

## Phytochemical Profiling and Bioactivities of *Pholidota pallida* Lindl

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**Abstract:** In the present work, *Pholidota pallida* was explored for its phytochemicals along with its bioactivities. The qualitative screening of plant extract confirmed the presence of alkaloids, flavonoids, terpenoids, tannins, steroids and traces of quinone and coumarin in it. Four fractions viz. Methanol (FM), n-Hexane (FH), Butanol-1 (BW) and Dichloromethane (FD) of methanolic crude of its leaf, stem and root were investigated for free radical scavenging, anti-inflammatory and anti-microbial activities. The responses were very unique for different dose and plant parts. The highest scavenging activity exhibited by the BW of bulb was 98.94% at 50 µg/ml dose. The highest anti-inflammatory activity was observed in FD of the plant leaf which was 79.16%. The inhibitory effect of different parts of the plant against four pathogenic bacteria was varied i.e. for *S. aureus* (FD of root; 17.5 mm), for *B. subtilis* (FD of bulb; 16.5 mm), for *S. typhi* (BW of root; 12mm), for *B. cereus* (BW of root; 9.5 mm) and root was found most effective part. The investigation indicates that, *Pholidota pallida* is a medicative plant having bountiful phytochemicals with antioxidant, anti-inflammatory and antimicrobial actions.

**Keywords:** Phytochemicals, anti-microbial, anti-oxidant, anti-inflammatory.

### 1. INTRODUCTION

Orchidaceae family has huge therapeutic potential and such plants are utilized as therapeutics since ancient times<sup>[1]</sup>. Orchids have been also used in traditional system of medicine for various ailments<sup>[2]</sup>. The tubers and pseudobulbs of *Orchis latifolia*, *Orchis mascula*, *Cymbidium aloifolium*, *Zeuxine strateumatica*, and some species of *Dendrobium*, *Eulophia* and *Habenaria* are restorative and prescript for various diseases<sup>[3]</sup>. *Vanda* is a well-known anti-inflammatory plant<sup>[4-5]</sup> and anti-proliferative against various cancer types<sup>[6]</sup>. Contrariwise, biological studies also apprise that, orchids harbor phytochemicals physic for fatal diseases<sup>[7]</sup>. Thence, orchid derived compounds have been receiving tremendous attention as therapeutics<sup>[8]</sup>.

Bangladesh is also a reservoir of 178 orchid species of which 26 were reported to have ethnomedicinal uses<sup>[9]</sup>. *Pholidota pallida* is an indigenous epiphytic orchid of Bangladesh with the pseudobulbs occurring in the plains and as well as in elevation<sup>[10]</sup>. It is a traditional haemostate and healing of insomnia, fractured bones, nasal, abdominal and rheumatic pains<sup>[11-18]</sup>. But, none of above mentioned therapeutic properties of this orchid has yet been studied regarding its pharmacological value or active chemical constituents. A study on the pharmaceutical value of this medicinal orchid would be the step to explore a possible sustainable use of it. Considering all these factors, *Pholidota pallida* was picked for exploring its phytochemicals and bioactivities through standard methods.

### 2. MATERIALS AND METHODS

Leaf, stem and root extracts of *Pholidota pallida* Lindl. were used in the present work. For extraction, naturally grown plant sample collected from Teknaf, Cox's bazar was cleaned, chopped, air dried at room temperature and finally ground into coarse powder. It was then ground into coarse powder by using grinding machine and stored in airtight container for further investigation. Mixing of one part with another was carefully avoided. Extract was filtered through Whatman No.1 filter paper and evaporated to dryness under vacuum below 50 °C to get the blackish extract. Then the concentrated crude extract was separated into four different solvent systems (Methanol, n-Hexane, Butanol-1,

Dichloromethane) following the Kupchan method. Standard methods of Sofowara <sup>[19]</sup>, Trease and Evans <sup>[20]</sup>, Harbrone <sup>[21]</sup> were followed for qualitative assessment of different secondary metabolites viz. Alkaloids, Phlobatannins, Flavonoids, Saponins, Tannins, Terpenoids, Steroids, Glycosides, Anthroquinone, Quinine and Coumarin. Antimicrobial activity of the plant extract was studied according to Bauer *et al* <sup>[22]</sup>, antioxidant activity was determined according to Brand-Williams *et al.* <sup>[23]</sup>, using DPPH and anti-inflammatory activity on egg albumin was assessed according to Shinde *et al.* <sup>[24]</sup>. Each set of experiment was replicated thrice.

