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ANTI-INFLAMMATORY, ANTIOXIDANT, AND ANTIBACTERIAL POTENTIAL OF *ACAMPE PRAEMORSA* (ROXB.) BLATT. & McCANN- AN INDIGENOUS MEDICINAL ORCHID

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Abstract

Plant parts such as leaf, stem, and root of *Acampe praemorsa* (Roxb.) Blatt. & McCann were investigated for anti-inflammatory, antioxidant, and antibacterial activities. Anti-inflammatory property was evaluated using heat induced albumin denaturation assay. In methanolic crude extracts, the highest anti-inflammatory activity was 76.87% in stem and the least (44.89%) in the leaf. Amongst the four fractions, the highest anti-inflammatory activity was 88.17% in Dichloromethane (DCM) fractions of leaf and the lowest was 21.77% in n-Hexane fractions of leaf. Antioxidant potentiality was determined using both methanolic crude extract and four fractions *i.e.* DCM, Butanol-1, n-Hexane, and Methanol of all the three plant parts *i.e.* leaf, stem, and root. In crude extract, the highest scavenging activity was 84.32% in leaf at concentration of 250 μgml^{-1}. On the other hand, amongst four fractions, methanolic fraction of root showed the maximum scavenging activity (97.84%) at concentration of 250 μgml^{-1} and the least antioxidant activity was 46.31% in n-Hexane fraction of root at concentration of 50 μgml^{-1}. To evaluate antibacterial activity, experiment was carried out against four human pathogenic bacteria *i.e.* gram-positive (*Bacillus subtilis, Staphylococcus aureus*) and gram-negative (*Escherichia coli, Pseudomonas aeruginosa*). The highest inhibition activity (17.4 mm) against *S. aureus* was found in stem extracts at 200 concentration. Inhibition zone of 16.2 mm was observed in stem extracts at concentration 100 against *P. aeruginosa*. The least inhibition activity (9.8 mm) was showed in stem extracts at 100 concentration against *P. aeruginosa* and *S. aureus*. The present findings revealed that *Acampe praemorsa* can be used as a potential source of new drug development in future.

Introduction

THE FAMILY Orchidaceae is a diverse and widespread family of flowering plants, with cosmopolitan distribution. The orchids are well known for their medicinal properties and a number of plant-derived medicines have been used since ages without any adverse effects. These plants represent a large natural source of useful compounds that might serve for the development of novel drugs. It is therefore necessary to make efforts to explore and study orchids for their medicinal properties. In this regard, traditional medicine has been paid great attention because of their cheap value, availability, and little side effects which vindicates WHO that around 80% of the world population still rely mainly on plant-based drugs (Kumara, 2001).

The inflammation is a local response of animals towards chemical or physical injury or bacterial invasion characterized by the formation of edema, leucocytes infiltration and granuloma formation, tissue injury, and repair in living tissues (Bairagi *et al.*, 2012). The inflammation accompanied by chemical mediators include histamine, serotonin, slow reacting substances of anaphylaxis (SRS-A), TNF-α, interleukins, prostaglandins, and some plasma enzyme systems undergo activation at the onset of inflammation to cause increased vasodilatation and permeability of blood vessels (Perianayagam *et al.*, 2006; Ullah *et al.*, 2014). Anti-inflammatory agents are capable of inhibiting the cyclo-oxygenase COX-1 and COX-2 pathway of arachidonic acid metabolism, which produces prostaglandins (Ullah *et al.*, 2014). Prostaglandins are one of the important biomolecules, which play a key role in the induction of inflammatory response as their biosynthesis is significantly increased during inflammation. However, sustained inflammation can lead to undesired health effect (Tasleem *et al.*, 2014). Although the modern drugs used for the suppression and relief of inflammation include non-steroidal anti-inflammatory drugs (NSAIDs), opiates, immunosuppressants, corticosteroids, and histamines are associated with serious adverse side effects such as gastrointestinal disturbances, renal damage, respiratory depression, and possible dependence (Choi *et al.*, 2009; Faujdar *et al.*, 2016). Anti-inflammatory activity has also been reported in *Dendrobium crepidatum* (Paul *et al.,* 2022), *D. moschatum* (Tahli *et al.,* 2021), and *Pelatantheria insectifera* (Hoque *et al.,* 2021a). Hence, there is a need for searching new, safe, and effective anti-inflammatory drugs from natural sources and medicinal plants possibly with fewer side effects.

