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ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACTS OBTAINED FROM *CHAETOCARPUS CASTANOCARPUS* ROXB THW. AGAINST HUMAN PATHOGENS.

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ABSTRACT

An attempt was made to investigate the antibacterial and antifungal activities of Petroleum ether, carbon tetrachloride, chloroform and ethyl alcohol extracts from *Chaetocarpus castanocarpus* against ten human pathogenic bacteria viz., *Shigella dysenteriae, Salmonella typhi, S. paratyphi, Bacillus subtilis, B. cereus, B. megaterium, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Vibrio cholerae* and three human pathogenic fungi viz., *Aspergillus niger, A. ochraceus,* and *A. ustus* using disc diffusion and poisoned food method respectively. The chloroform and ethyl alcohol extracts showed good antibacterial and antifungal activity against the tested organisms. The crude extract of chloroform exhibited the largest zone of inhibition (26 mm in diameter with 2000 μg/disc extract) against *S. dysenteriae*. In case of fungi, the highest percentage of inhibition (42.5.0% with 100 μg/ml medium) was recorded against *A. ustus*. The MICs were determined by broth macrodilution method. The lowest MIC (750 μg/ml) was recorded against *B. megaterium* towards chloroform and ethanol extract, and *V. cholerae* towards chloroform extract. In case of fungi, chloroform and ethanol extract exhibited the lowest MIC (2000 μg/ml) against *A. ustus*, and chloroform extract against *A. niger*.

Key words: Antimicrobial activity, *Chaetocarpus castanocarpus*, Crude extract, Leaf.

INTRODUCTION

Medicinal plants are important natural wealth of a country. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. Most of these medicinal plants are also rich sources of bioactive compounds and often serves as important raw materials for drug production and new drug development. In modern China, the traditional medicines are used widely as a significant instrument of health care. It is well known that infectious diseases account for high proportion of health problems,

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specially in the developing countries. So, there is a continuing need for new antimicrobial agents since none of the available drugs is free from adverse effects and limitation. But medicinal plants possess various remedial properties with worthless materials and it is important to separate the worthless materials from the good. Plant metabolites were proved to be the most important group of compounds that showed wide range of antimicrobial activity (Al Shamma *et al*. 1982, Rahman *et al*. 1999, Sohrab *et al*. 2001, Rahman *et al.* 2004). Now-a-days, the natural products have been interesting and important sources of biologically active (antimicrobial) substances and the major sources of which are still left undiscovered. Much of the work has been done in India and other countries. But very little work is done in this field from Bangladesh.

Chaetocarpus castanocarpus Roxb Thw. is a small evergreen tree belongs to the family Euphorbiaceae, grows commonly in the areas hilly areas of Sylhet and Chittagong, Bangladesh. The plant is used in diabetes and to stop bleeding. In Malaysia young leaves are used as vegetables. There is no report on antimicrobial activity of this plant.

MATERIALS AND METHODS

Collection and extraction of plant material

Leaves of *Caetocarpus castanocarpus* Roxb Thw. were collected in fresh condition from Rangamati Hill Tracts (Bangladesh). The collected and cleaned samples were cut into small pieces (1-2 cm), dried in air. The samples were ground to fine powder mechanically and then 100 g of the dried powder was kept steeped thrice overnight in petroleum ether, chloroform, carbon tetrachloride and ethyl alcohol. The extracts thus obtained were filtered, centrifuged at 2000 rpm for 15 minutes and then concentrated to a gummy material under reduced pressure at 50^0C by rotary vacuum evaporator. The gummy materials were then transferred to small vials and dried as usual. The extracts thus obtained were termed as crude extract.

Test organisms

The crude extracts obtained from *C. castanocarpus* were tested for their antibacterial activity against ten human pathogenic bacteria viz., *Shigella dysenteriae* AE 14396, *Salmonella typhi* AE 14612, *S. paratyphi* AE 14613 , *Bacillus subtilis* BTCC 17, *B. cereus* BTCC 19, *B. megaterium* BTCC 18, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* CRL(ICDDR'B), *Escherichia coli* ATCC 25922 and *Vibrio cholerae* AE 14748 and three human pathogenic fungi viz., *Aspergillus niger, A. ochraceus, and A. ustus.* The test

organisms were collected from Department of Microbiology, University of Chittagong, Bangladesh.

