

# Determination Of Tannin and Saponin Level in *Plagiochasma Appendiculatum* and *Targionia Hypophylla*

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**Abstract**—The bryophytes are a division of plants that includes all non-vascular land plants and can be split into three groups: Mosses, hornworts, and liverworts (Onele et al., 2018). Bryophytes are an important group of lower plants known for the presence of diverse secondary metabolites with significant biological activities. Among these metabolites, tannins and saponins play crucial roles due to their antioxidant, antimicrobial, and therapeutic properties. The present study was undertaken to determine and compare the total tannin and saponin content in two liverwort species, *Plagiochasma appendiculatum* and *Targionia hypophylla*. Quantitative estimation revealed that *Targionia hypophylla* exhibited higher total saponin content ( $3.84 \pm 1.92\%$ ) compared to *Plagiochasma appendiculatum* ( $2.50 \pm 0.22\%$ ). In contrast, total tannin content was significantly higher in *Plagiochasma appendiculatum* ( $163.39 \pm 0.69 \mu\text{g/g}$ ) than in *Targionia hypophylla* ( $57.09 \pm 2.47 \mu\text{g/g}$ ). The variation in phytochemical content may be attributed to species-specific metabolic pathways and ecological adaptations. The findings highlight the pharmaceutical potential of liverworts and support their further exploration as sources of bioactive compounds.

**Index Terms**—Bryophytes, Liverworts, Tannins, Saponins, *Plagiochasma*, *Targionia*

## I. INTRODUCTION

Bryophytes have demonstrated significant pharmacological potential along with diverse biological activities. Traditionally, they have been employed in the treatment of cuts, bone fractures, burns, contusions, open wounds, inflammatory conditions, and even respiratory ailments such as pneumonia [1]. Bryophytes represent a comparatively underexplored lineage of terrestrial plants that have historically played a role in traditional healing systems due to their medicinal potential. Owing to their distinctive structural and physiological characteristics,

they have been employed by numerous indigenous communities to manage a variety of ailments. These plants are rich in a wide spectrum of secondary metabolites, such as alkaloids, flavonoids, terpenoids, and polyphenolic compounds, which are largely responsible for their therapeutic value. Scientific investigations have demonstrated that bryophytes possess multiple pharmacological activities, including anti-inflammatory, analgesic, antimicrobial, and antifungal properties [2]. Studies on the phytochemical constituents of *Plagiochasma articulata* and *Targionia hypophylla* have revealed the presence of alkaloids, flavonoids, coumarins, phenols, tannins, steroids, and sugars [3]. Crude aqueous extracts of *Plagiochasma appendiculatum*, *Plagiochasma intermedium*, and *Fissidens* were screened for bioactive compounds and tested against *Xanthomonas citri* and *Pseudomonas syringae* using standard antimicrobial assays and exhibited significant antibacterial activity, with *P. appendiculatum* showing the highest efficacy compared to standard antibiotics [4]. Traditionally, bryophytes have been employed in Asian systems of medicine for treating skin disorders, wound healing, and certain cardiovascular conditions. Contemporary investigations have expanded this perspective, focusing on their diverse pharmacological applications. Recent studies particularly highlight their antimicrobial and antioxidant activities, including effects on insect larvae and honeybees [5]. However, phytochemical studies on Indian bryophytes are limited. No comparative studies on contents of tannin and saponin have been reported. Therefore, the present investigation focuses on the quantitative estimation of tannin and saponin content in two commonly occurring liverwort species, *Plagiochasma appendiculatum* and *Targionia hypophylla*.

## II. MATERIALS AND METHODS

Collection of plant and Preparation of Crude extract  
*Plagiochasma appendiculatum* and *Targionia hypophylla* collected from their natural habitat from Melghat forest in rainy and winter season. Collected plants cleaned and shade -dried for 7-8 days and then ground into a fine powder. Extraction is done with soxhlet extractor. Methanol was used as solvent. Methanolic extract was filtered and evaporated to dryness by gentle evaporation over a water bath at 45°C to obtained crude extract.