Antioxidants are widely used as ingredients in dietary supplements and are exploited to maintain health and prevent oxidative stress-mediated disease such as cancer, atherosclerosis, diabetics, inflammation and

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ageing, malaria, rheumatoid arthritis, neuro-degenerative disorders (Vasconcelos *et al.*, 2007). An antioxidant is a molecule capable of preventing the oxidation of other molecules such as free radicals or reactive oxygen species (ROS). Synthetic antioxidants such as Butylated Hydroxytoluene (BHT) and Butylated Hydroxyanisole (BHA) have recently been reported to be dangerous for human health. In this connection, some antioxidants have been isolated from different plant materials and search for effective, non-toxic natural compounds with antioxidant activity has been intensified. A few orchids have been reported to act as antioxidant agents. Isoamoenylin, a dihydrostilbene isolated from roots of *Dendrobium amoenum* var. *denneanum*, showed moderate antioxidative and weak antibacterial activities (Venkateswarlu *et al.*, 2002). On the other hand, three 2-glucosyloxycinnamic acid derivates, namely, cismelilotoside, dihydromelilotoside, and trans-melilotoside have been isolated from stem of *D. aurantiacum,* which exhibited potent antioxidant activities (Yang *et al.*, 2007). Recently, anti-oxidant activity has also been reported in *Acampe praemorsa* (Rahman *et al.,* 2023), *Aerides multiflora* (Sharma and Pathak, 2024), *Dendrobium crepidatum* (Paul *et al.,* 2022), *D. moschatum* (Tahli *et al.,* 2021), *Eria lasiopetala* (Hoque *et al.,* 2021b), and *Vanda cristata* (Pathak *et al.,* 2023).

The leaves of *Vanilla planifolia* have been reported to act as an antimicrobial agent (Kamath *et al.*, 2022). Methanolic extract of leaves of *Spiranthes mauritianum* showed antibacterial activity against gram-positive bacteria and have anti-inflammatory activity (Matu and Van Staden, 2003). Luo *et al.* (2007) has also observed that *Bletilla striata* possessed antioxidant and antimicrobial capacity.

Bangladesh is one of the naturally orchid growing countries in the world. As per the latest reports available, there are 188 orchid species (Rahman *et al.*, 2017). *Acampe praemorsa* (Roxb.) Blatt. & McCann, an indigenous orchid of Bangladesh is used to treat fever, earache, injury, male and female problems (Huda *et al.*, 2006), rheumatism, sciatica, and neuralgia (Chowdhery, 1998) and also used as a cooling agent (Yusuf *et al.*, 1994). The present work was undertaken to investigate the anti-inflammatory, antioxidant, and antibacterial properties of leaf, stem, and root of the selected orchid species, *Acampe praemorsa*.

Material and Methods

Collection and Identification of Plant Material

Acampe praemorsa was collected from Manikchari of Khagrachari district, Bangladesh. The orchid species was identified and authenticated by consulting relevant literature and critical examination of the herbarium. The voucher specimen (Accession no. 3272) has been preserved at the Herbarium of Chittagong University (HCU). Leaf, stem, and root of disease free and fresh plants of the species were used for different assays.

Processing, Extraction, and Fractionation of Samples

The freshly collected leaf, stem, and root of *Acampe praemorsa* were washed with water and cut into small pieces and dried in oven at 60°C. After grinding into powder, the samples were stored separately in airtight containers. Methanol (50 ml) was added to the 25 gm of sample from each of part (leaf, stem, and root) in a conical flask, shaken very well for 30 min, kept for overnight, shaken again and sonnicated for 10 min and then filtered using Whatman filter paper. The process was repeated 3 times with methanol and the extract was rotavaporated below 51°C and dried. The dried sample was kept as crude sample for each part (leaf, stem, and root). The whole process was repeated three times and finally, concentrated plant extract was kept in the refrigerator at 4°C. For fractionation of samples, the concentrated plant extracts were separated into four different solvents (n-Hexane, Dichloromethane, Methanol, and Butanol-1) by following Kupchan method.

