Phytochemical study of Usheera (*Vetiveria zizanioides* (Linn) Nash)

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Abstract:
Ayurveda is the art and science of life. It is one of our richest heritage gifted to us by our ancestors. In modern scientific era, pharmacognostical, phytochemical, experimental and statistical validation plays an important role in acceptance of medicines. To screen their phyto constituents an attempt was made Usheera Moola *Vetiveria zizanioides* (Linn) Nash. Phytochemical study, extract of *Vetiveria zizanioides* (Linn) Nash done using benzene as solvent. Then some part of extract was used for preliminary phytochemical tests. Positive results were seen for sterols, protien, carbohydrate, alkaliods, saponins, tannins and flavoniod, indicating the presence of their respective chemical compounds.

Key Words: Usheera, phytochemical, *Vetiveria zizanioides* (Linn) Nash

Introduction
Ayurveda, the knowledge of life science bestowed health and longevity in the form of preventive and curative measures. The curative aspects are mainly covered by Dravya chikitsa (Treatment using drugs). As diseases are born with human there is always a search for safest and curative drugs.

In the present era, the attraction towards Ayurveda is increasing day by day due to less unwanted side effects. On account of increasing urbanization, increasing demand of medicine for population, shortage of authentic material and also tendency of profiteering, there is a need for statutory control and development of pharmacopoeal standards.

According to Charaka qualities of drug should be

"Bahuta tatra yogyatwamanekavidha kalpana ,
Sampachet chatushkoyam dravyanam guna uchate”.

Means drug should have free availability, effectiveness, capability of being subjected to various forms of pharmaceutical processing and it should be in excellent condition i.e. without insect etc and which required rasapanchakas etc. The term sampat is very important to make many formulations of many drugs or single drug. It should go through the standardization parameters along with its authentic sources.

The roots of *Vetiveria zizanioides* (Linn) Nash were taken for present study. Analysis of sample was done to evolve suitable parameters for checking the quality.

On scientific background the present drug Usheera was subjected to different studies to know its external and internal structure and different chemical constituents in the selected part of plant and to evaluate its efficacy in diuretic activity.

Most of Ayurvedic drugs or formulations are known for their safety and efficacy. Hence literary research is done to specify a medicine that can act as a diuretic. Lots of drugs are mentioned as Mootravirechaniya (diuretic) in Ayurvedic classics. Here Mootravirechaniya is also called as Bastishodhana and Mootrala. They are having sheeta veerya or ushna veerya, madhura, amla, lavana rasas, drava and upakledi properties. The selected drug Usheera is having sheeta and madhura properties.
Review of Literature:

Sanskrit Name:
Usheera – The fragrant root of the plant

Botanical Name:
Vetiveria zizanioides (Linn) Nash → Vetiveria = the root i.e dug up
zizanioids = Zizania like

Vernacular Names:

<table>
<thead>
<tr>
<th>Language</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>English</td>
<td>Cuscus, Khuskhus, koosa</td>
</tr>
<tr>
<td>Gujarati</td>
<td>Valo</td>
</tr>
<tr>
<td>Hindi</td>
<td>Bala, Balah, Bena, Ganrar, Khas, Onei panni</td>
</tr>
<tr>
<td>Kannada</td>
<td>Lamancha hullu, Balada beru</td>
</tr>
<tr>
<td>Malayalam</td>
<td>Ramachehamver, Vettiver</td>
</tr>
<tr>
<td>Marathi</td>
<td>Vala</td>
</tr>
<tr>
<td>Tamil</td>
<td>Ilamichamver, Vettiver, Vilhalver, Viranam</td>
</tr>
<tr>
<td>Telgu</td>
<td>Avurugaddiveru, Lamajjakaumveru, Vettiveru, Vidavaliveru</td>
</tr>
</tbody>
</table>

TYPES: Table No:3

<table>
<thead>
<tr>
<th>Name of the text</th>
<th>Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raj-nighantu</td>
<td>Usheera/Lamajjaka</td>
</tr>
<tr>
<td>Kaiyadeva-nighantu</td>
<td>Usheera/ Lamajjaka</td>
</tr>
<tr>
<td>The wealth of India</td>
<td>Flowering /Non flowering</td>
</tr>
</tbody>
</table>

