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Short communication

Phytochemical screening of three medicinally important epiphytic orchids of Bangladesh

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Orchids are the largest and most diverse group among the angiosperms and they are mainly cultivated for beautiful flowers (Chowdhery, 1998). They are widely known for their horticultural and commercial importance but less for their medicinal value. Recently there has been tremendous progress in medicinal plants research; however orchids have not been exploited fully for their medicinal application. Medicinal plants produce a vast array of therapeutically important secondary metabolite of the classes, alkaloids, terpenoids and phenols (Hess, 1975; Goodwin and Mercer, 1986; Hopkins, 1999) and have strong physiological activities in the animal systems. For this reason, plants containing secondary metabolites are very important to us as potential ingredient of herbal and many modern medicines.

Traditional Chinese medicine widely utilizes orchids in medicines. Some orchid species like *Dendrobium crumenative, Eulophia campestris, Orchis latifolia, Vanda roxburghii* and *Vanda tessellata* have been documented for their medicinal value (Chowdhery, 1998). Phytochemically some orchids have been reported to contain alkaloids, terpenoids, flavonoids and stilbenoids (Singh & Sanjiv, 2009) and more than 44 orchid species of 34 genera have medicinal value (Ghanaksha & Kaushik, 1993). Orchids are also commercially important for its glycosidal value where four kinds of glycosides have been reported to be present in some orchids (Bose & Yadav, 1989). Loroglosin from *Loroglossum,* coumarin from *Angraceum fragrance* and saponin from *Paphiopedilum javanium* (Bose & Yadav, 1989) are commercially important glycosides. Another important and the most popular phenolic aldehyde is vanillin. *Vanilla planifolia* is the main source of commercial vanilla flavor. In Bangladesh there are 178 orchid taxa and some of these are medicinally important (Huda, 2008). . Of them, 26 species of orchids also been used by the tribal people of Bangladesh to treat different diseases (Huda *et al*., 2006). The main objective of the present work is to determine the qualitative status of phytochemical properties of three medicinally important epiphytic orchid species which were selected based on the frequent uses of these three species by the tribal people of Chittagong Hill tracts. Three medicinally important epiphytic orchid species viz *Acampe papillosa* (Lindl.), Lindl., *Cymbidium aloifolium* (L.) Sw. and *Rhyncostylis retusa* (L) Blume have been chosen investigated to study their phytochemical properties. Qualitative assessment was performed to study the presence of different secondary metabolites like alkaloids, flavonoids, tannins, saponins, glycosides etc.

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Collection, extraction and phytochemical screening of samples: The leaves of three medicinally important epiphytic orchid species viz, *Acampe papillosa, Cymbidium aloifolium* and *Rhyncostylis retusa* were used for the qualitative estimation of alkaloids and seven other secondary metabolites; flavonoids, steriods, phlobatannins, terpenoids, tannins, saponins and glycosides. Firstly these three species viz. *Acampe papillosa, Cymbidium aloifolium* and *Rhyncostylis retusa* were collected from field and identified consulting the literatures and confirmed after comparing with the existing herbarium preserved at the Herbarium of Chittagong University (HCU). The voucher specimens of the studied species (accession number AM 7 for *Acampe papillosa,* AM 9 for *Cymbidium aloifolium* and AM 3 for *Rhyncostylis retusa*) have been preserved at the HCU. The leaves of the studied species were thoroughly washed with water and dried in oven at 60^0 C for 48 hours. It was then ground into coarse powder by using grinding machine and stored in airtight container for further investigation. Mixing of one species with other was carefully avoided. 25 gm of samples from each of the species were used for phytochemical analysis.

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 Sofowara (1993), Trease & Evans (1989), and Harborne (1973). For qualitative test of alkaloids, the most reliable and rapid testing method was developed by Webb (1949) and the method was slightly modified by Aplin & Canon (1971). Phytochemical screenings of the three epiphytic orchid species for secondary metabolites were analyzed following standard methods. Test for phlobatannins and flavonoids were following Edeoga *et al*., 2005, test for saponins were following Kapoor *et al*., 1969, test for tannins was following Harborne, 1973, test for terpenoids and steroids were following Kolawole *et al*., 2006 and test for glycosides was following Harborne, 1973.

In the present work, the presence of relative alkaloid contents and seven other secondary metabolites in the extract of test plants and/or their organs were expressed by '+' sign ranging in the order of '+', '++' and '+++' signifying it's presence in degrees ('+' minimum to '+++', the highest quantity). Absence of the secondary metabolites was denoted by '–' sign. Results are given in the Table 1 by arranging the plant species in alphabetic order.

