Research Article

PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF EXTRACT OF LEAVES OF PERGULARIA DAEMIA LINN.

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ABSTRACT
Pergularia daemia Linn. is an important plant described in Ayurveda. This plant is used for treatment of a number of ailments like urinary disorders and cardiac problems. The leaves of Pergularia daemia Linn. was extracted with different organic solvents in increasing order of polarity. The results of the preliminary investigation revealed the presence of alkaloids, steroids, flavonoids, terpenoids, glycosides, & carbohydrates. The Diethyl ether and Acetone extract were studied phytochemical and four compounds i.e. ß- sitosterol, Stigmesterol were isolated by using thin layer and column chromatography. The chemical structures of the isolated compounds were established by spectroscopic techniques such as UV, IR and NMR spectroscopy. This was again confirmed by coTLC with standard sample.

KEYWORDS Pergularia daemia Linn, leaves, Phytochemical and Spectroscopy.

INTRODUCTION
Ayurveda an ancient system of Indian medicine has recommended in number of drugs from indigenous plant/animal sources for the treatment of several diseases or disorders. [1] Use of plant products, as medicine is inherent in Ayurveda, the ancient Indian system of health care.[2] In industrialized countries, herbal medicine are become increase in popular, However the expanded use of herbal medicine has lead to concerns relating to assurance of safety, quality and efficacy.[3] Secondly many of the antibiotics and synthetic drugs have shown sensitization reaction and other undesirable side effects and there is feeling that the herbal drugs are comparatively safer[4]

Pergularia daemia Linn.(Asclepiadaceae)
Vernacular name. English: Pergularia, Marathi:Utarni.[6,7] A foetid smelling lactiferous twinner found in the plains throughout the hotter parts of the India, ascending to an altitude of 1,000 m. in the Himalayas.[8,9] Widely distributed in the Old World tropics and sub-tropics from southern and tropical Africa through Arabia to Afghanistan, India and Sri-lanka.[10,11] Flowering may occur many times each year between Aug. and Jan. in central India.[12,13] Juice of leaves-used as expectorant in catarrhal affections, in infantile diarrohea given in asthma; applied to rheum. Swellings in combination with lime or ginger; in snakebite.[14, 15] Decoction of leaves used in asthma, their juice in infantile diarrohea combined with lime.[16,17]
This plant explore very little for phytochemical constituents. Thus through worthwhile we are studying this plant for phytochemical investigation.

MATERIAL AND METHODS:
Procurement of drug:
Leaves were collected from local areas of Aurangabad region.

Authentication of drug
Botanical survey of India Pune.
Drying, size reduction & Extraction:
The leaves of *Pergularia daemia* were dried in shade and subjected to reduction to a coarse powder using a grinding mill. Extraction, Phytochemical and Physicochemical Screening: The coarse powder was extracted successively with different organic solvents in increasing order of polarity (i.e., petroleum ether, diethyl ether, chloroform, acetone, and ethanol). The crude extracts were evaporated to dryness in a Rota evaporator under low temperature and reduced pressure. The percentage yield (w/w) of various dried extracts were as: Petroleum ether extract = 0.533 gm, Diethyl ether extract = 1.099 gm, Chloroform extract = 0.314 gm, Acetone extract = 16.70 gm, and Ethanol extract = 14.500 gm. The different extracts of the leaves of *Pergularia daemia* were subjected to preliminary phytochemical screening and physicochemical testing of leaves of *Pergularia daemia*. The qualitative chemical test of all the extracts and physicochemical parameters of the leaves were carried out by using standard procedure (3-8). The results so obtained are mentioned in the table-1&2. The UV spectrum was recorded on ELICO SL-160, India. Infra Red spectrum was recorded in Kbr disc on a Jasco FTIR Spectrophotometer. NMR spectra were recorded on a CPD32 Bruker in MeOD using TMS as internal standard.

