The family is done on leaves of medicinal plants the literature survey reveals that very less work on flavonoids. The stem bark possesses antifungal activity. Phytochemical investigation showed the presence of different classes of compounds e.g. astringent. The plant material used for each extract was separated and purified by very advanced technique flash chromatography and analyzed using GC-MS (gas chromatography-mass spectroscopy). It revealed 36 most probable steroid compounds. The major constituents were Beta sitosterol, stigmasterol acetate, Beta sitosterol acetate, Cholesterol, Cholest-3,5-diene, Dihydrotachysterol, Gamma Sitosterol, Stigmaster-3,5-diene, 26-hydroxycholesterol, Retinol.

Key words: Butea monosperma, chloroform extract, GC-MS analysis, stigmasterol acetate.

INTRODUCTION

Literature on traditional medicine is very merge. In India plenty of plants are being used as drugs due to their medicinal properties. The plant kingdom still holds many species of plant contains substance of medicinal values which are yet to be discovered\(^1\). One of them is leaves of Butea monosperma. It is medium sized tree with 20-40 feet height belonging to the family fabaceae. It is found in mountain region of India, Burma and few Asian countries \(^3\). This plant is extensively used in India to treat various diseases. The flowers are used in the treatment of hepatic disorders, viral hepatitis, diarrhea, anticonvulsive agent and tonic\(^4\). The roots are useful in treatment of night blindness, piles, ulcers, tumor\(^5\). The gum is powerful astringent. The stem bark possesses antifungal activity. Phytochemical investigation showed the presence of different classes of compounds e.g. flavonoids\(^8\) from flowers, sterols\(^8\) from stem bark. In continuation of our studies on medicinal plants the literature survey reveals that very less work is done on leaves of Butea monosperma. In the present investigation, we are reporting the probable compounds present in the chloroform extract separated by column chromatography and flash chromatography of the chloroform extract of leaves of Butea monosperma.

MATERIALS AND METHODS

Plant material

The Butea monosperma leaves were handpicked in the summer season of year 2007 from the region, which is near to Village-Mhasawad, District-Nandurbar Maharashtra, India. This region is a part of famous Satpuda ranges. The plant specimen were identified and authenticated from Botanical Survey of India, Pune. A voucher specimen (No.BSI/WRC/Tech/2010/1035) has been deposited at the department of botanical Survey of India, Pune.

ABSTRACT

India has rich heritage in medicinal plants in the Ayurvedic and Unani systems of medicine besides use of many plants in folk remedies. Butea monosperma, commonly known as flame of forest, Palas is medicinally important plant of family fabaceae, all part of the tree have medicinal properties. Taking in to consideration the medicinal importance of the plant, the chloroform extract of the plant leaves was first time separated by column chromatography and very advanced technique flash chromatography and analyzed using GC-MS (gas chromatography-mass spectroscopy). It revealed 36 most probable steroid compounds. The major constituents were Beta sitosterol, stigmasterol acetate, Beta sitosterol acetate, Cholesterol, Cholest-3,5-diene, Dihydrotachysterol, Gamma Sitosterol, Stigmaster-3,5-diene, 26-hydroxycholesterol, Retinol.

Extraction and isolation\(^10\)

The powder of Butea monosperma leaves was extracted by Soxhlet apparatus using solvent petroleum ether (60-80°C) and followed by chloroform and methanol. Accurately weighed 50 gm of leaves powder was loaded in Soxhlet’s extractor and defatted with petroleum ether (60-80°C) in 20 batches (50-60 cycles in each batch). Four sets of the Soxhlet extractor were carried out simultaneously. The progress of the extraction was evaluated by applying spot of extract on thin layer chromatography plate. The thin layer chromatography was performed using silica gel plate and the plate was visualized in UV-chamber followed by iodine chamber. The extract thus obtained was filtered and concentrated by rotary evaporator and finally dried at very low pressure. The defatted marc thus obtained was air dried and successively extracted with chloroform and methanol. These extracts were concentrated separately under reduced pressure. The phytochemical tests were performed for each extract\(^1\)\(^\text{-}10\).

