



RESEARCH ARTICLE

# *In vitro* studies on quality assessment and ethnobotany of *Acampe rigida* (Buch.-Ham. ex Sm.) P.F.Hunt encountered in Ultapani Forest Range, Assam

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## Abstract

*Acampe rigida* (Buch.-Hem. ex Sm.) P.F.Hunt (Family: Orchidaceae), locally known as 'Miral Baha' among the Santhal community of Ultapani Forest Range, BTR, Assam, blooms in the month of July to November. The present study was aimed to carry out the ethnobotanical studies, macroscopic, pharmacognostic, preliminary phytochemical and heavy metal analysis of *A. rigida*. Apart from being an eye-catching ornamental plant, it has a number of medicinal potentialities as folk medicine among the Santhal community where the leaves are used for recovering throat cancer and epilepsy and the paste helps in relieving rheumatism, healing wound, as tonic to strengthen the body and increase blood in the body. Parenchyma cells, stomata, fibre were observed in the microscopic examination of powdered plant part. The pulverised leaves colour, odour, flavor/taste and texture were all satisfactory. Preliminary phytochemical screening of *A. rigida* leaves aqueous extract confirmed the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, steroids, saponins, tannins, terpenoids, whereas phlobatannins was absent. Heavy metal tests revealed that the samples were free from bismuth, cadmium and lead. The present studies might be significant to establish the pharmacognostic and phytochemical standards of this orchid species and may help in the identification, to ensure quality and purity and standardization of the herbal drug.

## Keywords

*Acampe rigida*, ethnobotany, folk medicine, pharmacognostic, phytochemical screening, traditional community healers.

## Introduction

Orchidaceae has been recognised as one of the most beautiful families comprising of ornamental and medicinal plants (1, 2). The Orchidaceae is one of the highly advanced family of flowering plants and exhibit great diversity. Several studies have been done in different levels in number of countries around the world viz. China, Thailand, Bangladesh (3, 4). Apart from other countries, Orchidaceae shows its existence in India in enormous numbers and fortunately it has been encountered in Ultapani Forest Range which is geographically situated within Kokrajhar District of Assam. Ultapani is significant because of its ecosystem and rich biodiversity. The family Orchidaceae forms one of the most attractive captivating factors. As per the first Orchid census of India performed and published by Botanical Survey of India (BSI) in 2019, India is blessed with 1,256 orchid species or taxa belonging to 155 genera. Of which, 388 species are reported to be endemic (5, 6). Inter-

estingly, around 398 specific and 6 intraspecific orchids are documented in Assam (7).

Ultapani Forest Range is an evergreen and semi-deciduous type of forest with extensive forest cover. This area is slowly earning popularity amongst tourist because of its impressive biodiversity. It is one of the four territorial ranges under Haltugaon Forest division under Kokrajhar district. The geographical area of Ultapani Forest Range is 224.64 sq. Km. (approx.) with N26°52'13.5", E90°20'37.1"; N26°51'26.6", E90°15'23.4"; N26°44'24.0", E90°14'04.7"; N26°39'41.2", E90°17'49.0"; N26°39'18.1", E90°21'28.0" (8). During the field survey it was observed that this area was covered with various species of epiphytic orchids.

*Acampe rigida* (Buch.-Ham. ex Sm.) P.F.Hunt is one such beautiful orchid which is encountered in this forest range. It is an epiphytic orchid having 40-60 cm long stout stems which are mostly unbranched. The phenology of the vegetative phase of *A. rigida* revealed that the new shoots are formed during April to May and new leaves emerge through May to November, whereas senescence is initiated in January and during January- March, no growth is observed. The plant blooms in the month of July to November. As per phenology of the pod set phase, it was observed that the pod set begins in April and seed dispersal occurs from January to March (after 10-12 months) once the pod is matured.

Apart from being an ornamental plant, *A. rigida* is used amongst a group of people residing around the globe for its medicinal values. The Batakese tribe residing in Indonesia uses the decoction of *A. rigida* whole plant as a tonic to strengthen body (9). Similarly, the leaf and root are reported to stimulate blood circulation and relief from joint and muscular pain (10). However, no scientific study has been done till date on *A. rigida* of Ultapani Forest Range region to specify its medicinal value that is being practised by the Santhal community traditional healers. The present study was carried out to reveal the pharmacognostic and phytochemical data, morphological characterization for identification of this species of orchid, to ensure quality and purity and standardization of herbal drug.

