Pharmacognostic and phytochemical evaluation of eight crude extracts used in complementary and alternative system of medicine

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Abstract
The current study focuses the pharmacognostic evaluation of Arnica montana, Cicuta virosa, Digitalis purpurea, Sambucus nigra, Thuja occidentalis, Urtica urens, Arctostaphylos uva-ursi and Apis mellifera. Microscopic and histological examination, color reaction tests; thin-layer chromatography and fourier transform infra-red spectroscopy were carried out on crude extracts. Microscopic and histological examination exhibited the presence and absence of different cells or tissues (diagnostic characters). The presence of carbohydrates, proteins, steroids, saponins, tannins, alkaloids and tri-terpenes were detected in crude extracts of under studied plants and insect. In thin-layer chromatography (TLC) the presence of different chemical compounds were observed, their Rf values were calculated. Fourier transform infra-red (FT-IR) spectroscopy revealed the presence of major functional groups in each extract. Each plant material was also observed under microscope and all major diagnostic characters were recorded.

Keywords: Thin-layer chromatography, fourier transform infra-red spectroscopy, morphological studies, microscopic studies, chemical constituents

Introduction
Drugs of natural origin have played a significant role in prevention and alleviation of different pathologies afflicting mankind since ancient times. The natural origin drugs are preferred more nowadays due to the lesser side-effects associated with its use, more structural diversity leading to the new drug molecules discovery and present validated techniques for pharmacognostic evaluation as recommended by World Health Organization. Pharmacognostic and phytochemical evaluation is mandatory for understanding the therapeutic efficacy of natural origin drugs and validating the efficacy and safety profile of the drugs [14].

The exploration of hidden properties of phyto-medicine is essential for emerging trends in medicine. Therefore preliminary pharmacognostic and phytochemical analysis of crude extracts of Apis mellifera (Apidae), Arctostaphylos uva-ursi (Ericaceae), Arnica montana L. (Asteraceae), Cicuta virosa (Apiaceae), Digitalis purpurea L. (Scrophulariaceae), Sambucus nigra L. (Caprifoliaceae), Thuja occidentalis L. (Cupressaceae) and Urtica urens L. (Urticaceae) were carried out.

Material & Method
Collection and Preparation of Crude extract
The medicinal plants; Arctostaphylos uva-ursi, Urtica urens, Arnica montana, Cicuta virosa, Digitalis purpurea, Sambucus nigra, Thuja occidentalis and insect drug Apis mellifera were collected from different places; they were identified by Prof. Dr. Mansoor Ahmad, Research Institute of Pharmaceutical Sciences, University of Karachi, Karachi-Pakistan and the voucher specimen (FSMP-02-09) was deposited in the herbarium of Research Institute of Pharmaceutical Sciences, University of Karachi. The plants and insect drugs were shade dried, chopped and soaked in 3L of ethanol. Alcoholic extract was prepared by percolation. This procedure was repeated thrice. Extract was concentrated by rota-evaporator (Buchi-Rotary Evaporator, Switzerland, model # B490) at 40 °C. The yield of the extract was used for further experiments.

Chemicals & Reagents
All the chemicals and reagents used were of analytical grade and purchased from Merck (Germany).
Microscopic examination of crude drugs
Microscopic examination of crude drugs in entire and powdered form was carried out using microscope to detect diagnostic microscopic characters like cellular tissues, calcium oxalate crystals, trichomes, stomata, starch granules. Chloral hydrate, glycerin and iodine solutions were used to differentiate cellular structures [2, 5].

Chemical Identification Tests
For the detection of chemical constituents the following tests were performed, terpenes, alkaloids, tannins (lead acetate and Phenazone), saponins (froth and precipitate tests), carbohydrates (Molish test), sterols and proteins tests [6].

Thin Layer Chromatography (TLC)
Thin-layer chromatography was carried out using two solvent systems: ethyl acetate-methanol-water (100: 16.5: 13.5) and chloroform-methanol-water (80: 20: 2) respectively. The chromatograms were observed under UV lamp and Rf values were calculated [7].

FT-IR spectroscopy
FT-IR analysis was performed on Nicolet Avatar 330 FT-IR, USA [8].

Results
The results of the phytochemical analysis of eight drug extracts are as follows:

Macroscopic and microscopic evaluation
Macroscopic and microscopic evaluation of an insect drug *Apis mellifera* and plant drugs; *Arctostaphylos uva-ursi*, *Urtica urens*, *Arnica montana*, *Cicuta virosa*, *Digitalis purpurea*, *Sambucus nigra*, *Thuja occidentalis* were carried out and are shown in figures 1-8 respectively.

**Fig 1:** *Apis mellifera* L. and its diagnostic features: (a) Head (front) (b) Brain of *A. mellifera* (c) *A. mellifera* head showing distinct compound eyes and antennae (d) Macroscopic view of *A. mellifera* (e) Microscopic over view of a whole *A. mellifera* section.