### 3. RESULTS AND DISCUSSION

The therapeutic properties of medicinal plants arise from the secondary metabolites present in them <sup>[25]</sup>. Hence, the secondary metabolites i.e alkaloids, Phlobatannins, Flavonoids, Saponins, Tanins. Terpenoids, Steroids, Glycosides, Anthroquinone, Quinine and Coumarin contents were assessed qualitatively in the leaf, bulb and root extract of *P. pallida* (Table 1 and 2). In the qualitative test of alkaloids (Table 1) Dragendroff's, Wagner's and Tannic acid reagents appeared more effective to which plant extracts exerted their moderate (2+ and 3+) response while, Hager's and Mayer's reagents seemed to be less effective to which they responded very mild (+) or negative (-).

**Table1.** Qualitative test for alkaloids of *Pholidota pallida* Lindl

Plant parts used	Qualitative estimation of alkaloids by different reagents				
	D	H	M	W	T
Leaf	+++	-	+	+++	+++
Bulb	+++	-	-	+++	+++
Root	-	-	-	+++	++

**Notes:** Name of the reagents, D-Dragendroff's reagent, H-Hager's reagent, M-Mayer's reagent, W-Wagner's reagent and Tannic acid reagent.

Table-2 illustrates that, Tanin is present but Phlobatannins, and Anthroquinone are absent in every part. Flavonoids, Saponins, and Steroids are present in leaf and bulb but absent in root. Terpinoids and Coumarins are present in leaf and root but absent in bulb. Quinine is absent in leaf but present in bulb and leaf. Glycoside is only present in bulbs. Nagananda *et al.* <sup>[26]</sup> also screened *Pholidota pallida* Lindl from India and noticed alkaloids, flavonoid, phytosterols, and phenols in it.

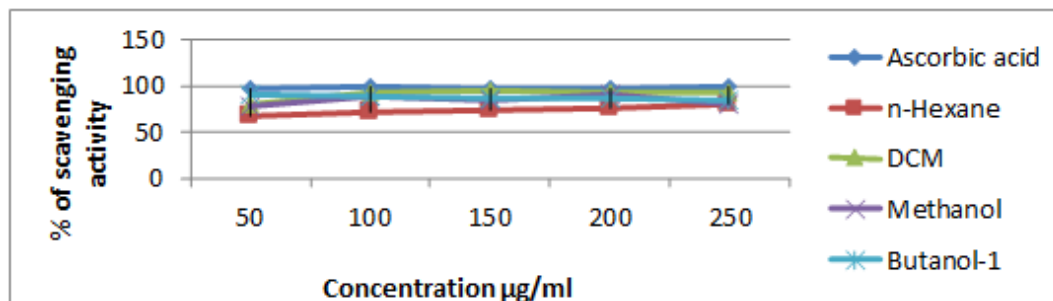
**Table2.** Qualitative test for ten other secondary metabolites of *Pholidota pallida* Lindl

Plant parts used	Secondary metabolites (% of coloration)									
	Phl.	Flv.	Sap.	Tan.	Ter.	Str.	Gly.	Anthr.	Qui.	Cou.
Leaf	-	+	+	+	+	+	-	-	-	+
Bulb	-	+	+	+	-	+	+	-	+	-
Root	-	-	-	+	+	-	-	-	+	+

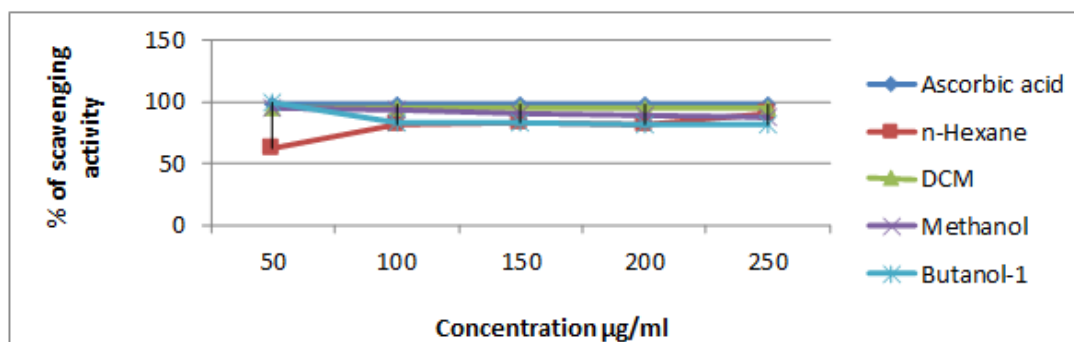
**Notes:** Phl= Phlobatannins, Flv= Flavonoids, Sap= Saponins, Tan= Tanins. Ter= Terpenoids, Str= Steroids, Gly= Glycosides, Anthr= Anthroquinone, Qui= Quinine, Cou= Coumarin.