Isolation and Separation of compounds:
On the basis of TLC observation of different extracts, Diethyl ether and Acetone extracts of leaves of *Pergularia daemia* were selected for isolation of pure compounds. The total four pure compounds out of which three from diethyl ether & one from acetone extracts were isolated and the structure of these compounds established by chemical and spectroscopic analysis (16,17,18). Diethyl ether extract (10.14 gm) of the leaves of *Pergularia daemia* was subjected to extensive column chromatography over silica gel (60-120 mesh) eluted with solvent Petroleum ether: Chloroform (different ratio). The eluted compounds number 1-3 has the Rf values of 0.651, 0.641 and 0.680 in (Petroleum ether :Diethyl ether, 6:4) respectively (19,20,21). Acetone extract (10.00 gm) of the leaves of *Pergularia daemia* was subjected to extensive column chromatography over silica gel (60-120 mesh) eluted with solvent Petroleum ether: Chloroform (different ratio). The eluted compound number 4 has the Rf value of 0.672 in (Petroleum ether: Diethyl ether, 95:17.5). Finally with the help of spectroscopic techniques like UV, IR, NMR and co-TLC we conclude the results (22,23,24).

RESULTS:
On the basis of phytochemical screening of different successive extracts of the leaves of *Pergularia daemia* we find that this plant have a number of phytochemical constituents like: alkaloids, steroids, flavonoids, terpenoids, glycosides, fats, carbohydrates, and (as shown in table-1). Whereas, on the basis of physicochemical evaluation of the leaves of *Pergularia daemia*, we observed that the plant have total ash value (6.77 % w/w), acid-insoluble ash value (0.33 %w/w), acid-soluble ash value (5.91 %w/w), water-soluble ash value (5.98 % w/w), sulphated ash value (3.44 %w/w), water-soluble extractive value (31.22%w/w), ethanol-soluble extractive value (39.56 %w/w) and moisture content (7.66 %w/w of dry weight), (as shown in
Compound No. 1: The compound to be a sterol as it absorbs the UV light at the wavelength of 257.4 nm, 231.1 nm and the IR spectra of the compound showed characteristic absorption at 3858.81, 3764.39, 3556.73 cm⁻¹ (for hydroxyl group stretching), 2911.23, 2865.81 cm⁻¹ (for CH₂ asym, str), 2349.59 cm⁻¹ (for long side branched substituted alkane), 1750.30 cm⁻¹ (for C=O str), 1656.21 cm⁻¹ (for C=C str), 1542.33 cm⁻¹ (for C-H bend), 1448.34, 1374.21 cm⁻¹ (for CH₃ assy, bend, vib), 1085.49 cm⁻¹ (for alcoholic group), 668.66 cm⁻¹ (for OH bend). The ¹H NMR spectra of the compound revealed the presence of signals at 3.813 ppm (for angular methyl group), 4.136 ppm (for methylene or methine type proton), 5.507 – 5.510 ppm (for OH proton), 5.521 – 5.534 ppm (for methylene type proton), 6.291 – 6.292 ppm (for CH₃ type proton), 6.211 – 6.213 ppm (for protons of sterol nucleus), 6.220 – 6.224 ppm (for sterol nucleus). The ¹C spectra of the compound revealed the presence of signals at 102.78 ppm (for carbon of nucleus at which hydroxyl group is attached), 106.97 ppm (for carbon of nucleus at which side chain is attached), 130.11 ppm (for carbon of nucleus at which unsaturation is present) and a signal at 158.84 ppm (for carbon at which substitution of methyl group is present). On the basis of the above observation, the compound was identified as β-sitosterol and it was further confirmed by peak-by-peak correlation of the IR and NMR spectra of the same compound.

Table No 1: Preliminary Phytochemical Screening of Different Extracts of leaves of *Pergularia daemia*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Extracts</th>
<th>Phytoconstituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Petroleum Ether</td>
<td>Fats, Steroids, Terpenoids</td>
</tr>
<tr>
<td>2</td>
<td>Diethyl Ether</td>
<td>Alkaloids, Steroids,</td>
</tr>
<tr>
<td>3</td>
<td>Chloroform</td>
<td>Flavonoids, Tannins, Glycosides</td>
</tr>
<tr>
<td>4</td>
<td>Acetone</td>
<td>Alkaloids, Flavonoids, Glycosides</td>
</tr>
<tr>
<td>5</td>
<td>Ethanol</td>
<td>Alkaloids, Glycosides, Steroids, Flavonoids, Carbohydrates</td>
</tr>
</tbody>
</table>