Determination of antimicrobial activity

The *in vitro* antibacterial and antifungal activities of the crude extract of the plant were determined by disc diffusion method (Bauer *et al.* 1966) and poisoned food technique (Miah *et al.* 1990) respectively. Mueller-Hinton (agar and broth) medium was used for culture of bacteria and Sabouraud (agar and broth) medium was used for culture of fungi. Ten percent ethanolic solution of the crude extract was used as the test material. All the results were compared with the standard antibacterial antibiotic ampicillin [20µg/disc, BEXIMCO Pharma Bangladesh Ltd.] and antifungal antibiotic nystatin [100µg/ml medium, BEXIMCO Pharma Bangladesh Ltd.]. MICs of the crude extract were determined by macro-dilution broth technique (Jones *et al.* 1985).

RESULTS AND DISCUSSION

Different crude extracts obtained from *Chaetocarpus castanocarpus* were screened for their antibacterial activity against ten human pathogenic bacteria and compared to that of standard antibacterial antibiotic ampicillin. The results of the sensitivity test are presented in Table 1. The chloroform and ethanol extracts showed better antibacterial activity compared to that of other extracts. Petroleum ether and carbon tetrachloride extracts showed poor activity or no activity with 2000 µg/disc extract against all the tested bacterial strains. The chloroform and ethyl alcohol extracts exhibited zone of inhibitions from 8 to 16 mm in diameter and 8 to 15 mm in diameter respectively. The largest zone of inhibition (16 mm) was recorded against *Shigella dysenteriae* with the crude extract of chloroform. Incase of ethanol extract, the largest zone of inhibition (15 mm) was found against *Bacillus megaterium*. Antibacterial antibiotic ampicillin (20µg/disc) was also found to be active against all the bacteria tested herein. The MIC values of the crude extract obtained from *C. castanocarpus* are summarized in Table 2. From Table 2, it appeared that the chloroform and ethyl alcohol extracts exhibited the lowest MIC values. The range of MIC value of the chloroform and ethanol extracts was 750 µg/ml to 2000 µg/ml. The lowest MIC (750µg/ml) was recorded against *B. megaterium* and *V. cholerae* with chloroform extracts. Incase of ethanol extract, the lowest MIC value (750µg/ml) was found against *B. megaterium*. Similar antibacterial activities of other plant extracts has been reported previously (Brantner and Grein 1994, Perez and Anesin 1994, Sakanaka *et al*. 1989, Sohrab *et al*. 2001).

TABLE 1: ANTIBACTERIAL ACTIVITY OF CRUDE EXTRACTS OF *CHAETOCARPUS CASTANOCARPUS.*

Name of bacteria	Diameter of inhibition zone in mm. (Crude extract 2000µg/disc)				Ampicillin* 20µg/disc
	Petroleum ether	Carbon tetrachloride	Chloroform	Ethanol	
Bacillus cereus	08	10	11	10	18
B. megaterium	07	07	15	15	16
B. subtilis	-	-	08	09	19
Escherichia. coli	-	-	09	08	10
Pseudomonas aeruginosa	09	07	10	12	12
Salmonella paratyphi	-	07	12	13	17
S. typhi	-	08	10	11	20
Shigella dysenteriae	07	09	16	14	22
Staphylococcus aureus	08	-	12	10	22
Vibrio cholerae	07	07	13	14	15

*Standard antibacterial antibiotic; - mean no inhibition.

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TABLE 2: MICS OF CRUDE EXTRACTS OF *CHAETOCARPUS CASTANOCARPUS.*

Name of Bacteria	MIC (Crude extract µg/ml medium)			
	Petroleum. ether	Carbon tetrachloride	Chloroform	Ethyl alcohol
Bacillus cereus	3500	2500	1000	1500
B. megaterium	3500	3500	750	750
Bacillus subtilis	NF	NF	2000	2000
Escherichia coli	NF	NF	1500	2000
Pseudomonas aeruginosa	3000	3500	1500	1500
Salmonella paratyphi	NF	NF	1000	1000
S. typhi	NF	NF	1500	1500
Shigella dysenteriae	NF	NF	1000	1000
Staphylococcus aureus	NF	NF	1000	1000
Vibrio cholerae	NF	3500	750	1000

NF = not found up to 3500 µg/ml

The antifungal activity of the crude extract (100 µg/ml medium) against three pathogenic fungi were studied and compared to that of standard antifungal antibiotic nystatin. The results of the inhibition of fungal mycelial growth are summarized in Table 3. From Table 3, it appeared that all the crude extracts obtained from *C. castanocarpus* inhibited the mycelial growth of all the test fungi to varied degrees (21.5% – 42.5%). The chloroform and ethanol extracts showed prominent inhibition (more than 40 %) of radial mycelial growth against *A. ustus.* The highest inhibition (42.5%) of fungal mycelial growth was recorded against *A. ustus*. Antifungal antibiotic nystatin (100µg/ml medium) exhibited inhibitions from 36.0–42.0% of mycelial growth of the fungi.

