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*RESEARCH ARTICLE*

# A CRITICAL REVIEW AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF GOKSHUR RASAYANA

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## Abstract

*Rasayanachikitsa* is one among the *asthangas* of ayurveda (one among the 8 fields). Acharya Vagbhatt has placed it on the 7th rank in the chronology of these eight fields. Further Acharya Arundatt has specified that *Rasayanas* are not just used for prolonging life and improving quality of life, but they also help in removing the effect of toxins from the individual's body. Thus, considering the same we realize how important and diverse is the effect of *Rasayanas*in maintaining health of the individual. Further in the chapter dedicated to *Rasayanas* in Ashtang Hriday, Acharya Vagbhatt has mentioned about *GokshurRasayana*. It is a simple *Rasayana* formulation which has not been worked up upon and thus, in this article we will understand the preparation and utility of this*Rasayana*and perform preliminary phytochemical examinations on the same.



## INTRODUCTION: -

Ayurveda, or the science of longevity, has two primary aims elucidated for mankind. First and the primary aim is of maintainace of health followed by its secondary aim which is of treating the unhealthy or diseased.[1] Ayurveda has mentioned a separate class of drugs called *Rasayanas* specially for maintenance and promotion of health of the individual.[2]

### RASAYANA IN BRIHATTRAYE

Acharya Charak in *Chikitsa-sthan*dedicated the very first chapter to *Rasayanas*. This chapter has been mentioned in four parts (called padas).[3] Acharya Sushrut has dedicated 4 chapters to *Rasayanas* in *Chikitsasthan* (Chapter 27-30).[4] Acharya Vagbhatt in Uttartantra, has dedicated the second last chapter to *Rasayanas* (Chapter 39- *Rasayan-adhyay*). Acharya Charak in Sutra sthan chapter 25, verse 40 has mentioned about *Ksheer* and *Ghrit* as best among the *RasayanaDravyas*(*ksheer-ghritabhyasorasayana naam*).[5]

### GOKSHUR RASAYANA

*GokshurRasayana* is a unique formulation mentioned by Acharya Vagbhatt. [6] The verse of this *Rasayana*inAshtang Hriday, Uttartantra(verse56-57)reads as-

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'फलोन्मुख़ो गोक्षुरकः समूलश्छायाविशुष्कः सुविचूर्णिताङ्गः |
सुभवितः स्वेन रसेन तस्मान्मात्रां परां प्रासृतिकीं पिवेद्यः ||५६||
क्षीरेण तेनैव च शालिमश्नन्जीर्णे भवेत्स द्वितुलोपयोगात् |
शक्तः सुरूपः सुभगः शतायुःकामी कुकुद्मानिव गोकुलस्थः ||५७|'

According to this verse, *Gokshura* plant, identified as *Tribulus terrestris Linn.* by Ayurvedic Pharmacopeia of India (API) [7]must be collected in fresh form along with roots, just before maturation of fruits (when the plant is bearing tender fruits). The same must be dried in shade and then given *Bhavana*(trituration) with the *Swarasa* (juice) of fresh *Gokshura* plants. The *Rasayana* thus prepared will be in *Kalka* (paste) form. This *Rasayana* on administration with milk in two *tola* (24 grams) quantity and when the individual is only on shaali rice diet bears enormous benefits to health.[6]

It increases strength, causes youthfulness, makes the individual beautiful, appealing, enlightens the lamp of fortune in life, prolongs life of an individual to a hundred years, at the same time increases his sexual power and abilities such that he is able to copulate like a bull standing in the herd of cows.[6]

Acharya Charaka, describes *Gokshura* as an *Agrya Dravya 'mutrakrichhranilaharanam'* (It is used in urinary disorders and balances vitiated Vata).[8]

*Gokshura* as a single drug has been well established for its Anti-urolithiatic, Antimicrobial, Antihelminthic, Cardiotonic, Anti-inflammatory, Hypolipidemic, Immunomodulatory, Antispasmodic, Analgesic, Aphrodisiac, Antidiabetic, Anti-tumour, Hepato-protective, Anti-oxidant, CNS modulator properties.[9]

## MATERIALS AND METHODOLOGY:-

Standard reference books like API and Quality Standards of Indian Medicinal Plants (by Central Council for Research in Ayurvedic Sciences) have set standards only for the root and fruit of the plant.[10,12]Since traditionally only these two plant parts have been employed for medicinal use [7,11] but in the context of *GokshurRasayana* we need standards for the powder of the whole plant, thus entire profiling was done.

