ABSTRACT

Medicinal plants are still important source for drug discovery. Herbal medicines have gained importance in recent years because of their efficacy and cost effectiveness. The objective of the present study is to investigate the phytochemical present in the Wrightia tinctoria. The phytochemical analysis was done by preliminary phytochemical test for secondary metabolites and LC-MS for non volatile constituents. The phytochemical test confirms the presents of alkaloids, flavanoids, terpenoids, tannins etc. the LC-MS analysis shows the presence of compounds in which most of them have the medicinal value. The present study on Wrightia tinctoria reveals the presence of various phytochemical constituents like β- amyrin, lupeol, archidoic acid, sitosterol, Uricosuric acid, indirubin and tryptanthrin. Wrightia tinctoria may be a potential source for antibacterial, anticancer, antiulcer, antiinflammatory and larvicidal drug discovery.

KEYWORDS- Wrightia tinctoria, Pharmacognostical analysis, Phytochemical analysis, LC-MS.

INTRODUCTION

Plant still contains a major source for drug discovery in development of synthetic molecules. The use of traditional plant extract in the treatment of various diseases has been flourished. In the early 19th century, when chemical analysis first became available, researchers began to extract and modify the active ingredients from plants. WHO estimated that about 80% of the
world population relays on herbal medicines. Herbal medicines have got importance in recent years because of their efficacy and cost effectiveness. These drugs are invariable single plant extract of mixture of extracts from different parts, which have been carefully standardized for their safety and efficacy. Substances derived from the plants remain the basis for large proportion of the commercial medications used today for heart disease, high blood pressure, pain, asthma and infectious diseases \(^1\). Now days medicinal plants receive more attention to researchers because of their safety and curative property which is due to complex mixtures secondary metabolites.

Wrightia Tinctoria is a small deciduous, perennial tree. It is distributed in south India specifically in Annamalai and Kanyakumari regions of Tamilnadu. It is wildly available in subtropical and tropical areas of the world. Plant grows well near costal areas. Plant belongs to the family, Apocynaceae. Commonly known as dudhi in hindi and asita kutuj in Sanskrit. Leaves of the plant are green in color with acute apex and entire margin and flowers are white in color.\(^2\)-\(^3\)

Ethnobotanical Survey of the Wrightia Tinctoria plant reveals that it is used traditionally as hepatoprotective, antitumour, antifungal, antiepileptic antihypertensive, analgesic, antiinflammatory, antidiarrhoeal, colon protective and antidiabetic.\(^4\)-\(^8\)

Based on the literature review there is no scientific reports on phytochemical investigations of chemical constituents of Wrightia tinctoria leaf. The present study has made an attempt to identify the chemical constituents from areal part of Wrightia tinctoria through LC-MS.

**MATERIALS AND METHODS**

The leaves of Wrightia tinctoria was collected from ghatigaon, Gwalior district, Madhya Pradesh, India and it has been identified and authenticated by Dr A. Arjariya, Professor, Govt. Maharaja PG College, Chatarpur, Madhya Pradesh, India.

The areal parts of the Wrightia tinctoria were collected during October-November month and washed with water. Then the plant material was shade dried for 10 days. The dried plant materials have been powdered using mechanical grinder to get uniform coarse particles. The powdered plant material was stored in poly-ethene air tight container at room temperature for further use.
Preparation of the crude plant extract
The shade dried coarse powdered leaves of Wrightia tinctoria (250 g) was packed in the soxhlet extraction apparatus and extracted with 2 liter of methanol at a temperature of 40-50°C for 72 hrs. The extract was filtered and filtered extract was then concentrated to dryness in rotary evaporator under reduced pressure at temperature of 40°C. the resultant green colour residue was stored in a desiccator for use in subsequent experiments and considered as crude extract. The yield of methanolic extract was 2.9%.

Phytochemical analysis
The preliminary phytochemical screening was carried out in ethanolic extract of Wrightia tinctoria to find out the nature of chemical compounds as per the standard procedure\textsuperscript{[9]} and the phytoconstituents were indentified through LC-MS (Table 1-2).

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Name of the compounds</th>
<th>Name of the test</th>
<th>Status of the substances</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous Extract</td>
<td>Methanol Extract</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Carbohydrates</td>
<td>Fehling's Solution</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Benedict's Solution</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>Mayer’s Solution</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Hager’s Solution</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>Wagner’s Solution</td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Dragendorff’s solution</td>
<td></td>
<td>++++</td>
</tr>
<tr>
<td>3</td>
<td>Steroids</td>
<td>Chloroform+ Acetic Acid+H\textsubscript{2}SO\textsubscript{4}</td>
<td>+++</td>
</tr>
<tr>
<td>4</td>
<td>Tannins and Phenols</td>
<td>10% lead acetate</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>5% Ferric chloride</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>1% gelatin</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponin</td>
<td>Foam test</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Fixed oils &amp; Fats</td>
<td>Spot test</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Gums &amp; Mucilage</td>
<td>Alcoholic Precipitation</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Proteins</td>
<td>Biuret test</td>
<td>++</td>
</tr>
<tr>
<td>9</td>
<td>Flavanoid</td>
<td>NaOH/HCl</td>
<td>+++</td>
</tr>
<tr>
<td>10</td>
<td>Volatile oils</td>
<td>Hydrodistillation method</td>
<td>-</td>
</tr>
</tbody>
</table>


LC-MS Specification
LC column: Reverse PhaseC- 18PUMP; SPD10AVP
Mobile Phase: Water: Methanol
Ionization Mode: Electronic Spray Ionization
Mode: Positive or Negative  
Injection Volume: 10 microlitre  
Flow rate: 2ml/min  
Column temperature: 250°C  
Column Phenomenex RP 18  
Column Dimension: 25 cm×2.5 mm  
LC detection: 254 nm  
m/z range: 50-1000  
Software: Bruker data analysis 4.0

RESULT

Wrightia tinctoria is a small deciduous trees; height 5-8m with young parts glabrous or puberlous. The leaves variable, 6-15 x 3-6cm, elliptic-lanceolate or oblong-lanceolate, acuminate, glabrous or the young leaves puberous beneath, base acute or rounded; main nerves, 6-12 pairs; petioles 3-4mm long. The juice of the tender leaves is used efficaciously in jaundice; also crushed fresh leaves when filled in the cavity of decayed tooth relieve toothache. In Siddha system of medicine, it is used for psoriasis and other skin diseases.

