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Propagation and Proximate Analysis of *Homalomena aromatica* (Spreng.) Schott of Manipur, India

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ABSTRACT: *Homalomena aromatica* (Spreng.) Schott is a valuable medicinal aromatic plant. The present study aimed to evaluate both *in vitro* and vegetative propagation strategies as well as proximate composition of the rhizome, leaf lamina and petiole of mature *H. aromatica* plants. *In vitro* shoot induction was achieved by inoculating rhizome buds in MS basal medium supplemented with different hormone combinations. For vegetative propagation, rhizome buds were planted in nursery bags in a mixture of sand, garden soil and perlite in the ratio of 1:2:1. Proximate analysis was conducted using standard procedures. The highest rate of shoot induction was observed in MS BAP 2 mg/mL. Vegetative propagation showed bud break rate of 51.39% in four weeks. The moisture, fat, nitrogen and protein content were highest in the leaf lamina whereas the rhizome showed highest contents of reducing sugars, starch, carbohydrate and ascorbic acid. The petiole showed highest content of ash and fibre.

Keywords: *Homalomena aromatica*, *in vitro* shoot induction, vegetative propagation, proximate analysis.

INTRODUCTION

Homalomena aromatica (Spreng.) Schott is a valuable medicinal aromatic plant belonging to the family Araceae, which has been traditionally used for centuries in Southeast Asian folk medicine and ethnobotany. This plant has wide-ranging applications in ethnobotany, pharmacology, perfumery, and the flavor industry. Traditional medicine practitioners have utilized various parts of *H. aromatica*, including leaves and rhizomes, for the treatment of joint pain, skin diseases, colds, asthma, diarrhea, and jaundice (Policegoudra *et al*., 2012; Raomai *et al*., 2013; Tamang *et al*., 2025). The rhizome yields a rich essential oil which has both pharmaceutical and commercial significance (Policegoudra *et al*., 2012; Tamang *et al*., 2025). In light of the commercial and medicinal value of the plant, the present study aimed to evaluate both *in vitro* and vegetative propagation strategies. A previous study has reported an *in vitro* micropropagation protocol (Raomai *et al*., 2013). Therefore, this study integrated previously unexplored hormonal combinations and evaluated the shoot induction response of *H. aromatica* rhizome bud explants. In addition, *H. aromatica* was reported as a non-conventional vegetable in Bangladesh and the parts used are described as stems and leaves (Abdullah *et al*., 2020). Therefore, this study evaluated the proximate composition of the rhizome, leaf lamina and petiole of mature *H. aromatica* plants.

MATERIALS AND METHODS

A. Collection of plants

Plants were collected from Jiribam district of Manipur, India in April, 2019 and identified at the Department of Life Sciences (Botany), Manipur University, Manipur, India. The plants were maintained in a nursery until substantial rhizomes could be collected. The present study was conducted in 2021.

B. In vitro shoot induction

Rhizomes were thoroughly washed in running tap water to remove soil particles and scales. The buds were excised from the rhizomes and were treated with detergent for 10 minutes, then rinsed three times in tap water. The buds were then placed under running tap water for one hour to remove any residual soil particles. They were then treated with 1% Bavistin (w/v) for 15 minutes, then washed under running tap water for five minutes. In the laminar hood, they were rinsed three times with sterile distilled water for thirty seconds. They were then treated with 0.1% $HgCl_2$ (w/v) and 0.1% Tween20 (v/v) for eight minutes, then rinsed five times with sterile distilled water for thirty seconds. The sterilized buds were then inoculated in Murashige and Skoog's (1962) basal medium (MS) supplemented with 3% sucrose (w/v), 0.01% inositol (w/v), and different hormones and hormone combinations. 0.26% CleriGel (w/v) was added after adjusting the pH to 5.8. The media was sterilized by autoclaving at 15 psi at 121ºC

for 15 minutes. Three replicates were taken for each treatment. The cultures were subcultured to fresh media of same composition every two weeks. Contaminated cultures were subcultured to fresh media of same composition at the first observance of contamination and discarded at second observance.