**Physico- chemical evaluation [10,12]**

**Determination of moisture content (via gravimetric method - Loss on drying)**

10 g of sample was placed in tared evaporating dish. It was dried at 105˚C for 5 hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample.

**Determination of Total Ash**

2 g of sample was incinerated in a tared platinum crucible at temperature not exceeding 450˚C until carbon free ash is obtained. Percentage of ash was calculated with reference to weight of the sample.

**Determination of Acid insoluble Ash**

To the crucible containing total ash, add 25ml of dilute HCl and boil. Collect the insoluble matter on ashless filter paper (Whatman 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccator for 30 mins and weigh without delay. Calculate the content of acid insoluble ash with reference to the air-dried drug.

**Determination of Water-soluble ash**

Boil the ash for 5 min with 25 ml of water; collect insoluble matter on an ashless filter paper, wash with hot water, and ignite for 15 min at a temperature not exceeding 450˚C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash with reference to the air-dried sample.

**Determination of Alcohol soluble extractive**

Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled Alcohol (approximately 95%). Shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette

out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours, cool in desiccator for 30 minutes and weigh. Calculate the percentage of Alcohol extractable matter of the sample. Repeat the experiment twice and take the average value.

**Determination of Water-soluble extractive**:
Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled water, shake occasionally for 6 hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105°C for 6 hours. Cool in a desiccator and weigh. Repeat the experiment twice. Take the average value.

**Determination of phytochemicals** [10,13]

**Preliminary phytochemical tests**

**Tests for alkaloids**
***Dragendroff's test:***
To a few mg of extract dissolved in alcohol, a few drops of acetic acid and Dragendroff's reagent were added and shaken well. An orange red precipitate formed indicates the presence of alkaloids.

***Wagners's test:***
To a few mg of extract dissolved in acetic acid, a few drops of Wagner's reagent was added. A reddish-brown precipitate formed indicates the presence of alkaloids.

***Mayer's test:***
To a few mg of extract dissolved in acetic acid, a few drops of Mayer's reagent was added. A dull white precipitate formed indicates the presence of alkaloids.

***Hager's test:***
To a few mg of extract dissolved in acetic acid, 3 ml of Hager's reagent was added, the formation of yellow precipitate indicates the presence of alkaloids.

**Tests for carbohydrates**
***Molisch's test:***
To the extract, 1 ml of α-naphthol solution and conc. sulphuricacid were added along the sides of test tube. Violet colour formed at the junction of the two liquids indicates the presence of carbohydrates.

***Fehling's test:***
A few mg of extract was mixed with equal quantities of Fehling's solution A and B. The mixture was warmed on a water bath. The formation of a brick red precipitate indicates the presence of carbohydrates.

***Benedict's test:***
To 5 ml of Benedict's reagent, a few mg of extract was added, and boiled for two minutes and cooled. Formation of a red precipitate indicates the presence of carbohydrates.

**Test for steroids**
***Libermann-Burchard test:***
To the extract was dissolved in chloroform, 1 ml of acetic acid and 1 ml of acetic anhydride were added, then heated on a water bath and cooled. Few drops of conc. Sulphuric acid were added along the sides of the test tube. Appearance of bluish green colour indicates the presence of steroids.

***Salkowski test:***
The extract was dissolved in chloroform and equal volume of conc. Sulphuric acid was added. Formation of bluish red to cherry red colour in chloroform layer and green fluorescence in the acid layer indicates the presence of steroids.

**Test for Saponins**
To a few mg of extract, distilled water was added and shaken. Stable froth formation indicates the presence of saponin.