Anti-Inflammatory Activity

Methanolic crude extract and fractionated samples of leaf, stem, and root were used for anti-inflammatory test following the method developed by Shinde *et al.* (1999). The activity was calculated by using the equation:

Percentage of inhibition $=\left(\frac{A-B}{A}\right) X\ 100$

where, A= Absorbance of control (5% egg albumin solution and methanol);

B= Absorbance of test group (5% egg albumin solution and plant extract) or, Absorbance of standard solution (5% egg albumin solution and acetyl salicylic acid.

LC_{50} was determined by generating an equation Y=mx+C by "% of inhibition" results at different concentrations. By putting these values in excel and inserting graph trend line was added. After selecting linear equation, Y=mx+C was found. Where, Y= % of inhibition; m= Coefficient; x= Concentration; C= Constant.

Antioxidant Activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) was used to evaluate the free radical scavenging activity of various parts of the studied plant. Methanol (2 ml) solution of plant extract or standard at different concentrations was taken in a test tube. Methanol (3 ml) solution in DPPH was added into the test tube. The test tube was incubated at room temperature for 30 min in dark place to complete the reaction. Then the absorbance of the solution was measured at 517 nm using a spectrophotometer against blank. A typical blank solution contained all reagents except plant extract or standard solution. The percentage (%) inhibition activity was calculated from the following equation. Scavenging activity % = $(1 - A_1/A_o) \times 100$, where, A_o is the absorbance of the control, and A_1 is the absorbance of the extract/standard.

Antibacterial Activity

Different concentrations such as 100, 200, 300, 400, 500 μgml^{-1} of plant samples were considered for the test. At first, 1 ml of broth culture of each bacterium (*Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*) was spread over the nutrient agar taken in glass petri-dish aseptically. The extract impregnated discs, blank paper discs impregnated with sterile solvents (methanol) were placed on the bacterium inoculated agar in the petri-dishes and incubated at 37°C. After 24 hr incubation, the zones of inhibition of bacterial growth around the discs were measured. The experiment was repeated thrice.

Statistical Analysis

The correlation among three activities in different parts of selected orchid species was analyzed statistically using XLSTAT 2020 statistic program software.

Results

Anti-Inflammatory Activity

The results of anti-inflammatory activity of methanolic crude extracts and fractionated samples of leaf, stem, and root of *A. praemorsa* are presented in Fig. 1. The highest (88.17%) inhibition of egg albumin denaturation was shown for DCM fraction of leaf and the lowest fraction showed n-Hexane (21.77%) inhibition. In case of stem, the highest (87.28%) inhibition egg albumin denaturation was found for methanolic fraction and the least (53.06%) anti-inflammatory activity was found for n-Hexane fraction. Whereas, the highest (88.96%) inhibition of egg albumin denaturation was shown for n-Hexane fraction of the root and methanol fraction exhibited the lowest (46.26%) inhibition. Butanol-1 fraction showed overall better performance and methanol fraction reported as worst for all the three samples (leaf, stem, and root) studied.

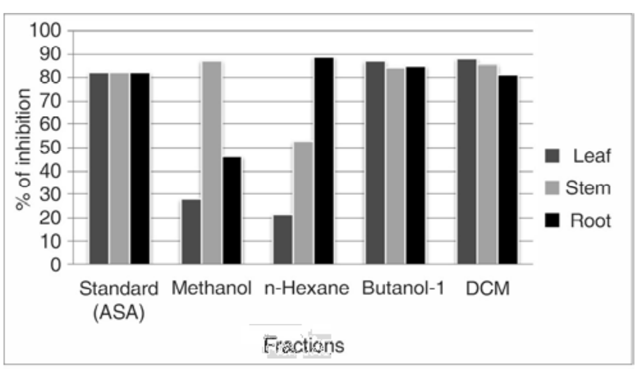

Fig. 1. Anti-inflammatory activity of leaf, stem, and root of *Acampe praemorsa* at different fractions.

