Original Research Article

PHYTOCHEMISTRY OF AERIAL PARTS OF *ARAUCARIA COLUMNARIS*

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ABSTRACT

The current article is designed for the extraction of chemical compound. Dichloromethane and methanol were applied to extract the chemical compound so that maximal number of compounds can be drawn out. Thin layer chromatography is applied as Analytical technique for the isolation and identification of the various compounds. Phytochemical analysis of aerial parts showed the presence of Tannins and cardiac glycosides. Cardioactive glycosides and Tannin and were reported in aerial parts of *Araucaria columnaris* for the first time. Therefore *Araucaria columnaris* can be used for further isolation and structural determination of cardioactive compounds.

***Keyword** : Araucaria columnaris*, cardioactive glycosides, Tannin, phytochemical study

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INTRODUCTION

The basic needs of life for human being are shelter, clothing, food, flavors and fragrances as not the least, medicines. The common source among all is plant. Plants have shaped the foundation of refined traditional medicine structures among which are Ayurvedic, Unani, and Chinese amongst others. Some important drugs that are still in use today are because of traditional medicine structures. The search for novel drugs, nowadays, has taken a somewhat diverse route where the science of ethnobotany and ethnopharmacognosy are being used as guide, which led towards different sources and classes of compounds [1].

Nowadays, studies on structure-activity relationships and their impact on the design of novel drugs have rendered them one of the utmost valuable and thus significant accomplishments of phytochemistry, an advance constituent in the group of pharmaceutical sciences [2].

Araucaria columnaris (Figure 1) belong to family Araucariaceae and genus Araucaria. It is distributed throughout the New Caledonia and Peshawar [3]. It is commonly used as ornamental plant all around the world [4]. Abietanes is compound isolated from *Araucaria columnaris* [5]. *Cupressus columnaris J.R. Forst.* is the synonym of *Araucaria columnaris* [3].

The purpose of the current paper is phyto-chemical analysis of the *Araucaria columnaris* (aerial part) for isolation and structure elucidation of pharmacologically active compounds.

Figure 1: *Araucaria columnaris*: Sketch of aerial parts, leaves and bark

Figure 3: *Araucaria columnaris*: Bark

Figure 2: *Araucaria columnaris*: Sketch of leaves

MATERIAL AND METHOD

Material:

Rotary Evaporator, Extraction bottle, Dichloromethane (DCM), Methanol, Ultrasonic bath, Dragendorff'sreagent, dilute ammonia solution, separating funnel, chloroform, acetic acid, Mayer's reagent, carbontetrachloride (CCl4), Five silica gel 60 F^{254} TLC plates (20x20cm) (Merck),

Collection of Plant material:

Araucaria columnaris was collected from an ornamental shop and identified by Dr. Altaf Hussain Dasti, Professor, Institute of pure and applied Biology, Bahauddin Zakariya University, Multan giving the Catalog number 12-fci. Total wet weight of plant collected was 6 kg. It was then reduced to 3 kg of dried plant. The plant was then ground till it become powder. The total weight of powder drug was 600 grams.

Extraction:

Maceration is the technique for extraction for finely ground plant material. Measured quantity of plant material (500gm) was taken in a glass bottle. After that quantified volume of dichloromethane was added to it with constant sonication in ultrasonic bath. It takes 24 hours to be settle down and then filtration was performed. Repeat the process three times with dichloromethane and then methanol. The Dichloromethane used during first, second and third soaking was 1000 ml, 700ml and 600 ml respectively and 600 ml, 400ml and 400 ml for methanol respectively. Rotary evaporator was used for the concentration of both extracts under reduced pressure labeled with codes as ACAPD and ACAPM respectively.