**Test for Tannins**
To the extract, a few drops of dilute solution of ferric chloride was added, formation of dark blue colour shows the presence of tannins.

**Test for Flavonoids**
***Shinoda's test:***
To the extract in alcohol, a few magnesium turnings and few drops of conc. hydrochloric acid were added and heated on a water bath. Formation of red to pink colour indicates the presence of flavonoids.

**Test for Phenol**
To the extract in alcohol, added two drops of alcoholic ferric chloride. Formation of blue to blue-black indicates the presence of phenol.

**Test for Coumarins**
To the extract in alcohol,a few drops of 2 N Sodium hydroxide solution was added. Dark yellow colour formation indicates the presence of coumarins.

**Test for Triterpenoids**
The extract was warmed with tin bits and few drops of thionyl chloride. Formation of pink colour indicates the presence of triterpenoids.

**Test for Amino acids**
To the extract, add few drops of Nin-hydrine reagent, purple colour indicates the presence of amino acids.

**Test for Carboxylic Acid**
Extract dissolved in water is treated with sodium bicarbonate. Brisk effervescence indicates the presence of carboxylic acid.

**Test for Resin**
Few mg of the sample was mixed with water and acetone. Turbidity indicates the presence of resins.

**Test for Quinone**
A few mg of alcohol extract was treated with 0.5% of sodium hydroxide. Deep coloration like pink, purple or red indicates the presence of quinone.

## OBSERVATIONS AND RESULTS:-

Observations and results of performing all the physico-chemical investigations are as follows-

| PARAMETER | Gokshura whole plant |
|---|---|
| **Loss on drying** | 9.81 ± 0.01 |
| **Total Ash** | 15.89 ± 1.32 |
| **Acid Insoluble Ash** | 3.95 ± 0.00 |
| **Water soluble Ash** | 5.51 ± 0.00 |
| **Alcohol soluble extractive value** | 10.58 ± 0.00 |
| **Water soluble extractive value** | 19.99 ± 0.01 |
| **Methanol soluble extractive value** | 28.00 ± 0.00 |
| **Chloroform soluble extractive value** | 4.99 ± 0.01 |
| **Pet ether soluble extractive value** | 9.39 ± 0.00 |
| **pH** | 6.11 |

**Table 1:-** Results of standardization parameters of Gokshura whole plant.
**[n = 3 %w/w; Avg. ± SD]**

| Tests | Color if positive | Gokshura plant |
|---|---|---|
| **Alkaloids** | | |
| **Dragendroff's test** | Orange red precipitate | Orange red precipitate |
| **Wagner's test** | Reddish brown precipitate | Reddish brown precipitate |
| **Mayer's test** | Dull white precipitate | Dull white precipitate |
| **Hager's test** | Yellow precipitate | Yellow precipitate |

**Table 2:-** Observations of alkaloid analysis of ethanolic extract of *Gokshura* powder

| **Steroids** | | |
|---|---|---|
| **Liebermann-Buchard test** | Bluish green colour | No bluish green colour |
| **Salkowski test** | Bluish red to cherry red colour in chloroform layer and green fluorescence in acid layer | No bluish red to cherry red colour in chloroform layer and green fluorescence in acid layer |

**Table 3:-** Observations of steroid analysis of ethanolic extract of *Gokshura* powder.

| **Carbohydrate** | | |
|---|---|---|
| **Molish test** | Violet ring | Violet ring |
| **Fehlings test** | Brick red precipitate | Brick red precipitate |
| **Benedicts test** | Red precipitate | Red precipitate |

**Table 4:-** Observations of carbohydrate analysis of ethanolic extract of *Gokshura* powder.

| **Tannin** | | |
|---|---|---|
| **With $FeCl_3$** | Dark blue or green or brown | Dark green colour |

**Table 5:-** Observations of Tannin analysis of ethanolic extract of *Gokshura* powder.

| **Flavanoids** | | |
|---|---|---|
| **Shinoda's test** | Red or pink | Red colour |

