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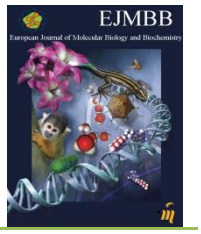

PHYTOCHEMICAL AND THERAPEUTIC EFFECT OF *ARAUCARIA COLUMNARIS* RESIN EXTRACTS ON CLINICAL PATHOGENS

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ABSTRACT
Araucaria columnaris is one of the ornamental plants of all over the world. It is commonly known as Christmas tree. In the present study tree exudates – resin from the bark of the *Araucaria columnaris* belonging to the family Araucariaceae were investigated for phytochemical and antibacterial activity. The resin was separately extracted with different solvents based on its polarity, water, methanol, ethyl acetate and benzene. The extracts were screened for phyto-chemical analysis, the result shows that methanolic extract involved in extraction of large number of phytochemicals when compared with other extracts and subjected to antibacterial assay against major human pathogens. Methanolic extract act as good inhibitory agent against major microorganisms. This finding revealed that methanolic resin extract of *Araucaria columnaris* had significant potential for the control of dreadful diseases causing human pathogens.

INTRODUCTION

Ornamental plants are mainly cultivated for decoration, adornments and to enhance the appearance of houses, gardens, road dies and also for commercial purposes such as flower decorations in the form of bouquet and wreaths. However, only very few of these plants species have found use in medicine and little or no literature exist on their chemical biological activities [1-2]. The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller [3]. Diseases that remain most challenging for today's health care system tend to be more complex than could be treated by current combination therapies. However plant based drugs contain a mixture of multiple components which saves the effective control of disease [4]. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency [5]. Resin is a hydrocarbon secreted by many plants, particularly coniferous trees, valued for its chemical constituents and uses such as in varnishes and adhesives. Plants produce resins for various reasons whose relative importance is debated. In this paper the phytochemicals and antimicrobial activity of *Araucaria columnaris* resin has been studied as part of the exploration for new and novel bio-active compounds for various therapeutic uses.

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MATERIALS AND METHODS

Identification and collection of plant materials

Araucaria columnaris resin samples were collected from garden of Nehru Arts and Science College, T.M. Palayam, Coimbatore, Tamil Nadu, India during the

month of October – December. The plant *Araucaria columnaris* was identified taxonomically by the Institute of forest genetics and tree breeding center (IFGTB), R.S. Puram, Coimbatore. The resin samples were shade dried, powdered and stored in polypropylene air-tight containers under proper conditions for further uses.

Extracts preparation

The crude powdered samples were weighed and subjected to solvent extraction for 8-10 hrs repeatedly in different solvents of different polarity that is distilled water, methanol, ethyl acetate and benzene, using Soxhelt apparatus. The extracts were then concentrated at 40-45 °C and air dried. The dried samples were then stored in air tight bottles at 4 °C for further analysis.

PRELIMINARY PHYTOCHEMICAL SCREENING

The preliminary phytochemical screening of different extracts of resin was carried out using the standard procedure described by [6-7].

Test for Tannins

The samples (1g) were separately boiled with 20 ml distilled water for five minutes in a water bath and were filtered. 1 ml of cool filtrate was distilled to 5 ml with distilled water and a few drops (2-3) of 10 % ferric chloride were added and observed for formation of precipitate and colour change. A bluish-black or brownish-green precipitate indicated the presence of tannins.

Test for Saponins (Frothing Activity Test)

The samples (5g) were mixed with 5ml of distilled water and shaken vigorously for about 5 minutes. Frothing persisted for 15 minutes indicated a positive reaction for saponin.

Test for Steroid and Terpenoids (Libermann-Burchard Test)

To 0.2g samples, 2ml of acetic acid was added, and then the solution was cooled in ice, followed by addition of concentrated sulphuric acid through the sides of the test tube. A violet to blue or bluish green colour ring indicated the presence of steroid and terpenoids.

Test for Flavonoids

The samples (1g) were boiled with 10 ml of distilled water for 5 minutes and filtered while hot. Few drops of 20 % sodium hydroxide solution were added to 1 ml of cooled filtrates. The yellow colour formed which on addition of acid changed to colourless, depicted the presence of flavonoids.