Antioxidant Activity

DPPH free radical scavenging method was used for the determination of the scavenging activities of the methanolic extracts of the leaf, stem, and root of *A. praemorsa* and then compared with the standard

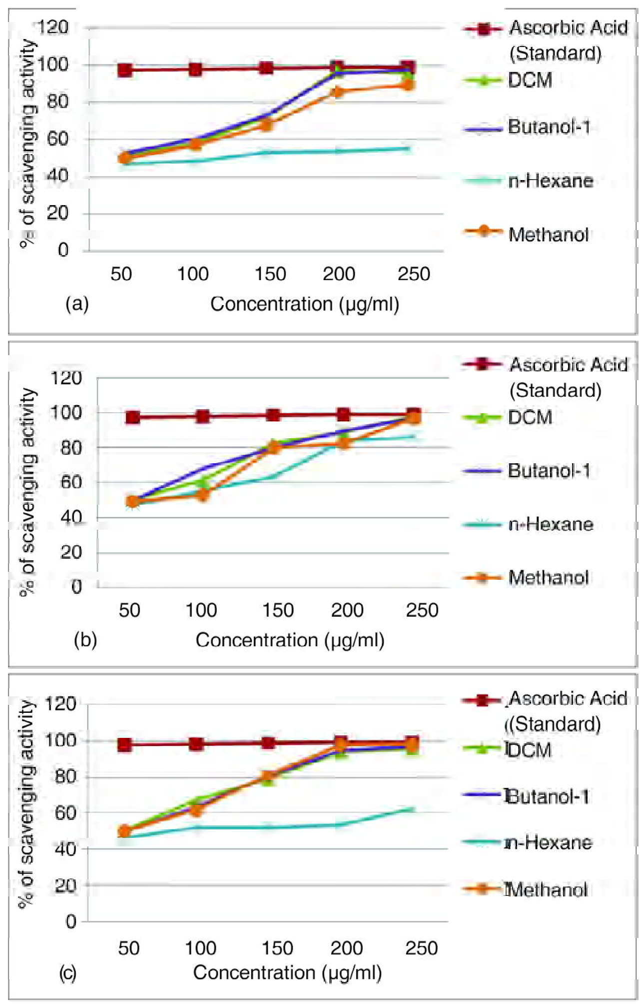

Fig. 2a-c. Relative % of scavenging activity or % inhibition of standard antioxidant ascorbic acid and methanolic crude extracts of *Acampe praemorsa* in different plant parts: a, leaf; b, stem; c, root.

antioxidant ascorbic acid. Amongst the five different concentrations used in the study (50, 100, 150, 200, and 250 µgml^{-1}), ascorbic acid showed the highest scavenging activity (96.40%) at 250 µgml^{-1} concentration and the lowest scavenging activity (95.14%) was at 50 µgml^{-1}. Crude extract of leaf of *A. praemorsa* showed 25.95%, 45.41%, 70.45%, 76.04%, 84.32% at 50, 100, 150, 200, 250 µgml^{-1} concentrations, respectively (Fig. 2a), where the highest scavenging activity was observed as 84.32% at 250 µgml^{-1} concentration and the lowest scavenging activity was 25.95% at 50 µgml^{-1}concentration. IC_{50} (µgml^{-1}) of leaf was 114.60. Extract of stem of *A. praemorsa* showed the highest scavenging activity (83.423 %) at concentration 250 µgml^{-1} and the lowest scavenging activity was 31.532% at concentration of 50 µgml^{-1}. IC_{50} of stem was 68.04 µgml^{-1} (Fig. 2b.) On the other hand, crude extract of root showed the highest (83.42 %) scavenging activity at 250 µgml^{-1} concentration and the lowest (19.64%) was at 50 µgml^{-1}. IC_{50} of root was 116.73 µgml^{-1} (Fig. 2c). The highest IC_{50} value was 116.73 µgml^{-1} in root, followed by leaf (114.60 µgml^{-1}) and stem (68.04 µgml^{-1}).

