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Research Article

COMPARATIVE PHYTOCHEMICAL STUDIES IN SELECTED ACACIA SPECIES

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ABSTRACT

Three Acacia species from south India, *Acacia catechu, Acacia lucophloea and Acacia nilotica* were taken for comparative phytochemical analysis. The chemical pattern of three species was compared using thin layer chromatography. The physico chemical parameters of each extract were analysed. TLC method was standardized using epicatechin as marker compound. Total tannins of all the species were determined spectrophotometrically. Gas Chromatographic analysis was carried out in n-hexane cold macerated leaf extracts of three species and compared the volatile constituents.

Keywords: Acacia, TLC, GC, Total tannins.

INTRODUCTION

Medicinal plants and plant- derived medicines are widely used in traditional cultures all over the world and they are becoming increasingly popular in modern society as natural alternatives to synthetic chemical[1]. Nearly all cultures from ancient times have used plants as a source of medicine. The World Health Organization (WHO) has listed 21,000 plants worldwide, reported to have medicinal uses. India is the largest producer of medicinal herbs and is called the *botanical garden of the world* [2]. Standardization is an important aspect for establishing the quality and/or efficacy of medicinal plants. Generally, two approaches being used for standardization are fingerprint analysis by HPLC/HPTLC and quantitation of individual chemical markers. It ensures reproducible pharmaceutical quality of herbal products the question of drug standardization is an important issue demanding immediate attention from all those involved with the ayurvedic industry[3]. The herbal formulation in general can be standardize schematically as to formulate the medicament using raw materials collected from different localities and a comparative chemical efficacy of different batches of formulation are to be observed. The preparations with better clinical efficacy are to be selected. After all the routine physical, chemical and pharmacological parameters are to be checked for all the batches to select the final finished product and to validate the whole manufacturing process[4].

Chemical and chromatographic techniques can be used to aid in identification of an herbal material or extract. Chromatographic technique such as HPLC, TLC, GC and spectroscopic methods such as IR and UV-may also is used for fingerprinting. Markers compounds may be used to identify herbal materials, set specifications for raw materials, standardize botanical preparations during all aspects of manufacturing processes and obtain stability profiles[5]. Acacia is the second largest genus in the Leguminosae family, comprising more than 1200 species. This species contains variety of bioactive components such as phenolic acids, alkaloids, Terpenes, tannins and flavonoids which are responsible for numerous biological and pharmacological properties[6].

MATERIALS AND METHODS

Preparation of Plant Extract

10 g of dried stem bark of each, *Acacia catechu, Acacia lucophloea and Acacia nilotica* was taken and suspended in 100 ml of 50% aqueous ethanol solution and subjected extraction by refluxing. The aqueous alcoholic extract obtained was filtered and the process was repeated for four days. The resulting filtrates were pooled for further processing. This pooled aqueous ethanolic extract was concentrated to 50 ml on rotavapour and it is taken for the study. 5 g of fresh leaf of all the species were cold macerated separately with n-hexane for Gas Chromatographic analysis.

Determination of Physico chemical Parameters

Qualitative analysis for Physico chemical Parameters were carried out in triplicate according to prescribed standard methods in Indian Pharmacopeia[7].

Estimation of Total Tannins

100 mg of tannic acid was dissolved in 100 ml of distilled water. 1 ml of this solution was diluted into 100 ml in distilled water to give 10 µg/ml tannic acid solutions.

A series of calibrated 10 ml volumetric flask were taken and working standards of 5- 45 µg solutions were taken. To each flask 0.5 ml Folin-Denis reagent[8] and 1 ml sodium carbonate solution were added, the volume is made up to 10 ml by distilled water. The solution without tannic acid was used as blank. The blue colored complex thus produced is measured at 775 nm.

1 ml of each extract is made up to 10 ml in similar manner. From the calibration curve the corresponding concentration of tannins were calculated. It was expressed as gram equivalent of Tannic acid.

Thin layer chromatographic profile

TLC of all extracts with epicatechin as marker was carried out on a pre-coated silica gel $60F_{254}$ TLC plate (Merck India) using toluene, ethyl acetate and formic acid as mobile phase in the ratio of 5:2:1. The plate was developed over a distance of 9 cm and visualized under visible light after spraying with Anisaldehyde sulphuric acid reagent followed by heating at 105^0C for 5 minutes.

Gas Chromatographic analysis

The cold macerated n-hexane extract was subjected to GC analysis on Agilent 6890 network GC, with a HP-5 column and Flame Ionisation Detector (FID). The injector temperature was set at 80^o C and that of detector was 220^o C. The temperature of the column was programmed as 0-5, 80^o C, 5-15, 100^o (held 5 minutes), 15-25, 120^o with an increase of 5^oC per ramp.