Table 1. **Qualitative test for alkaloids and seven secondary metabolites in the leaves of three epiphytic orchid species**

Name of the species	Qualitative estimation of alkaloids (by different reagents)					Secondary metabolites						
	D	H	M	T	W	Gly.	Flv.	Phl.	Sap.	Tan.	Ter.	Str.
Acampe papillosa	+++	+	+	++	+	+	+	-	+++	++	-	+
Cymbidium aloifolium	++	+	+	+++	++	-	+	-	++	+	++	+
Rhyncostylis retusa	+++	+	++	+++	++	+	+++	++	+	++	+++	+

Note: D=Dragendroff's reagent, H=Hager's reagent, M=Mayer's reagent, T=Tannic acid reagent and W=Wagner's reagent; Gly=Glycosides, Flv=Flavonoids, Phl=Phlobatannins, Sap=Saponins, Tan=Tannins, Ter=Terpenoids and Str=Steroids

The results of the Table 1 indicated that, different species of orchids showed positive response for different type of reagents. In *Acampe papillosa*, Dragendroff's reagent (D) showed '+++', Hager's reagent (H) '+', Mayer's reagent (M) '+', Tannic acid reagent (T) '++' and Wagner's reagent (W) '+' indicating presence of alkaloids. In *Cymbidium aloifolium*, Dragendroff's reagent (D) showed '++', Hager's reagent (H) '+', Mayer's reagent (M) '+', Tannic acid reagent (T) '+++' and Wagner's reagent (W) '++' also indicating presence of alkaloids. In *Rhyncostylis retusa*, Dragendroff's reagent (D), Hager's reagent (H), Mayer's reagent (M), Tannic acid reagent (T) and Wagner's reagent (W) showed '+' to '+++' revealed the presence of alkaloids.

The results of the table 1 also indicated that, *Acampe papillosa* exhibited the presence of different secondary metabolites with their intensity following '+++' with saponins, '++' with tannins, '+' with glycosides, flavonoids and steroids. Phlobatannins and terpenoids were absent in *Acampe papillosa.* Whereas, *Cymbidium aloifolium* showed '++' with saponins and terpenoids, '+' with flavonoids, tannins and steroids. On the other hand, glycosides and phlobatannins were absent in *Cymbidium aloifolium. Rhyncostylis retusa* expressed '+++' with flavonoids and terpenoids, '++' with phlobatannins and tannins and '+' with glycosides, saponins and steroids.

Different species showed different test for alkaloids with one or another type of reagents such as: Dragendroff's reagent, Hager's reagent, Mayer's reagent, Tannic acid reagent and Wagner's reagent. *Acampe papillosa*, showed a total of eight '+', *Cymbidium aloifolium* showed seven '+' and *Rhyncostylis retusa* showed eleven '+' indicating the different level of presence of alkaloids. All the three species exhibited positive response for alkaloids but among them, *Rhyncostylis retusa* showed higher presence of alkaloids.

Edeoga *et al.* (2005) examined and compared tannins, saponins, steroids, terpenoids, flavonoids, phlobatannin and cardiac glyciside distribution in ten medicinal plants (e.g- *Cleome rutidosperma, Emilia coccinea, Scoparia dulcis, Sida acuta, Stachytarpheta cayennensis, Tridax procumbens* etc.). All contained tannins and flavonoids except *S. acuta* and *S. cayennnsis* where tannins and flavonoids were absent, respectively. Uneven distribution of all these secondary metabolites in medicinal plants was also reported by others (Krishnaniah *et al.* 2009, Ayeni *et al*, 2010, Koche *et al*, 2010). The occurrence of different secondary metabolites suggested a wide range of biological application of the medicinal plants (Tanrisever *et al*, 1988). Secondary metabolites may be responsible for many pharmacological action like wound healing (Shivhare *et al,* 2010), cholesterol lowering (Sharmila *et al,* 2007) and antidiabetic activities (Rai *et al*, 2008).

In addition to alkaloids, qualitative assessment for seven other secondary metabolites, e.g. tannin, flavonoids, steroids, phlobatannins, saponins, glycosides and terpenoids were done in three selected epiphytic orchid species. Three epiphytic orchid species such as *Acampe papillosa, Cymbidium aloifolium* and *Rhyncostylis retusa* showed different tests for the seven secondary metabolites. *Acampe papillosa* showed presence of saponins, tannins, glycosides, flavonoids and steroids whereas, *Cymbidium aloifolium* showed less presence of saponins, terpenoids, flavonoids, tannins and steroids. *Rhyncostylis retusa*

revealed better presence of flavonoids, terpenoids, phlobatannins and tannins and less presence of glycosides, saponins and steroids. Phlobatannins and terpenoids were absent in *Acampe papillosa*, whereas, glycosides and phlobatannins were absent in *Cymbidium aloifolium.* Among three orchid species, *Rhyncostylis retusa* exhibited relatively higher presence of seven secondary metabolites (tannin, flavonoids, steroids phlobatannins, saponins, glycosides and terpenoids).

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