Blumea lacera (burn. F) D.C. known as Kukraunda is an important drug described in Ayurveda as bitter, thermo-genic, ant-inflammatory, styptic ophthalamic, digestive, anthelmintic, liver tonic, expectorant, febrifuge, anti-pyretic, diuretic and stimulant. The whole plant was extracted with hexane followed by ethanol, the extract so obtained was fractionated using chloroform to obtain chloroform soluble fraction. Four compounds were isolated from chloroform soluble fraction using column chromatography and characterized by techniques like Infrared spectroscopy (FTIR) and Nuclear magnetic resonance (1H NMR). Isolated compounds were screened for antibacterial activity. The compounds showed significant antibacterial activity against B. subtilis and E. coli.

**INTRODUCTION**

Nature has been a source of medicinal treatment for thousands of years. Now days, the search for new chemotherapeutic agents has been expanded to the whole biodiversity. An increasing number of chemotherapeutic agents are discovered as a result of chemical studies directed towards the isolation of the active substances from plants used in traditional medicine. Despite the great success achieved in natural products chemistry and drug development, we have barely begun to tap the potential of our molecular diversity.

*Blumea lacera* (burn. F) D.C. in an important Ayurvedic, Homeopathic, Unani drug entering into several formulations. The drug is known by the name of *Kukraunda* in India. Blumea in described in Ayurveda as bitter, thermo-genic, anti-inflammatory, styptic ophthalamic, digestive, anthelmintic, liver tonic, expectorant, febrifuge, anti-pyretic, diuretic and stimulant. The plant is used to drive away fleas and other insects in several parts of India. It is described as an anti-scorbutic in West Africa, essential oils from *Blumeahas* been shown analgesic, hypothermic and tranquilizing activities. The phytoconstituents separated in leaves are 5-hydroxy-3,6,7,3’,4’-pentamerthoxy flavone, 5,3’,4’-trihydroxy flavone and an unidentified flavone and in aerial parts compestrol has been described as an anti-scorbutic in West Africa, essential oils from *Blumeahas* been shown analgesic, hypothermic and tranquilizing activities.

**MATERIALS AND METHODS**

**Plant material**

Whole plants were collected from the fields around the campus of Indira Gandhi Krishi Vishwavidyalaya (Agriculture University) Raipur, Chhattisgarh, Indi during the months of November and December 2007, identified and authenticated by Dr. Pushpa Patel, Govt. PG College, Khargon, MP.

**Chemical and reagent**

All the chemicals and reagents used in the study were of analytical grade obtained from MERCK and their boiling points were compared with the available literature values.

**Extraction and fractionation**

The collected plant material was segregated from the extraneous material and dried in shade to prevent the loss of active ingredients. About 1 kg of shade dried coarsely powdered drug was extracted with n-hexane followed by 95% ethanol in a soxhlet apparatus by continuous hot extraction. Ethanol extract was filtered and concentrated to dryness under reduced pressure to obtain semisolid mass. A portion of ethanol extract was fractioned with chloroform. The solvent was removed by distillation to obtain the fraction. Ethanol and chloroform fraction were subjected to preliminary phytochemical analysis to detect the presence of various types of phytoconstituents.

**Spectral analysis and structural elucidation**

The identification of a molecule was done through the interpretation of the data obtained from spectroscopic analysis. The functional groups were identified by Infrared spectroscopy (FTIR). 1H NMR analysis was performed to determine the chemical shift values of the protons as well as the number of neighboring protons from the splitting of the multiplets. The determination of carbon, hydrogen,
and nitrogen (CHN analysis) was performed to check sample purity, and in conjunction with mass spectrometry and NMR data, to characterize the compound.

Antibacterial activity

All isolated phytoconstituents were screened for in vitro antimicrobial activity by agar diffusion cup plate method following standard protocol. All the compounds were screened for antibacterial activity at 50 and 100 µg/ml concentrations against the strains Escherichia coli and Bacillus subtilis. The melted nutrient agar was cooled to 45°C, mixed with culture media by gentle shaking and then poured onto a sterilized petri dish and allowed to solidify. Cups were made by punching the agar surface with the help of a sterile cork borer (6 mm). Suspension of isolated compounds in the strength of 50 and 100 µg/ml was prepared using distilled water as solvent and added to each bore. The drug was allowed to diffuse for about 4 h into the agar medium before the addition of bacterial suspension. Norfloxacin was used as standard drug. The inoculated plates were then incubated at 37°C for 48 h. The zone of inhibition for each isolated compound was recorded and measured in millimeters (mm).