Morphology:
A densely tufted perennial grass, root stock, branching with spongy aromatic roots, culms stout, upto over 1.8 cm high, usually sheathed all along, leaf sheaths compressed, especially the lower which are sharply keeled and fan like, imbricate, very smooth, firm, ligules reduced to a scarious rim: blades narrowly linear, acute, 30-90 cm long 4.2 -10.6 mm wide erect, rigid, firm or some what spongy, usually glabrous, rarely more or less hairy downwards on the face, pale green, midrib slender, lateral nerves close, 6 or more on each side, rather stout slightly prominent, margin spinously rough, panicle oblong, upto over 30 cm, long, usually contracted rhachis stout, smooth; whorls 6-10 with upto 20 rays branches oblique to suberect, naked for upto 5 cm filiform slightly rough, racemes upto 5 (rarely 7.5) cm long, very slender, joints about a slong as the sessile spikelets or sometimes distinctly exceeding them, smooth or more or les rough, minutely and unequally ciliolate at the slightly oblique tips, pedicels similar but shorter, sessile spikelet linear – lanceolate to almost linear acute or subacute 4.2-4.8 mm long; Yellowish, olive or violet – brown or purplish to almost black; callus obtuse, under 1mm long glabrous, involueral glumes, acute, coriaceous, lower muriculate, all over the black, 5 nerved, lateral nerves close, very fine, upper spinulously muricate on the keel; lower floral glume as long as the involucral glums, acute, reversely ciliolate upper upto 3.3mm long, narrow ablong – lanceolate mucronulate; ciliolate, lodicules 2, quadrate and conspicuous, though, small, styles and stigmas short stigmas purple, anthers 2.33 mm long pedicelled spikelet sparingly aculeolate or almost smooth, upper floral glume entire acute.

Properties:
Rasa: Tikta  Guna: Sheeta, Snigdha
Verya: Sheeta  Vipaka: Katu
Useful Part: Root

Solubility of *Vetiveria zizanioides* (Linn) Nash:
Materials: Fine root powder of *Vetiveria zizanioides* (Linn) Nash

Methodology:
A different solvent mixed with fine powder of roots *Vetiveria zizanioides* (Linn) Nash and was filtered in different funnels using different filter papers. The solvent giving minimum residue was selected. Because this solvent is may having maximum solubility.

Extraction:

Material:
Drug: Coarse powder of root *Vetiveria zizanioides* (Linn) Nash
Equipments: Soxhlet apparatus of 1000ml round bottom flask, Water Condenser with distillation apparatus, Breaker 500 ml, Measuring Cylinder, Thermostat (Heater), Stand, Electronic Weighing Machine, Filter paper, Magnate stirrer, Boiling chips etc.
Chemicals: Benzene

Methods: The coarse powder of *Vetiveria zizanioides* (Linn) Nash roots was subjected to exhaustive extraction by Soxhlet apparatus with Benzene. After extraction the solvent was distilled off, to obtain semisolid extract then concentrated on magnetic stirrer and the weights of each batch extract were recorded.

Determination of pH:

Material:
Drug- Extract of root *Vetiveria zizanioides* (Linn) Nash
Equipment: Digital calibrate pH
Methods: 50 ml distilled water was taken in a beaker, digital pH was immersed up to the maximum immersion level. Allowed the reading to stabilize and screw driver was used to turn the pH calibration trimmer to read 7.0.

Then 0.5gms of *Vetiveria zizanioides* (Linn) Nash extract was added to 50ml of distilled water in a beaker, stirred well with glass rod gently, at uniform suspension digital pH meter was immersed, observed the maximum immersion level and reading was recorded.

2) Preliminary phytochemical test:

Materials:

Drug: Extractive sample of roots of *Vetiveria zizanioides* (Linn) Nash.
Equipments: Test tube, Test tube holder, Test tube stand, Spirit lamp, Pipette, Glass rods, Beaker 50 ml to 250 ml conical flask, Water bath, Burner, Stand.

