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Phytochemical studies of leaves and roots (*In vitro*) of endemic orchid *Arachnis senapatiana* (Phukan & A. A. Mao) Kocyan & Schuiteman

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Abstract

Plants and its derived products are backbone of traditional medicines around the world. The extracts of different orchid species have been used in curing different diseases. *Arachnis senapatiana* is an endemic orchid distributed in isolated places of Nagaland, Manipur and Darjeeling hills which is an accepted name of the most commonly known *Armadorum senapatianum* Phukan & A. A. Mao. The aim of the present study was to investigate the presence of phytochemicals in *in vitro* raised leaves and roots of this endemic orchid. Qualitative phytochemical tests were conducted for confirming the presence of carbohydrates, protein, glycosides, flavanoids, phenol and tannins, saponins, steroids and terpenoids. The present finding provided evidence that crude aqueous extracts of the tested plant material contain medicinally important bioactive compounds. Further work need to be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants.

Keywords: *Arachnis senapatiana*, endemic, phytochemicals

Introduction

Orchidaceae is the largest family of Angiosperm. Most of the orchids are known for its ornamental and horticulture importance. Unlike other family of the angiosperms, its knowledge on medicinal aspect is less known by common people though there is use of medicinal orchids from ancient times. The genera which are having medicinal properties are Acampe, Calanthe, Coelogyne, Cymbidium, Cypripedium, Dendrobium, Ephemerantha, Eulophia, Eria, Galeola, Gastrodia, Gymnadenia, Habenaria, Luisia, Ludisia, Nervilia, Orchis, Vanda and Thunia etc. In an Ayurvedic herbal formulation "Astavarga" few orchids like *Microstylis wallichii*, *Habenaria acuminata*, *Habenaria intermedia*, *Habenaria edgeworthii* are the ingredients (Handa, 1986; Singh and Duggal, 2009) [4, 6]. The first record of Indian Orchids as ayurvedic medicine was first mentioned in "Charaka Samhita" written by Charaka in Sanskrit. Charaka discussed about the medicinal orchids *Eulophia dabia*, *Flickingeria nodosa* and *Malaxis rheedii*. "Jeevanti" a common name for *Flickingeria macraei* is used in Ayurveda as astringent to the bowels, aphrodisiac and in asthma and bronchitis (Kirtiker and Basu, 1975) [6]. Being largest family of the angiosperm and high alkaloids and glycosides content, research on orchid is full of potential (Jagtap *et al*, 2014) [5].

Armadorum senapatianum was first described as new species by Phukan & A. A. Mao (2002) [9]. Later Kocyan & Schuiteman (2013) [8] merged *Armadorum* to *Arachnis* based on the molecular studies. Therefore by molecular studies the accepted name is *Arachnis senapatiana* (Phukan & A. A. Mao) Kocyan & Schuiteman. In India including *Arachnis senapatiana*, there are 02 species. There are 16 species in the world distributed in South East Asia (www.ipni.org). *Arachnis senapatiana* is a R.E.T. plant distributed in isolated places of Nagaland, Manipur and Darjeeling hills which is an accepted name of the most commonly known *Armadorum senapatianum* Phukan & A. A. Mao. The present work was done to find out the medicinal aspects through qualitative phytochemical studies. The samples were collected from the micro propagated plants of *Arachnis senapatiana* done during 2013-2015 by the authors.

Materials and Methods**Preparation of plant extracts**

Leaves of *in vitro* grown *Arachnis senapatiana* was collected and kept in hot air oven for drying. After drying, the weight of the sample was taken. 5 gm of both samples were taken and crushed in mortar and pestle separately. In each sample, 200 ml of distilled water was added slowly and mixture was filtered to obtain filtrate.

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The extract was heated in hot plate with continuous stirring at 30 – 40 °C for 20 minutes. Then the extract was cooled in room temperature and stored in refrigerator for later use. The extract was used in different tests for qualitative phytochemical studies. Similarly, crude extract was taken out from the roots of *in vitro* grown *Arachnis senapati*.

The qualitative tests

Test for proteins

Millon's test

The extract was mixed with 2ml of Millon's reagent, white precipitate appeared and it turned red on heating gently. It confirmed the presence of protein.

Ninhydrin test

Presence of protein in the extract was done by ninhydrin test in which extract was boiled with 2 ml of 0.2% solution of ninhydrin and appearance of violet colour confirm the presence of amino acids and proteins.

