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RESEARCH ARTICLE

# Standardization of Snow Mountain Garlic

**Payal Kesharwani, Rahul Kaushik*, Kanika Sharma, Kartik Khetarpal, Tanya Goyal**


## ABSTRACT

**Background:** Snow mountain garlic (SMG) is also known as Kashmiri garlic and ek pothi lahsun, which grows in high altitude and low-temperature conditions. It has shown beneficial effects in breathing discomfort like common cold, asthma, and flu and refines blood circulation. Various standardization parameters have been recorded in this study.

**Objectives:** The present study focuses on standardization of SMG.

**Materials and Methods:** The standardization parameter follow WHO Guidelines of herbal drugs. Standardization parameter like macroscopic characters, extractive value (hot extraction), ash value (total ash, insoluble acid ash and water-soluble ash), bitterness value, swelling Index, phytochemical screening have been studied.

**Results:** The pharmacognostic studies shows that SMG is semi-obtuse in shape and have pale brown outer covering and inner colour is white with little sticky latex. It has a characteristic odor and slight astringent taste. The physiochemical analysis shows different extractive values in solvents, with maximum extractives obtained in hydro alcohol (45%). total ash value observed was 1.038%, insoluble acid ash 0%, water-soluble ash 0.16%, bitterness value of 0.030mg/mL, swelling index of 1.06, and the foaming index was found to be less than 100.

**Conclusion:** The Standardization of snow mountain garlic was performed and the data obtained can be further utilized for establishing the identity and purity of the drug.


**Keywords:** Kashmiri garlic, Physiochemical analysis, Phytochemical evaluation, Snow mountain garlic, Standardization parameter.

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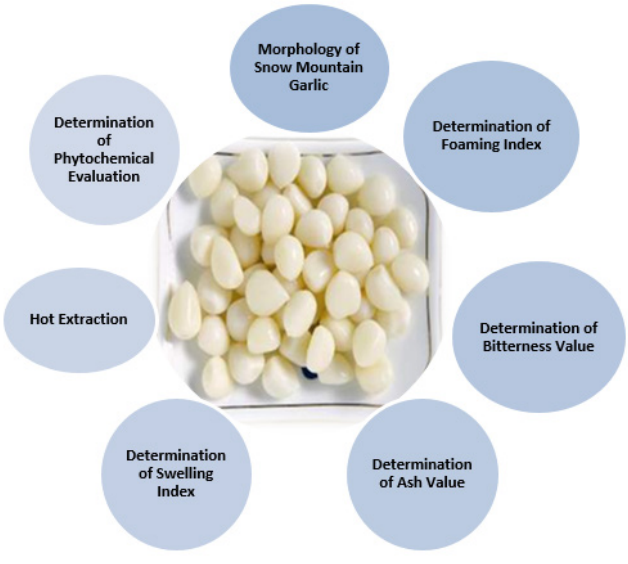
## GRAPHICAL ABSTRACT

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## INTRODUCTION

Snow Mountain Garlic (*Allium sativum L, Allium schoenoprasum),* belongs to the family Alliaceae and is also known as Kashmiri Garlic and ek pothi lahsun. Snow Mountain Garlic is found in the Himalaya mountain at the height of 6000 feet above sea level (Shaw, N.C *et al*., 2014). The plant is found to survive in extremely low weather (-10°C) and less amount oxygen. The ancient use of snow mountain garlic is that it increases the energy level.[2] Anciently, Snow Mountain garlic has been used to increase the energy level.[2] The lava layer was eventually covered with thick layers of snow. Due to the high altitude and geographical situation, the soil, air, and water are naturally organic in ancient days; mountaineers use to gulp snow mountain garlic to raise their energy level, keeping peripheral circulation intact, increasing oxygenation capacity, and detoxifying the body in extremely cold weather.[6]