RESULTS AND DISCUSSION

The physico chemical parameters such as water soluble extractive, alcohol soluble extractive, total ash, acid insoluble ash and water soluble ash were calculated (Table 1). The water soluble extractive was found to be 22-25% (W/V) for *A.catechu*, 19-21% for *A.nilotica* and total 23-25 % for *A. lucophloea.* The water soluble extractive and alcohol soluble extractive were found to be more for *A.nilotica*. The alcoholic soluble extractive is less compared to water soluble extractive. The P^H of water extracts vary from 6.2 to 6.6.

The total tannin is expressed in mg equivalent of Tannic acid per gram of extract (Fig: 1.1).The highest tannin content was found in *A.nilotica* (0.18 mg E TA/ g) The thin layer chromatographic profile

showed the comparative chemical pattern of three species (Fig 1.2). The TLC tracks 1 is epicatechin, 2, 3 and 4 correspond to *A. lucophloea, A.nilotica*, and *A.catechu* respectively. Epicatechin is present both in *A.nilotica* and *A.catechu* but it is absent in *A. lucophloea*. *Acacia nilotica* and *A.catechu* showed almost similar chemical profile.

Table 1

Physico chemical parameters	***A.catechu***	***A.nilotica***	**A. *lucophloea***
Water soluble extractive	22-25%	19-21%	23-25 %
Alcohol soluble extractive	19-21%	18-20%	19-22 %
Total ash	1.8-2.1%	1.6-1.8%	1.5-2.1%
Acid insoluble ash	0.2-0.5%	0.18-0.3 %	0.12-0.24 %
Water soluble ash	0.06-0.18%	0.05-0.09 %	0.02-0.06 %
PH of water extract	6.2	6.4	6.6
Loss on drying at 105 ^{0}C	7 -9 %	8-11%	6-8 %

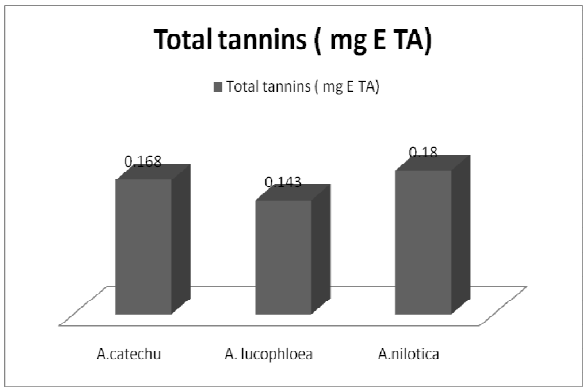

Fig. 1.1: Total Tannins

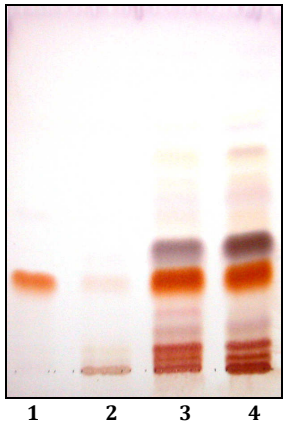

Fig. 1.2: TLC Profile

The Gas Chromatographic profile showed common peaks at R_t 12.46, 14.167, 14.88 and 19.30 with varying peak area which indicates the quantitative variation of volatile constituents.

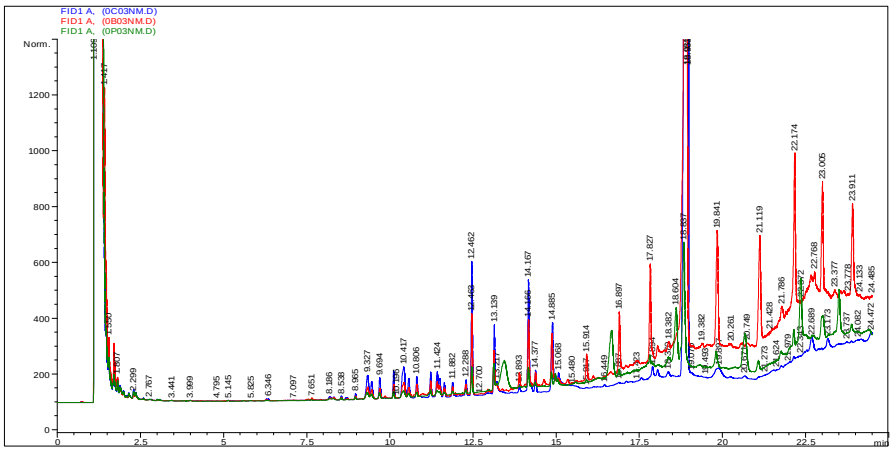

Fig. 1.3: Overlaid GC Profile

The physico chemical parameters, quantitative analysis and TLC Finger print can be used for quality evaluation of the selected Acacia species. The distinguishing bands in TLC Profile and the presence of marker compound epicatechin may be used as marker parameters for the Quality standardisation.

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