Antibacterial Activity

The anti-bacterial activity of leaf, stem, and root of *A. praemorsa* was tested at different concentrations (100, 200, 300, 400, and 500 µgml^{-1}) against four human pathogenic bacteria *i.e. Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The results are shown in Table 1.

The methanolic extracts of leaf exhibited positive activity against all the test organisms. The highest zone of inhibition was 15.4 mm at 300 µgml^{-1} concentration against *Bacillus subtilis* and the lowest zone of inhibition was 11 mm at 100 µgml^{-1} concentration against *Staphylococcus aureus*. In case of crude extract of stem, the highest zone of inhibition was 17.4 mm at 200 µgml^{-1} concentration against *Staphylococcus aureus* and the lowest zone was 9.8 mm at 100 µgml^{-1} concentration against *Staphylococcus aureus*, *Bacillus subtilis* and 500 µgml^{-1} concentration against *Pseudomonas aeruginosa, Bacillus subtilis.* On the other hand, root extracts showed the highest zone of inhibition 16.4 mm at 100 µgml^{-1} concentration against *Escherichia coli* and the lowest was 10 at 200 µgml^{-1} concentration against *Pseudomonas aeruginosa*.

Fig. 3 shows the correlation amongst the studied three properties in different plant parts of *A. praemorsa* where the strongest correlation exists between anti-

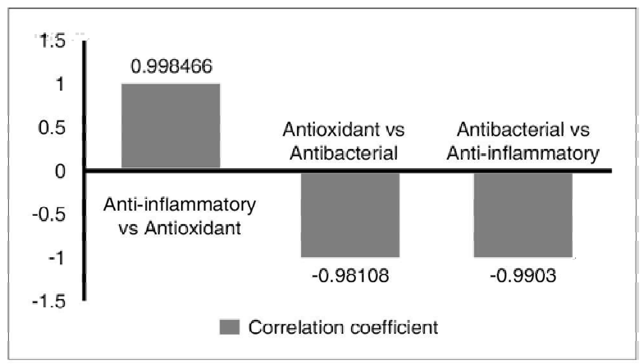

Fig. 3. Correlation amongst anti-inflammatory, antioxidant, and antibacterial properties of *Acampe praemorsa*.

inflammatory and antioxidant potentialities. On the other hand, antioxidant and antibacterial activities exhibited strong negative relation. The findings also reported that

Table 1. Varied zones of inhibition of different bacteria in leaf, stem, and root extracts of *Acampe praemorsa* at various concentrations.

Test organisms	Plant parts	Zone of inhibition (mm) at different concentrations (µgml^{-1})					
		100	200	300	400	500	Ampicillin
Escherichia coli	Leaf	15	14.6	12.6	14.8	12.2	
	Stem	10.6	10.8	12.8	11.4	12.4	15
	Root	16.4	11.2	10.6	15.4	13.6	
Pseudomonas aeruginosa	Leaf	12.4	13	12.4	14.4	11.8	
	Stem	16.2	11.4	10.6	11.6	9.8	24
	Root	12	10	14	11.8	12.6	
Staphylococcus aureus	Leaf	11	11.2	13	15.4	13	
	Stem	9.8	17.4	14.2	14.4	16	16
	Root	10.6	10.8	12.2	10	12.6	
Bacillus subtilis	Leaf	11.2	13.8	15.4	15.2	11.8	
	Stem	9.8	10.8	10.2	11.6	9.8	16
	Root	12.4	8.8	12.4	12.4	10.6	

the strongest negative relationship was present between antibacterial and anti-inflammatory properties.