Above outcomes indicate that, distribution of secondary metabolites is sporadic and uneven in various parts of the plant. Similar results were also observed by Rahman *et al.* <sup>[27]</sup>. Plants having metabolites seemed to exert antimicrobial, antioxidant, antifungal, anticancer and anti-inflammatory properties in several bioassays <sup>[28-29]</sup>. Thence, the plant was prompted to carry out further bioactive studies (antioxidant, Anti-inflammatory and antimicrobial tests) on it; <sup>[30]</sup>. Moreover, orchids are well known for having bioactivities and potential sources of antioxidants <sup>[31]</sup>. Different fractions at different concentrations (50, 100, 150, 200 and 250 µg/ml) also approved it (Fig 1-3) during their scavenging activity compared to ascorbic acid (98.39% at 50 µg/ml). BW fractions at low concentrations (50µg/ml) showed strong scavenging activities which were 98.94% for bulb, 95.17% for root and 91.40% for leaf. FM parts were also found abuzz at the same dose for root (95.02%) and bulb (94.45%) but for leaf (91.80%) it required higher dose (200 µg/ml). But, FD became vivid at higher concentration i.e. at 150 µg/ml for root (96.22%) and leaf (94.21%) and bulb (95.66%) at 100µg/ml. On the other hand, FH disclosed their utility at maximum dose i.e. root (95.90% at 200µg/ml), and leaf (80.79% at 250 µg/ml), except bulb (90.19%) at 50µg/ml. According to Jun *et al* <sup>[32]</sup>, extract having scavenging activity below the range of 250µg/ml dose is the indication of have significant antioxidant properties. From the above result it was inferred that, this plant has prominent antioxidant profile. Schinella *et al* <sup>[33]</sup> reported that, antioxidant plants are also effective as anti-inflammatory

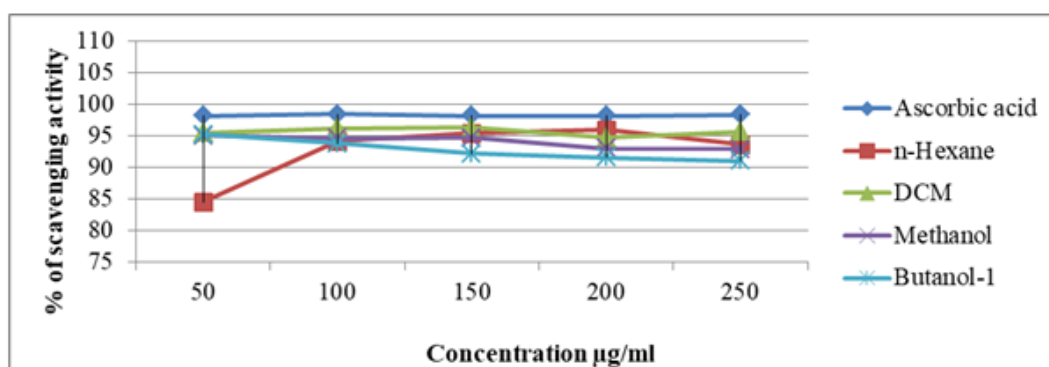
being. So, to detect this finding, all fractions were screened for their anti-inflammatory entity (Fig 4-9). Among the fractions of plant parts, the responses of different fractions were also unique. In case of FD, strong (79.16 % for leaf), and mild (21.89% for root and 10.21% for bulb) anti-inflammatory effects were observed. But in FM, bulb (69.80%) was found more effective than leaf (40.63%) and root (14.30%). In FH, leaf (44.80%) was found effective than bulb (28.59%) and root (15.63%). Consequently, in BW parts, root (43.75%) exerted more inhibitory effects than leaf (22.92%) and bulb (18.38%).