## Materials and Methods

### Collection and identification

Field survey was carried out in Ultapani Forest Range, Kokrajhar, Assam, India during 2019 to 2021 and the plant species was collected from its natural habitat. The voucher specimen was prepared following the standard method (11). The preliminary identification of the collected species was done by consulting the literature and specimens deposited at the Department of Botany, Bodoland University. However, the orchid species was identified and authenticated by Botanical Survey of India (BSI), Howrah having specimen number BUSD-04 dated 30.11.2021.

### Preparation of plant material

Leaves of *A. rigida* were washed thoroughly with water and dried at room temperature. The plant material was powdered by means of an electric grinder. The powdered sam-

ple was subjected to extraction using double distilled water (1:10 w/v) for 24 hrs at room temperature. The solution was filtered through Whatman filter paper No. 1 and was stored at 4°C in an airtight glass bottle for further analysis.

### Macroscopic study

The macroscopic features of the plant material were recorded by visual and physical examination of the whole plant and its parts placing it on black paper.

### Ethnobotanical survey

The local residents and the traditional community healers were interacted and questionnaires were prepared and distributed among them to record the indigenous knowledge and the medicinal uses of *A. rigida*.

### Pharmacognostic analysis

#### Organoleptic parameters

Organoleptic tests were carried out to record the colour, odour, flavor/taste and texture of the pulverized leaves by following the standard method (12).

#### Microscopic study

The powdered orchid leaf was mounted in water and safranin on clean slides and observed under microscope for various characteristics in fragmented form using binocular microscope (LaboMed vision 2000) (13, 14).

#### Preliminary phytochemical screening

Qualitative tests for alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins, tannins, terpenoids, steroids and phlobatannins were carried out using aqueous extract by following the standard methods as described earlier (15-19).

#### Heavy Metal Test

The orchid leaf extract was also evaluated for the presence or absence of heavy metals like cadmium, bismuth and lead following the standard protocol (20). Two tests were performed for each heavy metal as described below:

##### Test for bismuth

Process 1: Both test sample and control were mixed with H<sub>2</sub>S individually and observed for brown precipitation.

Process 2: Both test sample and control were mixed with NH<sub>4</sub>OH individually and observed for white precipitation.

##### Test for cadmium

Process 1: Both test sample and control were mixed with NH<sub>4</sub>OH individually to detect the presence or absence of cadmium based on white precipitation.

Process 2: Both test sample and control were mixed with Potassium Ferrocyanide individually to detect the presence or absence of cadmium based on white precipitation.

##### Test for lead

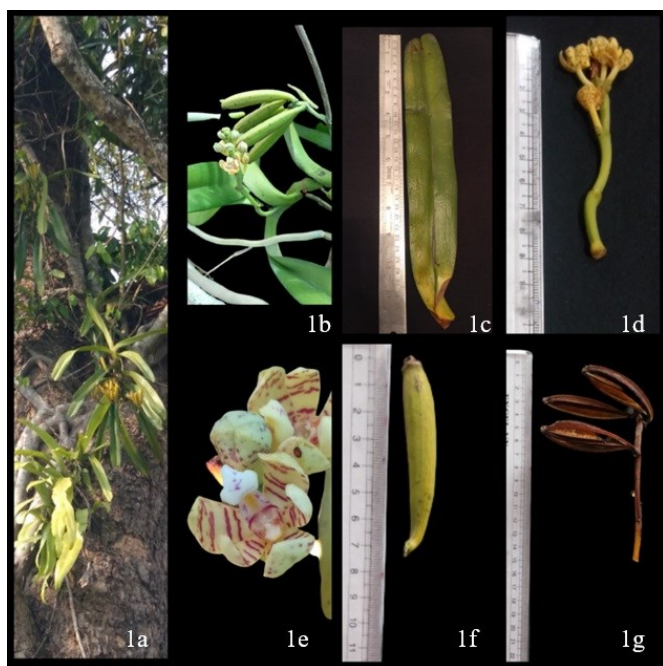
Process 1: Both test sample and control were mixed with dilute HCl (37%) individually and observed for white precipitate.

Process 2: Both test sample and control were mixed with dilute potassium iodide (KI) individually and observed for yellow precipitate.