### Estimation of Total Saponin Content

The sample was extracted by immersion using 96% ethanol solvent and allowed to stand in beaker for 24 hrs. It was stirred with a shaker at speed of 120 rpm for 2 hrs, and filtered with filter paper to separate the filtrate and residue. This residue obtain was macerated again with the same solvent until the filtrate was clear. The Saponin content was determined gravimetrically using a multi-step extraction and precipitation method. After filtering a 5 mL sample, the resulting residue was concentrated using a hot air oven. To begin the analysis, the concentrated extract was refluxed with 15 mL of petroleum ether at 60–80°C for 30 minutes to remove non-polar substances. Once cooled, the petroleum ether layer was discarded, and the remaining residue was dissolved in 15 mL of ethyl acetate. This solution was transferred to a separatory funnel, and the ethyl acetate phase was separated. The residue was then sequentially extracted three times with 15 mL of n-butanol. The combined butanol extracts were evaporated in a hot air oven at 70°C until dryness. The resulting concentrate was dissolved in 5 mL of methanol, and this solution was added dropwise into 5 mL of diethyl ether with constant stirring to precipitate the saponins. The precipitate formed was transferred onto a pre-weighed petri dish. After drying, the petri dish was weighed again until a constant weight was achieved. The saponin content was calculated by subtracting the weight of the empty petri dish from the final weight containing the dried saponin precipitate.

The weight of the saponins was calculated using the formula:

Saponin content (g/100g) = (Weight of saponin / Weight of extract) × 100.

### Estimation of Total Tannin Content

The sample was extracted by immersion using methanol solvent in 5g sample in 20ml methanol and allowed to stand in a beaker for 24 hrs. It was then stirred with a shaker at a speed of 120 rpm for 2 hrs and filter with filter paper to separate the filtrate. After filter then allowed to evaporate by submerging the flask in a water bath heated 100°C. The extract was dried for 30 min in a hot air oven at 100°C, then was allowed to cool in a desiccator and the dry extract was collect. And prepared the 1mg/ml extract for the tannin content was determined using the Folin-Ciocalteu colorimetric method. In this procedure, 500 µL(1mg/ml) of the sample was homogenized with 500 µL of ethanol. From this mixture, 1 mL was taken and diluted with 5 mL of distilled water. To this solution, Folin-Ciocalteu reagent and 20% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) were added, and the mixture was allowed to stand for 2 hours to ensure complete color development. The absorbance of the resulting solution was then measured at 748.5 nm using a spectrophotometer. The tannin content was calculated based on a standard calibration curve and expressed as milligrams of gallic acid equivalents (GAE) per gram of extract. The concentration of tannins was determined using the linear regression equation  $Y = mx + c$ , which was rearranged to solve for x as  $x = (y - c) / m$ , where y is the absorbance, m is the slope, and c is the intercept of the standard curve.

Total tannin content was determined using a colorimetric method and expressed as micrograms per gram (µg/g) of dry plant material. All experiments were performed in triplicates.

## III. RESULTS AND DISCUSSION

The quantitative analysis of tannin and saponin content in the selected liverworts showed notable variation between the two species. The results indicate that *Targionia hypophylla* possesses a higher saponin content compared to *Plagiochasma appendiculatum*, suggesting stronger surface-active and defensive properties (Table No.1). Conversely the significantly higher tannin content observed in *Plagiochasma appendiculatum* points towards its greater antioxidant and antimicrobial potential.

Table 1: Tannin and Saponin Level of *Plagiochasma appendiculatum* and *Targionia hypophylla*

Sr.No.	Species	Tannin Level (Mean $\pm$ SD) (g/100gm)	Saponin Level (Mean $\pm$ SD) (g/100gm)
1	<i>Plagiochasma appendiculatum</i>	163.39 $\pm$ 0.69	2.50 $\pm$ 0.22
2	<i>Targionia hypophylla</i>	57.09 $\pm$ 2.47	3.84 $\pm$ 1.92

Such interspecific variation in secondary metabolites among liverworts may be influenced by genetic factors, habitat conditions, and ecological adaptations. Similar variations in phytochemical profiles of bryophytes have been reported earlier, emphasizing their chemical diversity and medicinal importance.

#### IV. CONCLUSION

The present study confirms that both *Plagiochasma appendiculatum* and *Targionia hypophylla* are rich sources of bioactive compounds, particularly tannins and saponins. *Plagiochasma appendiculatum* is a promising source of tannins, while *Targionia hypophylla* exhibits higher saponin content. These findings support the potential application of liverworts in pharmacological and natural product research and encourage further detailed biochemical and biological studies.

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