A PHYTOCHEMICAL STUDY OF ANUPA AND JANGALA DESHASTHA SHITIVARAKA (*Celosia argentea* Linn.).

NITIN NAGNATH LAVATE

Associate Prof. & H.O.D. Department of Dravyaguna,

Rural Institute of Ayurveda Research college and hospital, A/P - Mayani Dist. Satara

Email-vdnitin678@gmail.com Ph.9763875828

ABSTRACT

Since long the subject of standardization of Ayurvedic drugs is hot. Today it has become a must to give attention towards all the factors which affect the potency of the drug. After lot of observation it was found that the geographical variation and climate of the place of origin of the drug may be the major factor in influencing the potency of drug. Hence for current study drug Shitivaraka is collected from Anupa and Jangala regions; and this drug is well known for its Mootrala (diuretic) property, it is mentioned and utilized by the Brahrayi physicians for the treatment.

The role of research in Ayurveda is not only to elucidate the principles of Ayurveda but also, to explain them in terms of modern parameters. Group-A(anupa), Group-B (Jangala), powdered drug is used for phytochemical study.

KEY WORDS: Anupa-Jangala Desha, Shitivaraka, Phytochemical

INTRODUCTION:

In this century the Ayurveda has come back into picture and propagation as tomorrow’s medical science; But some discriminations have been made with ayurveda. The reason spelled behind this is the use of impure and heavy metal containing Ayurvedic drugs. These drugs have been banned by some European countries. This degradation of drug is not only persecuting Herbo-mineral compounds but also medicinal plants too.

If we contemplate ourselves then we may be unable to give clarification regarding the drug and its unreliable therapeutic action. This is the reason for standardization of Ayurvedic drug. Since long we are discussing on standardization
and till now discussion is in process but there has been no any fruitful out come. The main base of our formulation and single preparation that is raw material the medicinal plants has been ignored or given less attention towards that. Till today the manufacturers companies as well as Ayurvedic Physicians use raw material available in market.

So for better therapeutic results only abundant availability of drug is not enough because the definition of ideal Medicine is given in Ch.Su.9/7. To over come this problem we have to give attention towards the area where the drug grown up. Only collection of drug is not sufficient but also their way of production is also important and surprisingly we found lots of discussion were held on environmental factors and there effect on efficacy of drug in Brhatryi Granthas that’s why the concept of Triveda Desha was developed. Today we have to uphold this ignored and untouched part of Ayurveda; and give attention on not only availability of drug but also environmental factors like soil, rainfall, temperature, season and their various effects on drug. So, here an attempt has been made to find out the reality behind the effect of different regions on quality and containt of drug which are collected from different region with the help of Phytochemical study.

AIMS AND OBJECTIVES

(1) To asses the authenticity of Trivida Desha concept and its effect on drug Shitivaraka.

(2) To collect the drug Shitivaraka from Anupa and Jangala Desha.

(3) To study Anupa and Jangala Deshastha Shitivaraka samples Phytochemically.

MATERIALS AND METHODS

The test drug Shitivaraka was personally collected from two different regions of India and was authenticated Botanically by expert’s, then submitted in pharmacy of IPGT & RA, GAU, Jamnagar for further processing. The two test drug samples were –

Sample-A- Anupa Deshastha Shitivaraka

Sample-B- Jangala Deshastha Shitivaraka

Collected from pharmacy in powdered (120#) and was used for the present study.

3.1 PHYSIO-CHEMICAL PARAMETERS

1. LOSS ON DRYING (LOD)

The moisture content of a drug should be determined for the percentage of its active chemical constituents because its
percentage depends upon air dried basis. So the moisture content of the drug should be minimized in order to prevent decomposition of the crude drugs either due to chemical change or microbial contamination.

**Procedure**

1 gram of drug sample was taken in a pre weighed dried petridish. It was dried in an oven at 105ºC untill reaching a constant weight. The petridish was taken out, self cooled and weighed immediately. The weight loss i.e. loss on drying was calculated and expressed as % w/w.

2. **ASH VALUE (AV)**

   This test was conducted to evaluate the percentage of inorganic salts, naturally occurring in the drug or adhering to it or deliberately added as a form of adulteration.

   **Procedure**

   1 gram accurately weighed sample was taken in a pre weighed dried crucible. It was incinerated in a muffle furnace up to 450ºC. The crucible was taken out, self cooled and weighed immediately. From the weight of the ash, the ash value was derived with reference to the air dried drug. It was calculated and expressed as % w/w.