Test for Cardiac Glycosides (Keller Kiliani Test)

Samples (1g) were treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. To this 1ml of concentrated sulphuric acid was added. A brown ring at the interface indicated the deoxysugar characteristics of cardenolides.

Test for Alkaloid

Samples (1g) were separately boiled with water and 10 ml hydrochloric acid on a water bath and filtered. The pH of the filtrates was adjusted with ammonia to about 6-7. Small quantity of the reagents picric acid solution, Mayer's reagent (Potassium mercuric iodide solution) were added separately to about 0.5 ml of the filtrate in a different test tube and were observed for colored precipitate which indicated the presence of alkaloids.

Test for Carbohydrate

Add two drops of Molisch's reagent to 1g of samples and mixed thoroughly. Poured 5ml of concentrated sulphuric acid along the sides of the test tube. A purple ring formed in the junction of two layers indicated the presence of carbohydrates.

Test for Protein (Ninhydrin test)

To 1g of sample added few drops of ninhydrin reagent and was heated for 2 minutes. It was observed for the presence of purple colour which indicated the presence of protein.

ANTIBACTERIAL ASSAY

Test Pathogens used in study

The cultures used in present study were *Bacillus cereus*, *Corynebacterium diptheriae*, *Enterococcus* sp., *E. coli*, *Klebsiella pneumonia*, *Proteus vulgaris.*, *Salmonella typhi* and *Staphylococcus aureus*. The isolates were sub cultured and stored at 4^0C.

Screening for Antibacterial Activity

Based upon Perez *et al*, 1990 [8], agar well diffusion method was carried out. Sterile Muller Hinton agar plates were prepared and lawn cultures of the organisms were spread on each plate. Three wells of 5mm size were cut in the agar plates with the help of sterile cork borer and the wells were loaded with resin extracts and control (distilled water) was also used. All the plates were incubated at 37^0 C for 24-48 hours. After incubation, the plates were observed for the formation of zone of inhibition and the zone sizes were measured.

RESULT AND DISCUSSION

Plants produce a diverse range of bioactive molecules making them rich source of diverse types of medicines. Chemical screening is performed to target isolation of new or useful type of constituents having potential activity. The discovery of a potent remedy from plant origin will be a great advancement in bacterial infection therapies.

In case of phytochemical screening the presence of flavanoids, steroid and cardiac glycosides were found to be in large amount in methanol extract when compared to

others and it was represented in Table 1. Nakayoma and Yamada, 1995, [9] reported that, the flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical property, which are useful in the treatment of diseases associated with the heart properties. The presence of flavonoids, exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties [10]. Quinlan *et al.*, 2000, [11] worked on steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates. Schneider and Wolfling, 2004, [12] says that cardiac glycosides, improves cardiac output and reduces distention of heart, thus are used in the treatment of congestive heart failure and cardiac arrhythmia. Saponin was present only in aqueous extract of resin and not in any other extracts of resin. Just *et al.*, 1998, [13] revealed the inhibitory effect of saponins on inflamed cells. In medicine, it is used in hyper cholestrolaemia, hyperglycemia, anti-oxidant, anticancer and anti-inflammatory etc. The occurrence of carbohydrate and protein was also found to be high in aqueous extract of resin. The moderate level of carbohydrate was found in methanolic extract. The Alkaloid, Tannin and Anthraquinone was found to be absent in all the extracts.

