Phytochemical Screening and Analgesic activity of “Kantkari”

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Solanum xanthocarpum schrad. & wendl., commonly known as Kantkari, belonging to Family Solanaceae, contains steroidal glycoalkaloid solasodine, β-solamargine and solasonine. The indigenous uses of plants also indicate anti-inflammatory, Antispermatogenic, Antidiabetic, Antiasthmatic, Molluscidal activity, useful in infantile atopic dermatitis, Cytoprotective, anticancer, insecticidal, diuretic activities of plant. The present studies showed that Petroleum Ether Extract (PEE) and Alcoholic Extract (AE) were investigated by TLC, HPTLC, IR and NMR.

Keyword: Solanum xanthocarpum, Phytochemical Screening, β-solamargine, Antidiabetic activity.

1. Introduction
The origin of Ayurveda has been lost in pre historic antiquity, but their characteristic concepts appear to have been nurtured between 2500 and 500 BC in India[1]. Solanum xanthocarpum schrad. & wendl., commonly known as Kantkari, belonging to Family Solanaceae. It is distributed in all districts in the plains & low hills throughout India; also grows as a weed along roadside and wasteland. The indigenous uses of plants also indicate Antispermatogenic[2], Antidiabetic[3], Antiasthmatic[4], cytoprotective[5], anticancer[6] activity of plant. It contains steroidal glycoalkaloid solasodine (about 0.2%), steroidal saponine, solamargine, β-solamargine, solasonine and solacarpidine.

1.1 Vernacular name

<table>
<thead>
<tr>
<th>Language</th>
<th>Vernacular name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanskrit</td>
<td>Kantakari, Nicigadhika</td>
</tr>
<tr>
<td>Bengali</td>
<td>Kantakari</td>
</tr>
<tr>
<td>Gujarati</td>
<td>Bhoiringani</td>
</tr>
<tr>
<td>Hindi</td>
<td>Kateli</td>
</tr>
<tr>
<td>Kannada</td>
<td>Nelagulla</td>
</tr>
<tr>
<td>Malayalam</td>
<td>Kantakkattiri</td>
</tr>
<tr>
<td>Marathi</td>
<td>Bhuiiringani</td>
</tr>
<tr>
<td>Oriya</td>
<td>Ankanti, Bheji begun</td>
</tr>
<tr>
<td>Tamil</td>
<td>Kandan-Kattin</td>
</tr>
<tr>
<td>Telegu</td>
<td>Pinna –mulaka</td>
</tr>
</tbody>
</table>

1.2 Macroscopic Character
Diffuse herb with prickly stem, leaves and calyx. Roots are almost cylindrical and
tapering. Fracture is short and taste bitter with no characteristic odour. Leaves are ovate–oblong, acute, pinnately 7-11 lobed, sparsely stellate pubescent. Odour and taste is not distinct. Stem-nodes and internodes are prominent. Fracture is short to slightly fibrous. Flower purple in few flowered axillary cymes. Fruits a glabrous, globular berry, green and white stripes when young, yellow when mature. Seeds smooth, compressed, reniform, taste bitter [7].

1.4 Chemical Constituents
Solanum xanthocarpum Schrad. & Wendl. contains steroidal glycoalkaloid solasodine (about 0.2%), steroidal saponine, solamargine, β-solamargine, solasonine, solacarpidine, sterol, viz, cycloartenol, nor carpestrol, cholesterol and their derivatives [10].

Fig 1: Solanum xanthocarpum (Flower, fruits and leaves)

1.3 Microscopic character
Root contains cork comprising of 3-6 layers of thin walled, rectangular and tangentially elongated cells. Secondary phloem composed of sieve elements and phloem parenchyma transversed by medullary rays. Stone cells single or in groupes of 2-20. Xylems compose tracheids, vessels, fibre tracheids and parenchyma. Vessels and tracheids with borderpits and fibers. In young stem epidermis covering the cortex, remain intact for a long time. Secondary cortex consists of 7-11 layers of parenchymatous cells. Some cells forming stone cells. Secondary phloem consists of sieve elements; parenchyma, fibers and stone cells. Inner phloem devoid of fibers. Vessels and tracheids with border pits. Fibers much elongated, thick-walled, lignified with tapering and pointed ends, some having bifurcations at one or both ends. Leaves contain epidermis wavy in outline. Stellate hairs and anisocytic stomata on both the surfaces. Bicollateral vascular bundles are also present [8-9].

2. Phytochemical Screening
2.1 Identification, Collection & drying
The plant material was collected from Orai & Oraiya district in September and dried in shade. The authenticity of plant was confirmed by Dr. R.K. Agrawal, Head, Department of Botany, Bundelkhand University, Jhansi (ref: BUJ/06).

