PHYTOCHEMICAL INVESTIGATION AND GC-MS ANALYSIS OF ETHANOLIC EXTRACT OF *Acacia leucophloea* (Roxb.) WILLDBARK

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ABSTRACT

Acacia leucophloea (Roxb.) Willd belongs to the plant family Mimosacese. The plant distributed in most of the regions of India and it has potential medicinal value and used as anthelmintic, vulnerary, demulcent, constipating, expectorant, and antipyretic. The present study was done to investigate the preliminary phytochemical and GC-MS analysis of bark extract of the plant. Phytochemical investigation of bark extract in different solvents revealed that the presence of tannins, flavonoids, alkaloids, phenols, terpenoids, steroids and phytosterols. The Six compounds were identified in the ethanol extract of bark by GC-MS analysis.

Keywords: *GC-MS, Extract, Phytochemical, Medicine*

Introduction

Medicinal plants are utilized by human being since ages in ancient medication because of their therapeutic potential and the search on medicinal plants have led to the discovery of novel drug candidates used against diverse diseases. On the report of World Health Organization (WHO) in 2008, more than 80% of the population of the world relies on traditional medicine for their primary healthcare needs (Pierangeli et al., 2009). Higher plants as sources of bioactive compounds still play a dominant role in the maintenance of human health. The sacintific data information available on higher green plants represent a reservoir of effectual chemotherapeutants, these are non-phytotoxic, more systemic and easily biodegradable (Vyas, 1999; Kaushik et al., 2002; ChamanLal and Verma, 2006). Plants are an abundant source of secondary metabolites with many biological activities. These secondary metabolites are an important source with a variety of forms and properties (de-Fatima et al., 2006). The Painganga forest is one of the phytogeographicallyrich areas of the region with greater endemism. The area is a treasure trove of medicinal plants and wild relatives of cultivated crops. (Kulkarni and Sontakke, 2020).

Traditionally, the bark of *Acacia leucophloea* is used as an astringent, bitter, anthelmintic, vulnerary, demulcent, constipating, expectorant, and antipyretic. It is also used for treating cough, bronchitis, vomiting, wounds, diarrhoea, dysentery, ulcers, internal and external haemorrhages, dental caries, oral ulcers and stomatitis (Bhadoria and Gupta 1981; Vijayakumari et al 1994). GC-MS is the best technique to identify the bioactive constituents of alkaloids, steroids, long-chain hydrocarbons, alcohols, acids, esters, amino and nitro compounds etc. (Karuppasamy et al, 2012). Although A. leucophloea (Roxb.)Willd. is being used as tribal medicine, the chemistry of this plant is not yet explored. The present study was carried out to identify some of the phytochemical present in the methanolic extract of the stem bark of *Acacia leucophloea* (Roxb.) Willd. by GC-MS technique, to discover the medicinal properties of the plant.

Material and Methods

Plant Material and preparation of crude extract

The bark of *Acacia leucophloea* (Roxb.) Willd. The plant was collected from Painganga forest region of Dist. Yavatmal(MS) India. The plant identification were done by using flora and relevant literature. The plant material that were collected and washed thoroughly with running tap water and shade dried. After shade drying the plant material was grinded and stored in powder form in plastic bottles for further use. The 30 gm of dried powder was extracted with 300 ml ethanol as solvent using Soxhlet apparatus for 24 hrs. The extract were lyophilized and stored in 4O C. (Sontakke and Shinde, 2019)

Phytochemical Analysis

Preliminary qualitative phytochemical screening was carried out with the following methods with minor modifications (Sontakke and Shinde, 2020).

Test for carbohydrates

Molisch's Test: Small Quantity of Petroleum ether, Benzene, Acetone, Chloroform, Ethanol, Distil water extract were taken. 10 ml of distil water and two drops of Ethanolicnaphthol (20%) and 2ml of concentrated Sulphuric acid were added, formation of reddish violet ring at the junction indicates the presence of carbohydrates.

Test for Saponins

Foam Test: 2 ml of Petroleum ether, Benzene, Acetone, Chloroform, Ethanol, Distil water extract were taken and added to an equal amount of distil water and shaken in a graduated cylinder for 15 minutes lengthwise. The formation of a 1 cm layer of foam indicates the presence of saponins (Kumar et al., 2009).