Discussion

Different bioactive compounds have been reported to be responsible for the anti-inflammatory activity in plants. Recent literature reports that flavonoids possess potent anti-inflammatory properties. Flavonoids have the ability to inhibit arachidonic acid metabolism and also enzyme prostaglandin synthetase involved in the production of chemical mediators of inflammation (Aquila *et al.*, 2009; Havsteen, 2002; Middleton *et al.*, 2000; Oweyele *et al.*, 2005). Phytosterols and triterpenoid are attributed to reducing pain, swelling, and inflammatory joint diseases with aging and stress (Tatiya *et al.*, 2017). Flavonoids, phenolic compounds, tannins, and saponins of some orchids have been reported toshow anti-inflammatory activity (Sukumaran and Yadav, 2016). In present investigation, amongst the three plant parts (leaf, stem, and root) of *Acampe praemorsa,* the highest (76.87%) inhibition of egg albumin denaturation was shown for stem and the lowest (44.89%) was shown for leaf. Standard drug ASA (Acetyl Salicylic Acid) showed 82.31% inhibition.

Several compounds with anti-inflammatory activity were isolated from *Dendrobium monoliforme* and *Gastrodia elata* has also also been a good source of compounds with anti-inflammatory, analgesic, and anti-angiogenic activity (Singh *et al.*, 2012). Yang *et al.* (2006) worked on *Dendrobium chrysanthum* to evaluate anti-inflammatory activity. Kalaiarasan and Ahmed (2012) have studied *Bulbophyllum kaitense* to investigate anti-inflammatory activity by HRBC **(**Human red blood cell membrane stabilization assay) method and found that ethanolic extract of *Bulbophyllum kaitense* pseudobulbs possess good anti-inflammatory activity. The petroleum ether, chloroform, and aqueous extracts have more or less anti-inflammatory activity. Sukumaran and Yadav (2016) investigated anti-inflammatory potential of *Dendrobium macrostachyum* and observed that the ethanol and water extract were highly effective as albumin denaturation inhibitors (LC_{50} = 114.13 and 135.818 μgml^{-1} respectively) and proteinase inhibitors (LC_{50} = 72.49 and 129.681 μgml^{-1}, respectively).

The present study revealed that in methanolic crude extracts, amongst five different concentrations used in the study (50, 100, 150, 200, and 250 μgml^{-1}), the highest scavenging activity of standard ascorbic acid was 96.396% at 250 μgml^{-1} concentration and the lowest was 95.135% at concentration 50 μgml^{-1}. Whereas in fractioned samples, the maximum scavenging activity was 99.09% at concentration of 250 μgml^{-1} and the lowest scavenging activity was 97.48% at 50 μgml^{-1}.

Mukherjee *et al.* (2012) found that the *in vitro* aqueous extract of *Dendrobium aqueum* showed increase in the percentage free radical scavenging potential in a dose dependent manner with the highest activity of 49% at a dose of 100 μgml^{-1} which was significantly less than the standard at the same concentration (93.3% at 100 μgml^{-1}). Sukumaran and Yadav (2016) studied general antioxidant potential of stem and leaf extracts of *Dendrobium macrostachyum* by *in vitro* methods. The antioxidant activity was determined by assays based on the decolourization of the radical monocation of DPPH, ABTS, and reducing power. The stem ethanolic extracts exhibited significant IC_{50} value of 10.21 μgml^{-1}, 31.54 μgml^{-1}, and 142.97 μgml^{-1}, respectively, for DPPH, ABTS radical scavenging, and reducing power activity. Suja and Willams (2016) worked on antioxidant potential of a wild epiphytic orchid *Acampe praemorsa* and reported DPPH radical scavenging activity varied from the minimum inhibition of 60.37 ± 0.011% (at 25μl) to the maximum inhibition of 69.74 ± 0.010% (at 100μl). Whereas, aqueous extract varied from the minimum inhibition of 56.00 ± 0.005% (at 25μl) to the maximum inhibition of 58.83 ± 0.011% (at 100μl) and the ethanol extract varied from the minimum inhibition of 51.01 ± 0.015% (at 25 μl) to the maximum inhibition of 54.93 ± 0.010% (at 100 μl).