The different treatments given are as follows:

1. MS (Control)
2. MS BAP ((Benzylaminopurine) 0.5 mg/L (M1)
3. MS BAP 1 mg/L (M2)
4. MS BAP 1.5 mg/L (M3)
5. MS BAP 2 mg/L (M4)
6. MS BAP 1 mg/L + IAA (Indole-3-acetic acid) 0.5 mg/L (M5)
7. MS BAP 1 mg/L + IBA (Indole-3-butyric acid) 0.5 mg/L (M6)
8. MS BAP 1 mg/L + IBA 1 mg/L (M7)

C. Vegetative propagation

72 rhizome buds were planted in 36 nursery bags, two in each bag. A mixture of sand, garden soil and perlite in the ratio of 1:2:1 was used. Irrigation was done every alternate day. The plantations were maintained in a shade net house.

D. Proximate analysis

The plants were divided into three parts, namely leaf, petiole and rhizomes. The different plant parts were thoroughly washed under running tap water and finally rinsed with distilled water. The different plant parts were cut into small pieces and allowed to shade dry until completely dried. They were then ground to a fine powder and used for further analysis. Experiments were done in triplicate. All experiments were repeated thrice.

Determination of moisture content

Moisture content was analyzed following the AOAC 2001.11, 21st Ed. 2019 (AOAC, 2019). 2 g each of HAR, HAL and HAP were taken in a dish of known weight. The samples were then dried in a hot air oven to a constant weight. Weight of the sample after drying is recorded.

Moisture content is calculated using the following equation:

$$Moisture\ (\%) = \frac{Initial\ weight - Final\ dry\ weight}{Initial\ weight} \times 100$$

Determination of ash content

Ash content was estimated following the AOAC 2001.11, 21st Ed. 2019 (AOAC, 2019). 2 g each of HAR, HAL and HAP were taken in crucibles and incinerated in a muffle furnace at 550°C for six hours until a uniform white ash was obtained. Weight of the ash was recorded.

Ash content was calculated using the following equation:

$$Ash\ (\%) = \frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100$$

Estimation of crude fibre

Crude fibre was estimated following the AOAC 2001.11, 21st Ed. 2019 (AOAC, 2019). 2 g each of HAR, HAL and HAP were acid-digested using 1.25% sulfuric acid for thirty minutes. The residue was then filtered and thoroughly washed with hot distilled water until free of acid. The residue was then digested with 1.25% sodium hydroxide solution for thirty minutes, after which the residue was repeatedly washed with hot distilled water followed by ethanol to remove residual alkali and moisture. The residue was then transferred to a previously weighed crucible and dried in a hot air oven to a constant weight. The dried residue was subsequently incinerated in a muffle furnace at 550°C until a white ash was obtained, after which the weight of the crucible was recorded.

The crude fibre content was calculated using the following equation:

$$\%Crude\ fibre = \frac{Weight\ of\ dried\ residue - Weight\ of\ ash}{Weight\ of\ sample} \times 100$$

Fat content estimation

The crude fat content was estimated following the AOAC 2001.11, 21st Ed. 2019 (AOAC, 2019). 2 g each of HAR, HAL and HAP were taken in a cellulose thimble and loaded into the Soxhlet apparatus. Petroleum ether was used as the extraction solvent. The samples were subjected to eight hours of continuous extraction, after which the solvent containing the extracted fats was evaporated. The flasks were further dried in a hot air oven to remove residual solvent until they weighed to a constant mass.

Fat content was calculated using the following equation:

$$\%Fat\ content = \frac{Weight\ of\ extracted\ fats\ (g)}{Weight\ of\ sample\ (g)} \times 100$$

Determination of nitrogen content

The nitrogen content was analyzed using the Micro-Kjeldahl method (Sadasivam & Manickam 2008). 100 mg each of HAR, HAL and HAP were taken in a digestion flask and digested with concentrated sulfuric acid along with potassium sulfate and copper sulfate until the solution became colorless. It was then cooled and diluted with a small amount of distilled water and transferred to the distillation apparatus. 10 mL of sodium hydroxide-sodium thiosulfate solution was added to the test solution and distilled. The generated ammonia was collected on boric acid containing a few drops of mixed indicator (two parts of methyl red solution and one part of methyl blue solution). The solution was then titrated against 0.02 N HCl until the first appearance of violet color. A reagent blank was run in parallel.

