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Marjahan Akter
Department of Botany, University of Chittagong, Chittagong, Bangladesh

Mohammed Kamrul Huda
Department of Botany, University of Chittagong, Chittagong, Bangladesh

Mohammed Mozammel Hoque
Department of Botany, University of Chittagong, Chittagong, Bangladesh

Correspondence
Mohammed Mozammel Hoque
Department of Botany, University of Chittagong, Chittagong, Bangladesh

Investigation of secondary metabolites of nine medicinally important orchids of Bangladesh

Marjahan Akter, Mohammed Kamrul Huda and Mohammed Mozammel Hoque

Abstract
Phytochemical investigation of nine medicinal orchdids species viz. *Acampe papillosa* (Lindl.) Lindl., *Aerides odoratum* Lour., *Bulbophyllum lilacinum* Ridl., *Cymbidium aloifolium* (L.) Sw., *Dendrobium aphyllum* (Roxb.) C.E.C. Fisch., *Eria tomentosa* (Koen.) Hook. f., *Geodorum densiflorum* (Lam.) Schltr., *Papilionanthe teres* (Roxb.) Schltr, and *Rhynchostylis retusa* (L.) Blume. Has been performed based on ethno-medicinally potentiality. Secondary metabolitez like alkaloids, flavonoids, tannins, coumarin, quinine, steroids, terpinoids are present in all species but phlobatannins absent in all species among 10 secondary metabolites. On the basis of overall phytochemical screening, *Aerides odoratum* Lour. Was found the most potential medicinal orchid which showed the presence of eight secondary metabolites among ten tested. On the other hand *Acampe papillosa* (Lindl.) Lindl, *Bulbophyllum lilacinum* Ridl. And *Papilionanthe teres* (Roxb.) Schltr. showed the presence of six secondary metabolites. The lowest activity of secondary metabolites observed in *Dendrobium aphyllum* (Roxb.) C.E.C.Fisch. Further detailed phytochemical study and isolation of bioactive compounds from these nine orchid species are needed to discover new drugs and medicine.

Keywords: Phytochemical screening, secondary metabolites, orchid of Bangladesh

1. Introduction
Orchids are well known for their beautiful attractive flowers and are among the most highly prized ornamental plants. In addition to their ornamental value, Orchids are also known for their traditional usage especially in the traditional systems of medicine [15]. It is believed that the Chinese were the first to describe Orchids for medicinal uses [5].
Orchidaceae is a highly evolved and widely distributed monocotyledonous family with a large number of epiphytic, terrestrial and saprophytic species. It comprises more than 30,000 species in approximately 750 genera [18]. The largest number of Orchids is found in tropical America (360 genera and 8,266 species) while tropical Asia come second with 250 genera and 6800 species [7]. Bangladesh is also rich in orchids, with 169 species and 2 varieties under 63 genera [14]. Then 177 species was reported under 70 genera [13]. About 26 species of orchids also been used by the tribal people of Bangladesh to treat different diseases [12]. These species are distributed mainly in the hilly areas of greater Sylhet Chittagong and Mymensing district [30, 3].
Phytochemicals are chemical compounds that occur naturally in plants. Phytochemicals may have biological significance, for example carotenoids or flavonoids, but are not established as essential nutrients [10]. There may be as many as 4,000 different phytochemicals [10]. Phytochemical-based dietary supplements can also be purchased [1]. According to the American Cancer Society, "Available scientific evidence does not support claims that taking phytochemical supplements is as good for long-term health as consuming the fruits, vegetables, beans, and grains from which they are taken"[1].
Medicinal plants have been playing a significant role as sources of raw materials in various traditional as well as modern systems of medicine in the developed and developing countries. Therapeutic importance of different medicinal plants is determined by the types of the secondary metabolites (therapeutic ingredients) they contain and their biological activities [23].
Secondary metabolites are organic compounds that are not directly involved in the normal growth, development or reproduction of an organism [9]. Secondary metabolites often play an important role in plant defense against herbivore and other interspecies defense. Humans use secondary metabolites as medicines, flavorings and recreational drugs.
Plants have been used for the treatment of diseases all over the world before the introduction of modern clinical drugs. Natural phytochemicals are known to contain substance that can be used for therapeutic purposes or as precursor for the synthesis of novel useful drugs. Use of plant as a source of medicine has been inherited and is an important componen of the health

