HPTLC Finger Print Profile of Dried Fruit of *Physalis alkekengi* Linn.

**Rasheed NMA**, **Shareef MA**, **Mushtaq Ahmad**, **Gupta VC**, **Shamsul Arfin** and **Shamshad AK**

**INTRODUCTION**

The dried fruits of *Physalis alkekengi* Linn (Fam. Solanaceae) are known differently in different languages such as Urdu: Kaknaj, Papotan; English: Winter cherry, Bladder cherry; Hindi: Kakanaja and it occurs in Southern Europe through China to Japan but does not occur in India, even though fruits are available in the Indian markets, in the name of Kaknaj.¹²,³ A diffuse perennial, with glabrous or slightly pubescent stems, whitish flowers and reddish fruits, 4–12 cm long, with blood red, inflated calyx, often grown as an ornamental plant.

The berries are very juicy and have an acidulous bitter taste. The fruits as well as leaves contain an amorphous bitter principle.⁴-⁶ The fruits also contain vitamin C, a carotenoid pigment (physalein) and probably an alkaloid.⁷ They are reported to be diuretic, febrifuge, hydroagogue and vermifuge.⁸,⁹ Berries contain malic and citric acids, a volatile matter, sugar, mucilage, pectin, woody fibre and water. They act on liver and are diuretic, alterative, anthelmintic and laxative, useful in kidney and urinary diseases and also in skin diseases.¹⁰

Castellani and Browning tried the use of an ethereal extract of berries in 5 grain doses, 3–4 times a day in cases of typical sprue in conjunction with the usual milk diet and alkaline treatment and found improvement in general condition of patient.

**ABSTRACT:** Dried fruit of *Physalis alkekengi* Linn. (Fam. Solanaceae) is called as Kaknaj in the Unani system of medicine and used as diuretic, antisepctic, corrective of liver and sedative. Standardization of this drug is the key factor in regulating the therapeutic efficacy. Organoletic parameters are not enough in establishing the standards of herbal drugs. Instrumental analysis of herbal drugs, which gives a more concrete picture regarding the qualitative and quantitative aspects of bioactive molecules, is widely accepted in the quality assessment of herbal drugs. However, such work related to traditional herbal medicines is lacking or in infantile stage. In the present study, morphological and physicochemical parameters and HPTLC finger print studies of *Physalis alkekengi* have been carried out and the results provide referential information for standardization.

**Keywords:** Fluorescence analysis, HPTLC analysis, Physicochemical parameters.

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*Author for Correspondence:* Email: rasheed_chem@yahoo.co.in; Phone: +91-9959840785

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Berries are remedy for anemia and rheumatism as they contain salicylic salts and rich in alkaline and mineral salts, viz., lime and phosphates.[11]

However no scientific standard parameters are available to determine the quality and genuineness of the drug. In the present study an attempt was made to standardize the drug with respect to HPTLC technique to provide beneficial information in regarding the standardization according to WHO guidelines.

**MATERIALS AND METHODS**

**Collection of Material**

Genuine *Physalis alkekengi* Linn was collected from the authorized agent in the local market of Hyderabad in Andhra Pradesh state of India. After confirmation of its botanical identity with the help of botanist, the fruits were subjected for morphological, physicochemical and HPTLC finger print studies.

**Morphological studies:** Routine procedures were followed for studying the external morphology of the drug including macroscopic and microscopic studies.

**Physicochemical parameters:** Physicochemical parameters such as ash and extractive values were determined according to the methods described in Unani Pharmacopoeia of India, 2008.[1]

**Fluorescence analysis:** Fluorescence analysis was carried out as per the method described by Trease and Evans.[12]

**HPTLC Apparatus**

HPTLC system composed of an automatic TLC applicator, basic marathon autosampler, densitometer CD 60 of DESAGA Sarstedt Gruppe system and UV-Vis Cabinet for recognition of spots. The chromatographic and the integrated data were recorded using computer based software DESAGA ProQuant 1.6 Version.

**Preparation of extract of the drug sample for HPTLC:**

The dried fruit was crushed with mortar and pestle to form coarse powder. Five grams powder of drug was macerated in 100 ml of methanol in a stoppered conical flask and was kept for 2 hours with gentle shaking in regular intervals. Later the contents were filtered through Whatman no. 41 filter paper and the filtrate was evaporated to get 20 ml of solution. The solution thus obtained was used as sample for the determination of components.