The nitrogen contents of the samples were calculated using the following equation:

$$\%\ Nitrogen = \frac{(Volume\ of\ HCl - Volume\ of\ blank) \times 14 \times\ Normality\ of\ HCl}{Weight\ of\ sample} \times 100$$

Determination of protein content

The protein content of the samples was calculated using the following equation:

$$\% \text{ Protein} = 6.25 \times \text{Nitrogen content}$$

Determination of reducing sugars

The presence of reducing sugars was determined by the Nelson-Somogyi method (Sadasivam & Manickam 2008). Briefly, 100 mg each of ground rhizome (HAR), leaf (HAL) and petiole (HAP) were taken and the sugars were extracted using 5 mL of 80% of hot ethanol twice. The supernatant was collected and evaporated on a water bath at 80°C. Then, 10 mL of distilled water was added to dissolve the sugars. Different concentrations of the samples and standard glucose were prepared in a final volume of 2 mL. Then, 1 mL of alkaline copper tartrate was addSed and boiled for 10 minutes. After cooling, 1 mL of arsenomolybolic acid was added and the volume was made up to 10 mL with distilled water. After incubation for 10 minutes, absorbance was measured at 620 nm using a UV/Visible spectrophotometer. The amount of reducing sugars present in the samples were calculated using the standard curve.

Determination of starch content

Starch content was estimated using the anthrone method (Sadasivam & Manickam 2008). 300 mg each of HAR, HAL and HAP were homogenized in hot 80% ethanol to remove sugars, then centrifuged at 10 g for ten minutes and the residue was retained. The residue was repeatedly washed with hot 80% ethanol till the washings do not give color with anthrone reagent. The residue was then dried over a water bath. Then, 5 mL of water and 6.5 mL of 52% perchloric acid were added to the residue, then extracted at 0°C for 20 minutes. The solution was then centrifuged at 10 g for ten minutes and the supernatant was collected. The extraction was repeated with fresh perchloric acid and the supernatants were pooled and the volume was made up to 100 mL. 0.1 mL of the supernatant was taken and the volume was made up to 1 mL with distilled water. Then, 4 mL of anthrone reagent was added and boiled for eight minutes, then immediately transferred to an ice bath. Absorbance was measured at 630 nm using a UV/Visible spectrophotometer. Glucose was used as standard. Amount of glucose content in the samples were calculated from the standard curve. Starch content in the samples was determined using the following equation:

$$Starch\ content = Glucose\ content\ in\ the\ sample \times 0.9$$

Determination of total carbohydrate

The total carbohydrate content was determined using the anthrone method (Sadasivam & Manickam, 2008). 100 mg each of HAR, HAL and HAP were hydrolyzed by boiling for three hours with 5 mL of 2.5 N HCl and cooled to room temperature, and then neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 50 mL and centrifuged at 10 g for then minutes. The supernatant was collected and used for analysis. Glucose was used as standard. Different aliquots of the samples and standard were prepared with a final volume of 1 mL. Then, 4 mL of anthrone reagent was added and boiled for eight minutes and then immediately transferred to an ice bath. Absorbance was measured at 630 nm using a UV/Visible spectrophotometer. The amount of total carbohydrate present in the samples were calculated from the standard curve.

$$Amount\ of\ total\ carbohydrate\ present\ in\ 100\ mg\ of\ the\ sample = \frac{mg\ of\ glucose}{Volume\ of\ test\ sample} \times 100$$

Determination of ascorbic acid content

The ascorbic acid content was determined using volumetric method (Sadasivam & Manickam 2008). 5 mL of 1 mg/mL ascorbic acid prepared in 4% oxalic acid solution was mixed with 10 mL of 4% oxalic acid solution. The resulting solution was titrated against 2,6-dichlorophenol indophenol dye until the appearance of a pink color which persists for a few minutes. The amount of the dye consumed is equivalent to the amount of ascorbic acid.