care system [2]. In recent years, the scientific evaluation constructed an overlay for the discovery of number of life saving drugs. Pharmaceutical companies have spent lot of time and money in developing natural products extracted from plants to produce more cost effective remedies that are affordable to the population. Currently, plant derived bioactive compounds have received considerable attention due to their therapeutic potentials as antimicrobial, anti-inflammatory, anticancer and anti-oxidant activities [24]. But phytochemical study on orchids are not yet explored, so present orchid species used as herbal medicines for different purposes of health have been selected for phytochemical investigation and which may explore new sources of drugs of herbal origin in future.

2. Materials and Methods

2.1 Collection of plant materials

The leaves and pseudobulbs were used for the qualitative estimation of alkaloids and other secondary metabolites. The orchid species were collected from Cox's Bazar and Hill Tracts Districts areas of Bangladesh. *Acampe papillosa* (Lindl.) Lindl, *Aerides odoratum* Lour, *Bulbophyllum lilacinum* Ridl., *Cymbidium aloifolium* (L.) Sw., *Dendrobium aphyllum* (Roxb.) C.E.C.Fisch., *Eria tomentosa* (Koen.) Hook. f., *Geodorum densiflorum* (Lam.) Schltr, *Papilionanthe teres* (Roxb.) Schltr., and *Rhynchostylis retusa* (L.) Blume. Has been selected for phytochmical investigation based on ethnobotanical information, the leaves and pseudobulbs of studied samples were thoroughly washed with clean water and chopped into small pieces.

2.2 Preparation of plant extract

25 gm of fresh chopped sample from each of plant were taken for further analysis. 50 ml of Methanol was added to the 25 gm of samples in a conical flask. Shaken very well for 30 minutes and then kept overnight and then shaken again and then filtered using Whatman filter paper. The process was repeated for 3 times with Methanol and the extract was then rotavaporated below 40^{o} C and dried. The dried sample was kept as crude sample for each plant.

2.3 Phytochemical screening

For the qualitative test (spot test) of alkaloids, 5 alkaloid detecting reagents were used. These were –Dragendroff's reagent, Hager's reagent, Mayer's reagent, Wagner's reagent, Tannic acid reagent.These reagents were prepared following the methods of Crownwell [6]. Qualitative tests were carried out on the fresh sample, powdered specimens and methanol extracted crude using standard procedures to identify the constituents as described by Sofowarar [26], Trease and Evans [28] and Harborne [11]. Qualitative test for the following secondary metabolites were done viz. Alkaloids, Phlobatannins, Flavonoids, Saponins, Tannins, Terpinoids, Steriods, Glycosides, Anthroquinone, Quinine, Coumarin

2.4 Test of alkaloids: For qualitative test of alkaloid, the most reliable and rapid testing method was develop by Webb [29] and the method was slightly modified by Aplin and Canon [4]. For the qualitative test of alkaloid, five alkaloid detecting reagents were used. These are Dragendroff's reagent (D), Hager's reagent (H), Mayer's reagent (M), Wagner's reagent (W) and Tannic acid reagent (T). These reagents were prepared following the methods of Crownwell [6].

5 gm fresh finely chopped and pasted plant material was mixed up to moisten with 10 ml 2% HCL and heated in water bath of 60 ^{o}C for one hour. After cooling the extract was filtered through Whatman No. 1 filter paper. Two drops of extract were put on a microscopic groove slide with one drop of the alkaloid detecting reagent. The relative abundance of precipitate, if any formed in the plant extract with the reagent was considered as an index of the quality of the presence of alkaloid and was expressed by'+', '++' and '+++' signs which mean slight, moderate, substantial to heavy amount respectively. No precipitate was indicated by '- '(negative sign) and stood for the absence of alkaloid in the plant extract. Phytochemical screening of orchid species for secondary metabolites were analyzed following standard methods.

2.5 Test for phlobatannins: Deposition of a red precipitate when an aqueos extract of each plant sample was boiled with 1% aqueous hydrochloric acid (HCL) was taken as evidence for the presence of phlobatannins [8].

