IJPSR (2012), Vol. 3, Issue 04 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 26 November, 2011; received in revised form 25 January, 2012; accepted 28 March, 2012

SCREENING OF PHYTOCHEMICALS AND IMMUNOMODULATORY POTENTIAL OF A MEDICINAL PLANT, *CINNAMOMUM TAMALA*

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Keywords:
Cinnamomum tamala,
phytochemical analysis,
Butanol extract,
Pneumonae,
E. coli,
S. aureus

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ABSTRACT

Cinnamomum tamala is found in tropical and sub-tropical Himalayas and in some other places. Phytochemical screening of the plant extracts were done to screen their active constituents. Antimicrobial activity of this plant extract was investigated by Kirby-Bayer methodology against four pathogenic organisms: *K. Pneumonae, E. coli, S. aureus,* and *P. aeroginosa.* The plant Butanol extract showed inhibitory activity against all the tested organisms and it has enhanced immunomodulatory activity also. Butanol extracts containing remarkable positive results for phytochemicals compared to other solvent extracts. The higher concentration (100mg/ml) of butanol extracts shows maximum inhibition. Proliferation and activation of lymphocytes was also identified. The study scientifically validates the use of plant in traditional medicine.

INTRODUCTION: The emergence of multi-drug resistant bacterial strains throughout the globe limits the effectiveness of current drugs and significantly limits treatment, leading to prolonged infections [1]. The increasing resistance of bacteria to antibiotics is kindled due to the misuse and over prescription of the drugs. As microbial resistance to antibiotics spreads, the development of new antimicrobial agents has to be expedited if the problem is to be contained. Thus there is a need to develop new antibiotics to delay or prevent the arrival of post-antibiotic era [2].

Thus, the search for newer sources of antibiotics is a global challenge in preoccupying research institutions, pharmaceutical companies and academia [3]. However, the past record of rapid, widespread and emergence of existence to newly introduced antibiotics indicates that even new families of antibiotics are expected to have a short life [4]. This situation, coupled with the undesirable side effects of certain antibiotics and the emergence of previously uncommon infections is now one of the serious medical problem [5]. The problem posed by the high cost, adulteration and increasing side effects of these synthetic drugs coupled with their inadequacy in disease treatment found more especially in the developing countries should also be emphasized [6].

Plants have great potential for producing new drugs for human benefit. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and even infectious diseases [7]. According to World Health organization [8], more than 80% of the population is based on the traditional medicine.

Traditionally usages of plant based drugs are based on the experience and superstition passed from generation to generation. Plants have provided a source of inspiration of novel drug compounds as plant derived medicine have made large contributions to human health. Their role is twofold in the development of new drugs [9].

Plants show enormous versatility in synthesizing complex materials which have no immediate obvious growth or metabolic functions. Green plant posses the broadest spectrum of synthetic activity and have been the source of many useful compounds [10]. Coincidently, the last decade has also witnessed increasing intensive studies on extracts and biologically active compounds isolated from plants species used for natural therapies or herbal medicine [11].

Cinnamomum tamala is found in tropical and sub-tropical Himalayas and Jaintia hills and in eastern Bengal, India, and belongs to the family Lauraceae. The leaf extract of this plant is used as antidiarrheal property, hypoglycemic activity and for the preparation of various kinds of ailments likely for anorexia, dryness of mouth, bladder disorders, etc., [12].

MATERIALS AND METHODS:

Plant Material Collection and Preparation: The leaves used for investigation were collected from the markets. The leaves were cleaned and powdered with the help of mixer grinder. The powder was sieved through a 1mm mesh and was stored in an air-tight container for future use. The powdered sample used for preparation of extract. In the present study, the plant extract prepared with different organic solvents (low polar to high polar). Hexane, butanol, ethanol, chloroform and aqueous solvents used for extract preparation by Soxhlet method.

Phytochemical Screening: Phytochemical constituents of the plant were screened by using standard procedures described by [13, 14, 15].

Test Organisms: *E. coli, K. pneumoniae, S. aerus* and *P. aeruginosa* were used for the study of antimicrobial activity and these samples were isolated from nosocomical infected patients. A 24hrs fresh culture was prepared in nutrient broth and was used for the antimicrobial testing.

Antibacterial Activity: Antibacterial activities of the selected microorganisms were checked against Butanol extract of Cinnamum tamala by Kirby-Bayer methodology.

Immunomodulatory Activity: Immunological reflection in health system was analyzed in normal and treated animals by two different methods like, humoral and cellular immune response.

Immunization: Inject approximately 0.5ml of the leaf extract into the mice through intramuscularly. Repeat the injection after two weeks, being carefully to inject into the opposite side to that previously used. After two weeks later, take the blood sample from the immunized mice by bleeding techniques. The blood samples were collected for four weeks for the conformation of the lymphocytes presence. The B and T lymphocytes were enumerated from the test animal by B-Cell resetting assay and T-cell resetting assay methodology.