**Fig 2:** *A. uva-ursi* L. and its diagnostic features: (a) mesophylls near mid-rib containing calcium oxalate crystals; (b) sunken stomata in epidermis; (c) calcium oxalate crystals; (d) flower; (e) longitudinal section of leaf; (f) fibres; (g) gynoecium; (h) twig with flower; (i) unicellular trichomes.

**Fig 3:** *Arnica montana* L. and its diagnostic features: (a) pollen grains; (b) ray florate and disc florate; (c) pollen grains; (d) pappus borste; (e) unicellular trichomes; (f) fruit knot on epidermis; (g) multicellular trichome (h) glandular trichomes; (i) multicellular trichomes; (j) epidermis of corolla containing oil globules; (k) endothecium; (l) fruit peel with phytomelan.

Fig 3: *Arnica montana* L. and its diagnostic features: (a) pollen grains; (b) ray florate and disc florate; (c) pollen grains; (d) pappus borste; (e) unicellular trichomes; (f) fruit knot on epidermis; (g) multicellular trichome (h) glandular trichomes; (i) multicellular trichomes; (j) epidermis of corolla containing oil globules; (k) endothecium; (l) fruit peel with phytomelan.
**Fig 4:** *Cicuta virosa* L. and its diagnostic features: (a) Transverse section of *C. virosa* root; (b) transverse section of endosperm; (c) endosperm; (d) fruit; (e) twig with leaves, flowers and fruits; (f) longitudinal section of *C. virosa* root.

**Fig 5:** *Digitalis purpurea* and its diagnostic features: (a) transverse section of midrib (b) transverse section of *D. purpurea* leaf (c) glandular trichome with uniserrate stalk (d) glandular trichome with bicellular heads (e) lower epidermis (f) leaf (g) upper epidermis

**Fig 6:** *Sambucus nigra* and its diagnostic features: (a) epidermis containing sieve tubes, mesophyll cells, calcium oxalate crystals and starch grains; (b) endothecium; (c) unicellular and grandular trichomes; (d) the vascular system; (e) sunken stomata in lower epidermis; (f) *S. nigra* plant; (g) pollens.

**Fig 7:** *Thuja occidentalis* L. and its diagnostic features: (a) twig of *T. occidentalis*; (b) sieve tubes; (c) transverse section of leaf; (d) sunken stomata in epidermis; (e) schizogenous cavities.
Fig 8: *Urtica urens* L. and its diagnostic features: (a) transverse section of leaf containing sieve tubes and schizogenous cavities; (b) different types of trichomes; (c) trichomes with epidermis; (d) sieve tubes; (e) schizogenous cavities; (f) sunken stomata in lower epidermis; (g) whole plant.

Fig 9: Shows the chromatogram of the eight drugs (extracts) in solvent system chloroform-methanol-water (80:20:2) at short wave length 254 nm.

Fig 10: Shows the chromatogram of the eight drugs (extracts) in solvent system ethyl acetate-methanol-water (100:16.5:13.5) at long wave length 366 nm.

Fig 11: Shows the FT-IR spectra of *A. mellifera* drug extract (significant functional groups peaks include OH, CH, benzene, C-O-C).
Fig 12: Shows the FT-IR spectra of *A. uva-ursi* drug extract (significant functional groups peaks include OH, CH, benzene, C-O-C).

Fig 13: Shows the FT-IR spectra of *A. montana* drug extract (significant functional groups peaks include OH alcoholic, =C-H, CH, OH acidic, C=O, benzene ring, C-O-C).

Fig 14: Shows the FT-IR spectra of *C. virosa* drug extract (significant functional groups peaks include OH, CH, benzene, C-O-C).
Fig 15: Shows the FT-IR spectra of *D. purpurea* drug extract (significant functional groups peaks include OH, CH, benzene, C-O-C).

Fig 16: Shows the FT-IR spectra of *S. nigra* drug extract (significant functional groups peaks include OH, CH, benzene, C-O-C).

Fig 17: Shows the FT-IR spectra of *T. occidentalis* drug extract (significant functional groups peaks include OH, CH, benzene, C-O-C).
Fig 18: Shows the FT-IR spectra of *U. urens* drug extract. (Significant functional groups peaks include OH, CH, benzene, C-O-C).