**Development of HPTLC technique:**

**Development and determination of the solvent system:**

- **Sample applied:** Sample drug solution of *Physalis alkekengi* about 10 µl.
- **Solvent system:** Toluene: ethyl acetate: methanol (7: 2: 1)
- **Migration distance:** 95 mm
- **Scanning wavelength:** 366 nm

The sample was spotted with the help of automatic TLC applicator system of the DESAGA Sarstedt Gruppe on precoated aluminum sheets of silica gel 60 F$_{254}$ (Merck). After trying with various solvent systems with variable volume ratios, the suitable solvent system as stated above is selected in its proportional ratio and developed in the twin-trough glass TLC chamber to the

**TABLE 1 :** Peak area and $R_f$ values of the components of the drug.

<table>
<thead>
<tr>
<th>PEAK NO.</th>
<th>NAME</th>
<th>MIGRATION DISTANCE (mm)</th>
<th>AREA PERCENTAGE (%)</th>
<th>HEIGHT (mm)</th>
<th>$R_f$ VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Component 1</td>
<td>15.0</td>
<td>81.4</td>
<td>1351.32</td>
<td>0.06</td>
</tr>
<tr>
<td>2</td>
<td>Component 2</td>
<td>26.3</td>
<td>1.4</td>
<td>81.70</td>
<td>0.20</td>
</tr>
<tr>
<td>3</td>
<td>Component 3</td>
<td>36.4</td>
<td>0.3</td>
<td>8.31</td>
<td>0.31</td>
</tr>
<tr>
<td>4</td>
<td>Component 4</td>
<td>44.5</td>
<td>2.0</td>
<td>53.25</td>
<td>0.41</td>
</tr>
<tr>
<td>5</td>
<td>Component 5</td>
<td>51.2</td>
<td>0.4</td>
<td>9.91</td>
<td>0.48</td>
</tr>
<tr>
<td>6</td>
<td>Component 6</td>
<td>55.6</td>
<td>0.2</td>
<td>9.62</td>
<td>0.54</td>
</tr>
<tr>
<td>7</td>
<td>Component 7</td>
<td>71.1</td>
<td>4.5</td>
<td>110.09</td>
<td>0.72</td>
</tr>
<tr>
<td>8</td>
<td>Component 8</td>
<td>76.7</td>
<td>1.1</td>
<td>96.25</td>
<td>0.78</td>
</tr>
<tr>
<td>9</td>
<td>Component 9</td>
<td>84.2</td>
<td>2.4</td>
<td>73.43</td>
<td>0.87</td>
</tr>
<tr>
<td>10</td>
<td>Component 10</td>
<td>90.1</td>
<td>6.4</td>
<td>163.95</td>
<td>0.94</td>
</tr>
</tbody>
</table>
maximum height of the plate so that the components are separated on the polar phase of silica gel and mobile phase of solvent system.

After developing, the TLC plate was dried completely and the spots were observed with UV cabinet system for detection of spots at 366 nm. Further it was scanned with densitometer CD60 of DESAGA Sarstedt Gruppe system under the UV range of 366 nm for maximum number of components. A corresponding densitogram was obtained in which peaks were appeared for the spots corresponding to \( R_f \) values of each component.

**RESULTS AND DISCUSSION**

**Organoleptic parameters**

The fruit of *Physalis alkekengi* Linn is reddish brown in colour, bitter in taste with characteristic odour.

**Morphology of Physalis alkekengi Linn-fruit**

**Macroscopic**: Red coloured berry, globose, about 1 to 1.5 cm in diameter, outer surface wrinkled, with dried flesh; unilocular, completely packed with seeds, overlapping, centrally oriented, insignificant placenta present; seeds 1.8 to 2.2 mm, numerous, flat, with curved embryo, hilum in the concavity; fruit sweet and sour in taste (Fig. 1).

**Microscopic**: Cuticle present; fruit wall not distinguishable as epicarp, mesocarp and endocarp clearly; the outer layer consists of a single layer of non lignified, thin walled cell with brown contents; below this are a few layers of horizontally oriented cells with orange contents and loosely arranged layers of parenchyma, with mucilage cells; inner layers of the fruit wall and the placentae proliferate into the locule packed with minute seeds.

**Powder**: The powder is brownish-orange in colour; shows sclereids, parenchymatous cells, endospermic parenchymatous cells rich in oil and aleurone grains.