## Results and Discussion

### Macroscopic study

Macroscopic studies indicated that the non-pseudo bulbous, monopodial, unbranched sometime branched wild orchid was epiphytic in nature having 40-60 cm long stout stems. Leaves were spirally arranged, deep green, leathery, lorate, uneven shortly 2-lobed, 14-35×3-5.5 cm; inflorescence axillary, about 9-21 cm long, stout, peduncle with racemes, suberect, shortly branched, many flowered; flowers were fragrant, bowl-shaped, yellow with purplish brown transverse stripes, 1.0-1.8 cm broad; sepals and petals are fleshy; lip saccate, hairy, white with purplish brown or longitudinal stripes above, channelled in the middle, sac short, column short; fruit-set long ribbed, 7.5-10×1.5-2 cm, green colour while young, dark brown colour when dried. (Fig. 1)



**Fig. 1.** *Acampe rigida* plant. **a)** Habit of *A. rigida*; **b)** Fruit along with flower; **c)** Leathery, deep green leaf; **d)** Inflorescence; **e)** Fleshy yellow flower with hairy white lip; **f)** Young fruit; **g)** Dried fruit

### Ethnobotanical survey

Ethnobotanical information collected by means of interviews and questionnaires among the traditional community healers revealed that *A. rigida* leaf is popular among the Santhal community and has a number of beneficial effects as mentioned in Table 1. It was reported that the juice

(boiled) of *A. rigida* is effective as tonic to strengthen body when taken orally on daily basis among the Lake Toba community, Indonesia which is in compliance with those of the Santhal community traditional healers, though the processing and dose is different (9). Similarly, the ethnomedicinal use of the whole plant of *A. rigida* was documented in Thailand as body tonic (21).

The roots and leaves of *A. rigida* is used in China to get rid of pain and enhance blood circulation which is similar to the information provided by Mr. Tibru Hembram and Mr. Basu Murmu, traditional healers among the Santhal community respectively for *A. rigida* leaves (22). However, the use of *A. rigida* leaves to treat throat cancer, epilepsy and wound healing has been documented for the first time.

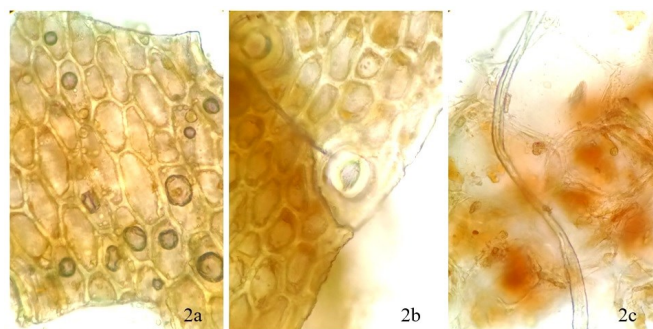
### Pharmacognostic analysis

#### Organoleptic evaluation

The study done by using organ senses to evaluate the colour, odour, flavor/taste and texture of the pulverised leaves showed that it was peanut brown in colour having oily odour. The texture was spongy granular with bitter taste.

#### Microscopic study

The powder microscopic analysis of *A. rigida* leaf showed the presence of parenchyma cells, stomata and fibre in the powdered plant part (Fig. 2). The parenchyma cells pre-



**Fig. 2.** Microscopy of powdered *A. rigida* leaf (40X). **a)** Parenchyma cells; **b)** Stomata; **c)** Fibre.

sent in the plants are associated with most of the metabolic functions such as repairing damaged cells, photosynthetic processes, exchange of gases and storage of energy and unwanted products (23). Fibres are supporting tissue that gives mechanical support (12) whereas stomata helps in gaseous exchange (24).

**Table 1.** Ethnobotanical information collected from Santhal community traditional healers

Scientific name	Part used	Other ingredient	Medicinal uses	Processing	Dosage	Herbalist	Preventive measure
<i>Acampe rigida</i> (Buch.-Ham. ex Sm.) P.F.Hunt	Leaves	Nil	Recovers throat cancer	Crushed and made into tablets	2 tablets per day	TH	
		<i>Scleromitron diffusum</i> (Willd.) R.J. Wang leaves (40%)	Treats epilepsy	Crushed and made into tablets	1 tablet per day	TH	Intake of egg, fish, meat or any other allergenic food items is prohibited during medication.
		Sand from bird's nest (10%) along with <i>Scleromitron diffusum</i> (Willd.) R.J. Wang leaves (30%)	Recovers Rheumatism	Crushed	The paste is applied on affected area	TH	
		Nil	Used as a tonic	Juiced	1 tablespoon juice twice a day	BM	