2.2 Material and Methods
Identified plant (S. xanthocarpum Schard. & Wendl.) cut in to pieces and shade dried at room temperature and to make coarse powder by using dry grinder and packed into Soxhlet apparatus and extracted successively. All the extracts were dried at water bath till solid to solid to semisolid mass was obtained and were stored in airtight containers in refrigerator below 10°C.
2.2.1 Preparation of extract

About 400 gms of dried powder was extracted with petroleum ether at 60°C - 80°C by continuous hot percolation using soxhlet apparatus. The extraction was continued for 72 hours. A dark green waxy residue approximate 4 gms was obtained. The mark left after petroleum ether extract was taken and it was again extracted with ethanol up to 72 hours in soxhlet apparatus. A dark green residue about 5.165 gms was obtained.

Table 1: (Extractive values of different solvents) PhytoChemical Test of *S. xanthocarpum*

<table>
<thead>
<tr>
<th>Drug</th>
<th>Solvent</th>
<th>Extractive values (w/w) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder</td>
<td>Alcohol (Ethanol)</td>
<td>10.18</td>
</tr>
<tr>
<td>Powder</td>
<td>Petroleum Ether</td>
<td>1.02</td>
</tr>
<tr>
<td>Powder</td>
<td>Aqueous</td>
<td>13.88</td>
</tr>
</tbody>
</table>

Table 2: (+) Presence of constituents, (-) Absence of constituents HPTLC Profile of *Solanum xanthocarpum* Finger printing for Petroleum Ether extract Stationary phase-HPTLC Plate

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Test</th>
<th>Petroleum ether extract</th>
<th>Alcoholic Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbohydrates</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Glycosides</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Fixed oils and fats</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Proteins and amino acids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Phenolic and amino acids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Phyto sterols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Alkaloids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Saponins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Gums and mucilages</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

*Mobile phase-Benzene:Ethyl acetate (90:10)
Tank saturation-15 minute with filter paper
Spraying agent-Vanillin-Sulphuric acid
Scanning-Track 3 scanned at 200nm and at 580nm.*
HPTLC Profile of *Solanum xanthocarpum*

Finger printing for Petroleum Ether extract

**Stationary phase-HPTLC Plate**
- Mobile phase: Benzene: Ethyl acetate (90:10)
- Tank saturation: 15 minute with filter paper
- Spraying agent: Vanillin-Sulphuric acid
- Scanning: Track 3 scanned at 200nm and at 580nm.

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HPTLC Profile of *Solanum xanthocarpum*

Finger printing for alcoholic extract

**Stationary phase-HPTLC Plate**
- Mobile phase: Methanol: Benzene (80:20)
- Tank saturation: 15 minute with filter paper
- Spraying agent: Anisaldehyde-Sulphuric acid
- Scanning: Track 3 scanned at 200nm and at 580nm.
Graph No. 3

HPTLC Profile of *Solanum xanthocarpum*

Finger printing for alcoholic extract

Stationary phase-HPTLC Plate

Mobile phase-Methanol: Benzene (80:20)

Tank saturation-15 minute with filter paper

Spraying agent-Anisaldehyde-Sulphuric acid

Scanning-Track 3 scanned at 200nm and at 580nm.

Graph No. 4
2.3 Characterization of isolated compound A by I.R. spectra and $^1$HNMR

2.3.1 I.R. Spectra

3423-(N-H) str secondary amide, 2909-(C-H) str CH$_3$, CH$_2$, 2850-(C-H) str in CH$_3$-CH$_2$, 1625-(C=C) str (nonconjugated diene/conjugated diene), 1377-[C (CH$_3$)$_2$] in gemdimethyl, a doublet, 1318-(C-O) (O-H) tertiary alcohols, 1242-(C-O-C=C=O) C-O str, 781-(C-H) Aromatic hydrocarbon, 667-(C-H) def disubstituted(meta)B, 516-(C-I).

![Graph 6: IR Spectra of Petroleum Ether extract](image)

2.3.2 $^1$H NMR

0.89(d,3H), 0.62(d,3H), 1.15(d,3H), 1.44(d,3H), 4.5(d,1H), 5.10(d, 1H), 5.33(d, 1H), 6.83(1H)

![Graph 7: NMR Spectra of Petroleum Ether Extract -I](image)
2.4 Characterization of isolated compound B by I.R. spectra and 1H NMR

2.4.1 I.R. Spectra

3366-(N-H) stretching Primary amide, 2920-(C-H) stretching hydrocarbon, 2851-(O-H) stretching, 2362-(C=C), 1633-(C=C) stretching (nonconjugated), 1382-(N=O) str Nitrocompound Ar-NO₂, 1044-(C-O-) alcohols, ethers, carboxylic acid, 815-(C-H) stretching aromatic dissubstituted (para), 775-(C-H) aromatic disubstituted (meta), 719-(C-H) methylene rocking, 634-monosubstituted acetylenes occur at 650-610.

