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## Determination of "Saponin in Reetha"

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### Abstract

The present study focuses on the quantitative determination of saponin content in Reetha (*Sapindus mukorossi*) using a UV-Visible spectrophotometric method. Saponins are naturally occurring glycosides known for their surface-active, antimicrobial, and medicinal properties. Due to their significant commercial and pharmacological applications, accurate estimation of their concentration is essential. In this study, dried Reetha fruit powder was subjected to methanolic extraction, followed by purification using diethyl ether and n-butanol partitioning. A series of standard saponin solutions were prepared and analyzed spectrophotometrically at 285 nm to construct a calibration curve. The absorbance of the purified Reetha saponin content determined by comparison with the standard curve.

The results demonstrated a clear linear relationship between absorbance and concentration, indicating the reliability of the method. The spectrophotometric approach proved to be simple, efficient, and suitable for routine analysis of saponins in natural plant materials.

### Introduction

Saponins are a diverse group of naturally occurring glycosides widely distributed in the plant kingdom. These compounds are known for their surface-active properties, which allow them to form stable foams in aqueous solutions. Saponins possess a range of biological and pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, and hemolytic effects. Because of these properties, they are of increasing interest in pharmaceutical, cosmetic, and agricultural industries. *Sapindus mukorossi*, commonly known as Reetha or soapnut, is the plant of the sapindaceae family, traditionally used in Indian and other Asian cultures for its cleansing and medicinal properties. The fruits of Reetha are rich in saponins, which are responsible for its foaming ability and therapeutic potential. Quantifying the saponin content in Reetha is essential for standardizing its use in herbal formulations and evaluating its economic and pharmacological value. Among various analytical methods available, UV-Visible spectrophotometry offers a simple, rapid, and cost-effective approach to saponin quantification. This method involves extracting saponins from the plant material, purifying them using solvent partitioning, and measuring absorbance at a specific wavelength—typically around 285 nm where saponins show characteristic UV absorption. The study aims to determine the saponin content in *sapindus mukorossi* fruits using a spectrophotometric method. The procedure includes methanolic extraction, purification with diethyl ether and n-butanol, and quantification through a calibration curve generated from a standard saponin solution.

### Objective

To quantitatively determine the saponin content in the fruits of Reetha (*sapindus mukorossi*) using a UV-Visible spectrophotometric method.

1. To extract saponins from Reetha fruit powder using methanol as the solvent.
2. To purify the saponin extract through liquid-liquid partitioning using diethyl ether and n-butanol.
3. To prepare a standard calibration curve using known concentration of standard saponin.
4. To measure the absorbance of the purified sample and standard solutions at 285 nm.
5. To calculate the saponin content in the extract based on the calibration curve.
6. To evaluate the efficiency of the extraction and purification method.

### Literature Review

Reetha, also known as *Sapindus mukorossi*, has been a subject of interest in various studies due to its versatile Medicinal Properties.

### Medicinal Properties and Uses

- Reetha is traditionally used in India for its detoxifying properties and as a natural hair cleanser.
- It contains saponins, which are responsible for its cleansing and foaming properties.
- The fruit, shells, shell powder, roots, and bark of Reetha are used in various Ayurvedic preparations.
- Reetha is indicated in treating skin diseases, psychiatric disorders, and respiratory disorders

### Chemical Constituents

- Reetha contains saponins, including hederagenin, oleanolic acid, and sopindic acid.
  - The saponins present in Reetha have been found to have antimicrobial and antiseptic properties.

### Pharmacological Effects

- Reetha has been found to have anti-ulcer activity, with a maximum anti-ulcer activity found at 400 mg/kg of aqueous extract.
- The saponins present in Reetha have been found to have moderate activity against Epstein Barr virus early antigen activation assay.

### Phytochemistry

Reetha saponins are a complex mixture of triterpenoid saponins, which are composed of a hydrophobic nucleus linked to one or more hydrophilic sugar chains (Kumar et al., 2017). The main saponins present in Reetha are hederagenin, oleanolic acid, and sopindic acid (Singh et al., 2018). These saponins have been found to possess a range of biological activities, including antimicrobial, anti-inflammatory, and antioxidant activities.

### Pharmacology

Reetha saponins have been found to possess a range of pharmacological activities, including:

#### Antimicrobial activity

Reetha saponins have been found to exhibit antimicrobial activity against a range of microorganisms, including bacteria, fungi, and viruses (Kumar et al., 2017).