3. **WATER SOLUBLE EXTRACTIVE (WSE)**

   This test was carried out to determine the water soluble extractive and approximate measures of their chemical constituents of the test drug.

   **Procedure**

   5 gm. of the sample was weighed accurately. To it 50 ml of distilled water was added and kept covered overnight. It was stirred intermittently in the initial period. Next day, it was filtered. 20 ml of the filtrate was accurately measured with a pipette and transferred to the already weighed evaporating dish. The evaporating dish was placed on a water bath for evaporation of the water. After evaporation of the water it was dried in an oven, allowed cooling and weighed immediately. From the weight of the residue obtained, the percentage of water soluble extractive was calculated and expressed as % w/w.

4. **METHANOL SOLUBLE EXTRACTIVE (MST)**

   This test was carried out to determine the methanol soluble extractive of the test drug.

   **Procedure**
The method adopted for this experiment was same as that of water-soluble extract but by using methanol instead of water. Percentage of methanol soluble extract was calculated and expressed as % w/w.

5. PH VALUE

This test is carried out to determine the pH of the test drug with the help of pH meter

Procedure

10g of test drug sample was weighted and taken in a conical flask. Then add 50 ml accurately measured water and stirred well for few minutes; kept this solution for some time and then filtered it through filter paper. Take the filtered solution in a beaker. Standardize the pH meter and electrodes with buffer solution of known pH i.e. 7pH. Rinse the electrodes with distilled water and introduce into the test solution contained in a small beaker. Read the pH value of solution.

6. PARTICLE CONSISTENCY

This test was conducted to evaluate the different type of particle size of the test drug, by using a set of sieves which have mesh’s (60-85-120) that’s fineness gradually increasing.

Procedure

Arrange the sieves according to their mesh size. Take a 10g weight of the test drug’s and place on top sieve i.e. in 60# then cover it with lid. Shake the set of sieves continuously until it is clear that no further separation occurs. Weight the amount left on each sieve and in the pan, calculate the percentage with reference to the amount of total drug taken and expressed as % w/w.

3.2. QUALITATIVE TESTS FOR VARIOUS FUNCTIONAL GROUPS

1. ALKALOIDS

1. With Mayer’s reagent

The methanol extract of the samples was taken in a watch glass, solvent was evaporated. It was added with 2N HCl and few drops of Mayer’s reagent when alkaloids gave a white precipitate.

With Dragendorff’s reagent

The methanol extract of the sample was evaporated. On addition of a few drops of dilute 2N HCl and Dragendorff’s reagent alkaloids gave orange precipitate.

2. ANTHRAQUINONES

Modified Borntrager’s test

1 gm of sample was extracted by boiling with 40% H$_2$SO$_4$ and filtered. The filtrate was extracted with ether thereafter. When ether fraction was mixed with equal portion of NH$_3$, anthraquinones gave pink color in NH$_3$ layer.

3. CARBOHYDRATE

Molisch test

2ml of test drugs were taken in test tubes. To it, 2 drops of fresh 10% a-Naphthol reagent and 2ml of conc. H$_2$SO$_4$ were added and mixed so as to form a layer
below the mixture. A red violet ring indicates the presence of carbohydrate.

4. TANNINS

1. The aqueous extract of test samples was taken in test tubes. A very dilute solution of ferric chloride was added into the test samples. Development of blue/olive green color indicates the presence of tannins.

2. The aqueous extract of test samples was taken in test tubes, by adding 5% lead acetate solution, it gives precipitate which turns red on addition of KOH solution. Addition of excess KOH dissolved the precipitate.

5. TRITERPENOIDS

1. Liebermann-Burchard reaction
A chloroform solution of test drugs was taken in a test tube. On addition of acetic anhydride and conc. H2SO4 the solution turns green, orange, blue or purple red color.

2. Salkowski test
A chloroform solution of test drugs was taken in a test tube. On addition of equal volume of conc. H2SO4, the solution turns purple in color.

3.3. ANALYSIS FOR MINERALS AND MICROELEMENTS

1. NITROGEN-Kjeldhal Method
The test drug sample is heated in presence of conc. H2SO4 K2SO4 etc. till solution becomes clear. Then solution is kept for self cooling, then diluted the solution and made alkaline; and distilled in boric acid. The ammonia was distilled and this solution was determined titrimetrically.

2. PHOSPHOROUS-Olesen blue method
The test drug is mixed with Ammonium molybdate; which reacts with phosphorous of the drug sample in present of an acidic medium to form heteropoly acid molybdophosphoric acid; which is reduced to colored complex molybdenum blue by SnCl2. Then color conc. is measured by spectrophotometer by 690 nm.