Phytochemical Analysis

Test for Alkaloids:

Powdered drug (0.5-1g) was boiled with dilute hydrochloric acid (10 ml) in a test tube for one minute; it was allowed to cool and fragments to settle down. Filtered the supernatant liquid into other test tube. Pour three drops of Dragendorff's reagent. Clear precipitate or turbidity seemed, representing the occurrence of alkaloids. To confirm the existence of alkaloids, the remaining part of solution was made alkaline to litmus paper with dilute NH_3 solution. It was then extracted with $CHCL_3$ (5ml) by shaking it gradually and permit the layers to isolated. The lower $CHCL_3$ layer was detached and extracted with dilute CH_3COOH (10 ml). The $CHCL_3$ layer was cast-off. The extract was distributed into 4 parts and adds few drops of Wagner's reagent, Mayer's reagent and Dragendorff's reagent separately to each of 3 parts while the 4th parts worked as untreated control. An observation of turbidity or precipitate compared with untreated control with either or all reagents confirmed occurrence of alkaloids [6].

Test for Anthraquinone glycosides:

Borntrager's test:

Ground drug (0.1g) was extracted with hot H_2O (10 ml) for five minutes. Filter the solution when it was hot. Cool afterward and extracted with CCl_4 (10ml). The $CCl_4$was detached, washed with water (5ml) and shaken with dilute NH3 solution (5ml). Absence

of pink to cherry red color showed the absence of free anthraquinone Ground drug (0.1g) was dissolved with Iron (III) chloride (10 ml) and HCl (5ml). Δ the solution on heated bath for ten minutes. Filter the solution when it was hot. Cool afterward and extracted with carbon tetrachloride (10ml). The carbon tetrachloride was detached, washed with water (5ml) and shaken with dilute NH_3 solution (5ml). Absence of pink to cherry red color showed the absence of bound anthraquinone [6].

Test for Cardioactive glycosides:

Keller Kiliani test:

Drug used for analysis was crushed then taking 1g. 10 ml of alcohol (70%) with 1g of crushed drug was boiled on water bath for two minutes. Filter the extract and add distilled water (twice amount) to dilute the filtrate. After then lead sub acetate was added. Filter it. $CHCL_3$ or CCL_4 was used for extraction of filtrate by vigorous shaking. Transfer the organic portion in a crucible. Evaporate the organic portion and add 3ml of Iron (III) chloride (3.5 %) in a residue. Transfer the portion in a test tube and then H_2SO_4 was added cautiously along the wall of test tube [6].

Test for Tannins:

Lead acetate test:

Powder of plant was dissolved in distilled water and boiled. After boiling filter the solution and added lead acetate in the filtrate which gave precipitate, are indicating the presence of tannin.

Test for Saponin:

Grounded drug (0.5g) was shaken with H_2O. Consistent foam showed presence of saponin [6].

Thin Layer Chromatography

Requirements:

Test samples, organic solvents (chloroform, methanol, ethyl acetate, n-hexane and isopropyl alcohol), Spotting capillary, coated TLC plates, TLC tank, oven and UV illuminator.

Spotting and Development of TLC plates:

Ten mg of each methanolic and dichloromethane extracts of aerial parts were dissolved in 1ml of methanol and dichloromethane (HPLC grade), respectively. Five silica gel 60 F254 TLC plates (20x20cm) were marked at 1cm from each side and cut into smaller plates. 5-10ul of sample was applied by capillary on the line marked. Samples were applied at equal distance for simple TLC and spot was no more than 6mm in diameter.

Visualization of TLC plates:

TLC plates were first visualized in the UV light (254nm and 366nm) and visualized spots were marked for the determination of Rf value. Subsequently sprayed with the Godin's reagent.

Godin reagent:

This reagent was prepared by mixing equivalent volumes of 1% vanillin in ethanol and 3% perchloric acid in water. TLC plates were sprayed with this mixture and then with 10% sulphuric acid in ethanol. Sprayed TLC plates were then heated at 100C°. Different spots were observed [7].

Measurement of Rf value:

Both distances, covered by mobile phase and substances were measured and Rf value was calculated

as:

Rf = Distance traveled by the component / distance traveled by the solvent front

RESULTS

Extraction:

For extraction of Aerial part of *Araucaria columnaris* maceration process was adopted. The solvent used for extraction were methanol and dichloromethane. The results are shown in the table 1

Table 1: Results of extraction of plant material with different solvents.