#### Anti-inflammatory activity

Reetha saponins have been found to exhibit anti-inflammatory activity by inhibiting the production of pro-inflammatory cytokines and enzymes (Singh et al., 2018).

#### Antioxidant activity

Reetha saponins have been found to exhibit antioxidant activity by scavenging free radicals and inhibiting lipid peroxidation (Sharma et al., 2019).

### Industrial Applications

Reetha saponins have a range of industrial applications, including:

#### Cosmetics

Reetha saponins are used in the manufacture of cosmetics, such as skin cleansers, shampoos, and conditioners.

#### Pharmaceuticals

Reetha saponins are used in the manufacture of pharmaceuticals, such as anti-inflammatory and antimicrobial agents.

#### Food industry

Reetha saponins are used as a natural emulsifier and stabilizer in the food industry.

### Conclusion

Reetha saponins are a complex mixture of triterpenoid saponins that possess a range of biological activities, including antimicrobial, anti-inflammatory, and antioxidant activities. The saponins have a range of industrial applications, including cosmetics, pharmaceuticals, and food industry. Further research is needed to fully explore the potential of Reetha saponins. Reetha is a versatile medicinal plant with a range of pharmacological effects. Its saponins have been found to have antimicrobial, antiseptic, and anti-ulcer activities. Further research is needed to fully explore the potential of Reetha in various therapeutic applications.

### Experimental Work

**Aim:** To determine the saponin content in Ritha (Saponin mukorossi) using a spectrophotometric method.

**Theory:** Saponins are natural glycosides composed of a hydrophobic aglycone (sapogenin) linked to one or more hydrophilic sugar chains. This amphiphilic nature impacts surface-active properties, enabling them to form stable foams in aqueous solutions. The spectrophotometric method for saponin determination is based on their ability to absorb ultraviolet (UV) light at a specific wavelength, typically around 285nm. This absorption arises from the chromophoric groups present in the sapogenin moiety. By measuring the absorbance of a solution containing saponins at this wavelength, their concentration can be quantitatively estimated using Beer-Lambert's law, which relates absorbance to concentration. Extraction with methanol dissolves the saponins from plant material, while purification using liquid-liquid extraction with diethyl ether and n-butanol removes impurities such as fats, chlorophyll, and other non-polar substances that could interfere with UV absorbance measurements. The purified saponin extracts absorbance is then compared against a standard calibration curve prepared from known concentration of pure saponin.

This method provides a simple, rapid, and cost-effective approach to saponin quantification without requiring complex instrumentation or reagents.

### Procedure

#### Extraction of saponin



1. Weigh 10g of Reetha powder and place it in a clean beaker.
2. Add 100ml of methanol to the powder.
3. Place the beaker in water bath at 60-70°C.
4. Heat for 2 hours with stirring occasionally.
5. Cool slightly and then filter the mixture using filter paper.
6. Collect the filtrate (liquid) this contains saponins.
7. Keep the residue (solid) for second extraction.
8. Repeat extraction with fresh methanol combined filtrates.
9. Distillation separates liquids based on their boiling points.
10. In this case, the methanol-saponin mixture is heated.
11. Methanol, with a lower boiling point than saponins, will evaporate first and be condensed and collected separately.
12. The saponins will remain in the flask as a residue.

#### **Purification of saponins**

1. Dissolve the dried extract in 50mL of distilled water.
2. Add 50mL of diethyl ether to the solution and stir well.
3. Shake the funnel allow the layer to separate.  
Upper layer = Diethyl ether (non-polar) – discard this layer Lower layer = Aqueous (contains saponins) – retain this layer.
4. Repeat the ether extraction.
5. Add 50mL of n-butanol to the aqueous layer and stir well.
6. Separate the butanol layer and collect it.  
Upper layer = n-Butanol (contains saponins) – collect this layer Lower layer = Aqueous discard this layer.
7. Repeat the n-butanol extraction with fresh n-butanol.
8. Combine the butanol layers and evaporate the solvent using distillation method.
9. In this case, the methanol-saponin mixture is heated.
10. Methanol, with a lower boiling point than saponins, will evaporate First and be condensed and collected separately
11. The saponins will remain in the flask as a residue.

#### **Preparation of standard saponin solution**

1. Weigh 10mg of standard saponin and transfer it to a 100mL volumetric flask.
2. Add 50mL of methanol to the flask and stir well.
3. Dilute the solution to 100mL with distilled water.