3. POTASSIUM-Flame photometer
When the test drug sample solution is atomized led to burner and excited to spectral emission in flame. Since the intensity of radiation emitted by each element depends primarily on concentration of its atoms in the flame.

4. CALCIUM-EDTA titration (Titrimetric method)
The Ca is determined by titration with EDTA in alkaline medium.

5. MAGNESIUM EDTA titration (Titrimetric method)
The Mg is determined by titration with EDTA in alkaline medium.

6. FREE LIME- Titrimetric method
The test drug sample is treated with acid and then lime is determined by titration with alkali.
7. Fe, Mn, Zn,Cu – AAS (Atomic Absorption Spectrophotometer)

Combustion flame converts inorganic substances in the solution into free atoms in the flame, absorbed light emitted by cathode lamp. The absorption depends upon concentration of the element.

**OBSERVATION AND RESULTS**

1. PHYSIO-CHEMICAL PARAMETERS

<table>
<thead>
<tr>
<th>Sr.</th>
<th>Parameters / Samples</th>
<th>Anupa deshasth sample-A</th>
<th>Jangala deshasth sample-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Loss on drying</td>
<td>9.5 % w/w</td>
<td>5.4 % w/w</td>
</tr>
<tr>
<td>02</td>
<td>Ash value</td>
<td>8.05 % w/w</td>
<td>11.1 % w/w</td>
</tr>
<tr>
<td>03</td>
<td>Water soluble extractive</td>
<td>10.1 % w/w</td>
<td>16.4 % w/w</td>
</tr>
<tr>
<td>04</td>
<td>Methanol soluble extractive</td>
<td>6.1 % w/w</td>
<td>7.8 % w/w</td>
</tr>
<tr>
<td>05</td>
<td>pH value</td>
<td>6.30</td>
<td>5.99</td>
</tr>
<tr>
<td>06</td>
<td>Particle consistency</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A. above 60 mesh</td>
<td>17.01 % w/w</td>
<td>18.56 % w/w</td>
</tr>
<tr>
<td></td>
<td>B. between 60-85 mesh</td>
<td>21.71 % w/w</td>
<td>18.49 % w/w</td>
</tr>
<tr>
<td></td>
<td>C. between 85-120 mesh</td>
<td>50.53 % w/w</td>
<td>47.48 % w/w</td>
</tr>
<tr>
<td></td>
<td>D. below 120 mesh</td>
<td>08.51 % w/w</td>
<td>14.01 % w/w</td>
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</tbody>
</table>

2. QUALITATIVE TESTS

<table>
<thead>
<tr>
<th>Sr.</th>
<th>Tests</th>
<th>Name of reagents</th>
<th>Results</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sample-A</td>
</tr>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Mayer’s reagent</td>
<td>+’ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dragendroff’s reagent</td>
<td>+’ve</td>
</tr>
<tr>
<td>2</td>
<td>Anthraquinones</td>
<td>Modified Borntrager’s test</td>
<td>-’ve</td>
</tr>
<tr>
<td>3</td>
<td>Carbohydrate</td>
<td>Molisch test</td>
<td>-’ve</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ferric chloride reagent</td>
<td>+’ve</td>
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</table>
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3. ANALYSIS FOR MINERALS AND MICROELEMENTS

<table>
<thead>
<tr>
<th>Sr.</th>
<th>Parameters</th>
<th>Method Used</th>
<th>Sample-A</th>
<th>Sample-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nitrogen</td>
<td>Kjeldhal Method</td>
<td>1.17%</td>
<td>1.50%</td>
</tr>
<tr>
<td>2</td>
<td>Phosphorous-</td>
<td>Olesen blue method</td>
<td>0.80%</td>
<td>0.62%</td>
</tr>
<tr>
<td>3</td>
<td>Potassium-</td>
<td>Flame photometer</td>
<td>0.60%</td>
<td>0.78%</td>
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<tr>
<td>4</td>
<td>Calcium</td>
<td>EDTA titration (Titrimetric method)</td>
<td>2.50%</td>
<td>1.90%</td>
</tr>
<tr>
<td>5</td>
<td>Magnesium</td>
<td>EDTA titration (Titrimetric method)</td>
<td>0.92%</td>
<td>1.15%</td>
</tr>
<tr>
<td>6</td>
<td>Free Lime</td>
<td>Titrimetric method</td>
<td>0.80%</td>
<td>0.40%</td>
</tr>
</tbody>
</table>