RESULTS AND DISCUSSION

Phytochemical and chromatographic studies

Preliminary phytochemical studies of Blumea lacera revealed the presence of alkaloids, carbohydrates, glycosides, phytosterols, phenolic compounds and flavonoids. Thin layer chromatographic studies of the chloroform fraction revealed 4 phytoconstituents of Rf 0.75, 0.56, 0.37 and 0.31 under sunlight and 7 phytoconstituents as Rf 0.82, 0.70, 0.58, 0.30, 0.27, 0.23 and 0.17 when exposed to iodine vapours.

Isolation of phytoconstituents

Four compounds were isolated by silica gel column chromatography from chloroform soluble fraction of ethanol extract of Blumea lacera. Compound 80 was isolated as a colourless oily liquid. Its IR peaks (cm⁻¹) at 3425 (free -OH of an aliphatic moiety), 2923 (aromatic C-H), 1734 (conjugated C=O) 1653, 1377 (aromatic C-C) 1460 (ά-CH₂), 1260 and 1094 (aryl ether C-O-) and NHMR values δ 4.12m br (-OCH₂CH₃), 4.17s, 6.14s (-OH) Confirmed that it is a derivative of protocatechuic acid with an aliphatic moiety having a free hydroxyl group.

Compound 81 gave positive test with dragendorff’s reagent and exhibited IR peaks (cm⁻¹) at 3429 (primary amide linkage), 2921 (aliphatic –CH₃), 2852(–CH₂), 1737 (-COO-NH₂ ), 1664 (-CONH₂ ), 1459, 1373 (CH₃–CH₂ bending) 1176, 1163 (C-N stretching) and 1051 (-C-O-C). Its ¹HNMR peaks were atΔH (ppm) 0.82dd (J=1.5, 1.0Hz, -CH₃), 1.29t (CH₂–CH₂), 2.15s (-COCH₃), 3.47m br (-CONH₂ ), 7.26s (Ar-H).

Compound 82 exhibited IR Peaks (cm⁻¹) at 3413, 2922, 2852, 1664,1379, 1077, 658 and 1 H NMR peaks (ppm) at δ 8.10 s, 0. 86s br, 1.25s br, 1.61s, 1.95s, 2.13s, 2.29s br, 2.51d (J=1.6 Hz), 2.77d br (J=14.0Hz), 3.7t (J=4.0) were shown some similarity to 82 The IR and 1HNMR values of these two compounds matched with published literature for compounds with primary and secondary amide moieties respectively.

Compound 83 showed IR peaks (cm⁻¹) 3362 (-OH), 1602-1003(Characteristic for flavonoids skeleton) characterized the compound shoving a flavonoids moiety39, and 1HNMR values δ 4.12m br (-OCH₂CH₃), 4.71S, 6.14S (-OH) Confirmed that it is a derivative of phytosterols with an aliphatic moiety having a free hydroxyl group.

Antimicrobial activity

The determination indicates whether the organism is 'sensitive' or 'resistant' to the agent. The organism being 'sensitive' means that the antimicrobial agent at clinically attainable concentration inhibits growth of the organism; 'resistant' means that the growth of the organism is not inhibited. The test results of this study are depicted in Table 1.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>Diameter of zone of inhibition in (mm)</th>
<th>B. subtilis (Gram +ve)</th>
<th>E. coli (Gram -ve)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>B. subtilis (Gram +ve)</td>
<td>E. coli (Gram -ve)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>50 µg/ml</td>
<td>100 µg/ml</td>
<td>50 µg/ml</td>
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<td>6</td>
<td>Control</td>
<td>NI</td>
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</tbody>
</table>

Table 1: Table shows antibacterial activity (zone of inhibition) of isolated compounds.

CONCLUSION

The plant Blacera (burm.f) D.C. compositae has been studied to compare and give report on phytochemical investigations. The preliminary phytochemical investigation showed the presence of alkaloids, carbohydrates, steroids phenol, flavonoids, and glycosides in different extracts further TLC was performed for extract for identification of constituents and compound were isolated from chloroform soluble fraction by using column chromatography. The spectral analysis (NMR, IR) indicate that the isolated fraction having constituents may be phytosterols, primary and secondary amide, derivatives of protocatechuic acid and flavonoids.

The phytochemicals study of Blacera reveals that the chloroform soluble fractions of its ethanol extract constituted major classes of bioactive constituents. The chloroform soluble fraction showed significant antimicrobial activity. Hence we may conclude that Blacera may prove to be a valuable medicinal plant with some bioactive constituents and through further studies we may able to isolate more phytoconstituents, which can be medicinally important.

ACKNOWLEDGEMENT

The authors thank Dr. Pushpa Patel of Govt PG College Khargone for authenticating the plant material The authors also thank GRY Institute of Pharmacy Khargone for support and help provided in carrying out this work.

REFERENCES