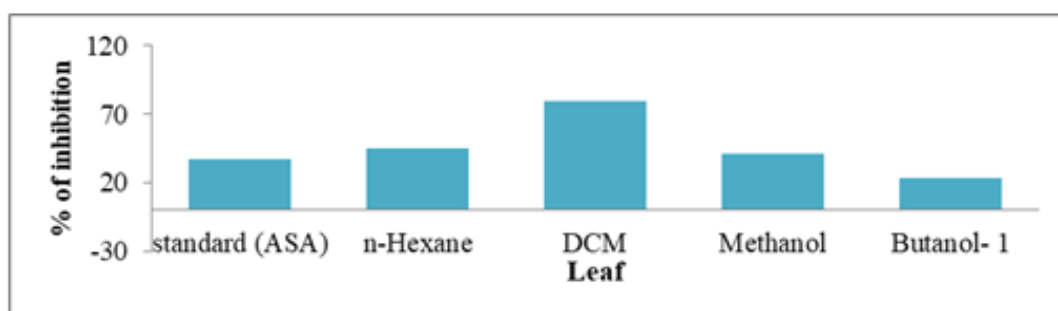
**Fig1.** Relative % of Scavenging activity or % inhibition of standard antioxidant Ascorbic acid and Methanol, n-Hexane, Butanol and DCM fraction of leaf of *Pholidota pallida* Lindl.



**Fig2.** Relative % of Scavenging activity or % inhibition of standard antioxidant Ascorbic acid and Methanol, n-Hexane, Butanol -1 and DCM fraction of bulb of *Pholidota pallida* Lindl.



**Fig3.** Relative % of Scavenging activity or % inhibition of standard antioxidant Ascorbic acid and Methanol, n-Hexane, Butanol-1 and DCM fraction of root of *Pholidota pallida* Lindl.



**Fig4.** Anti-inflammatory activity of different fractions of *Pholidota pallida* Lindl leaf

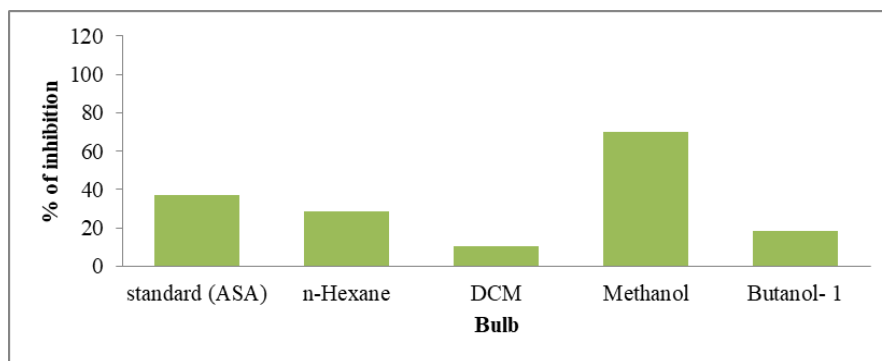


Fig5. Anti-inflammatory activity of different fractions of *Pholidota pallida* Lindl. bulb

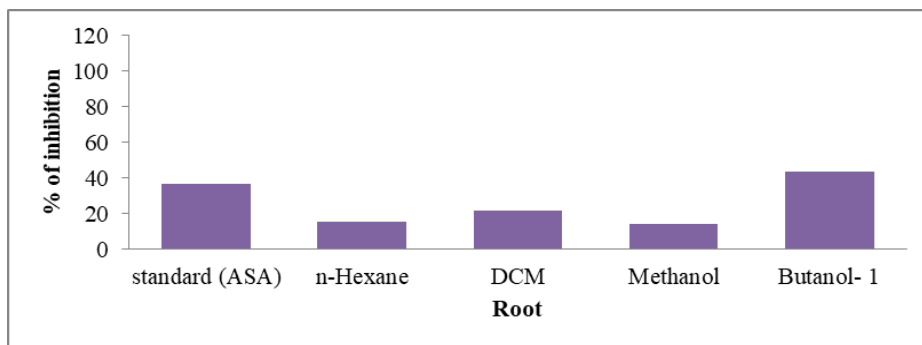


Fig6. Anti-inflammatory activity of different fractions of *Pholidota pallida* Lindl. root

