICAL AND IMMUNOMODULATION POTENTIAL OF THERAPEUTICALLY VALUABLE PLANT *(Cinnamomum tamala)*

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ABSTRACT

Traditionally used medicinal plants are a rich source of therapeutically valuable compounds. This project aims at the screening of such compounds in the therapeutically valuable plant *Cinnamomum tamala*, commonly called bay leaf, and studying its immunomodulation potential. This was carried out by obtaining the extract of the dried plant sample by hot extraction process, using soxhlet apparatus. The extract was used for the phytochemicals like sugars , sterol, phenolic compounds, alkaloids , flavonoids, saponins, tannins and amino acids by standard tests. Immunomodulation potential of the plant was studied by administering 0.5 %(v/v) concentration of the extract to experimental mice, blood was collected and 5 immunological assays such as antibody titration (N-normal, T-treated, N-3 $\log 2^{2,}$,T-5 $\log 2^{2,}$, differential count(increase in the number of immune cells) plaque forming cell count, lymphocyte migration, T-cell and B cell assay rosetting assays were performed. The active compounds were isolated using chromatography and its chemical structure was analyzed at the atomic level using nuclear magnetic resonance NMR, FT-IR and UV-Vis. This will be useful in the development of a cheap and useful and safe plant based drug.

Keywords: *Cinnamomum tamala,* immunomodulation and phytochemical activity.

INTRODUCTION

Cinnamomum tamala belongs to the family Lauraceae. Indian bay leaves are the leaves of a tree closely related to cinnamon. The tough, three veined leaves are very popular in northern India. But are little known elsewhere at least, earlier days [1]. They were well known to the Romans under the name malabathrum and used both for perfumery and in cooking; in recipes, they were often just referred to as folia leaves, which some cookbook editions misrender as bay leaves. Indian bay leaves were still available during the middle ages and used for beer brewing till the 16th century but later they fell victim to the multitude of new species available and were forgotten [2-3].

METHODS

Sample collection: The sample *Cinnamomum tamala* was collected from the local market manavalanagar, thiruvallur district, Tamilnadu state, India. Fresh samples of small healthy leaves were collected from the plant in the early morning for investigation. The collected leaf samples immediately transferred to the laboratory for shadow dryness.

Preparation of plant sample: The plant material was dried in shadow place after completely dry, the dried sample, powdered with the help of a mixer grinder. The powdered sample used for preparation of extract . In the present study the plant extract is prepared with different organic solvent (low polar to high polar). Hexane , butanol, ethanol, chloroform and aqueous solvents used for extract preparation by pestle and soxhlet method.

Soxhlet method: Extraction procedures are to separate phytochemicals from other constituents of the leaf extract. The soxhlet apparatus was completely sterilized and rinsed with selected solvents. Then the dried sample powder is about 10 g taken in soxhlet and also 100 ml of solvent used for extraction and identification of phytochemicals. The samples were extracted using different solvent systems starting with a low polar solvent to high polar solvent(for ex: diethyl ether, butanol, methanol etc). The contents were extracted till they reached the solvent initial color. Finally after completion of extraction, the solvents evaporated at room temperature, after complete evaporation of solvents the extract dried. The dried extracts are made into different concentrations (25,50 and 100µg/ml)using different test solvents. The extracts were used to screen the antibacterial properties of the test sample [4].

Antibacterial efficiency of *Cinnamomum tamala* was screened by disc diffusion methods and well plate method.The sterile nutrient agar plate was prepared and dried. The pathogenic culture was gram positive bacteria and gram negative bacteria were selected for analysis. The bacterial strains subcultured in sterile nutrient broth periodically and incubate at 37 º c for 18-24 hours. Each young culture lawn into dried sterile nutrient agar plates separately. Then the plates were incubated at 37 °C for 2-3 days. After the incubation period the zone of inhibition was measured and recorded. Similarly instead of plant extract a standard antibiotic, tetracycline used to detect antibacterial activity and compared to plant extracts [5].

The different solvent extracts of *C.tamala* were used to screen the following phytochemicals like sterol, sugar, alkaloids, phenolic compounds, flavonoids, tannins, saponins, amino acids and ascorbic acid. The test extracts were mixed with a minimum quantity of chloroform, 3 -4 drops of acetic anhydride and 1 drop of concentrated sulfuric acid. The test solvent content was purple color, it was changed into blue green indicating the presence of sterol. The test extracts were mixed with a minimum quantity of anthrone and few drops of concentrated sulfuric acid and heated. The test content contain sugar, it was changed to green to purple color.