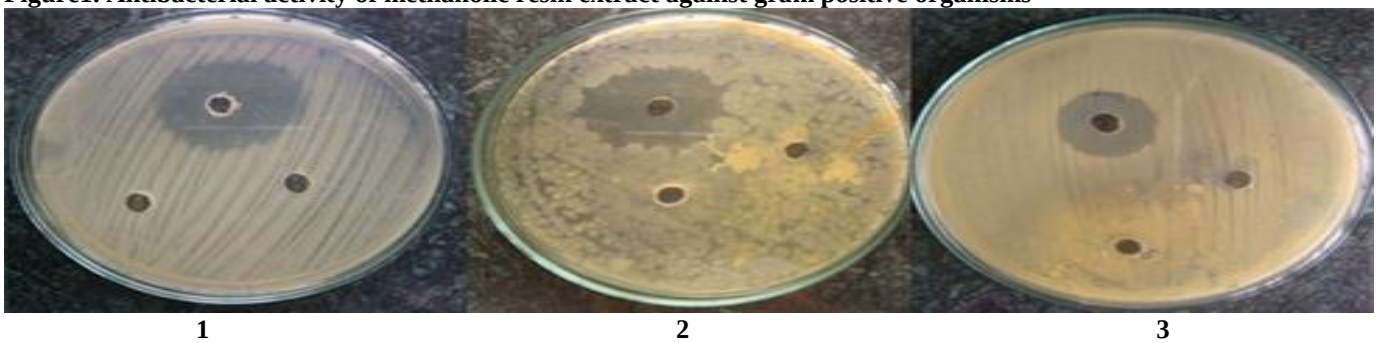
Different extracts of the resin tested against pathogenic bacteria showed varying degree of antibacterial activities was shown in graph1. The maximum zone of inhibition was found in methonal extract, when related to other extracts (Figure 1). The methanolic resin extract showed highest inhibition against Gram positive organism, *Corynebacterium diptheriae* (30mm) followed by *Bacillus cereus* (20mm) compared to Gram negative organisms. But in case of other three extracts no growth inhibition was found against gram negative organisms. The results of present investigation clearly indicate that the antibacterial activity may vary with the solvent used for extraction purpose. So this may be the reason for significant antibacterial activity of methanolic resin extract in this study. Nikaido and Vaara, 1985, [14] says the reason for the difference in sensitivity between Gram (+) and Gram (-) bacteria could be explained by the morphological difference between these microorganisms. Gram (-) bacteria have an outer phospholipid membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophillic solutes such as some plant products. Cowan, 1999, [15] reported the uses of various solvents used for extraction purposes and the high polar nature of methanol was involved in extraction of huge active phyto-components.

Table 1. Preliminary Phytochemical analysis of resin extract

Phytochemicals	Solvents used for extraction			
	Water	Methanol	Ethylacetate	Benzene
Tannin	-	-	-	-
Alkaloid	-	-	-	-
Flavanoid	++	+++	++	+
Steriod	++	+++	++	++
Glycosides	-	-	-	-
Cardiac glycosides	++	+++	++	++
Terpenoid	-	+	-	-
Saponin	+++	-	-	-
Anthraquinone	-	-	-	-
Protein	+++	-	-	-
Amino acid	+++	-	-	-
Carbohydrate	+++	++	-	-

Key: +++- high level, ++- moderate level, - - absence

Figure1. Antibacterial activity of methanolic resin extract against gram positive organisms



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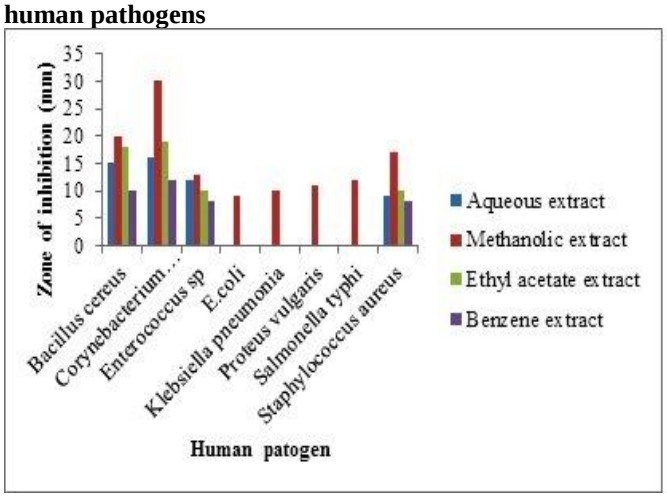
Legend: Zone of inhibition of methanolic resin extract towards (1) *Corynebacterium diptheriae* (2) *Bacillus cereus* (3) *Staphylococcus aureus*

Graph 1. Antibacterial activity of resin extracts against human pathogens



CONCLUSION

Considering the rich diversity of plants, it is expected that screening and scientific evaluation of plant resin extracts for their antimicrobial activity may provide new antimicrobial substance. Hence in the present study the antimicrobial activity of *Araucaria columnaris* resin and its nature of the active principle have been demonstrated for the first time against human pathogenic bacteria. The results suggest the possible exploitation of this plant in the management of harmful human pathogen.

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