Test for Tannins

Ferric Chloride Test: Small Quantity of Petroleum ether, Benzene, Acetone Chloroform, Ethanol, Distil water extract were taken separately in water and 2-3 drops of 5% ferric chloride were added. The formation of black or green colour indicates the presence of tannins.

Test for Flavonoids

Sulphuric Acid Test: A fraction of extract was treated with concentrated sulphuric acid and observed for the formation of orange colour.

Test for Alkaloids

Mayer's Test: 2ml of concentrated hydrochloric acid was added to 2ml of plant extract then a few drops of Mayer's reagent were added. The presence of white precipitate or green colour indicates the presence of alkaloids.

Test for Glycosides

Sulphuric Acid Test: To 2ml of plant extract, 1ml of glacial acetic acid and 5% ferric chloride was added then a few drops of concentrated sulphuric acid were added. The indication of greenish-blue colour shows the presence of glycosides.

Test for Proteins and Amino acids

Ninhydrin Test: To 2ml of plant extract, few drops of 0.2% Ninhydrin was added and heated for five minutes. The formation of blue colour indicates the presence of proteins.

Test for Steroids and phytosterols

Sulphuric Acid Test: To 1 ml of plant extract, an equal volume of chloroform and few drops of concentrated sulphuric acid were added. The formation of a brown ring indicates the presence of steroids and the formation of bluish-green colour indicates the presence of phytosterols.

Test for Phenols

Ferric Chloride Test: To 1 ml of plant extract, 2ml of distilled water followed by few drops of 10% ferric chloride was added. The formation of blue or green colour indicates the presence of phenols.

Test for Terpenoids

Salkowski test: 2ml of chloroform and 3ml of concentrated sulphuric acid was added to 2ml of plant extract which form a layer. Reddish-brown coloration at the interface is formed indicating the presence of Terpenoids.

GC-MS Analysis

The ethanolic extract were subjected to Gas Chromatography and Mass Spectroscopy for the determination of bioactive volatile compounds. GC-MS analysis of the samples were carried out using Perkin Elmerclarus 680 with mass spectrometer Clarus 600 (EI) using Turbo Massver 5.4.2 Software with NIST – 2008 Library ver. Helium was used as the carrier gas and the temperature of programming were set with the initial oven temperature at 600C and held for 2 min and final temperature of the oven was 3000 degrees centigrade with the rate at 100 degrees centigrade per min. A 2-µL sample were injected with split 50:1. Mass spectra were recorded over 35-650 amu range with electron impact ionization energy 70 eV; a scan interval of 2 min and fragments from 50 to 600 Da. The chemical components from the different extracts of plants were identified by comparing

the retention times of chromatographic peaks using the Quadra pole detector with NIST Library to relative retention indices. Quantitative determinations were made by relating respective peak areas to TIC areas from the GC-MS.

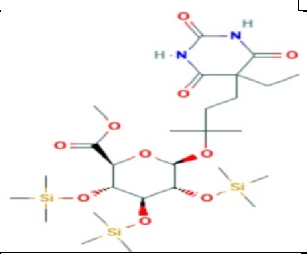
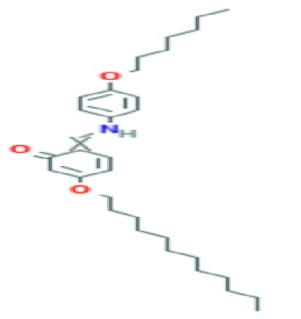
Results and Discussion

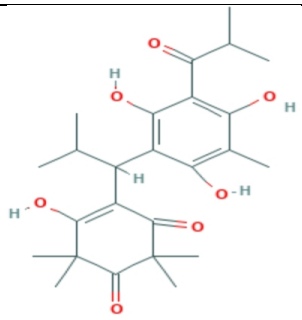
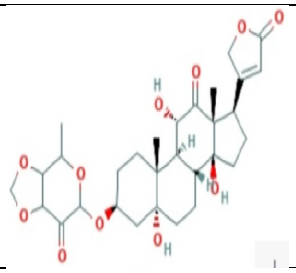
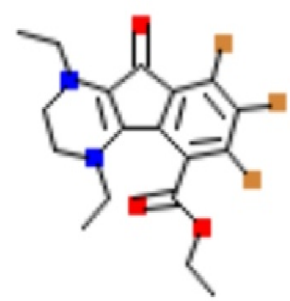
Table 1: Phytochemical Analysis of Different solvent extracts of *Acacia leucophloea*(Roxb.)Willdbark