2.6 Test for flavonoids: A portion of the crude powdered plant sample was heated with 10 ml of ethyl acetate over a stem bath for 3 min. The mixture was filtered and 4 ml of filtrate was shaken with 1 ml of dilute Ammonia solution. A yellow coloration was observed indicating a positive test for flavonoides [8].

2.7 Test for saponins: About 2 gm of crude powder was boiled with 20 ml of distilled water in a water bath and filtered. 10 ml of filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The persistent of froth indicates the presence of saponins [16].

2.8 Test for tannins: About 0.5 gm of the crude powdered samples boiled in 10 ml of distilled water in a test tube and filtered. A few drops of ferric chloride reagent added to the filtrate. A blue-black precipitate was taken as evidence for the presence of tannins [11].

2.9 Test for terpenoids: 0.5 gm of crude powder was dissolved in 5 ml of methanol. 5 ml of the extract was treated with 2 ml of chloroform in a test tube. 3 ml of concentrated sulphuric acid carefully added to the mixture to form a layer. An interface with a reddish brown coloration formed if terpenoid constituent is present [17].

2.10 Test for steroids: 0.5 gm of crude powder was dissolved in 5 ml of methanol 1 ml of the extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids [17].

2.11 Test for glycosides: 0.5gm crude powder was dissolved in 5 ml of methanol. 10 ml of 50% HCL was added to 2 ml of methanolic extract in a test tube. Then it was heated in a boiling water bath for 30 minutes. 5 ml of Fehling's solution was added to the mixture and the mixture was boiled for 5 minutes. A brick-red precipitate was taken as evidence for the presence of glycosides [11].

2.12 Anthroquinone

2 ml of solution was added with Magnesium acetate. Formation of pink color indicates the presence of Anthroquinones [26].

2.13 Quinine
1ml of extract, 1 ml of concentrated Sulfhuric acid was added and was allowed to and for some time to develop color. Development of red color shows the presence of Quinine [26].

2.14 Coumarin
1 ml of extract, 1 ml of 10% NaoH was added and was allowed to stand for some time development of yellow color shows the presence of Coumarin [26].

3. Results and Discussion
In the present work, secondary metabolites like alkaloids, flavonoids, glycosides, saponins, tannins, coumarin, steroids, terpinoids, anthroquinone, phlobatannins and quinine were considered for qualitative assessment. Alkaloids were assessed qualitatively with five different alkaloid detecting reagents. They were Dragendroff's reagent (D), Wagner's reagent (W), Mayer's reagent (M), Hager's reagent (H) and Tannic acid reagent (T). The presence of relative alkaloid contents and ten other secondary metabolites in the extract of test plants and/or their organs was expressed by '+' sign ranging in the order of '+', '++' and '+++' signifying its presence in degrees ('+' minimum to '+++', the highest quantity). Absent of the secondary metabolites was denoted by '-' sign.

Table 1: Qualitative test for alkaloids of nine orchid species.

Sl. No.	Name of plants	Plant parts Used	Qualitative estimation of alkaloids by different reagents				
			D	H	M	W	T
1	*Acampe papillosa* (Lindl.) Lindl.	Leaf	+++	++	+++	++	+++
2	*Aerides odoratum* Lour.	Leaf	+++	+++	++	+++	+++
3	*Bulbophyllum lilacinum* Ridl.	Pseudobulb	+++	++	+++	+++	+++
4	*Cymbidium aloifolium* (L.)	Leaf	+++	++	+++	++	+++
5	*Dendrobium aphyllum* (Roxb.) C. E. C. Fisch.	Leaf	++	++	+++	+++	+++
6	*Eria tomentosa* (Koen.) Hook. f	Leaf	++	++	++	+++	+++
7	*Geodorum densiflofum* (Lam.) Schltr.	Pseudobulb	++	+	++	+++	+++
8	*Papilionanthe teres* (Roxb.) Schltr.	Leaf	+	+	+	+++	+++
9	*Rhynchostylis retusa* (L.) Blume.	Leaf	+++	+	+	++	+++

Notes: Name of the reagents, D-Dragendroff's reagent, H-Hager's reagent, M-Mayer's reagent, W-Wagner's reagent and T- Tannic acid reagent. '+++'- High, '++'- Moderate, '+'- Low and '-'- Absence.

