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Research article

GC-MS ANALYSIS OF PHYTOCOMPONENTS IN RESIN OF *ARAUCARIA COLUMNARIS* (COOK PINE) AND ITS MEDICINAL USES

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ABSTRACT: Plants produce a diverse range of bioactive compounds making them rich source of different types of medicines. The plants are mostly cultivated for decoration and to enhance the appearance of houses, gardens, road sides, and also for commercial purposes such as floral decorations in form of bouquet. They are also source of fragrant oils for perfume making and cosmetic. However only very few of these plant species have found to be used in medicine and little or no literature exit on their chemical and biological activities. *Araucaria columnaris is* an ornamental plant, commonly known as Christmas tree, belonging to the family Araucariaceae. Plant resin was used for the present study, antibacterial activity and GC-MS analysis of *Araucaria columnaris* resin was carried out. The resin was subjected to solvent extraction using decreasing polarity solvents (aqueous, methanol, ethyl acetate and benzene). TLC profiling of all the extracts gives an idea about the presence of various phytochemicals and its fractions were checked for antibacterial activity against major clinical pathogens. It was found that fraction 4 (F4) of methanolic extact showed maximum zone of inhibition against Gram positive organisms. This fraction was subjected to GC-MS analysis; the result revealed the presence of a board range of many medicinal compounds and antioxidant activity of resin of *Araucaria columnaris* were identified.

Keyword: *Araucaria columnaris,* resin, GC-MS analysis, Thin layer chromatography, phytochemicals, antibacterial, antioxidant.

INTRODUCTION

India is endowed with a rich wealth of medicinal plants. India recognizes more than 2500 plant species which have medicinal values. Plants are like natural laboratories where a great number of chemicals are biosynthesized and fact they may be considered the most important source of chemical compounds (Kirtikar and Basu, 1995). World Health Organization 1998, has advocated traditional medicine as safe remedies for ailments of microbial and non- microbial origin. Nearly 50% of the drugs used in medicine are of plant origin. It is important to use phytochemical methods to screen and analyze bioactive components, not only for the quality control of crude drugs, but also for the elucidation of their therapeutic mechanisms. The genus *Araucaria* belongs to Araucariaceae comprises about 38 species. The presences of diverse secondary metabolites have been reported from species of the genus *Araucaria*. Moreover, the biological activities of *Araucaria* species such as antiulcer and antipyretic effect of oleoresins of *Araucaria bidwillii* (Anderson, 1972), gastroprotective and wound healing action of *Araucaria araucana* (Hirschmanna, *et al.,* 2005), antibacterial activity of *Araucaria angustifolia* and antimicrobial activity of *Araucaria cunninghamii* (Jia Chen *et al*., 2011), have been investigated, but there have not been much reports on the phytochemical components and biological activity of *Araucaria columnaris.* In this study, the bioactive components of *Araucaria columnaris* resin extracts have been evaluated using GC-MS. The *in vitro* screening methods could provide the needed preliminary observations essential to elect crude plant extracts with potentially useful properties for further chemical and pharmacological investigations.

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MATERIALS AND METHODS

Identification and collection of plant materials

Fresh sample of resin of the plant *Araucaria columnaris* was collected from herbal garden of Nehru Arts and Science College, T.M. Palayam, Coimbatore, Tamil Nadu, India, during the month of October – December 2011. The plant *Araucaria columnaris* was identified taxonomically and authenticated by the Institute of forest genetics and tree breeding center (IFGTB), R.S. Puram, Coimbatore, Tamil Nadu, India. The resin sample was shade dried, powdered and stored in polypropylene air-tight containers under proper conditions for further uses.

Preparation of Extracts

The crude powdered sample (20 g) (Resin) was weighed and subjected to solvent extraction for 8-10 hrs repeatedly extracted in different solvents of decreasing polarity that is distilled water (R1), methanol (R2), ethyl acetate (R3) and Benzene (R4) 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.

