### **Pharmacological Study**

# *In vitro* antimicrobial and brine shrimp lethality of *Allophylus cobbe* L.

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#### Abstract



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Plants are rich source of pharmacologically active agents, which could be explored in disease management. Methanol, ethanol, and petroleum ether extracts of the whole plant of *Allophylus cobbe* L. were evaluated for antimicrobial and cytotoxic activities. *In vitro* antimicrobial sensitivity by disk diffusion method was conducted against four Gram-positive and seven Gram-negative pathogenic bacteria and seven fungi. In the antibacterial and antifungal sensitivity tests, growth inhibition was found to be within the range of 10.0-17.67 mm. Strong zone of inhibition by the ethanol extract of *A. cobbe* (EEAC) was found against *Trichophyton* spp. With some exceptions, a mild to strong antimicrobial activity was observed in this study. Significant minimum inhibitory concentration (MIC;  $15.625 \ \mu g/ml$ ) was found against *Trichophyton* spp. Other detected MICs were within the range of  $31.25-125 \ \mu g/ml$ . The petroleum ether extract of the plant exhibited strong cytotoxicity in the brine shrimp lethality bioassay test.

Key words: Allophylus cobbe, antimicrobial, bioassay, brine shrimp lethality

### Introduction

Many of the plant materials used in traditional medicine are readily available in rural areas and this has made traditional medicine relatively cheaper than modern medicine.<sup>[1]</sup> Medicinal plants contain pharmacologically active principles which, over the years, have been explored in traditional medical practice for the treatment of various ailments.<sup>[2]</sup> Bangladesh is a developing country and it covers a large number of poor people who are unable to access the modern medical support. Most of them are usually dependent upon the Kabiraj (traditional medicine practitioners) for their health problems.

Allophylus cobbe L. (Family: Sapindaceae), a herb, grows wild in the hilly region of Bangladesh. It has anti-feedant activity and is used as oxytocic and antidiarrheal agents by the traditional practitioners in the East-West region of Bangladesh.<sup>[3]</sup> The aim of the present study is to evaluate the antimicrobial sensitivity and cytotoxic responses of the methanol, ethanol, and petroleum ether extracts of the targeted plant and to search logical evidence for its folk use and further exploitation.

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### **Materials and Methods**

#### Plant collection and identification

The plant under investigation was collected from the hilly area of Baluchara, Chittagong, Bangladesh, in January 2010 and was identified by the authority at Forest Research Institute, Chittagong, Bangladesh.

#### Extraction

The plant was subjected to shade dry. Then the crude dried plant was ground into coarse powder and subjected to hot extraction<sup>[4]</sup> with methanol, ethanol, and petroleum ether by the Soxhlet apparatus. The extraction was carried out for about 18 h and the extract was filtered through a cotton plug followed by Whatman filter paper, no. 1. The extracts were then concentrated by using a rotary evaporator.

#### Antimicrobial screening

The antibacterial and antifungal activities of the crude extractives were evaluated by the disk diffusion method<sup>[5]</sup> against four Gram-positive and seven Gram-negative pathogenic bacteria and seven fungi using ciprofloxacin (Ciprocin, 500 mg/tab., Square Pharmaceuticals Ltd.) and fluconazole (Flugal, 25 mg/cap., Square Pharmaceuticals Ltd., Dhaka, Bangladesh), respectively, as standards. The organisms were obtained as pure culture from the Faculty of Biology, University of Chittagong, Bangladesh. The antimicrobial activity of the test agents was expressed by measuring the diameter of zone of inhibition expressed in millimeters (mm). The experiments were carried out in triplicate.

#### Minimum inhibitory concentration

The minimum inhibitory concentrations (MICs) of the extractives were determined by the serial tube dilution test<sup>[6]</sup> in nutrient broth medium containing graded concentrations (500, 250, 125, 62.50, 31.25, 15.625, 7.8125, 3.90625, 1.953125, and 0.9765625  $\mu$ g/ml) of the crude extractives and inoculated test organisms. Also, the graded concentrations of ciprofloxacin and fluconazole were taken as standards for bacteria and fungi, respectively.

#### Brine shrimp lethality bioassay

Brine shrimp lethality bioassay method<sup>[7]</sup> was applied for determination of general toxic property of the plant extractives. Dimethyl sulfoxide (DMSO) solutions of the samples were applied against Artemia salina in a 1 d ex vivo assay. For the experiment, crude extracts were dissolved in DMSO and solutions of varying concentrations (10.5, 9.0, 7.5, 6.0, 4.4, 3.0, 1.5, 0.75, 0.375, and 0.1875  $\mu$ g/ml) were obtained by serial dilution. Vincristine sulphate (Vincristine, Richter Inj., powder for reconstitution, 1 mg vial, Gedeon Richter Ltd.) was used as reference standard.