The results shown in Table 1 indicate that in respect to qualitative test for the determination of the presence of alkaloids with one or another type of reagents. *A. papillosa* (Lindl.) Lindl, *A. odorata* Lou, *B. lilacinum* Ridl., *C. aloifolium* (L.) Sw. and *R. retusa* (L.) Blume, showed the highest alkaloids presence in Dragendroff's reagent (D). On the other hand, moderately presence of the alkaloids was found in *D. aphyllum* (Roxb.) C.E.C., *E. tomentosa* (Koen.) Hook. f. and *G. densiflorum* (Lam.) Schltr. and the lowest alkaloids was observed in t *P. teres* (Roxb.) Schltr. of the Dragendroff's reagent (D) test. In contrast, the presence of alkaloids was revealed to be higher through Hager's reagent (H) tests in of *A. odorata* Lour. And moderate in *A. papillosa* (Lindl.) Lindl, *B. lilacinum* Ridl, *C. aloifolium* (L.) Sw., *D. aphyllum* (Roxb.) C.E.C., and *E. tomentosa* (Koen.) Hook. f. of *G. densiflorum* (Lam.) Schltr., *P. teres* (Roxb.) Schltr. and *R. retusa* (L.) Blume. Indicated low alkaloids. In the study of Mayer's reagent (M) test highly positive result found in *A. papillosa* (Lindl.) Lindl, *B. lilacinum* Ridl., *C. aloifolium* (L.) Sw. and *D. aphyllum* (Roxb.) C.E.C. another plant of *E. tomentosa* (Koen.) Hook. F and *G. densiflorum* (Lam.) Schltr. indicated the moderate and lowest alkaloids presence of plant *P. teres* (Roxb.) Schltr. and *R. retusa* (L.) Blume. Qualitative test of the alkaloids Wagner's reagent (W) indicated the highly positive of *A. odorata* Lou, *B. lilacinum* Ridl., *D. aphyllum* (Roxb.) C.E.C., *E. tomentosa* (Koen.) Hook. F, *G densiflorum* (Lam.) Schltr. and *P. teres* (Roxb.) Schltr. Accordingly three plants viz. *A. papillosa* (Lindl.) Lindl., *C. aloifolium* (L.) Sw. and *R. retusa* (L.) Blume. Showed the moderate presence of alkaloids.The results of the qualitative test of Tannic acid reagent (T) indicated the remarkable presence of alkaloids in the nine studied orchids.

Table 2: Qualitative test for ten secondary metabolites of nine orchids.

Sl. No.	Name of plants	Plant parts used	Secondary metabolites (% of coloration)									
			Gly.	Flv.	Phl.	Sap.	Tan.	Ter.	Str.	Ant.	Qui.	Cou.
1	*Acampe papillosa* (Lindl.) Lindl.	Leaf	+++	+++	-	+	+++	+++	++	+	+++	+++
2	*Aerides odoratum* Lour.	Leaf	+++	+++	-	++	+++	+++	+++	+	+++	+++
3	*Bulbophyllum lilacinum* Ridl.	Pseudobulb	+++	+++	-	+	+++	++	+++	+	+++	+++
4	*Cymbidium aloifolium* (L.)	Leaf	+	++	-	++	+++	+++	+++	+	+++	+++
5	*Dendrobium aphyllum* (Roxb.) C. E. C. Fisch.	Leaf	+++	++	-	+	++	+++	++	-	+++	++
6	*Eria tomentosa* (Koen.) Hook. f	Leaf	++	+++	-	++	+++	+++	+++	+	+++	++
7	*Geodorum densiflofum* (Lam.) Schltr.	Pseudobulb	++	++	-	+	+++	+++	+++	+	+++	+++
8	*Papilionanthe teres* (Roxb.) Schltr.	Leaf	++	+++	-	++	+++	+++	+++	+	+++	+++
9	*Rhynchostylis retusa* (L.) Blume.	Leaf	++	++	-	++	+++	+++	+++	-	+++	++

Notes: Gly. = Glycosides, Flv.= Flavonoids, Phl. = Phlobatannins, Sap.= Saponins, Tan.= Tanins, Ter.= Terpinoids, Str.= Steroids, Ant.= Anthroquinone, Qui.= Quinine, Cou.= Coumarin. '+++'- High, '++'- Moderate, '+'- Low and '-'- Absence.