Thin Layer Chromatography

TLC glass plates of size 20 x 20 cm were washed in clean water and kept for drying. Silica gel G (Hi Media) of 60 to 120 meshes was used as stationary phase. The prepared plates were activated by heating at 110 °C for 1 ½ h in hot air oven. The plates were spotted with 10 µL extracts using capillary tubes. The plant extracts were allowed to dry and chloroform and methanol (29:0.3) was used as the solvent system (mobile phase). For visualization of phenolic compound, Folin-cioclaute reagent was used as the spraying agent. Various bands were appeared on TLC plate (Stahl, 1969) The RF values were calculated using the formula:

$$\text{RF value} = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}$$

Antibacterial assay

Test Organisms

The bacterial strains used for the screening were *E.coli, Salmonella* sp, *Staphylococcus aureus, Bacillus* sp and *Enterococcus* sp, *Klebsiella* sp, *Proteus* sp, *Corneybacterium diptheria.* The isolates were sub cultured and stored at 4^{o}C.

Antibacterial activity of TLC fractions of extracts

The TLC bands were scraped individually and were dispensed in appropriate solvent and filtered to get the pure sample. Further these samples were tested for antibacterial activity against selected human pathogens. Sterile Muller Hinton agar (MHA) plates were prepared and lawn cultures of the organisms were spread on each plate. 3 wells of 5 mm size were cut in the agar plates with the help of sterile cork borer and the wells were loaded with different extracts of various concentrations. The positive (Ampicillin) and negative controls (concern solvents) were also used. All the plates were incubated at 37^{o}C for 24-48 hours. After incubation, the plates were observed for the formation of zone of inhibition and the zone sizes were measured (Perez *et al.,* 1990).The fraction having maximum antibacterial efficacy was subjected to GC-MS analysis.

GC–MS Analysis of TLC Fraction

Based on the antimicrobial assay, the most effective fraction of the methanolic extracts of the resin (F4) was further used for the identification of bioactive constituents by GC– MS analysis, at The South India Textile Research Association (SITRA), Coimbatore, Tamil Nadu, India. Analysis by GC/MS was performed using a Thermo Gc - Trace Ultra Ver: 5.0, Pyrolysis auto sampler interfaced to a Perkin Elmer Turbomass Gold equipped with a fused silica capillary column (J & W; DBI; 30m length x 0.25 mm id. film thickness 0.25 µm). The fraction was pyrolysed at 610 °C and then introduced to the GC column. The transfer line was held at 280 °C and the source temperature was maintained at 180 °C and ionization energy was set at 70eV. Helium was employed as carrier gas (1 mL /m). The GC oven temperature was programmed: The column held initially at 70 °C / m (isothermal) and then increased by at 8 °C /m to 260°C / m min^{-1} (isothermal). Qualitative identification of the different constituents was performed by composition of the relative retention times and mass spectra with those of authentic reference compounds by retention indices (RI) and mass spectra.

Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unidentified component was compared with the spectrum of the identified components stored in the NIST08 and Wiley08 library. The name, molecular weight and structure of the components of the test materials were ascertained.

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RESULT AND DISCUSSION

In the present study, the screening of phytochemical constituents, evaluation of antimicrobial and identification of bioactive compounds using TLC and GC-MS of the *A. columanris* (Ornamental plant) resin extracts were performed. TLC was performed to isolated pure compounds from resin extracts. Totally 21 fractions were collected and methanolic F4 fraction showed maximum zone of inhibition against Gram positive organisms (*Bacillus* sp (20mm) and *Staphylococcus aureus* (17mm) respectively) compared with Gram negative organism(Graph 1). It was supported by the work done by Carlos *et al.,* 2006, in which it was reported that the methanol extract of *Araucaria araucana* bark showed the highest inhibitory activity against the tested Gram-positive bacteria and did not show the same activity against the tested Gram-negative bacteria. 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 (Nikaido and Vaara, 1985). 1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, Diisooctyl-phthalate, Phthalic acid, isobutyl isoporpyl ester are some of the compounds from the resin exhibiting biological properties (Figure 1, Table1). Benzenedicarboxylic acid bis(2-ethylhexyl) phthalate has been isolated from a marine alga, *Sargassum weightii*, and apart from its plasticizing ability it was also found to have antibacterial effect on a number of bacteria (Sastry and Rao, 1995). Phthalates are reported to have antimicrobial and other pharamacological activities (Table 2). Vegetable oil-derived plasticizers such as phthalates are benign and not only make plastic material flexible but they also offer benefits such as its resistance to migration, evaporation and leaching, and the stability to light and heat, thus offer environmental friendly plastic for future use (Ramalakshmi and Muthuchelian, 2011).Thus it could be concluded that *Araucaria columnaris* plant is of phytopharmaceutical importance.