500 mg each of HAR, HAL and HAP were extracted in 4% oxalic acid and the volume was made up to 35 mL for HAR and 20 mL for HAL and HAP and centrifuged at 10 g for ten minutes. Then, 10 mL of 4% oxalic acid was added to 5 mL of the supernatant and titrated against the dye.

The amount of ascorbic acid present in the sample was determined using the following equation:

$$\%Ascorbic\ acid\ content = \frac{Dye\ factor \times Titre\ value \times Total\ volume\ (mL)}{Volume\ taken\ (mL) \times weight\ of\ sample\ (g)} \times 100$$

Dye factor was determined using the following equation:

$$Dye\ factor = \frac{mg\ of\ standard\ ascorbic\ acid}{Titre\ value\ of\ standard\ ascorbic\ acid\ (mL)}$$

RESULTS AND DISCUSSION

A. In vitro shoot induction

The culture media supplemented with different hormones and hormone combinations showed varying effects of bud growth. MS media supplemented with BAP 1 mg/L (M_2) and 2 mg/L (M_4) showed visible response in three and four weeks (Table 1). M_1 (MS BAP 0.5 mg/L) and M_7 (MS BAP 1 mg/L + IBA 1 mg/L) showed some response at four weeks but contamination was observed. Contamination rate was found to be very high. 57.14% of the total cultures on average were eventually discarded due to contamination. The best response at four weeks was observed in M_4 (Fig. 1). These findings align with previous reports on the *in vitro* propagation of *H. aromatica* (Raomai *et al*., 2013). The previous study also reported a high contamination rate (Raomai *et al*., 2013).

This study evaluated previously unexplored hormonal combinations. However, we found that MS

supplemented with BAP 2 mg/mL is most suitable for *in vitro* shoot induction of *H. aromatica* rhizome buds as formerly reported. Therefore, no further studies under *in vitro* conditions were conducted.

The rhizome buds showed a considerably strong response in the vegetative propagation study, with a bud break rate of 51.39% at four weeks (Table 2). Fig. 2 shows the representative photographs of three years of growth.

Morphometric data of the plants were recorded (Table 3). A wide variation in petiole length and rhizome weight was observed among the plants.

Table 1: Response rates of *H. aromatica* rhizome buds in MS media supplemented with different hormone combinations.

Culture media	Basal Medium	Cytokinins	Auxins	Response*		
				Week 2	Week 3	Week 4
M_1	MS	BAP 0.5 mg/L	–	–	–	+
M_2	MS	BAP 1 mg/L	–	–	+	++
M_3	MS	BAP 1.5 mg/L	–	–	–	–
M_4	MS	BAP 2 mg/L	–	–	+++	+++
M_5	MS	BAP 1 mg/L	IAA 0.5 mg/L	–	–	–
M_6	MS	BAP 1 mg/L	IBA 0.5 mg/L	–	–	–
M_7	MS	BAP 1 mg/L	IBA 1 mg/L	–	–	+

* + indicates low, ++ moderate and +++ high response rates. – indicates no response.

Table 2: Response rates of *H. aromatica* rhizome buds in the vegetative propagation model.

Time	Bud break	Fully formed shoot with juvenile leaves	Dormant buds
2 weeks	25	0	47
3 weeks	46	6	20
4 weeks	37	23	12