The test extracts were taken with 2N HCl. the aqueous layer was formed. It was decanted and to which are added one or two of Mayer's reagents. The test content was changed into white turbidity or precipitated. The test extract in alcohol was taken with a bit of magnesium and 1 drop of neutral ferric chloride. The test content was changed into intense color and shows positive results for phenolic compounds. The test extract in alcohol mixed with a bit of magnesium and 1 or 2 drops of conc.HCl. The solution was heated. The test solution was changed into red or orange red color in the presence of flavonoids. The test extract was mixed with water and lead acetate. The test solution contains tannins; it was precipitated in white color. The test extracts were mixed with water and shook well. The test solution was changed into foamy leather, if the test sample contains saponins [6]. The test extracts were mixed with ninhydrin in alcohol. The solution contains amino acids, it was changed into blue or violet in color.

TLC plates washed in distilled water and dried. Then the TLC plate was coated with silicate. After coating with silica gel, the plate was incubated at 100 °C in a hot air oven for activation. The activated TLC plate is marked in the bottom and upper region. The bottom region makes a mark offering sample load. TLC studies in successive extraction of hexane, butanol and aqueous prepared by both mortar and pestle and soxhlet method. Each test extracts spotted in TLC plates. The samples reach up to the top marking region then spray ninhydrin solution for determination of spots. The Rf value was measured and recorded, was calculated by the following formula

Rf= distance traveled by solute/distance traveled by solvent

Immunological assay:

The Immunological assay should be followed by collecting blood in both antigens injected and plant extract injected mice. The effective plant extract solvent and the antibacterial activity was injected into mice. From the normal and antigens injected mice, serum samples taken, the antibody levels were estimated. Quantitation of serum antibody were carried out by antibody titre plate technique containing respective antigens. 25 µl of physiological saline was added into all wells of microtiter plate, and then 25 µl of antiserum added into the first well of microtiter plate, the antiserum was serially diluted in the well of the first row till the 11^{th} well of titre plate leaving the 12^{th} well as positive control. Then 25**µl** of 1% test antigen in saline was added to all the wells of the microtiter plate. The plate was handshaken for the effective mixing of reagents and incubated for an hour at 37° C. The highest dilution of the samples shows detectable agglutination expressed in log2 of the serum.

Blood cells collected from the test antigen and control mice using heparin pretreated vials. T cell counts in the blood were carried out up to loading of lymphocytes in a nylon wool column. Resuspended lymphocytes were loaded into an activated nylon wool column. Then the column was held vertically above the eppendorf tube, now hot saline about 60° C was dripped into the column. The hot saline passing that of the column was collected in the eppendorf tube, which contains T lymphocytes 0.2 ml of saline containing T lymphocytes was taken in a separate eppendorf tube to the 0.2 ml of 1% SRBC was added and then the mixture was centrifuged at 1600 rpm for 12 minutes. After centrifugation those samples were incubated in an ice box or refrigerator at 4 °C for 5 mins. After cold incubation the pellet in the eppendorf tube was re suspended by gentle flushing with a pasteur pipette. Then a drop of it was taken in a clean dry slide. Observed and enumerated T cells under microscope for rosettes. Number of rosettes formed per 100 lymphocytes observed.

RESULT AND DISCUSSION

The antibacterial activity and phytochemical screening of *Cinnamomum tamala* was analyzed and recorded. Antimicrobial screening of the leaf extract were analyzed using disc diffusion method. The plant leaves were extracted using hexane, butanol and aqueous by soxhlet method. Followed by cold method preparation is performed by loading the sample extract to disc with different conc. of 25, 50,100 µg / ml . Butanol extract of *C. tamala* shows max zone of

inhibition against pathogenic pest organisms. The other extracts of hexane and aqueous do not have much effect on bacterial growth. Whereas butanol extract shows predominant inhibition against gram + bacteria, (example – *S.aureus*) compared to gram- bacteria, (example – *S.typhi* ,*K.pneumoniae*). Increment of extract conc. leads to maximum inhibition compared to lower conc. Of extracts. All the solvent extract tested against bacterial pathogens, of which butanol *Oo*solvents extract shows remarkable antibacterial activity when compared to other solvent extracts and standard antibiotics.