**Table 1:** Chemical identification tests of the eight crude extracts using different chemical reagents.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>DRUG NAME</th>
<th>TANNINS (lead acetate test)</th>
<th>SAPONINS (froth formation)</th>
<th>ALKALOIDS</th>
<th>CHO (Molish test)</th>
<th>PROTEINS</th>
<th>STEROLS</th>
<th>STEROIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Apis mellifera</em></td>
<td>Very slight ppts</td>
<td>Froth formation</td>
<td>No orange ppts</td>
<td>Color changes to purple</td>
<td>Soluble</td>
<td>No ppts</td>
<td>No Purple ring formation Purple color Golden brown</td>
</tr>
<tr>
<td>2.</td>
<td><em>Arnica montana</em></td>
<td>Very slight ppts</td>
<td>No froth formation</td>
<td>No orange ppts</td>
<td>Color changes to purple</td>
<td>Soluble</td>
<td>No ppts</td>
<td>Purple ring formation Purple color Brown</td>
</tr>
<tr>
<td>3.</td>
<td><em>Cicuta virosa</em></td>
<td>No ppts</td>
<td>Froth formation</td>
<td>No orange ppts</td>
<td>Color changes to purple</td>
<td>Soluble</td>
<td>No ppts</td>
<td>Purple ring formation Purple color Golden yellow</td>
</tr>
<tr>
<td>4.</td>
<td><em>Digitalis purpurea</em></td>
<td>No ppts</td>
<td>Froth formation</td>
<td>No orange ppts</td>
<td>Color changes to purple</td>
<td>Soluble</td>
<td>No ppts</td>
<td>Purple ring formation Purple color Brown</td>
</tr>
<tr>
<td>5.</td>
<td><em>Sambucus nigra</em></td>
<td>Slight fine ppts</td>
<td>Froth formation</td>
<td>No orange ppts</td>
<td>Color changes to purple</td>
<td>Soluble</td>
<td>No ppts</td>
<td>Purple ring formation Blackish-green color Orange</td>
</tr>
<tr>
<td>6.</td>
<td><em>Thuja occidentalis</em></td>
<td>No ppts</td>
<td>Froth formation</td>
<td>No orange ppts</td>
<td>Color changes to purple</td>
<td>Soluble</td>
<td>No ppts</td>
<td>Purple ring formation Purple color Brown</td>
</tr>
<tr>
<td>7.</td>
<td><em>Urtica urens</em></td>
<td>No ppts</td>
<td>Excessive froth formation</td>
<td>Orange ppts</td>
<td>Color changes to purple</td>
<td>Soluble</td>
<td>No ppts</td>
<td>No Purple ring formation Blackish-purple color Brown</td>
</tr>
<tr>
<td>8.</td>
<td><em>Arctostaphylos uva-ursi</em></td>
<td>Fine ppts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2:** RF value of the eight crude extracts

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of drugs</th>
<th>RF value of drugs in different solvent systems</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHCl₃: MeOH: H₂O (80:20:2)</td>
<td>Ethyl acetate: MeOH: H₂O (100:16.5:13.5)</td>
</tr>
<tr>
<td></td>
<td>At 254 nm</td>
<td>At 365 nm</td>
</tr>
<tr>
<td>1</td>
<td><em>Arnica montana</em></td>
<td>0.03 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.07 0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.17 0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.26 0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.30 0.42</td>
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<tr>
<td></td>
<td></td>
<td>0.36 0.54</td>
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<tr>
<td></td>
<td></td>
<td>0.47 0.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.74 0.76</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.80 0.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.66 0.66</td>
</tr>
<tr>
<td>2</td>
<td><em>Thuja occidentalis</em></td>
<td>0.03 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.05 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.11 0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.16 0.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.28 0.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.33 0.56</td>
</tr>
</tbody>
</table>
Results of chemical identification tests performed on eight respective crude drugs are tabulated in table 1.

**Chemical Identification Tests**

**Thin-layer chromatography**

Chromatograms and Rf values of the eight crude extracts are shown in fig. 9-10 and table 2.

**Fourier Transform Infra-red Spectroscopy**

Fourier transform infra-red spectra exhibiting the functional groups of each studied crude drug are shown in figures 11-18.

**Discussion**

The crude extracts studied in our present research are being used in complementary and alternative system of medicine since long. This research work is carried out to meet the requirements of World Health Organization and other regulatory bodies concerning standardization of natural-origin drugs on same pattern as synthetic drugs. Preliminary pharmacognostic studies (macroscopy, microscopy, color reaction tests, thin layer chromatography and fourier transform infra-red spectroscopy) were carried out on the eight crude drugs respectively to authenticate the presence of chemical constituents in them reported by other researchers [9-16].

**Conclusion**

Our pharmacognostic and phytochemical evaluation study will facilitate in carrying out further pre-clinical and clinical trials on the selected eight drugs of natural origin.

**Abbreviations Used**

TLC = Thin-layer chromatography  
FT-IR = Fourier transform infra-red  
Rf = retardation factor  
HCl = hydrochloric acid  
H₂SO₄ = Sulphuric acid  
A. mellifera = *Apis mellifera*  
*A. uva-ursi* = *Arctostaphylos uva-ursi*  
A. montana = *Arnica montana*  
C. virosa = *Cicuta virosa*  
D. purpurea = *Digitalis purpurea*  
*S. nigra* = *Sambucus nigra*  
T. occidentalis = *Thuja occidentalis*  
U. urens = *Urtica urens*

**Conflict of Interest**

Authors declared no conflict of interest.
References