Test for carbohydrates

Fehling's test

The qualitative tests for carbohydrates were done by Fehling's test. Equal volume of Fehling A and Fehling B reagents were added and mixed. To the extract 2 ml of the mixed reagents was added and boiled gently. Appearance of brick red precipitate at the bottom of the test tube confirmed the presence of carbohydrates.

Benedict's test

The extract was mixed with 2 ml of Benedict's reagent and boiled. The formation of reddish brown precipitate confirmed the presence of carbohydrates.

Iodine test

Extract was mixed with 2ml of iodine solution. A dark blue or purple colour appears which indicate the presence of carbohydrates.

Test for phenols and tannins

For test for phenols and tannins 1 ml of extract was mixed with 2 ml of 2% solution of Ferric chloride and appearance of blue-green or black colour indicates the presence of phenols and tannins

Test for flavonoids

Alkaline reagent test

Alkaline reagent test was performed for flavonoids in which 1

ml of extract was mixed with 2 ml of 2% solution of NaOH and if intense yellow formed and turned colourless on addition of few drops of dilute acid indicate the presence of flavonoids.

Test for saponins

In the test for saponins to 1 ml of extract 5 ml of distilled water was added and shaken strongly and formation of stable foam confirmed the presence of saponins.

Test for glycosides

Liebermann's test

In Liebermann's test 1 ml of extract was mixed with 2 ml of Chloroform and 2 ml of Acetic acid and cooled in ice. Then slowly and carefully concentrated Sulphuric acid was added. Then a colour change from violet to blue to green was observed which indicated the presence glycone portion of the glycosides.

Salkowski's test

In Salkowski's test 1 ml of extract was mixed with 2 ml of Chloroform and to it 2 ml of concentrated sulphuric acid was added carefully, shaken gently. Appearance of reddish brown colour confirmed the presence of steroidal ring, i.e., glycone portion of the glycosides.

Keller-Kilani test

In Keller-Kilani test 1 ml of extract was mixed with 2 ml of glacial acetic acid which contained 1-2 drops of 2% solution of ferric chloride. Then the mixture was poured slowly and carefully into a test tube which contains 2 ml of concentrated sulphuric acid and appearance of a brown ring at the interphase confirmed the presence of cardiac glycosides.

Test for steroid

For test of steroid 1 ml of extract was mixed with 2 ml of chloroform and concentrated sulphuric acid was carefully added sidewise and appearance of red colour in the lower chloroform layer indicate the presence of steroid.

Test for terpenoids

For test for terpenoids 1 ml of extract was added in 2 ml of chloroform and evaporated to dryness using hotplate and 2 ml of concentrated Sulphuric acid was added and heated for few minutes. Appearance of greyish colour indicates the presence of terpenoids.

Results

Table 1: The result for phytochemical studies of *Armodorum senapati* was summarized in following table

Plant-parts	Carbohydrates	Protein	Glycosides	Flavonoids	Phenols and tannins	Saponins	Steroids	Terpenoids
Leaves	+	+	+	+	+	+	+	+
Roots	+	+	+	-	-	-	+	+

+ indicates presence,

- indicates absence

From the above studies it was found that all the test done in the leaves of *Arachnis senapati* were positive whereas test done in the roots out of 8 tests, 5 were positive and other 3 are negative. Tests showed that carbohydrates, protein, glycosides, flavonoids, phenols and tannins, saponins, steroids, terpenoids are present in the leaves of the plant while only carbohydrates, protein, glycosides, steroids, terpenoids are present in the roots of the plant. Tannins are responsible for the antimicrobial, anti-inflammatory, anti-bacterial

(Duguid *et al.*, 1989) ^[3] properties of the plants. The presence of terpenoids, flavonoids, tannins are responsible for the anti-microbial activity of the organic extracts. Analgesic properties are attributed to the alkaloids (Antherden, 1969) ^[2]. The presence of saponins in plants and have been reported to be responsible for the tonic and stimulating activities observed in Chinese and Japanese medical herbs (Alinnor, 2008) ^[1]. The results obtained in this study suggest that the identified phytochemical compounds may be the bioactive constituents

responsible for the efficacy of the leaves and roots of the plants studied.

Our study is first ever report to the best of our knowledge on qualitative study of *Arachnis senapatiensis*. As *Arachnis senapatiensis* is R.E.T. plants which contain important phytochemicals mentioned above and which can be beneficial for the mankind, therefore it should be conserved through *ex situ* and *in situ* methods. This orchid has potential as a medicinal plant and can be useful in the treatment of disease. Micro propagation should be done to increase its population and after acclimatization, replantation of it to its natural habitat to ensure its availability.

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