Chemicals: 10% cone H$_2$SO$_4$, Chloroform solution, Acetic-anhydride, Sulphur powder, Soda lime, Millions reagent, Mercuric sulphate 10%, Sulphuric acid 1%, Sodium nitrate 5%, Sodium hydroxide 17%, Copper sulphate (CuSO$_4$) 10%, Tannic acid, Acetic anhydride, Acetyl chloride, Zinc chloride, Mayer’s reagents, Wagner’s reagent (Iodine in potassium iodide), Hager’s reagent (saturated picric acid) solution, Dragendorff’s reagent (Potassium bismuth iodide), Ammonium Renikate, Molisch’s reagent, Barfords reagents, Benedicts reagent, Saponin, Ferric chloride fragments, Pieces of magnesium ribbon and Concentrated hydrochloric acid, Zinc dust, Sodium hydroxide, 10% Lead acetate, Bromine water, Ferric chloride, Lead acetate.
Methods:

1 Test for Sterols:
A Salkowski’s test: 2ml extract, 2ml chloroform and 2ml cons H₂SO₄ were added, shaked and allowed to stand.
B Sulphur test: A pinch of Sulphur was added to the extract.

1 Test for Proteins:
Preparation of test Solution:
0.5 gm of sample solution was added in 100 ml water and heated. This solution was used for following tests.
A. Biuret test: 3ml Test solution, 4% soda lime and few drops 1% CuSO₄ were mixed and allowed to stand.
B. Million’s test: 3ml Test solution and 5ml Million’s reagent was added.
C. Xanthoprotein test: 3ml test solution and 1ml Conc H₂SO₄ was added, boiled then added NH₄OH.

3 Test for Triterpenoids:
A. Liebermann-Burchardt test: 2ml of extract was mixed with 2ml Chloroform, Acetic anhydride and Conc Sulfuric acid was added from the sides of the test tube.
B. Tschugajew test: 2ml of Acetyl chloride and pinch of zinc chloride were added to the extract and boiled in water bath.

4 Test for Alkaloids:
Preparation of test solution: The benzene extract was evaporated to residue, dil HCl was added and shacked well then filtered by using filtrate. The following tests were performed.
A. Mayer’s Test: 2ml filtrate and few drops Mayer’s reagents i.e. potassium mercuric iodide, mixed and allowed to stand.
B. Wagner’s test: 2ml filtrate and Wagner’s reagents mixed and allowed to stand.
C. Hager’s test: 2ml filtrate and few drops Hager’s reagents mixed and allowed to stand.
D. Dragendroff’s test: 2ml filtrate with Dragendroff’s reagent mixed and allowed to stand.

5 Test for Carbohydrates:
A. Molisch’s test: 2ml extract with few drops of Molisch reagent were taken and shaked then 2 ml of concentrated 10% H₂SO₄ added slowly to the sides of the test tube.
B. Barford’s test (test for monosaccharides): Equal volume of sample solution and Barfords reagents were taken, boiled 2 minutes in the water bath.

6 Test for Saponins:
A. Foam test: The drug extract shacked vigorously with water.
B. Heamolysis test: The drug extract was added to 1 drop of blood on glass slide.

7. Test for Flavonoids:
A. Ferric chloride test: 2ml extract and few drops of Ferric chloride solution were mixed and allowed to stand.
B. Lead acetate test: 2ml extract was mixed with two drops of 10% lead acetate.
C. Bromine water test: 2ml extract was mixed with few drops bromine water and allowed to stand.

8. Test for Tannins:
A. Ferric chloride test: 2ml extract and few drops of Ferric chloride solution was mixed and allowed to stand.

B. Lead acetate test: 2ml extract was mixed with two drops of 10% lead acetate.

C. Bromine water test: 2ml extract was mixed with few drops bromine water and allowed to stand.

D. RESULTS OF PHYTOCHEMICAL STUDY:

E. SOLUBILITY TEST: Table No:10

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Soluble</th>
<th>Speringly soluble</th>
<th>Insoluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>_</td>
<td>+</td>
<td>_</td>
</tr>
<tr>
<td>Solvent Ether</td>
<td>_</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>Petroleum Ether</td>
<td>_</td>
<td>+</td>
<td>_</td>
</tr>
<tr>
<td>Acetone</td>
<td>_</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>Benzene</td>
<td>+</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Toluene</td>
<td>_</td>
<td>_</td>
<td>+</td>
</tr>
<tr>
<td>Chloroform</td>
<td>_</td>
<td>+</td>
<td>_</td>
</tr>
<tr>
<td>Ethyle Alcohol</td>
<td>_</td>
<td>+</td>
<td>_</td>
</tr>
<tr>
<td>Xylene</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Carban tetrachloride</td>
<td>_</td>
<td>_</td>
<td>+</td>
</tr>
</tbody>
</table>