#### Statistics

Experimentally obtained primary data were manipulated as the source of responses. All experiments were performed in duplicate and replicated at least three times. Data were presented as mean  $\pm$  standard deviation (SD) and were considered statistically significant when *P* values were <0.05.

#### **Observations and Results**

In the antibacterial and antifungal sensitivity test [Table 1], the highest zone of inhibition  $(17.67 \pm 0.47 \text{ mm})$  was found against *Microsporum* spp. with the Ethanol Extract of *Allophylus cobbe* (EEAC). This was followed by  $11.67 \pm 0.47$ ,  $11.0 \pm 0.82$ ,  $11.67 \pm 0.94$ ,  $10.33 \pm 1.25$ ,  $10.33 \pm 1.25$ , and  $10.33 \pm 0.47$  mm by the same extract against *Cryptococcus neoformans*, *Bacillus subtilis*, *Pityrosporum ovale*, *Salmonella paratyphi*, *Staphylococcus aureus*, and *Vibrio cholerae*, respectively. But the extract EEAC was inactive against *Bacillus megaterium*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Shigella dysenteriae*, *Shigella sonnei*, *Salmonella typhi*, *Aspergillus niger*, *Blastomyces dermatitidis*, *Candida albicans*, and *Trichophyton* spp.

The second highest zone of inhibition was found against Ca. albicans (15.33  $\pm$  1.25 mm) and Sh. dysenteriae (15.0  $\pm$  0.82 mm) by the Methanol Extract of Allophylus cobbe (MEAC). This was followed by 11.0  $\pm$  0.82, 10.33  $\pm$  0.47, 10.0  $\pm$  0.82, and 10.33  $\pm$  1.25 mm by the same extract against Ba. megaterium, As. niger, Cr. neoformans, and Sh. dysenteriae, respectively. But the extract MEAC was inactive against Ba. subtilis, Ba. cereus, St. aureus, Ps. aeruginosa, Sh. sonnei, Sa. typhi, Vi. cholerae, Sa. paratyphi, Bl. dermatitidis, Pi. ovale, Trichophyton spp., and Microsporum spp.

Again, the highest zone of inhibition  $(14.33 \pm 0.47 \text{ mm})$  of Petroleum Ether Extract of Allophylus cobbe (PEEAC) was produced against *Cr. neoformans*. This was followed by 12.33  $\pm$ 0.47, 12.33  $\pm$  0.47, 12.0  $\pm$  0.82, 11.67  $\pm$  0.47, 11.0  $\pm$  1.63, 10.33  $\pm$  0.47, 10.0  $\pm$  0.82, and 10.0  $\pm$  0.82 mm by the same extract

Test microorganisms	Diameter zone of inhibition (mm)				
	MEAC (50 μg/μl)	EEAC (50 μg/μl)	PEEAC (50 µg/µl)	STD (50 μg/μl)	
Gram-positive bacteria				CFN	
Bacillus subtilis	ND	11.00±0.82***	11.67±0.47**	16.00±0.82	
Bacillus megaterium	11.00±0.82***	ND	12.00±0.82**	17.33±1.25	
Bacillus cereus	ND	ND	10.33±0.47***	16.67±0.94	
Staphylococcus aureus	ND	10.33±1.25***	ND	18.67±0.94	
Gram-negative bacteria					
Pseudomonas aeruginosa	ND	ND	ND	18.00±1.63	
Escherichia coli	10.33±1.25**	ND	ND	17.67±1.25	
Shigella dysenteriae	15.00±0.82***	ND	ND	15.67±0.47	
Shigella sonnei	ND	ND	12.33±0.47***	20.67±0.47	
Salmonella typhi	ND	ND	ND	15.00±0.82	
Vibrio cholerae	ND	10.33±0.47***	10.00±0.82***	19.33±0.47	
Salmonella paratyphi	ND	10.33±1.25***	12.33±0.47**	17.33±0.47	
Fungi				FCN	
Aspergillus niger	10.33±0.47***	ND	ND	16.67±0.47	
Blastomyces dermatitidis	ND	ND	10.00±0.82***	16.00±0.82	
Candida albicans	15.33±1.25*	ND	11.00±1.63**	16.67±1.69	
Pityrosporum ovale	ND	11.67±0.47***	ND	17.33±0.47	
Trichophyton spp.	ND	ND	ND	16.33±1.25	
Microsporum spp.	ND	17.67±0.47***	ND	19.00±0.82	
Crvptococcus neoformans	10.00±0.82***	11.67±0.94***	14.33±0.47*	15.00±0.82	