It has a hard, golden-brown husk and looks like an individual clove of garlic. It has a rounded, bulbous shape with stiff, flattened portion on one side and forming a pointed tail at the end of the clove. The small single clove measure 1.5-4 centimeters in diameter. The hardened outer layers form a protective husk for the bulb while it develops in sub-zero degree temperatures. The clove beneath is a bright white to creamy-white color and offers a strong, pungent garlic flavor without the acidity present in other varieties.[3] Kashmiri garlic tends to be seven times more potent than commercial garlic in terms of beneficial compounds and properties.[4] Kashmiri garlic is available year-round with a peak season in the spring months.[5]

Snow mountain garlic is used to breathe discomfort like common cold, asthma, and flu, which also refines blood circulation. Thus, avoiding the risk of

Arteriosclerosis and Thrombosis. It is also beneficial in arresting the condition of diarrhea and dysentery, eases constipation and is a good antidote for reptile as snake bites.[7] It possesses anti-cancer properties, reduces the occurrence of heart troubles, and is also helpful in tackling Tuberculosis. It vitalizes the liver, treat typhoid, and combats Skin complaints, namely skin Eruptions like Acne and Wounds. It also counteracts intestine ailments like cholera, encourages the secretion of gastric juice and suppresses acid reflux. It also acts as an immunity booster and helps in the treatment of malaria.[8] Snow mountain garlic is a good source of Manganese, Vitamins B6 and C, as well as Copper, Selenium, and Phosphorus.[9] Along with this, it is also rich in Calcium and Vitamin B1.[10] Garlic contains the enzymes alliin and alliinase. Allicin is the compound that gives its pungent smell.[5]

The present study is aimed to evaluate the organoleptic, morphological and microscopical features, physicochemical parameters like extractive values, ash values, foaming and swelling index, bitterness values, etc. This study is conducted as per the guidelines of the World Health Organization (WHO) for quality control of herbal drug materials.[3]

## MATERIALS AND METHODS

### Plant Material

Cloves of snow mountain garlic were collected from Kashmir Valley and used for all the experiments.

### Chemical Reagent

All the chemicals used in the present study were of analytical grade and purchased from manufacturers: HCL(New Pradhav Chemicals Kanpur), Distilled Water (Sadbhavana chemicals Morbi), Petroleum ether (Qualitech Lab chemicals Agra), Chloroform (Ridhdhi sidhdhi chemicals Ankleshwar), Acetone (Prasol chemicals Pvt.Ltd Navi Mumbai), Methanol ,Potassium bismuth iodide, Potassium mercuric iodide (RFCL Ltd.), Picric acid (Indian platinum private limited), Iodine in potassium iodide (RFCL Ltd.), Ferric chloride, H2SO4 (RFCL Ltd.), Nitroprusside (RFCL Ltd.), NaHCO3 (RFCL Ltd.), Mlacetic anhydride (RFCL Ltd.), Lead acetate (Allan chemical corporation, Potassium dichromate (RFCL Ltd.), Bromine water (RFCL Ltd.), Potassium permanganate (RFCL Ltd.), Zinc chloride, Ninhydrin reagent (RFCL Ltd.), CuSO4 (Avon chemicals), HNO3, Millon's Reagent, Alpha Naphthol (Chandan intermediate and chemicals Pvt. Ltd Ahmedabad), Fehling solution A & B (RFCL Ltd.), Benedict reagent (RFCL Ltd.) and Iodine (Godo shigen co. ltd).

**Figure 1 :** Snow mountain garlic (Allium sativum)

### Standardization Parameter

WHO Guidelines were followed for standardization of herbal drugs. Various Standardization parameters like macroscopic characters, extractive value (hot extraction), ash value (total ash, insoluble acid ash, water-soluble ash), Bitterness value, Swelling Index, Phytochemical Screening. Microscopic characters of snow mountain garlic were observed for color, odor, taste, and shape. The observations and the outcomes for the morphological characters were recorded in Figure 1 and Table 1. Extraction value of drug was subjected to the hot and successive extraction. Methodologies for the determination of standardization parameter used in this research were adapted from those reported by (Atul D. *et.al*., 2011, Kaushik R *et al*., 2018).[11,12]

### Phytochemical Evaluation

After collection and authentication, the plant materials were shade dried and crushed separately. 2g drug was extracted separately in Soxhlet apparatus for 6 hr using twice the amount of solvent. The extract was evaporated to dryness under reduced pressure and controlled temperature (40-50°C).