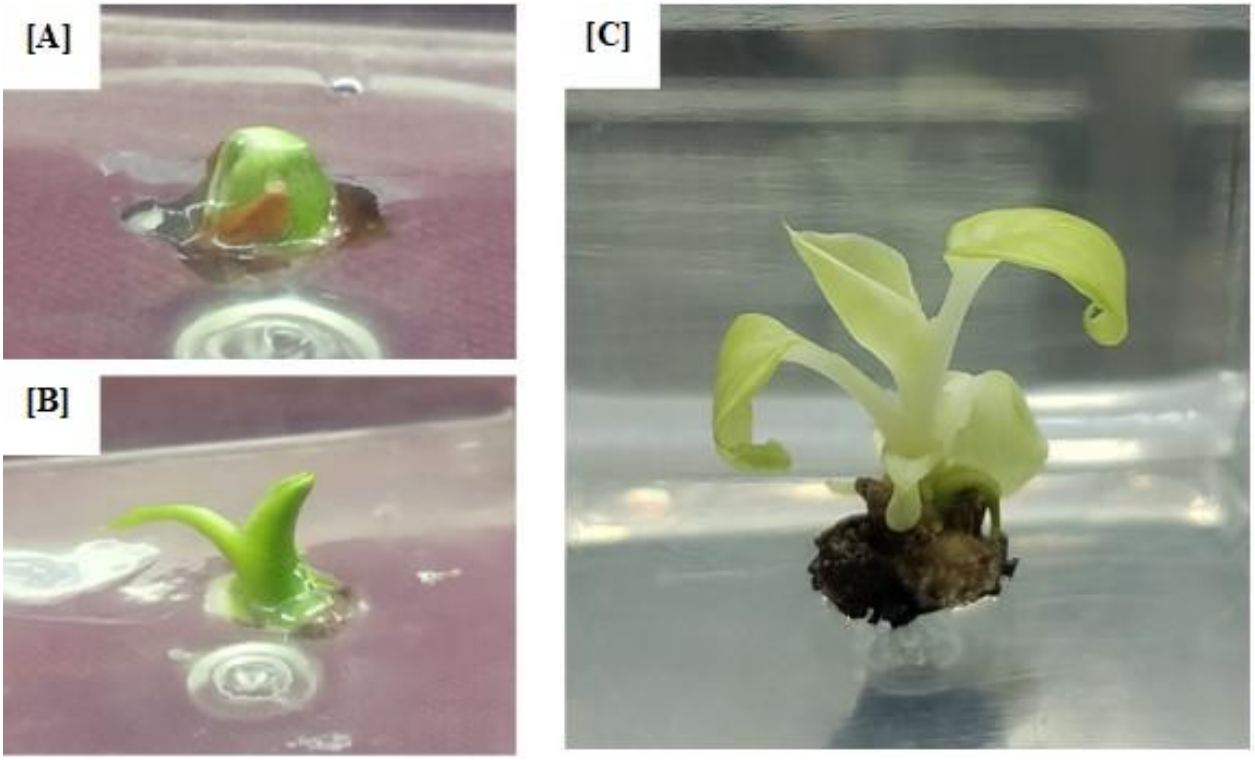

Fig. 1. Representative images of *in vitro* shoot induction using rhizome buds in MS media containing BAP 2 mg/mL. [A]: Inoculation stage; [B]: Initiation of shoot formation; [C]: Fully developed *in vitro* shoot.

Fig. 2. Representative images from vegetative propagation of *H. aromatica* from rhizome buds. Upper row (from left to right): Buds planted in nursery bags; bud break; development of young shoot; young plant; young plant showing visibly formed rhizome. Lower row (from left to right): Mature plant with fully formed rhizome; mature rhizomes harvested after three years; rhizome showing buds developing into new shoots; a young shoot developed from rhizome bud.

Table 3: Morphometric data of *H. aromatica* mature plants.

Plant part	Leaf			Rhizome			
Parameters	**Petiole length (cm)**	**Lamina length (cm)**	**Lamina breadth (cm)**	**Rhizome length (cm)**	**Rhizome diameter (cm)**	**Rhizome weight (g)**	**Nodes per rhizome**
Mean ± Standard deviation	47.87 ± 10.58	21.21 ± 3.05	17.40 ± 2.99	21.15 ± 3.32	3.29 ± 0.98	157.5 ± 74.25	6 ± 2.53

Proximate analysis of the mature plants was also conducted. It was observed that the moisture, fat, nitrogen and protein content were highest in the leaf lamina whereas the rhizome showed highest contents of reducing sugars, starch, carbohydrate and ascorbic acid (Table 4). The petiole showed highest content of ash and fibre (Table 4). These findings align with previous reports. Leaf lamina usually maintains higher water content to support stomatal conductance and carbon assimilation (Ievinsh, 2023). A large fraction of leaf nitrogen is invested in photosynthetic machinery and therefore, nitrogen-rich leaves also tend to have more photosynthetic proteins which might explain the relatively higher nitrogen and crude protein contents (Perchlik & Tegeder 2018, Patel *et al.*, 2023). Leaf lamina also contains a high amount of membrane lipids (chloroplast thylakoid and other cellular membranes) and surface-associated lipids (e.g., cuticular waxes), which might contribute to the solvent-extractable fraction measured as crude fat (Ikewuchi *et al.*, 2019).