The results of the qualitative test for ten secondary metabolites are presented in Table 2. The results demonstrated that glycosides were higher in *A. papillosa, A. odorata, B. lilacinum and D. aphyllum* whereas it was moderate in *E. tomentosa, G. Densiflorum, P. teres and R. retusa.* On the other hand Flavonoids were found to be highly positive in *A. papillosa, A. odorata, B.;ilacinum, E. tomentosa and P. teres and C. aloifolium, D. aphyllum, G. densiflorum and R. retusa was* moderately present. Phlobatannins and Saponins test indicated the absence and low secondary

metabolites in nine studies species. The qualitative test of Tanins, Terpinoids, Steroids, Quinine and Cumarin showed the highest positive in *A. papillosa, A. odorata, B. lilacinum, C. aAloifolium, E. tomentosa, G. densilforum, P. teres* and *R. retusa. Whereas* moderate presence in *D. aphyllum.* On the other hand Anthroquinone was absent in *B. Lilacinum, R. retusa and A. papillosa, A. odorata, B. lilacinum, C. aloifolium, E. tomentosa, G. densiflorum and P. teres*. Showed lowler presence. Marjoka [19] conducted phytochemical screening of three medicinally important epiphytic orchids of Bangladesh viz. *Acampe papillosa* (Lindl.) Lindl., *Cymbidium aloifolium* (L.) Sw. and *Rhynchostylis retusa* (L.) Blume. *Acampe papillosa* showed presence of saponons, tannins, glycosides, flavonoids and steroids whereas, *Cymbidium aloifolium* showed less presence of saponins, terpinoids, flavonoids, tannins and steroids. *Rhynchostylis retusa* revealed better presence of flavonoids, terpinoids, phlobatannins and tannins but less presence of glycosides, saponins and steroids. Phlobatannins and terpinoids were absent in *Acampe papillosa*, whereas glycosides and phlobatannins were absent in *Cymbidium aloifolium*. Among three orchid species, *Rhynchostylis retusa* exhibited relatively higher presence of seven metabolites (tannins, flavonoids, steroids, phlobatannins, saponins, glycosides and terpinoids. Shubha and Chowdappa [25] conducted phytochemical analysis in *Cymbidium aloifolium* (L.) Sw. The phytochemical analysis showed the presence of eleven phytochemicals. The leaf exhibited maximum number of phytochemicals like alkaloids, flavonoids, phenols, quinine, coumarin, saponins, carbohydrates, tannins etc. Radhika and Murthy [22] worked on preliminary phytochemical analysis in *Rhynchostylis retusa* (L.) Blume. A qualitative phytochemical analysis showed the presence of alkaloids, tannins, flavonoids, glycosides, saponins,coumarins etc. Theng and Korpenwar [27] studied phytochemical investigation in endangered orchid *Geodorum densiflorum* (Lam.) Schltr. Phytochemical screening revealed the presence of alkaloid, steroids, carbohydrates, saponons, phenol and tannin. Radhika [21] studied the preliminary phytochemical analysis in *Cymbidium aloifolium* (L.) Sw. Preliminary phytochemical screening of this plant revealed that the presence of alkaloids, tannins, flavonoids, anthroquinone, terpinoids etc.

Based on the results of the present study, it can be concluded that *Aerides odorata* Lour, *Acampe papillosa* (Lindl.) Lindl., *Bulbophyllum lilacinum* Ridl. And *Papilionanthe teres* (Roxb.) Schltr. was found the most potential medicinal orchid species and it showed the presence of six secondary metabolites among 10 secondary metabolites tested. Therefore, the findings of the present study have distinctly focused on the potential medicinal values of this plant and promoted the ongoing research of medicinal orchids in Bangladesh.

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