The petroleum ether (60-80°C), chloroform, acetone, methanol, and water extracts of the plant material were subjected to preliminary phytochemical screening to detect the phytochemical components- Alkaloids, Glucosides, Flavonoids, Saponins, and Sterols. Methodologies for the determination of phytochemical evaluation used in this research were adapted from those reported by (Divyakant P.*et.al*.,2008, Kaushik R *et al*., 2018).[12,13]

## RESULT

### Macroscopy

Table 1: Observations for Organoleptic characteristics of drug Allium sativum (Kashmiri Garlic)

| *S.No.* | *Parameters* | *Inference* |
|---|---|---|
| 1 | Color (Outer) | Pale brown covering |
| 2 | Color (Inside) | White with little sticky latex |
| 3 | Odor | Characteristic |
| 4 | Taste | Slight Astringent |
| 5 | Shape | Semi Obtuse |

## Hot Extraction

**Table 2:** Petroleum ether (60-80°C) extractive value

| S. No. | Wt. of Empty China Dish (g) | Wt. of China Dish + Extract(g) | Wt. of Extract (g/g) | % Extractive Value |
|---|---|---|---|---|
| 1 | 57.43 | 57.44 | 0.01 | 2% |
| 2 | 62.40 | 62.42 | 0.02 | 4% |
| 3 | 47.61 | 47.62 | 0.01 | 2% |
| Mean | | | 0.013 | 2.66% |

**Table 3:** Chloroform extractive value

| S. No. | Wt. of Empty China Dish(g) | Wt. of China Dish+ Extract(g) | Wt. of Extract (g/g) | % Extractive Value |
|---|---|---|---|---|
| 1 | 64.11 | 64.15 | 0.04 | 8% |
| 2 | 54.03 | 56.07 | 0.04 | 8% |
| 3 | 47.61 | 47.64 | 0.03 | 6% |
| Mean | | | 0.36 | 7.33% |

**Table 4:** Ethyl acetate extractive value

| S. No | Wt.of Empty China Dish (g) | Wt. of China Dish+ Extract(g) | Wt. of Extract (g/g) | % Extractive value |
|---|---|---|---|---|
| 1 | 57.43 | 57.44 | 0.01 | 2% |
| 2 | 47.61 | 47.62 | 0.01 | 2% |
| 3 | 62.40 | 62.42 | 0.02 | 4% |
| Mean | | | 0.133 | 2.66% |

**Table 5:** Methanolic extractive value

| S. No. | Wt. of Empty China Dish (g) | Wt.of China Dish + Extract (g) | Wt.of Extract (g/g) | % Extractive value |
|---|---|---|---|---|
| 1 | 64.81 | 64.89 | 0.08 | 16% |
| 2 | 64.20 | 64.25 | 0.05 | 10% |
| 3 | 62.40 | 62.44 | 0.04 | 8% |
| Mean | | | 0.056 | 11.3% |

**Table 6:** Aqueous extractive value

| S. No. | Wt. of China Dish+Extract (g) | Wt. of China Dish + Extract (g) | Wt.of Extract (g/g) | % Extractive value |
|---|---|---|---|---|
| 1 | 64.21 | 64.37 | 0.160 | 32% |
| 2 | 51.45 | 51.59 | 0.139 | 27.8% |
| 3 | 47.61 | 47.76 | 0.150 | 30% |
| Mean | | | 0.10 | 29.9% |