The petroleum ether extract confirmed the presence of sterols and triterpenes. Petroleum ether extract of leaves Butea monosperma resulted in the isolation and identification of two known compounds β-carotene and stigmasterol.\(^20\)

The chloroform extract confirms the presence of sterols, triterpenes as glycosides. The methanol extract confirms the presence of flavonoids and proteins.\(^21\)

The chloroform extract was separated by column chromatography using hexane ethyl acetate solvent system. One solid compound isolated by column chromatography while other compounds were semi solid and oily. The semi solid fraction was again separated and purified by very advanced technique flash chromatography.

Flash Chromatography

The purification of natural products remains challenging, lengthy, and tedious. Automated flash chromatography instruments made this process easy. Flash chromatography is basically an air pressure driven hybrid of medium pressure and short column chromatography. This approach was pioneered by W.C. Still at Columbia University\(^22\). Column performance is quite sensitive to the rate of elution and is best with relatively high eluant flow rates. The time required to elute the desired components from the
column is generally so fast (5-10 min) that we have abandoned automatic fraction collectors in favor of a simple rack holding test tubes. Small fractions are typically collected early in the elution with larger ones being collected toward the end of the chromatography. UV detector equipped with instrument conveniently detects separated components.

**GC-MS analysis.**

GC-MS is one of the best techniques to identify the constituents present in the semi solid and oily compounds. Gas chromatography Mass spectrometer Perkin Elmer USA model Auto system XL GC interfaced to a API 20 NL based packed column with flame ionization detector and analyzer- Quadrupole with prefilter was used for mass spectral identification of the isolated components. Equipped with Turbo mass range upto 1200 amu, PE 5MS capillary column (30 m x 0.25 mm x 0.25 μm film thick-nesses) were used for GC-MS. The oven temperature was maintained at 60 °C for 5 min then programmed to 240 °C at 5° per min. The carrier gas was helium, at a flow rate of 1 ml per min, and injection volume was 1 μl. In mass spectrometry electron impact ionization was performed at electron energy of 70 eV.

**Compound Identification.**

By using mass spectrometry the ions or atoms of a compound are separated by their mass. It was used to confirm the atomic structure of compound by comparing the achieved spectra with standard data. Column fractions and isolated compounds were identified by comparison of their mass by comparing the achieved spectra with standard data. Column fractions and isolated compounds were identified by comparison of their mass. It was used to confirm the atomic structure of compound by comparing the achieved spectra with standard data. Column fractions and isolated compounds were identified by comparison of their mass.
mass spectra and retention indices with those published in and contained in the NIST’68 computer library.

RESULTS AND DISCUSSION.

Three fractions separated by column chromatography were subjected for flash chromatographic separation. These are designated as BSP/CHI/1, BSP/CHI/2, BSP/CHI/3.

The fraction BSP/CHI/1 is subjected for GC-MS analysis. The results of GC-Ms analysis of BSP/CHI/1 showed six peaks of Relative Retention Time (Fig. 1). Two peak with RRT 31.87 and 32.86 comprised 18.14 and 69.81 % areas respectively and showed the presence of steriod. The most probable compounds in this area were analyzed using software Wiley 27SL database. The spectra of the compounds were matched with NIST and Willey library. They were confirmed by the study of classical fragmentation pattern, base peak and molecular ion peaks of the compounds. It revealed the presence of 20 compounds in RRT 31.87 (Table 1) and 16 compounds in RRT 32.86 (Table-2). The most probable steroidal compounds present are Beta sitosterol, stigmasterol acetate, Beta sitosterol acetate, Cholesterol, Cholesta-3, 5-diene, Dihydrotachysterol, Gamma Sistitoleterol, Stigmastan-3,5dien, 26-hydroxycholesterol, Retinol.

Beta-sitosterol is known as potent anti-inflammatory antipyretic drug. Dihydrotachysterol (DHT) is among of Vitamin D and was reported to act as systematic effectors of calcium metabolism and promotes calcification. However, high dose of DHT induce the pathogenic and butirien, the antihypertopotentiosic principles of Butea monosperma flowers. . Planta Med, 1986, 52(2), 77-79.

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