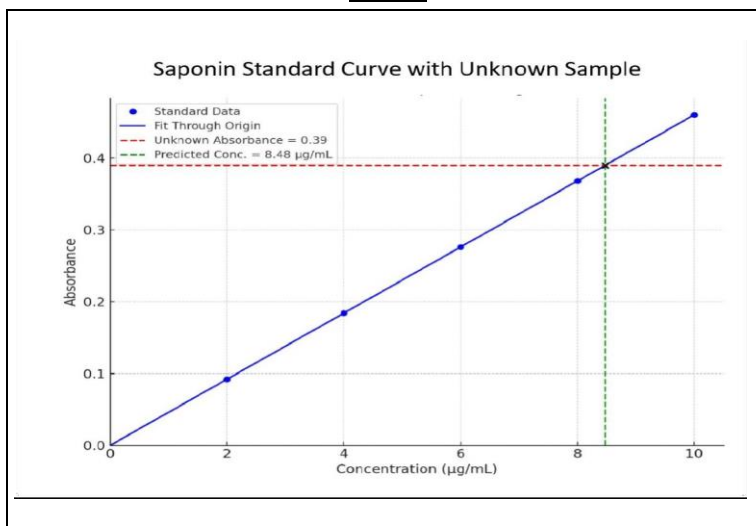
#### **Spectrophotometric Analysis**

1. Prepare a series of standard saponin solutions with concentrations ranging from 1-10mg/ML.
2. Measure the absorbance of each standard solution at 285nm using a UV-Vis spectrophotometer.
3. Plot a calibration curve of absorbance vs. concentration.
4. Weigh 2mg of the purified saponin extract and transfer it to a 10ml volumetric flask.
5. Add 5mL of methanol to the flask and stir well.
6. Dilute the solution to 10mL with distilled water.
7. Measure the absorbance of the sample solution at 285nm using a UV-Vis spectrophotometer.
8. Determine the concentration of saponin in the sample solution using the calibration curve.

**Observation Table**

| Concentration  | Absorbance |
|----------------|------------|
| 2              | 0.092      |
| 4              | 0.184      |
| 6              | 0.276      |
| 8              | 0.368      |
| 10             | 0.460      |
| Unknown Sample | 0.390      |

## GRAPH



## Calculation

Concentration = 8.48 mg/mol Volume = 10 ml

Weight = 2 mg = 2000 mg

Saponin content (%) = (concentration of saponin X volume of solution) / (weight of Reetha extract x 100)

Saponin content (%) =  $8.48 \times 10 / 2000 \times 100$

=  $84.8 / 2000 \times 100$

= 4.24 %

## Result

Percentage of saponin present in given sample of Reetha = **4.24%**

## Applications

### Pharmaceutical Applications

1. Anti-inflammatory agents: Reetha saponins have anti-inflammatory properties, making them potential candidates for developing anti-inflammatory drugs.
2. Antimicrobial agents: Reetha saponins have antimicrobial properties, making them potential candidates for developing antimicrobial drugs.
3. Anticancer agents: Reetha saponins have been shown to have anticancer properties, making them potential candidates for developing anticancer drugs.

### Cosmetic Applications

1. Skin care products: Reetha saponins can be used in skin care products due to their moisturizing and anti-inflammatory properties.
2. Hair care products: Reetha saponins can be used in hair care products due to their ability to cleanse and nourish the hair.
3. Oral care products: Reetha saponins can be used in oral care products due to their antimicrobial properties.

### Food Industry Applications

1. Food emulsifiers: Reetha saponins can be used as natural emulsifiers in food products.
2. Food stabilizers: Reetha saponins can be used as natural stabilizers in food products.
3. Food preservatives: Reetha saponins can be used as natural preservatives in food products.

### Agricultural Applications

1. Pest control: Reetha saponins can be used as natural pest control agents in agriculture.
2. Fertilizers: Reetha saponins can be used as natural fertilizers in agriculture.

### Other Applications

1. Detergents: Reetha saponins can be used in the manufacture of detergents.
2. Soaps: Reetha saponins can be used in the manufacture of soaps.
3. Personal care products: Reetha saponins can be used in the manufacture of personal care products, such as toothpaste and shampoo.

## Conclusion

The present study successfully extracted, purified, and quantified the saponin content in Reetha (*Sapindus mukorossi*) powder. The results demonstrated a significant amount of saponins present in Reetha powder, which has potential applications in pharmaceutical, cosmetic, and other industries. The extraction and purification methods used in this study were found to be effective, and the UV-Vis spectrophotometry method was suitable for quantifying the

saponin content. This study provides a basis for further research on the pharmacological and industrial applications of Reetha saponins.

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### Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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