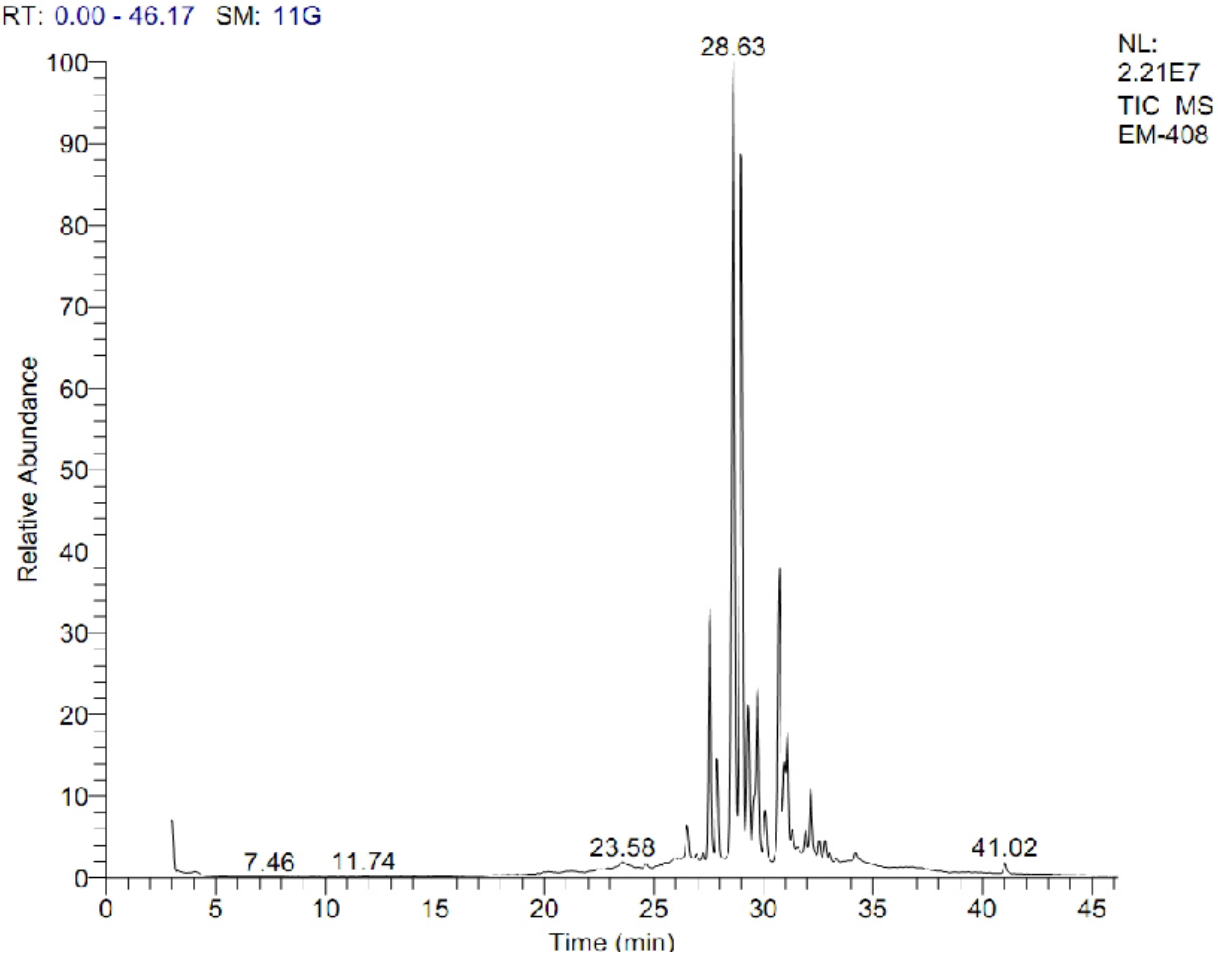

Figure 1: GC MS analysis of *Araucaria columnaris* methonolic resin extract

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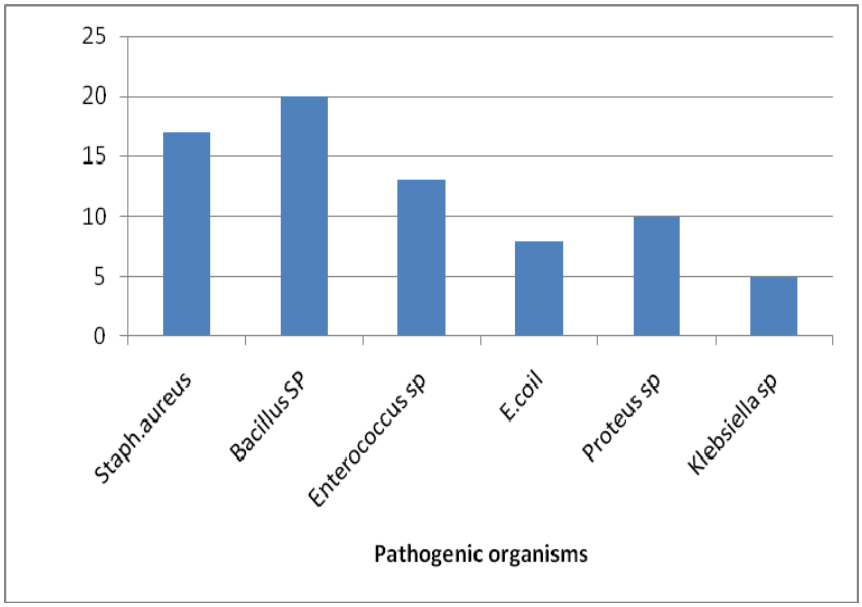

Graph 1: Antibacterial activity of methanolic (F4) fraction

Table 1: GC-MS analytical report of *Araucaria columnaris* resin extracts

S. No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %
1.	23.58	2-(2-Hydroxy-2-P-Chlorophenylethyl)-3,5 6-trimethyl pyrazine (terpenoid derivatives)	$C_{15}H_{17}ClN_2O$	276	0.22
2.	41.02	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390	0.35
3.	41.02	Diisooctyl-phthalate	$C_{24}H_{38}O_4$	390	0.35
4.	26.94	Phthalic acid, isobutyl isoporpyl ester	$C_{15}H_{20}O_4$	264	0.12
5.	41.02	Di-(2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$	390	0.35
6.	41.02	4-(3-Benzyl-2,4-dioxo-1-phenethyl-1,2,3,4-tetrahydro-5-pyrimidinylmethyl)benzamide	$C_{26}H_{33}N_3O_3$	435	0.35
7.	41.02	Dihydro-3-methylene-5-hexyl-2(3H)-furanone	$C_{11}H_{18}O_2$	182	0.35
8.	23.58	2-(2-HYDROXY-2-P-CHLOROPHENYLETHYL)-3,5, 6-TRIMETHYLPYRAZINE	$C_{15}H_{17}ClN_2O$	276	0.22
9.	23.58	Butane, 1,3-dichloro-3 methyl-(CAS)	$C_5H_{10}Cl_2$	140	0.22
10.	23.58	3-Cyclopentyl-1-propyne	C_8H_{12}	108	0.22
11.	28.63	N-Benzyl-2-(toluene-4-sulfonyl)acetamide	$C_{16}H_{17}NO_3S$	303	30.29

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Table 2: Biological properties of GC-MS compounds

S. No	Name of the compound	Biological properties
1.	2-(2-hydroxy-2-p-chlorophenylethyl)-3,5,6-trimethylpyrazine	Antimicrobial activity
2.	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	Antimicrobial activity, Anti inflammatory
3.	Diisooctyl-phthalate	Antimicrobial and other pharamacological activities.
4.	4-(3-Benzyl-2,4-dioxo-1-phenethyl-1,2,3,4-tetrahydro-5-pyrimidinylmethyl)benzamide	Antimicrobial activity, Anti oxidant

CONCLUSION

Therefore it is recommended that more work to be conducted to help optimally extract all the bioactive compounds in the plant and thus formulated into an appropriate dosage for the treatment of infectious diseases. The plant *Araucaria columnaris* holds promise for the production of novel pharmaceuticals as well as a nutraceutical. It would be worthwhile to further isolate the compounds and determine their specific activity and also to understand the synergistic effect of compounds for therapeutic roles.

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