Nil	Increases blood in body	Juiced	1 tablespoon juice in empty stomach during early morning	BM	Intake of egg, fish, meat or any other allergetic food items is prohibited during medication.
Sand from bird's nest (10%) along with <i>Scleromitron diffusum</i> (Willd.) R.J. Wang leaves (30%)	Used in wound healing	Crushed	The paste is applied on affected area	TH	

Herbalists: Mr. Tibru Hembram (TH), Mr. Basu Murmu (BM)

### Preliminary phytochemical analysis

Preliminary phytochemical screening of *A. rigida* leaves aqueous extracts confirmed the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins, tannins, terpenoids, steroids, whereas phlobatannins was

**Table 2.** Preliminary phytochemical screening of *A. rigida* leaves aqueous extract

Constituent	Chemical Test	Leaves of <i>A. rigida</i>
Alkaloids	Mayer's Test	-
	Wagner's Test	+
Carbohydrates	Molisch Test	++
	Fehling's Test	++
	Shinoda Test	+
Flavonoids	Pew's Test	+
	Alkaline Reagent Test	+
Glycosides	Keller-Kiliani Test	++
	Ellagic Acid Test	++
Phenols	Ferric Chloride Test	++
	Foam test	++
Saponins	Ferric Chloride Test	++
Tannins	Bromine Water Test	+
	Salkowski Test	++
Steroids	Salkowski Test	++
Phlobatannins	Hydrochloric acid Test	-

"++" sign indicates Present in High amount, "+" sign indicates Present in Average amount and "-" sign indicates the absence of constituent in the sample.

absent (Table 2). It is well defined that plant derived phytochemicals have the capacity to treat different diseases viz, alkaloids have the capacity to treat inflammation, allergy, disorders caused by bacteria and fungi, epilepsy, anti-cancerous activity (25-28); flavonoids can protect body from oxidative damage (29-31); reducing sugars can protect from cardiovascular disease (32), carbohydrates functions to provide energy to the body (33, 34).

Qualitative tests to identify the constituents were performed on methanol leaf extract of *A. rigida* from Tirunelveli hills (35). They reported the absence of reducing sugar, terpenoids and tannins in the methanolic leaf extract. However, we found that these constituents were present in the aqueous extract of powdered *A. rigida* leaf. The probable reason for the difference in the presence or absence of a particular secondary metabolite might be the

different solvent used for extraction.

**Table 3.** Heavy metal test for *A. rigida* leaves aqueous extract

Experiment	Observation	Results
<b>Test for Bismuth</b>		
H <sub>2</sub> S gas + sample solution	No dark brown precipitate	Bismuth absent
NH <sub>4</sub> OH + sample solution	No white precipitate	Bismuth absent
<b>Test for Cadmium</b>		
NH <sub>4</sub> OH + sample solution	No white precipitate	Cadmium absent
Potassium Ferrocyanide + sample solution	No white precipitate	Cadmium absent
<b>Test for Lead</b>		
Dilute HCl + sample solution	No white precipitate	Lead absent
KI + sample solution.	No yellow precipitate	Lead absent

### Heavy metal analysis

Heavy metal tests revealed that the samples were free from bismuth, cadmium and lead (Table 3). This suggests that the *A. rigida* leaves is safe and free from adulteration and thus can be used to develop herbal drugs either singly or in synergy.

### Conclusion

From above study it has been observed that *A. rigida* has been used for various ailments by the traditional healers belonging to the Santhal community residing in the Ultapani Forest Range. Thus, pharmacognostic diagnosis was carried out to standardize and characterize the leaves of *A. rigida* having therapeutic potential on the basis of macroscopy, qualitative phytochemicals, microscopy, organoleptic and heavy metal parameters. The presence of different components and absence of heavy metal ascertains that *A. rigida* is a potential candidate for developing leads for various ailments. It is suggested that further studies needs to be carried out to update unexploited medicinal value of this species which can help in discovery and preparation of herbal valued products.

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### Authors contributions

AKG conceptualized and designed the study. SD, SB, AKG carried out the research work, and acquired the data. SD and AKG the authors analyzed the data and wrote the first draft of the manuscript. Finally, all the authors edited the manuscript and approved the final version for submission.

### Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None

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