Table No 2: Physicochemical Screening of leaves of *Pergularia daemia*

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Experiment</th>
<th>Percentage Yield (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total ash value</td>
<td>6.77</td>
</tr>
<tr>
<td>2</td>
<td>Acid-insoluble ash value</td>
<td>0.33</td>
</tr>
<tr>
<td>3</td>
<td>Acid-soluble ash value</td>
<td>5.91</td>
</tr>
<tr>
<td>4</td>
<td>Water-soluble ash value</td>
<td>4.78</td>
</tr>
<tr>
<td>5</td>
<td>Sulphate ash value</td>
<td>3.44</td>
</tr>
<tr>
<td>6</td>
<td>Water-soluble extractive value</td>
<td>31.22</td>
</tr>
<tr>
<td>7</td>
<td>Ethanol-soluble extractive value</td>
<td>39.56</td>
</tr>
<tr>
<td>8</td>
<td>Moisture content</td>
<td>7.66s</td>
</tr>
</tbody>
</table>
ß-Sitosterol

It also gave Libermann-Buchard Test, indicating steroidal nature of compound. Further, it was confirmed by co-TLC with that of authentic firmed by peak-by-peak correlation of the IR and NMR spectra of the same compound. It also gave Libermann-Buchard Test, indicating steroidal nature of compound. Further, it was confirmed by co-TLC with that of authentic sample.

Compound No.2 The compound to be a sterol as it absorbs the UV light at the wavelength of 258.5 nm, 221.1 nm and the IR spectra of the compound showed characteristic absorption at 3431.87 cm⁻¹ (for OH group); 2931.65 cm⁻¹ (for alkane methylene group); 2841.65 cm⁻¹ (for CH₂ str in case of alkane); 2351.41 cm⁻¹ (for branched substituted chain); 1639.80 cm⁻¹ (for C=C aromatic stretching); 1372.52 cm⁻¹ (for CH bend, vib); 1167.6 cm⁻¹ (for C-Ostr); 679.69 cm⁻¹ (for out of plane bending of OH group). The ¹H NMR spectra analysis of the compound showed characteristic signals at 1.955 – 1.966 ppm (for equatorial protons of cyclic ring); 3.112 – 3.759 ppm (for angular methyl group type protons); 3.451 – 3.798 ppm (for methyl group attached at side chain); 3.9312 – 4.048 ppm (for methylene or methine type protons); 5.871 – 5.884 ppm (for protons of 3H methylene group); 6.188 - 6.194 ppm (for protons of 3H methyl group); 6.313 – 6.327 ppm (for protons of aromatic nucleus); 6.378 – 6.424 ppm (for protons of unsaturated carbon of nucleus). The ¹³C spectra analysis of the compound showed the characteristic signals at 104.10 ppm (for carbon of nucleus at which hydroxyl group is attached); 108.20 ppm (for carbon of nucleus at which hydroxyl group is attached); 112.08 ppm & 141.85 ppm (for unsaturated carbon of side chain); 122.34 ppm (for unsaturated carbon of nucleus); 139.07 ppm (for carbon at which substitution of –CH₃ group is present). On the basis of these observations, the compound under study is assumed as the stigmasterol. It was further confirmed by peak-by-peak correlation of IR and NMR spectra of same compound. It also gave Liebermann- Buchard test indicating steroidal nature of compound. Further it confirmed by co-TLC with that of authentic sample.
DISCUSSION

The present study is completely based on the phytochemical investigation leaves of *Pergularia daemia*. On the basis of phytochemical screening of different successive extracts of leaves of *Pergularia daemia* we conclude that this plant have a number of phytochemical constituents like: alkaloids, steroids, flavonoids, terpenoids, glycosides, fats, carbohydrates, (as shown in table-1). Whereas, on the basis of various physical evaluation we conclude that the plant have total ash value (6.77% w/w), acid-insoluble ash value (0.33%w/w), acid-soluble ash value (5.91 %w/w), water-soluble ash value (4.78%w/w), water-soluble extractive value (3.44%w/w), water-soluble extractive value (31.22%w/w), ethanol-soluble extractive value (39.56%w/w) and moisture content (7.66 %w/w of dry weight), (as shown in table-2). On further phytochemical analysis we separate out the four components in their pure form and on the basis of UV, IR and NMR spectroscopic analysis of these components we have the conclusion that the isolated compounds are sterols. Further on the basis of peak by peak correlation of IR and NMR, we have the confirmation that compound 1 is β-sitosterol, compound 2 is stigmasterol, Further these Compounds were confirmed by co-TLC with that of authentic samples. Further study is in progress to isolate more compounds.

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