The high levels of reducing sugars, starch, and total carbohydrates observed in the rhizome clearly indicate its role as a major storage organ. In many perennial plants, rhizomes act as reservoirs of non-structural carbohydrates that support basic metabolic needs during dormancy and provide the energy required for rapid sprouting and vegetative growth when conditions become favorable (Wang *et al.*, 2022). Starch represents the primary long-term energy store, whereas reducing sugars such as glucose and fructose serve as readily available energy sources and participate in sugar-mediated signaling processes that regulate growth and development (Rolland *et al.*, 2006).

The relatively high ash content in the petiole suggests a higher accumulation of inorganic mineral constituents, which is consistent with the role of petioles as transport conduits and structural support tissues. Petioles often serve as pathways for xylem- and phloem-mediated movement of water, nutrients, and minerals between roots and leaves, leading to higher concentrations of mineral elements such as calcium, potassium and magnesium (Wunnenberg *et al.*, 2021).

Table 4: Proximate composition of mature *H. aromatica* rhizome, leaf lamina and petiole.

Parameter		Mean	S.D.	S.E.M.
Moisture (%)	HAR	4.25	0.09	0.05
	HAL	7.31	0.05	0.03
	HAP	4.46	0.19	0.11
Ash (%)	HAR	6.75	1.06	0.61
	HAL	10.13	0.30	0.17
	HAP	12.94	1.03	0.60
Crude fibre (%)	HAR	10.2	0.14	0.08
	HAL	4.35	0.21	0.12
	HAP	13.40	0.28	0.16
Fat (%)	HAR	1.01	0.21	0.12
	HAL	6.60	3.44	1.99
	HAP	1.30	0.23	0.14
Nitrogen (%)	HAR	1.82	0.11	0.06
	HAL	2.51	0.15	0.09
	HAP	0.72	0.07	0.04
Protein (%)	HAR	11.38	0.70	0.40
	HAL	15.69	0.96	0.56
	HAP	4.49	0.44	0.25
Reducing sugar (mg GE/g sample)	HAR	15.79	0.30	0.17
	HAL	8.26	0.34	0.20
	HAP	6.09	0.21	0.12
Starch (g/g sample)	HAR	3.69	0.03	0.02
	HAL	0.63	0.02	0.01
	HAP	0.83	0.01	0.01
Total carbohydrate (mg GE/100 mg sample	HAR	131.23	3.24	1.87
	HAL	29.49	0.56	0.33
	HAP	75.78	0.81	0.47
Ascorbic acid content (%)	HAR	46.37	1.42	0.82
	HAL	26.32	1.63	0.94
	HAP	31.30	0.82	0.47

CONCLUSION

In this study, both *in vitro* shoot induction from rhizome buds and natural vegetative propagation were successful. Shoot induction from rhizome buds was achieved using a hormone combination consistent with previous reports, indicating reproducibility and reliability of the protocol. Vegetative propagation would be more practical for low-cost conservation. Lower input requirements and adaptability of this approach would make it favourable for community-based conservation programs. However, *in vitro* culture remains valuable for germplasm preservation and mass production from a small number of parent plants. Proximate analysis suggests that the plant may be a good nutraceutical ingredient, validating its use as an unconventional vegetable.

FUTURE SCOPE

Further studies to establish protocols for microrhizome induction would be crucial since the rhizome is the most commercially valued part of the plant. Further analysis of phytochemical contents and evaluation of bioactivities are important to study the pharmaceutical potential of the plant.

Author contributions. Yanglem S.: Conceived and designed the analysis; Collected the data; Contributed data or analysis tools; Performed the analysis; Wrote the paper
Waikhom B.S.: Contributed data or analysis tools
Maibam D.D.: Conceived and designed the analysis

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Conflict of interest. The authors declare that there is no conflict of interest.

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