**Table 6:-** Observations of Flavanoid analysis of ethanolic extract of *Gokshura* powder.

| **Saponins** | | |
|---|---|---|
| **With $NaHCO_3$** | Stable froth | Stable froth |

**Table 7:-** Observations of saponin analysis of ethanolic extract of *Gokshura* powder

| Triterpenoids | | |
|---|---|---|
| **Tin and thionyl chloride test** | Pink | No pink colour |

**Table 8:-** Observations of Triterpenoid analysis of ethanolic extract of *Gokshura* powder.

| Coumarins | | |
|---|---|---|
| **With 2 N NaOH** | Yellow | No yellow colour |

**Table 9:-** Observations of Coumarin analysis of ethanolic extract of *Gokshura* powder.

| Phenols | | |
|---|---|---|
| **With alcoholic ferric chloride** | Blue to blue-black | No blue to blue-black |

**Table 10:-** Observations of Phenol analysis of ethanolic extract of *Gokshura* powder.

| Carboxylic acid | | |
|---|---|---|
| **With water and $NaHCO_3$** | Brisk effervescence | Noeffervescence |

**Table 11:-** Observations of Carboxylic acid of ethanolic extract of *Gokshura* powder.

| Amino acid | | |
|---|---|---|
| **With ninhydrineReagent** | Purple colour | No purple colour |

**Table 12:-** Observations of Amino acid analysis of ethanolic extract of *Gokshura* powder.

| Resin | | |
|---|---|---|
| **With aqueous acetone** | Turbidity | No turbidity |

**Table 13:-**Observations of resin analysis of ethanolic extract of *Gokshura* powder.

| Quinone | | |
|---|---|---|
| **Conc. sulphuric acid** | Pink/purple/red | No pink/purple/red |

**Table 14:** Observations of Quinone analysis of ethanolic extract of *Gokshura*powder

| TEST | GOKSHURA WHOLE PLANT |
|---|---|
| **Alkaloid** | + |
| **Steroid** | - |
| **Carbohydrate** | + |
| **Tannin** | + |
| **Flavanoids** | + |
| **Saponins** | + |

| | |
|---|---|
| **Terpenoid** | - |
| **Coumarins** | - |
| **Phenols** | - |
| **Carboxylic acid** | - |
| **Amino acids** | - |
| **Resin** | - |
| **Quinone** | - |

**Table 15:-**Inference of preliminary phytochemical screening of ethanolic extract of *Gokshura*whole plant**.**

## DISCUSSION:-

*GokshurRasayana*, which is made of a single drug *Gokshura*, is an easy preparation and a palatable formulation (due to *madhur rasa* (sweet taste) of *Gokshura*) which can be utilised for strengthening the body's *dhatus*, since *Gokshura* itself is *balyakar*and *brihanya* and in this *Rasayana*form it gets potentiated further.

This *Rasayana* is a rich source of carbohydrates and an excellent source of phytochemicals like alkaloids, tannins and flavonoids which not just help in building and nourishing the body tissues but also possess diverse pharmacological activities which makes it a one stop solution for many conditions specially the ones related to fertility and ageing.

Thus, this *Rasayana* can be used for achieving both the goals of Ayurveda i.e. it helps in maintaining the health of an individual as well as helps in curing the diseased individuals.

## CONCLUSION:-

*Gokshura* is a highly reverbed medicinal plant in the Ayurvedic system of medicine. Many species of Tribulus are being sold on the name of *Gokshura* and thus it was incredibly important to make phytochemical analysis of the entire plant of *Tribulus terrestris Linn.*,which is the official source plant of*Gokshura*. Acharya Vagbhatt in Ashtang Hridaymentioned about*GokshurRasayana*- its preparation, dose, *anupan, sehpan*, and its diverse uses. Since this *Rasayana*is a simple preparation and *Gokshura* is an easily available medicinal plant growing like a weed in hot and sandy parts of the country, it can be easily brought to use in therapeutics.

## CONFLICT OF INTEREST

Nil.

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