\*P<0.5, \*\*P<0.1, \*\*\*P<0.01, the diameter of zone of inhibition is expressed as Mean±SD (n=3), a diameter less than 8 mm was considered inactive. ND - Not detected; STD: Standard drug, CFN: Ciprofloxacin, FCN: Fluconazole, MEAC: Methanol extract of Allophylus cobbe, EEAC: Ethanol extract of Allophylus cobbe, PEEAC: Petroleum ether extract of Allophylus cobbe

against Sh. sonnei, Sa. paratyphi, Ba. megaterium, Ba. subtilis, Ca. albicans, Ba. cereus, Vi. cholerae, and Bl. dermatitidis, respectively. Also, the extract was found to be inactive against St. aureus, Ps. aeruginosa, Ei. coli, Sh. dysenteriae, Sa. typhi, As. niger, Pi. ovale, Trichophyton spp., and Microsporum spp.

In MIC by serial tube dilution method, a potent MIC (15.625  $\mu$ g/ml) was shown by MEAC against *Microsporum* spp. [Table 2]. The MIC of 62.50  $\mu$ g/ml was found against *Ba. subtilis, Pi. ovale, Cr. neoformans* and 125  $\mu$ g/ml was found against *St. aureus, Vi. cholerae*, and *Sa. paratyphi*, respectively, with the same extract. MICs for the other test organisms were not detected.

For the extract MEAC, the MIC of  $31.25 \ \mu g/ml$  was found against *Sh. dysenteriae* and *Ca. albicans*. This was followed by 62.50  $\mu g/ml$  for *Ba. megaterium*, and 125  $\mu g/ml$  for *Ei. coli*, *As. niger*, and *Cr. neoformans*. MICs for the other test organisms were not detected.

Again, with some exceptions, the PEEAC produced mild to moderate MICs against the test organisms. The MIC of 31.25  $\mu$ g/ml was found against *Sh. sonnei*, *Sa. paratyphi*, and *Cr. neoformans*. This was followed by 62.50  $\mu$ g/ml for *Ba. subtilis*, *Ba. megaterium*, and *Ca. albicans*. On the other hand, the pathogens *Ba. cereus*, *Vi. cholerae*, and *Bl. dermatitidis* were inactivated at the MIC of 125  $\mu$ g/ml. But the extract was unable to inhibit the growth of other nine pathogens.

The growth of the test pathogen, *Microsporum* spp. was strongly inhibited by the ethanol extract of *Al. cobbe*. From the study, it was observed that mild to moderate MICs were produced by the extractives (with some exceptions), where the standard drugs were ciprofloxacin and fluconazole for the test bacteria and fungi, respectively.

In the cytotoxicity test [Table 3], the extract PEEAC showed significant cytotoxicity in comparison to the other two extractives. The  $LC_{50}$  and  $LC_{90}$  of the PEEAC, MEAC, and EEAC were 0.79 and 1.31, 6.13 and 10.32, and 6.51 and 12.50 µg/ml, respectively. The standard, vincristine sulfate, produced a potent  $LC_{50}$  and  $LC_{90}$  of 0.47 µg/ml and 0.67 µg/ml, respectively, which were compared with the extractives.

#### Discussion

A mild to moderate zone of inhibition was observed by all the extractives against *Cr. neoformans*. All the extractives were inactive against *Trichophyton* spp. and *Ps. aeruginosa*. A wide spectrum of antimicrobial activity was observed with the PEEAC. PEEAC was found to have a moderate antibacterial activity for the test Gram-positive species. Among the three extractives, PEEAC was found to be more active against *Ba. subtilis*, *Ba. megaterium*, *Ba. cereus*, *Sh. sonnei*, *Sa. paratyphi*, *Bl. dermatitidis*, *Ca. albicans*, and *Cr. neoformans*. The extractive MEAC was more active than the other two extractives against *E. coli*, *Sh. dysenteriae*, *As. niger*, and *Ca. albicans*. The test pathogens, *St. aureus*, *Vi. cholerae*, *Pi. ovale*, and *Microsporum* spp. were found to be more sensitive to the extractive EEAC than the others.

In the MIC test, EEAC strongly inhibited the growth of the test species, *Microsporum* spp. Moderate MICs (31.25–62.50 µg/ml)

were found against Sh. dysenteriae, Ca. albicans, Sh. sonnei, Sa. paratyphi, Cr. neoformans, Ba. subtilis, Ba. megaterium, and Pi. ovale by the all extractives. Also, the test extractives produced a mild growth inhibition (125  $\mu$ g/ml) against Ba. cereus, St. aureus, Ei. coli, Vi. cholerae, Sa. paratyphi, As. niger, Bl. dermatitidis, and Cr. neoformans. All the extractives produced no inhibition to Ps. aeruginosa, Trichophyton spp., and Sa. typhi.