As per our knowledge the some of the chemical constituents of Wrightia tinctoria was not yet scientifically reported. Moreover, identification of chemical constituents in the crude drugs is the basic goal to prove its pharmacological effect behind the folklore uses and ultimate discovery of novel therapeutics. In the present study phytochemical investigation on Wrightia tinctoria methanolic extract has been done by primary phytochemical screening and LCMS analysis. Our study reveals the presence of various natural bioactive compounds shown in table 1 and these chemical compounds also been found in other species of Wrightia tinctoria.

Pharmacological properties of Wrightia tinctoria

The phytochemical investigations on Wrightia tinctoria methanolic extract have revealed the presence of several natural compounds (table 1) and most of them have various biological activities.

Quercetin

Quercetin is a plant pigment (flavanoid). It is 3,3’,4’,5,7 Pent hydroxy flavones.
Flavanoid, plant pigment mostly derived from benzo γ-pyrene. All flavanoids drive 15-carbon skeleton (C₆-C₃-C₆) from two basic metabolites. It is found in many plants and foods such as red wine, onions, green tea, apples and barriers.

Quercetin is used for treating conditions of the heart and blood vessels including hardening of the arteries (atherosclerosis). It is also used for diabetes, caterates, hay fever, peptic ulcer and schizophrenia.

It protects the GI mucosa from acute lesions induced by various experimental models and against different necrotic agents including restraint stress, pylorus: lignation. In gastro-protective action mechanism involve endogenous PAF an increase in mucus production.

Quercetin modulates activity of several molecules such as DNA (for tumor treatment), leucotrienes and prostaglandins.

Lupeol
Lupeol, a phytosterol and triterpene, is widely found in edible fruits and vegetables. In various in vitro and preclinical animal studies suggest that lupeol has a potential to act as anti-inflammatory, anti-microbial, anti-protozoal, anti-proliferative, anti-hypertensive, hepatoprotective and cholesterol lowering agent. Employing various in-vitro and in-vivo models, lupeol has also been tested for its therapeutic efficiency against conditions including wound healing, diabetes, cardiovascular diseases and kidney disease. Lupeol has been found to be pharmacologically effective in treating various diseases under preclinical testing on animal model irrespective of varying route of administration like topical, oral, intraperitoneal and intravenous. It is worthy that lupeol has been reported to selectively target modulates the expression or activity of several molecules such as cytokines IL-2, IL-4, IL-5, ILβ, proteases, α-glucosidase, cFLIP, Bcl-2 and NFkB.

α-amyrin-
It is the Pentacyclic triterpenes, found in natural products. The chemical structure of α-amyrin (3β-hydroxy-urs-12-en-3-ol) is shown in figure. The molecular mass of α-amyrin is C₃₀H₅₀O. it presents an MS ion peak at m/z 426 (M⁺). α-amyrin reduced the mechanical hyperalgesia produced by i.pl. injection of carrageenan, capsaicin, bradykinin, substance P, prostaglandin E₂, 8-Br-Camp. α-amyrin inhibited both neurogenic and inflammatory phases of the overt nociception caused by intraplantar (i.pl.) injection of formalin. Likewise, α,-amyrin given by
i.p., p.o., i.t., or i.c.v. routes inhibits the neurogenic nociception induced by capsaicin. Moreover, i.p. treatment with α-amyrin was able to reduce the nociception produced by 8-bromo-cAMP (8-Br-cAMP) and by 12-O-tetradecanoylphorbol-13-acetate (TPA) or the hyperalgesia caused by glutamate.

β-amyrin-
It is the Pentacyclic triterpenes, found in natural products. The chemical structure of α-amyrin (3β-hydroxy-urs-12-en-3-ol) is shown in figure. The molecular mass of β-amyrin is C₃₀H₅₀O. it presents an MS ion peak at m/z 426 (M⁺). In our LC- MS spectra of derivative of β-amyrin i.e. β-amyrin acetate present which have molecular mass of 469. This is shown in figure 1.

β-amyrin produces consistent peripheral, spinal and supraspinal antinociception in rodents, especially when assessed in inflammatory models of pain. The mechanisms involved in their action are not completely understood but seem to involve the inhibition of protein kinase A- and protein kinase C-sensitive pathways.

Fig. i: LC-MS Spectra of Wrightia tinctoria leaf extract

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>NAME OF COMPOUND</th>
<th>MOLECULAR MASS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lupeol acetate</td>
<td>469.3109</td>
</tr>
<tr>
<td>2</td>
<td>Quercetin Hexaacetate</td>
<td>512.7655</td>
</tr>
<tr>
<td>3</td>
<td>α-amyrin</td>
<td>426.7873</td>
</tr>
<tr>
<td>4</td>
<td>β-amyrin</td>
<td>426.7873</td>
</tr>
</tbody>
</table>

CONCLUSION
The present study of Wrightia tinctoria reveals that various plant chemical constituents Quercetin lupeol, α-amyrin, β-amyrin were isolated from leaves of plant Wrightia tinctoria with the help chromatographic technique LC-MS.
REFERENCES