**Physicochemical Characters**

The physicochemical parameters of the drug such as total ash, water-soluble ash, acid insoluble ash, alcohol soluble matter and water-soluble matter (% w/w) were tabulated in table-2.

**Fluorescence analysis of powdered drug**

The fluorescence analysis of the powdered drug upon treatment with different reagents and observation in UV short and long wavelength regions and also in visible light showed corresponding colours in the solution as described in table-3.

**HPTLC ANALYSIS**

It is evident from table-1 that there are ten spots with \( R_f \) values 0.06 (dark brown), 0.20 (blue), 0.31 (Fig. 1).
TABLE 3: Fluorescence analysis of powdered drug upon treatment with different reagents.

<table>
<thead>
<tr>
<th>S. NO</th>
<th>REAGENTS</th>
<th>UV LIGHT</th>
<th>VISIBLE LIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SHORT 254 nm</td>
<td>LONG 366 nm</td>
</tr>
<tr>
<td>1.</td>
<td>Powder as such</td>
<td>Blackish</td>
<td>Light yellow</td>
</tr>
<tr>
<td>2.</td>
<td>Powder treated with 1N NaOH in methanol</td>
<td>Dark green</td>
<td>Light Blue</td>
</tr>
<tr>
<td>3.</td>
<td>Powder treated with 1N NaOH in water</td>
<td>Black</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>4.</td>
<td>Powder treated with 1N HCl</td>
<td>Dark black</td>
<td>Light green</td>
</tr>
<tr>
<td>5.</td>
<td>Powder treated with 50% HNO₃ aqueous</td>
<td>Grey</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>6.</td>
<td>Powder treated with 50% H₂SO₄ aqueous</td>
<td>Dark black</td>
<td>Light blue</td>
</tr>
</tbody>
</table>

TABLE 4: Fluorescence analysis of powdered drug extracts with different solvents.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>EXTRACTION SOLVENT</th>
<th>UV LIGHT</th>
<th>VISIBLE LIGHT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>254 nm</td>
<td>366 nm</td>
</tr>
<tr>
<td>1.</td>
<td>Petroleum ether</td>
<td>Yellow</td>
<td>Black</td>
</tr>
<tr>
<td>2.</td>
<td>Chloroform</td>
<td>Black</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>3.</td>
<td>Ethyl acetate</td>
<td>Black</td>
<td>Pale yellow</td>
</tr>
<tr>
<td>4.</td>
<td>Alcohol</td>
<td>Black</td>
<td>Blue</td>
</tr>
<tr>
<td>5.</td>
<td>Acetone</td>
<td>Black</td>
<td>Pale yellow</td>
</tr>
</tbody>
</table>

(brown), 0.41 (blue), 0.48 (light blue), 0.54 (blue), 0.72 (blue), 0.78 (light brown), 0.87 (light blue) and 0.94 (light green) as shown in figure 3 indicating the occurrence of at least ten different components in the methanolic extract. It is also clear from table-1 and the chromatogram as shown in figure-2, that out of ten components, the component with Rf value 0.06 (dark brown) and component at Rf value 0.94 (light green) were found to be more predominant as the percentage area is more with 81.4% and 6.4% respectively. And remaining components were found to be very less in quantity as the percentage area for all the spots was less than 4.5%. Thus the developed chromatogram will be specific.

FIGURE 2: Multiwavelength scan carried out in the range of 250 nm to 400 nm showing variation in number of peaks at different wavelengths.
with selected solvent system and constant R<sub>f</sub> values, and serve the better tool for standardization of the drug.

Chemical compounds, some of which are having therapeutic activities, are species specific and vary from species to species. These compounds can be visualized by developing chromatograms. Characteristic TLC/HPTLC finger printing of a particular plant species will not only help in the identification and quality control of a particular species but also provide basic information useful for the isolation, purification, characterization and identification of marker chemical compounds of the species. Thus the present study will provide sufficient information about therapeutic efficacy of the drug and also in the identification, standardization and quality control of medicinal plant.

**CONCLUSION**

In addition to organoleptic parameters, chromatographic finger printing of herbal medicines will be helpful in the identification and quality control of the drug and ensure therapeutic efficacy. HPTLC analysis of dried *Physalis alkekengi* Linn. can provide standard finger prints and can be used as a reference for the identification and quality control of the drug.

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**REFERENCES**