Anti-microbial properties of orchid plants also play an essential role for preventing traditional disorders. Anti-microbial is an agent that kills the microorganisms or inhibits their growth. The present work reported that all the concentrations (100, 200, 300, 400, and 500 μgml^{-1}) exhibited positive activity against the tested organisms *i.e. Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The highest zone of inhibition was 17.4 mm at 200 concentration against *S. aureus* and the lowest was 9.8 mm at 100 concentration against *S. aureus*, *B. subtilis at* 500 concentration against *B. subtilis.* Soumiya and Williams (2017) investigated the antimicrobial properties of leaf of *Cymbidium aloifolium* and revealed that the methanolic extract showed inhibition zone of (30 mm) against *Staphylococcus aureus*. A moderate anti-microbial activity was found in methanolic leaf extract of *Peristylus densus* against *Salmonella typhi* and *Staphylococcus aureus* by Jagtap and Satpute (2015). Hoque *et al.* (2016) while working on anti-micobial activities of three orchid species found the maximum inhibition zone (37.5 mm) in fresh ethanolic leaf extract of *Aerides odorata* against *Salmonella typhi*.

Antibacterial activity of *Coelogyne breviscapa, Dendrobium nutantiflorum, Luisia zeylanica,* and *Pholidota pallida,* was determined by Rashmi *et al.*

(2015) using Agar well diffusion method and showed that all orchids extracts inhibited both gram-positive and gram-negative bacteria. Amongst the all bacteria, *Bacillus subtilis* and *E. coli* were inhibited to higher extent and marked antibacterial activity was observed in case of *Arundina graminifolia, Coelogyne breviscapa*, *Eulophia promensis,* and *Geodorum densiflorum* which were tested for antibacterial potentiality by Alam (2012) and found that the methanolic extract of pseudobulbs of *G. densiflorum* showed positive activity against *Bacilllus cereus* and *Salmonella typhi*, but there was no activity reported against *Staphylococcus aureus* and *Vibrio cholerae*. On the other hand, pseudobulbs of *E. promensis* showed considerable activities against three bacteria except for *Bacillus cereus* and root extract of *Arundina graminifolia* showed considerable activity for all three organisms except for *Bacillus cereus*. Shubha and Chowdappa (2016) investigated the anti-bacterial activity of *Cymbidium aloifolium* and showed the methanolic and acetone extracts of capsule cover exhibited the maximum anti-bacterial activity against *E. coli, P. aeruginosa*, and *S. aureus* (16 mm) followed by *Bacillus subtilis* (14 mm) whereas, the capsule cover and root acetone extracts inhibited *Klebsiella pneumoniae.*

Phytochemical screening of *Acampe praemorsa* revealed the presence of several bioactive compounds including alkaloids, phlobatannins, saponins, tannins, terpenoids, steroids, glycosides, anthroquinone, quinine, coumarin, cardiac glycosides, protein, flavonoids, fixed oils, fats, and phenols (Basher, 2018). The two important phenanthropyrans, flavidinin and praemorsin have also been isolated from this species (Huda and Kashem, 2021). Thus, the presence of these compounds and their synergistic properties might be rendering the possible mechanism for anti-inflammatory, antioxidant, and antimicrobial activity, in the presently tested orchid. The current study revealed that the indigenous knowledge of medicinal orchids provides opportunities to validate their medicinal claims on scientific lines which are so far been untapped from their vast medicinal potential.

Conclusion

On the basis of the results obtained, it can be reported that methanolic crude extracts of stem and leaf of *Acampe praemorsa* showed the highest anti-inflammation and antioxidant effect respectively, while amongst different fractions, the n-Hexane and methanolic fraction of root was the maximum, whereas methanolic extract of stem showed the best antibacterial activity against *Staphylococcus aureus.* To conclude, different parts of *Acampe praemorsa* exhibit significant anti-inflammatory, antioxidant, and antimicrobial activity. Therefore, the present investigation has the findings on the efficacy and safety of the selected orchid species, *Acampe praemorsa* and promotes the continued searching for new leads for the development of better drugs against infectious diseases and other common ailments in Bangladesh.

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