## Ash Values

## Bitterness Value

$$\text{Bitterness value} = (2000 \times C)/(a \times b)$$

Where,

a = Concentration of stock solution in mg/mL = 36 mg/mL

b = Volume of stock solution in tube with threshold bitterness = 9 mL

c = Quantity of quinine HCL in mg = 0.005 mg

Bitterness Value = 0.030 mg/mL

**Table 7:** Hydro alcohol extractive value

| S. No. | Wt.of Empty China Dish | Wt.of China Dish + Extract (g/g) | Wt.of Extract (g/g) | % Extractive Value |
|---|---|---|---|---|
| 1 | 56.02 | 56.30 | 0.28 | 56% |
| 2 | 64.24 | 64.43 | 0.19 | 38% |
| 3 | 62.43 | 62.64 | 0.21 | 42% |
| Mean | | | 0.22 | 45% |

**Table 8:** Quantitative Estimation of Total Ash

| S. No. | Wt of Empty Cucible(g) | Wt of Crucible +Ash (g) | Wt. of Ash (g/g) | % Total Ash |
|---|---|---|---|---|
| 1 | 17.660 | 19.740 | 2.080 | 1.040% |
| 2 | 18.250 | 20.320 | 2.070 | 1.035% |
| 3 | 19.910 | 21.960 | 2.080 | 1.040% |
| Mean | | | 2.076 | 1.038% |

**Table 9:** Quantitative Estimation of Acid Insoluble Ash

| S. No. | Wt. of Empty Crucible (g) | Wt. of Crucible + Acid Insoluble Ash (g) | Wt. of Acid Insoluble Ash (g/g) | % Acid Insoluble Ash |
|---|---|---|---|---|
| 1 | 18.230 | 18.220 | 0.01 | 0.005% |
| 2 | 18.250 | 18.250 | 0.00 | 0% |
| 3 | 19.910 | 19.910 | 0.00 | 0% |
| Mean | | | 0.003 | 0.0% |

**Table 10:** Quantative Estimation of Water-Soluble Ash

| S. No | Wt. of Empty Crucible | Wt. of Empty Crucible+Ash (g/g) | Wt. of Water-Soluble Ash(g/g) | % Water Soluble Ash |
|---|---|---|---|---|
| 1 | 17.640 | 17.630 | 0.010 | 0.05% |
| 2 | 19.250 | 19.240 | 0.010 | 0.05% |
| 3 | 18.910 | 18.900 | 0.01 0 | 0.05% |
| Mean | | | 0.003 | 0.016% |

**Table 11:** Serial dilution for the initial test to determine bitterness value (Standard)

| | Tube Numbers | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Solution Used | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| Drinking Water | 5.8 | 5.6 | 5.4 | 5.2 | 5.0 | 4.8 | 4.6 | 4.4 | 4.2 |
| Stock solution Quinine HCl (mL) | 4.2 | 4.4 | 4.6 | 4.8 | 5.0 | 5.2 | 5.4 | 5.6 | 5.8 |
| Quinine HCl in 10mL solution (mg/mL) | 0.042 | 0.044 | 0.046 | 0.048 | 0.050 | 0.052 | 0.054 | 0.056 | 0.058 |

## Swelling Index

**Table 12:** Serial dilution for the second test to determine bitterness value (test sample)

| | *Test tube numbers* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Solution used* | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* | *9* | *10* |
| Drinking water (mL) | 9.0 | 8.0 | 7.0 | 6.0 | 5.0 | 4.0 | 3.0 | 2.0 | 1.0 | 0.0 |
| Stock sol. of herbal drug examined (mL) | 1.0 | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 | 7.0 | 8.0 | 9.0 | 10.0 |

**Table 13:** Quantitative Estimation of Swelling Index

| | *Tube Numbers* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Solution Used* | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *8* | *9* |
| Drinking Water | 5.8 | 5.6 | 5.4 | 5.2 | 5.0 | 4.8 | 4.6 | 4.4 | 4.2 |
| Stock solution Quinine HCl (mL) | 4.2 | 4.4 | 4.6 | 4.8 | 5.0 | 5.2 | 5.4 | 5.6 | 5.8 |
| Quinine HCl in 10mL solution (mg/mL) | 0.042 | 0.044 | 0.046 | 0.048 | 0.050 | 0.052 | 0.054 | 0.056 | 0.058 |