MICROELEMENT

<table>
<thead>
<tr>
<th>Sr.</th>
<th>Parameters</th>
<th>Method Used</th>
<th>Sample-A</th>
<th>Sample-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Iron</td>
<td>A.A.S.</td>
<td>840ppm</td>
<td>845ppm</td>
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<tr>
<td>02</td>
<td>Manganese</td>
<td>A.A.S.</td>
<td>040ppm</td>
<td>020ppm</td>
</tr>
<tr>
<td>03</td>
<td>Zinc</td>
<td>A.A.S.</td>
<td>105ppm</td>
<td>052ppm</td>
</tr>
<tr>
<td>04</td>
<td>Copper</td>
<td>A.A.S.</td>
<td>035ppm</td>
<td>025ppm</td>
</tr>
</tbody>
</table>

* A.A.S. (Atomic Absorption Spectrophotometer)

DISCUSSION

The drug Shitivaraka was collected from two different regions. The test samples (Sample-A = Anupa Deshastha Shitivaraka Churna and Sample-B = Jangala Deshastha Shitivaraka Churna)

1.) The loss on drying indicates the water and moisture content in samples. There is much variation in loss on drying of the samples; 9.5 % w/w to 5.4 % w/w in
Sample-A and Sample-B respectively; the sample-A is having more loss on drying which indicates that it has more % of water or moisture as compared to sample-B. It may be due to the fact that sample-A is collected from Anupa region which is considered to be heavy rain fall region as compared to sample-B collected region.

2.) Ash value indicates the presence of inorganic constituents in the sample and it is found that there are more inorganic constituents in sample-B 11.1 % w/w than sample-A 8.05 % w/w.

3.) The data also reveals that there is considerable difference in the extractive values, both water and Methanol soluble extractives. The WSE of Sample-B is higher (16.4 % w/w) as compared to that of Sample-A (10.1 % w/w). There is also variation in (MSE); in sample-B (7.8 % w/w) it is higher as compared to sample-A (6.1 % w/w). The higher extractive values indicates the more availability of drug content in that media. So the tests indicate that sample-B is having more bioavailability of drug constituents which may gives more good results in clinical trails of that drug.

4.) The pH value indicates the potential hydrogen ions available in particular substance. Both test samples are having slight acidic pH that is in sample-A (6.30) and sample-B (5.99) which is not having much variation.

5.) The Particle consistency of powdered is carried out to determine the different type of particles size present in that drug sample. The Particle size of test samples is not much variable except the particles below mesh no.120, the sample-B is having 14.01 % w/w particles and sample-A is having 08.51 % w/w.

6.) The qualitative test conducted by different reagents for Alkaloids, Tannin and Triterpenoids became positive and they were considered to be present of these three in both test samples. Anthraquinones was not detected in both samples in analytical test conducted for that. There is one reference found regarding presence of carbohydrate in leaves of test drug in 5.8% level (Ref. Wealth of India). But the qualitative test conducted for carbohydrate was negative, it may due to the fact that whole plant powder was used for analysis and the above ref. was regarding the level of carbohydrate in leaves.

7.) Minerals perform several vital functions which is absolutely essential for the very existence of the organism. They include calcification of bone, blood coagulation, neuromuscular irritability, acid-base equilibrium, fluid balance and...
osmotic regulation. Certain minerals are integral components of biologically important components such as hemoglobin (Fe), Insulin (Zn) and (Mg,Mn,Cu,Zn,K) participate as cofactors for enzymes in metabolism.

It is very difficult to correlate the Ayurvedic concepts with above elements. But in present times it is required to maintain the constant therapeutic effects and to compare different types of drug samples, It may become an evolutionary step and the problem of standardization may somewhat be solved with available tools.

CONCLUSION

1) More loss on drying was found in Anupa sample than Jangala sample and water soluble extractive of Jangala sample (16.4%w/w) was more as compared to Anupa sample (10.1 %w/w).

2) Both samples showed presence of alkaloids, tannins and triterpenoids chemically.

3) The percentage of minerals like Nitrogen, Potassium,Magnesium is more in Jangala Shitivaraka sample.

4) The percentage of microelements like Zinc, copper, Manganese is more in Anupa Shitivaraka sample.

Ayurvedic Pharmacopoeia of India has not mentioned Shitivaraka (Celosia argentea Linn.) Panchang. The Data evolved in the present study will be very useful and shall serve as reference for its routine analysis.

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