RESULTS AND DISCUSSION: The preliminary phytochemical analysis of *C. tamala* leaves showed the presence of sterol, sugar, alkaloids, phenolic compounds, flavonoids, Tannins, saponins and amino acids (**Table 1**). Among the five solvent extracts of C. tamala studied for the antibacterial activity responds against pathogens. Butanol extracts containing remarkable positive results of phytochemical compared to other solvent extracts. The butanol extract shows alkaloids flavonoids and tannins. The flavonoids and alkaloids have important antibacterial potential efficiency. Butanol extracts of *Cinnamomum tamala* adversely affect the growth of microorganisms. However the extent of concentration was more in case of test pathogens.

TABLE 1: PHYTOCHEMICAL SCREENING OF CHOSEN TEST PLANT

Phytochemicals	Diethyel ether	n-Butanol	Methanol	Chloroform	Water
Sterol	-	+	+	-	-
Sugar	+	+	+	+	+
Alkoloids	+	+	+	+	+
Phenolic compounds	+	+	+	+	+
Flavonoids	+	+	+	+	+
Tannins	+	+	+	+	+
Saponins	+	+	+	+	+
Aminoacids	+	+	+	+	+

"+" indicates presence, "-" indicates absence.

Whereas, in case of low concentration this indicates the presence of some toxic compounds in leaf extracts. The alkaloids and flavonoids were bioactive compounds, butanol soluble growth inhibitors that also play significant role in plant microbe's interactions [16, 17]. Similarly the higher concentration of butanol extracts inhibits maximum level compared to lower concentration. The higher concentration (100mg/ml) of butanol extract has the maximum inhibition against *K. Pneumonae, E.coli, S. aureus* and *P.aeroginosa* respectively (**Table 2**).

TABLE 2: ANTIBACTERIAL ACTIVITY OF BUTANOL EXTRACT AGAINST DIFFERENT BACTERIA

Sample cultures	Concentration of the extract in mg/ml/Zone of inhibition in mm			
	25mg/ml	50mg/ml	75mg/ml	100mg/ml
k.pneumoniae	0.3	3.7	8.5	17
E.coli	0.6	4.3	9	14
S.aerus	1.0	3.7	6.4	11
P.aeruginosa	0.8	2.6	7.3	9

B-Lymphocytes counts using rosette forming assay revealed increment significance in test probiotics exposed mice than control (**Table 3**). Decrement in B-Lymphocyte was much pronounced in test probiotics exposed mice in the first week and second week also had more or less similar impact on B-cell estimations. Present study; clearly confirm the decrement in B-cell number in mouse exposed to test probiotics. So the impact of test probiotics on the synthesis, proliferation and activation of lymphocytes was documented [17] reported the differentiation of B-cell counts affected by probiotics.

TABLE 3: ENUMERATION OF B AND T CELLS USING ROSETTE FORMING ASSAY IN NORMAL AND TEST PROBIOTICS EXPOSED MICE

Test chemical	I week		II week		III week	
	Lymphocyte estimation		Lymphocyte estimation		Lymphocyte estimation	
	% of B cell	% of T cell	% of B cell	% of T cell	% of B cell	% of T cell
Normal	19.7	57.6	20.1	59.2	26.8	64.6
1/10 conc.	23.6	70.8	19.6	77.2	26.8	76.8
1/20 conc.	29.8	60.2	26.2	64.3	30.2	69.6

CONCLUSION: The butanol extracts of Cinnamomum tamala leaves showed moderately antibacterial activity against *S. aureus, K. Pneumonae, P. aeroginosa* and *E. coli*. The higher dose of butanol extracts activity almost similar to standard antibiotics. Mostly, all the extracts remarkably inhibit gram positive bacteria than the gram negative bacteria. The phytochemical screening of leaf extracts found flavonoids, alkaloids, sugars, sterol, tannins, amino acids and saponins were present in n-Butanol and Methanol extracts.

This study discloses that n-Butanol extract of *Cinnamomum tamala* has phytochemically active substances in it. It could be very much helpful to fight against multidrug resistance of the worldwide emerging problem due to its immune power enrichment in the host system. These evidences suggest that *Cinnamomum tamala* could be beneficial for the protection and alleviation of diabetic complications, but further studies need to be carried out to define active principles.

Antimicrobial activity and immunomodulation activity of different solvent extracts of *C. tamala* indicates that the butanol extracts had greater inhibition against tested pathogens in the host Swiss albino mice by increasing its immune power. Hence, it could be used in antibacterial drug preparation. However, more detailed phytochemical analysis should be performed to isolate and characterize the active compounds which could be responsible for the antimicrobial and immunomodulatory effect.

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