The extract PEEAC was found to be potent cytotoxic agent to the brine shrimps as it produced strong  $LC_{50}$  and  $LC_{90}$ . The extractives MEAC and EEAC also produced a moderate cytotoxic activity in comparison to the standard, vincristine sulfate.

#### Conclusion

From the study it is evident that the extractives of *Al. cobbe* L. showed mild to strong antimicrobial activity and moderate to strong cytotoxicity. A significant antimicrobial and cytotoxic profile was observed by the crude PEEAC. Further investigation is required to isolate the bioactive moieties.

# Table 2: Minimum inhibitory concentrations (MICs) of Allophylus cobbe extracts

Test organisms	Minimum inhibitory concentrations (µg/ml)		
	PEEAC	EEAC	MEAC
Bacillus subtilis	62.50	62.50	ND
Bacillus megaterium	62.50	ND	62.50
Bacillus cereus	125	ND	ND
Staphylococcus aureus	ND	125	ND
Pseudomonas aeruginosa	ND	ND	nd
Escherichia coli	ND	ND	125
Shigella dysenteriae	ND	ND	31.25
Shigella sonnei	31.25	ND	ND
Salmonella typhi	ND	ND	ND
Vibrio cholerae	125	125	nd
Salmonella paratyphi	31.25	125	nd
Aspergillus niger	nd	nd	125
Blastomyces dermatitidis	125	nd	nd
Candida albicans	62.50	nd	31.25
Pityrosporum ovale	nd	62.50	nd
Trichophyton spp.	nd	nd	nd
Microsporum spp.	nd	15.63	nd
Cryptococcus neoformans	31.25	62.50	125

MICs: Minimum concentrations of the extractives that caused the inhibition of the organisms growth, MICs >125  $\mu$ g/ml were considered as inactive, ND: Not detected, MEAC: Methanol extract of Allophylus cobbe, EEAC: Ethanol extract of Allophylus cobbe, PEEAC: Petroleum ether extract of Allophylus cobbe

Table 3: Cytotoxic response of Allophylus cobbe

extracts					
Sample	LC <sub>50</sub> (μg/ml)	LC <sub>90</sub> (µg/ml)			
VS	0.47	00.67			
MEAC	6.13	10.32			
EEAC	6.51	12.50			
PEEAC	0.79	01.31			

VS:Vincristine sulfate, MEAC: Methanol extract of Allophylus cobbe, EEAC: Ethanol extract of Allophylus cobbe; PEEAC: Petroleum ether extract of Allophylus cobbe

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# हिन्दी सारांश

# Allophylus cobbe L. वनौषधि में antimicrobial एवं cytotoxicity गतिविधि का अध्ययन

## मुहम्मद तरिकुल इस्लाम, मुहम्मद अब्दुन नूर, विजय कैरों, रिवेलिल्सन मेन्डिस द फ्रीटस

पौधे pharmacologically सक्रिय एजेंटों की समृद्ध स्रोत हैं, जो मानव रोग प्रबंधन में प्रयोग किये जाते हैं । पूरे संयंत्र Allophylus cobbe मेथनॉल और पेट्रोलियम ईथर extractives का रोगाणुरोधी और cytotoxicity गतिविधि के लिए अध्ययन किया गया । इन विट्रो डिस्क प्रसार विधि द्वारा रोगाणुरोधी संवेदनशीलता ४ ग्राम सकारात्मक और ७ ग्राम नकारात्मक रोगजनक बैक्टीरिया और ७ कवक के विरुद्ध आयोजित किया गया है । जीवाणुरोधी और ऐंटिफंगल संवेदनशीलता परीक्षण में, विकास निषेध 90.0 – १७.६७ मि.मी. की सीमा के अन्तर्गत होना पाया गया । EFAC द्वारा निषेध का मजबूत क्षेत्र ट्रायकॉफ़ायटन एस.पी.पी. के विरुद्ध पाया गया । कुछ अपवादों के साथ हल्के से लेकर मजबूत स्तरीय रोगाणुरोधी गतिविधि इस अध्ययन में पायी गयी । महत्वपूर्ण MIC (१५.६२५ माइक्रो ग्राम/मिलीलीटर) ट्रायकॉफ़ायटन एस.पी.पी. के विरुद्ध पाया गया । वनौषधि के पेट्रोलियम ईथर घनसत्व ने brine shrimp lethality bioassay निकालने नमकीन चिंराट मारक bioassay परीक्षण में मजबूत cytotoxicity का प्रदर्शन किया ।