Similarly, the higher concentration of butanol extracts inhibits maximum level compared to lower concentration. The higher concentration 100µg/ml of butanol extract shows 12,12,8,8 and 11 cm of inhibition against *S.aureus*, *K.pneumoniuae*, *V.cholerae, E.Coli* respectively. On the basis of traditional use of the plants as a potent antibacterial and immunomodulate agent, the present study was carried out with butanol extract of *C. tamala* plant material to substantiate the folklore claim using different experimental models. Among the different antibacterial screening, butanol extract shows maximum antibacterial against the above mentioned pathogens. This plant species have been used traditionally by tribal people for the treatment of gastrointestinal and respiratory system in animals [7]

s.no	solvent	Conc. µg/ml	*E.coli*	*K. pneumoniae*	*S.typhi*	*V.cholerae*	*S.aureus*
1	control	0	0	0	0	0	0
2	tetracycline	50	10.4	13.5	11.8	10.2	14
3	hexane	25 50 100	2.3 4.8 4.9	2.6 3.5 4.2	2.6 3.6 4.1	3.1 4.2 4.6	2.3 3.5 4.6
4	butanol	25 50 100	9.0 10.8 11	4.6 8.8 12	4.5 6.2 8.0	4.8 6.8 8.0	5.2 9.1 12
5	ethanol	25 50 100	5.0 5.6 6.1	3.2 3.8 4.1	4.2 5.1 6.2	2.1 3.6 4.2	3.2 4.5 5.4
6	Chloroform	25 50 100	4.1 4.6 5.0	3.6 4.1 4.9	2.1 3.6 3.9	2.6 3.8 4.5	2.9 3.4 5.1

7	water	25 50 100	6.1 6.8 7.1	3.2 4.2 5.2	4.1 5.1 5.8	3.4 4.6 5.2	3.8 4.9 6.1

PHYTOCHEMICAL SCREENING:

Butanol extracts contain remarkable positive results of phytochemicals compared to other solvent extracts such as alkaloids, flavonoids and tannins. The flavonoids and alkaloids have important antibacterial potential efficiency [8-9].

s.no	phytochemical	hexane	butanol	ethanol	chloroform	Water
1	Sugar	-	+	+	+	-
2	Alkaloid	+	+	-	-	+
3	Phenolic compound	-	+	+	+	+
4	Flavonoids	+	+	+		+
5	Tannins	-	-	-		-
6	Saponins	-	-	-	+	-
7	Amino acid	-	+	-	-	-

THIN LAYER CHROMATOGRAPHY ANALYSIS:

From the phytochemical screening the result shows the number of phytochemicals present in the test sample. The phytochemical was confirmed with the help of TLC analysis.

Preparation of extract	observation	hexane	butanol	ethanol	chloroform	water
soxhlet	Rf value(cm) color	9.5 Light green	10.8 Dark brown	9.4 brown	8.9 green	10.2 Dark green
Mortar and pestle	Rf value(cm) color	11.2 green	12.3 brown	11.6 green	11.2 brown	10.6 Dark green

IMMUNOLOGICAL ASSAY:

T cells from antigen injected mice and plant extract injected mice. The percentage of T cells in antigen of *S.aureus* is 64, *S.pyogenes* is 67, *B.subtilis* is 62, *S.typhi* is 64, and *Shigella sonnei* is 67. The B cells from antigen injected mice and plant extract injected mice. The percentage of B cells present in antigen of *S.aureus* is 38, *S.pyogenes* is 43, *B.subtilis* is 37, *S.typhi* is 40, *Shigella sonnei* is 35.

s.no	sample	Lymphocyte subset population	
		B cell	t cell
1	Normal	27.3	58
2	treated	27.3	72.7

SUMMARY AND CONCLUSION:

From this study, it could be concluded that *Cinnamomum tamala* effect on pathogenic microorganisms by releasing butanol soluble phytochemicals that may be responsible for the observed reduction in bacterial growth. However, mostly all the extracts remarkably inhibit gram positive bacteria than the gram negative bacteria. Due to this readily soluble nature of *C.tamala* enhanced bacterial activity in butanol extract suggests the greater usability of *C.tamala* in the antibacterial drug preparation in alternative medicine.

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