**Table 14:** Foam Height of 1% Solution

| *S.No.* | *Tube No.* | *Height of Foam* |
|---|---|---|
| 1 | 1 | 0.20 |
| 2 | 2 | 0.35 |
| 3 | 3 | 0.50 |
| 4 | 4 | 0.60 |
| 5 | 5 | 0.70 |
| 6 | 6 | 0.85 |
| 7 | 7 | 0.90 |
| 8 | 8 | 1.05 |
| 9 | 9 | 1.20 |
| 10 | 10 | 1.90 |

## Foaming Index

Height of Foam produced in each tube less than 2 cm; hence Foaming Index is less than 100.

## Phytochemical Evaluation

**Table 15:** Preliminary phytochemical investigation of different extracts of (PE) Petroleum Ether; (C) Chloroform; (EA)Ethyl acetate; (M) Methanol ;(HA) Hydro alcohol; (W) Water; (+) Present; (–) Absent

| *S. No.* | *Chemical Test* | *PE* | *C* | *EA* | *M* | *W* | *HA* |
|---|---|---|---|---|---|---|---|
| 1. | Reducing sugars | | | | | | |
| A | Fehling's test | - | - | - | - | - | + |
| B | Benedict's test | - | - | - | + | + | + |
| 2. | Fats and oils | | | | | | |
| A | Solubility test | - | + | - | - | + | + |
| B | Filter paper stain test | - | - | - | - | + | + |
| 3. | Amino Acid | | | | | | |
| A | Ninhydrin test | - | - | - | - | - | - |
| 4. | Proteins | | | | | | |
| A | Biuret test | - | - | - | - | - | - |
| 5. | Alkaloids | | | | | | |
| A | Mayer's test | - | + | - | + | + | - |
| B | Hager's test | - | - | - | - | + | - |
| C | Wagner's test | - | - | + | - | - | - |
| D | Dragendroff's test | - | - | - | - | + | + |
| 6. | Flavanoids | | | | | | |
| A | Lead acetate test | + | + | + | - | + | - |
| 7. | Terpinoids | | | | | | |
| B | Filter paper test | - | - | - | - | + | + |
| 8. | Cardiac Glycosides | | | | | | |
| A | Keller killani test | - | - | - | - | + | + |
| B | Legal test | - | - | - | + | + | + |
| 9. | Anthraquinone Glycosides | | | | | | |
| A | Borntrager's test | - | - | + | - | - | - |
| B | Modified Borntrager test | + | - | - | + | - | - |
| 10. | Saponin Glycosides | | | | | | |
| A | Foam | - | + | + | - | + | + |

## DISCUSSION

Snow mountain garlic is known as *Allium sativum, Allium schoenoprasum, Kashmiri garlic and ek pothi lahsun*. It has hard, golden-brown husk and looks like an individual clove of garlic. It has rounded, bulbous shape with a stiff, flattened portion on one side forming a pointed tail at the end . The color of the clove's outer covering is pale brown and inside color of clove is white with little sticky latex and characteristic odor. Major chemical constituent present in allium sativum is allicin. Snow mountain garlic is a good herbal for breathing discomforts like common cold, asthma, flu also refine the blood circulation. The extractive value of snow mountain garlic was having a maximum extractive value obtained of 45% in hydroalcoholic. The minimum extractive value was 2.66% in petroleum ether and ethyl acetate, indicating a large number of phytoconstituents in the aqueous extract. A high level of ash value such as total ash 1.038%, acid insoluble ash 0% and water soluble ash of 0.16%. The foaming index was found to be less than 100 whereas

swelling index of 1 was observed. Snow mountain garlic, being less bitter, showed a bitterness value of 0.030.

## CONCLUSION

The organoleptic characteristics, hot extraction, bitterness value, ash value, swelling index, foaming index and phytochemical evaluation conducted in the study can help evaluate the identify and purity of the drug. Herbal manufacturing industries and laboratories can use the standards obtained in this study for quality control and assurance.

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