Sr. No	Phytochemical Test	Petroleum ether	Benzene	Acetone	Chloroform	Ethanol	Distil water
1	Carbohydrates	+	---	+	+++	++	+
2	Saponins	++	++	+	+	---	+
3	Tannins	---	---	+++	---	+++	++
4	Flavonoids	+	+	+++	++	+++	++
5	Alkaloids (Mayer's test)	++	++	+++	++	+++	++
6	Glycosides	---	---	+	---	+++	++
7	Proteins	---	+	+	---	---	---
8	Steroids &Phytosterol	---	---	+++	+	+++	++
9	Phenols	---	---	+++	---	+++	+
10	Terpenoids	---	---	++	---	+++	++

Preliminary phytochemical analysis of bark of *Acacia leucophloea* (Roxb.) Willd. plant was carried out by using different solvent extracts. The bark extract showed the presence of alkaloids, flavonoids, tannins, phenols, steroids, phytosterols and terpenoids. Most of the phytochemicals were found to be present in acetone, ethanol and water extracts while pertolium ether, benzene and chloroform extracts showed the lowest presence of alkaloids. (Table1).

Table2 : GC-MS Analysis of *Acacia leucophloea* (Roxb.)Willd

Sr. No.	R/T	Peak area (%)	Compound Analyzed	Molecular formula	Probable Structural Formula	Activity reported
1	9.13	2.57	1-Dodecane	$C_{12}H_{24}$		Ectoparaticide
2	11.70	0.76	a-D-Glucopuranosi-duronic acid	$C_{27}H_{52}N_2O_{10}Si_3$		Deoderant, Dermagenic, Dermatophyticide, Antieczemic, Antiitch, Antikeratolic
3	28.12	3.09	3 H-Cycloprop(1,2)-5-cholest-1-en-3-one, 1-carboethoxy-1-cyano-1-2-dihydro-	$C_{32}H_{49}NO_3$		Antifugal, Deodarant, Antibacterial, Anticandidosis, Antibiotic

4	29.89	0.75	(22S)-6a,11a,21-Trihydroxy-16a, 17a-propylmethyle nedioxypregna-1,4-diene-3,20-dione	$C_{25}H_{34}O_7$		Absorbant, Antivertilago, Acnegenic, Antiaging, Antiedemic, Antiitch, Antidermatitic
5	31.68	1.45	Card-20(22)-enolide	$C_{30}H_{40}O_{11}$		Antioxidant
6	32.44	0.69	5H-Indenol(1-2-b)pyrazin-5-one	$C_{18}H_{19}Br_3N_2O_3$		Antibacterial, Antifungal, Antiseptic, Candicide, Deodarant, Antidermatic

The chromatogram of Ethanol extract of bark of *Acacia leucophloea* (Roxb.) Willd. clearly shows the presence of six peaks indicating the presence of six related phytochemical compounds detected was shown in Table 2. The six phytoconstituents were characterized and identified on comparison of the mass spectra of the constituents provided by the NIST library.

Conclusion

The phytoconstituents of many plants can often be identified from the peak pattern of the chromatograms obtained directly from headspace analysis. Similarly, unique qualitative and quantitative patterns from a GC analysis will often help identify the phytoconstituents of plant extracts. The technique of fingerprint could really identify false herbal products. The construction of chromatographic fingerprints aims at evaluating the quality of Herbal Medicines (Yi-Zeng et al 2004). The fundamental reason for the quality control of herbal medicines is based on the concept of phytoequivalence of herbs, and then to use this conception to identify the real herbal medicine and the false one and also to check the quality. In the present investigation, six bioactive compounds have been identified from the Ethanol extract of bark of *Acacia leucophloea* (Roxb.) Willd. by Gas Chromatogram-Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds in *A. leucophloea* (Roxb.) Wild. proved that the pharmaceutical importance. Though, further studies will require finding out its bioactivity, toxicity profile.

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