Graph 8: IR Spectra of Alcoholic Extract

2.4.2 1H NMR
(300MHz, CDCl₃)
5.35(m,1H,H-3), 4.12(dt,1H,H-16), 4.07(dd,1H,H-26), 3.45(td,1H,H-26), 2.76(m,1H,H-20), 2.02(s,3H,CH₃ acetyl), 1.01(d,3H,H-21), 0.97(s,3H,H-19), 0.94(d,1H,H-27), 0.72(s,3H,H-18).

Graph 9: NMR Spectra of Alcoholic Extract –II
3. Analgesic Activity
Analgesia is defined as a state of reduced awareness to pain, analgesics are substances which decreases pain sensation by increasing threshold to painful stimuli[11]. The receptors for pain are present in most areas of the body in skin, viscera, blood vessels, skeletal muscles, cornea and other organs. There receptors may be activated by a wide variety of stimuli, like mechanical, thermal, electrical and chemical[12].

3.1 Acute Toxicity Studies
Healthy adult albino rats and albino mice of either sex, starved over night, were divided into groups (n = 6) and were orally fed with increasing doses (10, 40, 100, 400, 1000 and 4000 mg/kg body weight) of petroleum ether extract and alcoholic extract. The total extract administrated orally in dose up to 4000 mg/kg body weight did not produce any evident sign of toxicity and any mortality in rats when observed up to 14 days after administration.

3.2 Evaluation of Analgesic activity
Analgesic activity was evaluation in albino Wister rat using Eddy’s hot plate method. Albino rats of either sex, weighing between 100-150gm, are used. They are kept on the hot plate for a maximal period of 30 seconds. The hot plate, which is commercial available, consists of an electrically heated surface. The temperature is controlled for 55°C to 56°C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop–watch[13].

In the hot plate method, albino rats (100-150gm) were divided into 4 groups each consisting of 6 animals. One groups served as a negative control (received 5% gum acacia 5 ml/kg), while third & forth group received petroleum ether & alcoholic (respectively) extract of S. xanthocarpum (400mg/kg) orally. The basal reaction time was noted before and 1, 2, 3 hours after administration.

3.3 Statistical Analysis
Results expressed as Mean ± S.E.M., were evaluated by unpaired student t-test.

Table 3: Effect of Extracts of S. xanthocarpum on Thermic Stimulus Induced Pain (Hot Plate test) in Rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/Kg)</th>
<th>Reaction Time in second of time Hour's</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Control (5% gum acacia)</td>
<td>5ml/Kg</td>
<td>4.65 ± 0.18</td>
</tr>
<tr>
<td>Pentazoicin</td>
<td>5mg/Kg</td>
<td>4.75 ± 0.09</td>
</tr>
<tr>
<td>Petroleum Ether Extracts</td>
<td>400mg/Kg</td>
<td>5.22 ± 0.30*</td>
</tr>
<tr>
<td>Alcoholic Extracts</td>
<td>400mg/Kg</td>
<td>4.43 ± 0.07*</td>
</tr>
</tbody>
</table>

All Values are as Mean ± SEM * P < 0.001, Table 3 represented by this graph.
4. Result and Conclusion

*Solanum xanthocarpum* is distributed in all district in the planes and low hills throughout India, also grows as a weed along road side and waste land. The plant of *Solanum xanthocarpum* is collected in the months of September from Orai. A moderately coarse powder of whole plant was packed in a Soxhlet Apparatus and extracted with Petroleum Ether (60-80 °C) and later with Ethanol. Petroleum Ether Extract (PEE) and Alcoholic Extract (AE) were investigated by TLC and HPTLC. Best resolution of the PEE and AE was obtained when Benzene: Ethyl Acetate (90:10) and Methanol: Benzene (80:20) respectively, was a solvent system and Silica gel G as adsorbent. Fractionation of PEE into Methanol soluble and Methanol insoluble in isolation of one compound (A) that was subjected to spectral analysis. Fractionation of AE into Petroleum Ether soluble and Petroleum Ether insoluble in isolation of another compound (B) that was subjected to spectral analysis[14]. These compounds are isolated and characterized by IR and NMR. The spectrum studies suggest the Compound A is Solasodine (Glyco-alkaloid) and Compound B is Sapogenin (Steroidal Saponin). The both plant extract (PEE & AE) of *S. xanthocarpum* also showed analgesic activity and their activity were compared with Pentazocin and shown in Table No-3.

5. References

5. Prashanath KV, Cytoprotective role of *solanum nigrum* against gentamicin induced kidney cell (vero cells) damage in vitro, *Fitoterapia*, 2001; 72,481-86.