Ea)

A) EXTRACTION: Table No:11

<table>
<thead>
<tr>
<th>Roots of Vettiveria zizanioides (Linn) Nash</th>
<th>Solvent</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coarse roots powder 50 gms</td>
<td>650 ml</td>
<td>5gms</td>
</tr>
</tbody>
</table>

Eb) RESULTS OF PRELIMINARY PHYTOCHEMICAL TESTS: Table No:12

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Tests for Sterols</td>
<td></td>
</tr>
<tr>
<td>a) Salkowskí’s test</td>
<td>+ve</td>
</tr>
<tr>
<td>b) Sulphur test</td>
<td>+ve</td>
</tr>
<tr>
<td>2) Test for Proteins</td>
<td></td>
</tr>
<tr>
<td>a) Biuret test</td>
<td>−ve</td>
</tr>
<tr>
<td>b) Million’s test</td>
<td>+ve</td>
</tr>
<tr>
<td>c) Xanthoprotein test</td>
<td>+ve</td>
</tr>
<tr>
<td>3) Test for Triterpenoides</td>
<td></td>
</tr>
<tr>
<td>a) Liebermann’s test</td>
<td>+ve</td>
</tr>
<tr>
<td>b) Tschugajew test</td>
<td>+ve</td>
</tr>
<tr>
<td>a) Mayer’s test</td>
<td>+ve</td>
</tr>
</tbody>
</table>
b) Wagner’s test +ve

5) Test for Carbohydrates
a) Molish’s test +ve
b) Barfoed’s test -ve
c) Benedict’s test +ve

6) Test for Saponins
a) Foam test +ve
b) Haemolytic test +ve

7) Test for Flavonoids
a) Lead acetate +ve
b) Alkaline reagent test +ve
c) Ferric chloride test -ve
d) Bromine water test +ve
e) Zinc HCL reduction test +ve

8) Test for Tannins
a) Ferric chloride test -ve
b) Lead acetate test +ve
c) Bromine water test +ve

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**Results of pH Value:**

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled normal</td>
<td>7.0</td>
</tr>
<tr>
<td>Acidic media</td>
<td>0 to 7.0</td>
</tr>
<tr>
<td>Alkaline media</td>
<td>7.0 to 14.0</td>
</tr>
<tr>
<td><em>Vetiveria zizanioides</em> (Linn) Nash</td>
<td>7.8</td>
</tr>
</tbody>
</table>

**Discussion on Phytochemical Test:**

The medicinal value of plant is mainly attributed to the active chemical components such as alkaloid, sterols, triterpeniodes, saponins, tannins and flavonoids. In the present study all the phytochemical components of *Vetiveria zizanioides* (Linn) Nash was tested qualitatively by employing the specific chemical tests.

Before going through the tests, the dried powder of roots *Vetiveria zizanioides* (Linn) Nash was subjected to solubility tests and observed maximum solubility in Benzene and confirmed. Then it was subjected to exhaustive extraction by soxhlet apparatus around complete extraction of drug. The extractive sample of the test drug showed presence of Sterol, By Sakowski test, Chloroform layer appeared and acid layer showed greenish yellow colour and in Sulpher test it sanked. Like this Tschugajew test showed presence of Triterpenoids. In Foam test we got persistent foam and in Haemolytic test Haemolytic zone appeared indicating presence of Saponins. White ppt in Lead acetate test decoloration of Bromin water showed presence of Flavonoids respectively. So these tests indicate the presence of active chemical constituents responsible for diuretic effect of drug.

To know the acidic and alkaline neutralizing activity of roots is important to determine the pH value, it was 7.8. It showed that drug was slightly alkaline in nature.

**Conclusion:**

1. Usheera is an ideal drug, which shows the properties of dravya sampannata
2. In the phytochemical study the trial drug shows the presence of sterols, proteins, Tannins, Flavonoids and Triterpenoids.

References:

Bibliography