Plant Name	Part Used	Solvent Used	Extract obtained (gm)	Sample codes
Araucaria columnaris	Aerial part	Dichloromethane	9.0	ACAPD
		Methanol	15.9	ACAPM

Phytochemical analysis of crude extract :

Phytochemical studies were carried out for detection of secondary metabolites i.e. alkaloids, anthraquinone glycosides, cardiac glycosides, saponins and tannin; in plant material. The results of the study are shown in table 2.

Table 2: Results of identification of secondary metabolites in crude extract.

Plant Name	Part used	Alkaloids	Anthraquinones	Cardiac glycosides	Saponins	Tannins
Araucaria columnaris	Aerial part	-	-	+++	-	+++

+++ = Strongly positively results
- = No results

Chromatographic method:

Result of TLC of dichloromethane extract of *Araucaria columnaris:*

UV active components were observed at 254nm with Rf values as follows:
0.05, 0.16, 0.24, 0.36, 0.44, 0.5, 0.55, 0.79, 0.85, 0.90.
UV active components were observed at 366nm with Rf values as follows:
0.05, 0.16, 0.24, 0.36, 0.44, 0.5, 0.55, 0.66, 0.79, 0.85, 0.90.

Some colored components became visible lable as yellow "Y" following Godin reagent and 10% sulfuric acid spray respectively having Rf values given below:
0.16, 0.24, 0.36, 0.44, 0.5, 0.55, 0.90. The photograph of developed TLC plate of dichloromethane extract of Araucaria columnaris is shown in figure 2.

Figure 2: TLC of dichloromethane extract Aerial parts of *Araucaria columnaris*

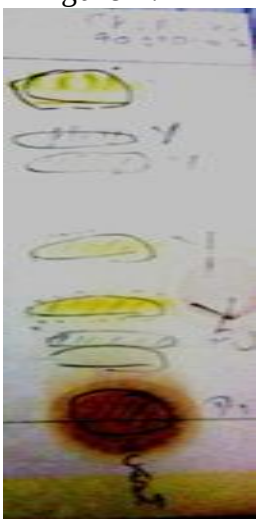

Figure 3: TLC of methanolic extract Aerial parts of *Araucaria columnaris*

Result of TLC of methanol extract of *Araucaria columnaris*:

UV active components were observed at 254nm with Rf values as follows:
0.02, 0.15, 0.28, 0.45, 0.68, 0.75, 0.91
UV active components were observed at 366nm with Rf values as follows:
0.28, 0.45, 0.91
Some colored components became visible lable as yellow "Y", pink "P.I" and purple "P.U" following Godin reagent and 10% sulfuric acid spray respectively having Rf values given below:
0.02, 0.2, 0.28, 0.45, 0.68, 0.75. The photograph of developed TLC plate of dichloromethane extract of Araucaria columnaris is shown in figure 3.

DISCUSSION

Current investigation deals with the phytochemical evaluation of *Araucaria columnaris* (Araucariaceae). The end result of phytochemical analysis of secondary metabolites displayed the occurrence of cardiac glycosides and tannin in aerial part of crude extract of *Araucaria columnaris*. There have been reports of the presence of biflavanoid, isoflavanoids, phenyl propanoids, furans, lignans, protein, terpenes, and polysaccharides in genus *Araucaria* [8]. In comparison with the crude extract of *Araucaria columnaris* under current investigations it has been found that these constituents were reported for the very first time which can be explored for further phytochemical studies and isolation of cardioactive glycosides. Thin Layer Chromatography (TLC) has been a good analytical technique for isolation and identification of the various compounds. Number of UV visible components from

dichloromethane and methanolic extract of aerial parts of *Araucaria columnaris has been* identified through their Rf value. It has been found the difference in Rf value in comparison between methanolic and dichloromethane extract.

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