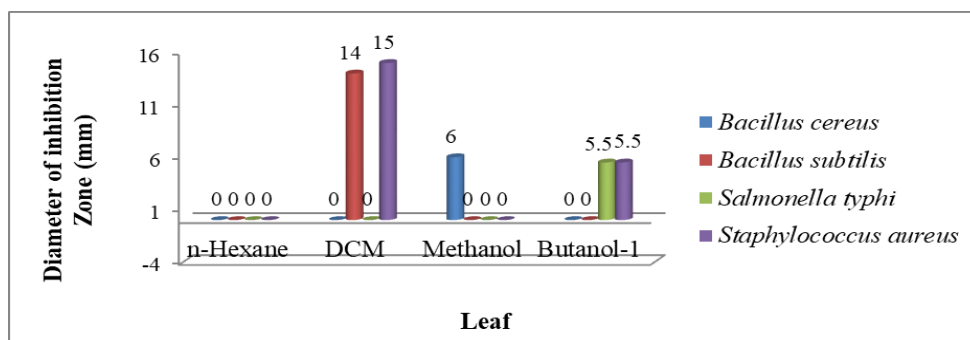


Fig7. Anti-bacterial activity of different fractions of *Pholidota pallida* Lindl. leaf

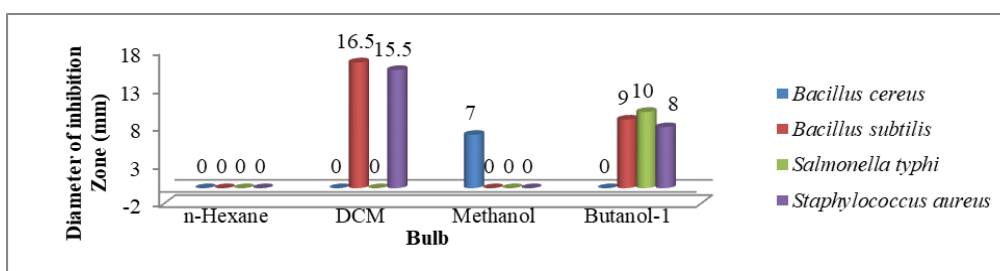


Fig8. Anti-bacterial activity of different fractions of *Pholidota pallida* Lindl. bulb

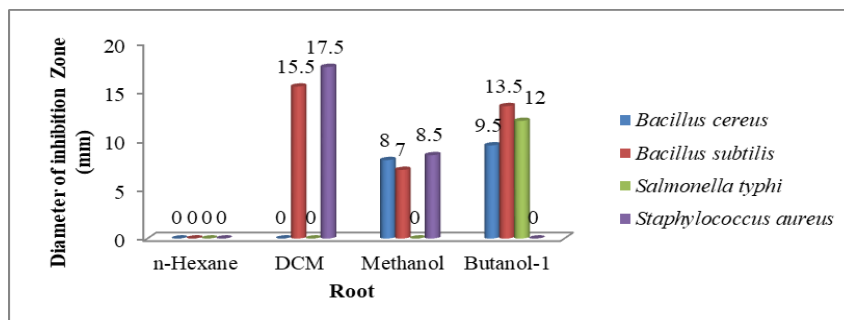


Fig9. Anti-bacterial activity of different fractions of *Pholidota pallida* Lindl. root

Chinsamy *et al.* [34] also observed incredible antioxidant and anti-inflammatory potency of orchids which were also harmonious to the present investigation while he was screening some indigenous orchids of South Africa. Such influential outcome amplified the plant to check its inhibition against bacteria as anti-inflammatory plants also possess antibacterial activity [35]. For investigating the antimicrobial potential, inhibitory effect of plant part fractions against four human pathogenic bacteria were cross examined compared to positive control (Ampicillin 25 µg/disc). During this investigation it was noticed that, FH fractions of the plant is inert against the four pathogenic bacteria and only BW part can inhibit *S. typhi*. On the other hand, FD of leaf (14 and 15mm), stem (16.5 and 15.5 mm) and root (15.5 and 17.5 mm) is highly effective against *B. subtilis* and *S. aureus* but unresisting against *B. cereus* and *S. typhi*. FM of all parts inhibited *B. cereus* in the range of 6-8 mm but failed to avert *S. typhi*. In FM part only bulb had resistance against *B. subtilis* (7mm) and *S. aureus* (8.5mm). BW of all part the plant exhibited inhibitory effect against *S. typhi* of which root showed the maximum (12 mm). Sandrasagaran *et al.* [36] experienced similar outcomes while working with an orchid (*Dendrobium crumenatum*) against 8 pathogenic bacteria. They found stem extract had the most potent antimicrobial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Enterobacter aerogenes* while root and stem extracts were active against *Streptococcus pneumonia*, *Shigella dysenteriae* and *Saccharomyces cerevisiae*.

#### 4. CONCLUSION

The combined outcome implies that, extracts of different parts of this epiphytic orchid have ample phytochemicals with anti-oxidant, anti-inflammatory and antibacterial properties which can be a vital source of relevant drugs in the future.

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