TABLE 3: ANTIFUNGAL ACTIVITY OF CRUDE EXTRACTS OF *CHAETOCARPUS CASTANOCARPUS.*

Name of fungi	Percent inhibition of fungal mycelial growth[a] (100 µg/ml medium)				
	Petroleum ether	Carbon tetrachloride	Chloroform	Ethanol	Nystatin*
Aspergillus niger	21.5	22.5	30.0	32.5	36.0
A. ochraceus	24.85	25.5	30.0	27.0	37.5
A. ustus	34.38	39.0	42.5	41.0	42.0

* Standard antifungal antibiotic ; - mean no inhibition
[a]Growth measured- radial growth in cm.

MIC values of the crude extract against fungal pathogens are presented in Table 4. From the Table 4, it was found that the chloroform and ethanol extracts exhibited the lower MIC values compared to that of other extracts. The MIC values of the chloroform extract were 2000 µg/ml, 2500 µg/ml, and 2000 µg/ml against *Aspergillus niger, A. ochraceus,* and *A. ustus* respectively. Incase of ethanol extract, the MIC values were 2500 µg/ml, 2500 µg/ml and 2000 µg/ml against *Aspergillus niger, A. ochraceus* and *A. ustus* respectively. The crude chloroform extract exhibited the lowest MIC (2000 µg/ml) against *A. niger* and *A. ustus.* Incase of ethanol extract, the lowest MIC was found as 2000 µg/ml against *A. ustus*. Similar antifungal activities on plant extracts of other plants have also been previously reported by Naidu and John 1981, Renu 1983, Rojas *et al*. 1992, Ali Shtayeh *et al.* 1997 and Anwar *et al.* 1994.

TABLE 4: MICS OF CRUDE EXTRACTS OF *CHAETOCARPUS CASTANOCARPUS* AGAINST FUNGI.

Name of Fungi	MIC (Crude extract µg/ml medium)			
	Petroleum ether	Carbon tetrachloride	Chloroform	Ethyl alcohol
Aspergillus niger	3000	3000	2000	2500
A. ochraceus	3500	3000	2500	2500
A. ustus	2000	2500	2000	2000

The finding of the active compound may be interesting in the search for new efficacious and safe antimicrobial agent against a variety of pathogenic bacteria and fungi. The present investigation confirms that there are antibacterial and antifungal properties in the crude extract of *C. castanocarpus*. Now it is evident from above discussion that the crude extracts obtained from *C. castanocarpus* exhibited good antibacterial and antifungal activity against the organisms tested herein. So, the plant can be used as a novel antimicrobial agent.

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REFERENCES

AL SHAMMA, A., DRAKE, S. D., GUAGLIARD, L. F., MITSCHAR, L. A. AND SWAZZ, J. K. 1982. Antimicrobial alkaloids from *Boehmeria cylindrica*. *Phytochemistry*. **21**(2): 445-487.

ALI-SHTAYEH, M. S., AL-NURI, M. A., YAGHMOUR, R. M. R. AND FAIDI, Y. R. 1997. Antimicrobial activity of *Micromeria nervosa* from the Palestinian area. *J. Ethnopharmacol*. **58** : 143-147.

ANWAR, M. N., SINGHA, P.,BEGUM, J. AND CHOWDHURY, J. U. 1994. Antifungal activity of some selected plant extracts on phytopathogenic fungi. *Bangladesh J. Life Sci*. **6**(2)**:** 23-26.

BAUER, A. W., KIRBY, M. M., SHERRIS, J. C. AND TURCK, M. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Amer. J. Clin. Path.* **45**: 493-496.

BRANTNER, A. AND GREIN, E. 1994. Antibacterial activity of plant extracts used externally in traditional medicine. *J. Ethnopharmacol*. **44:**35-40.

JONES, N. R., BARRY, L. A., GAVAN, L. T. AND WASHINGTON, J. A. 1985. *Manual of Clinical Microbiology*, pp. 972-976, 4th Ed. American Society for Microbiology, Washington D. C.

MIAH, M. A. T., AHMED, H. U., SHARMA, N. R., ALI, A. AND MIAH, S. A. 1990. Antifungal activity of some plant extracts. *Bangladesh J. Bot.* **19**(1)**:** 5-10.

NAIDU, A. D. AND JOHN, V. T. 1981. *In vitro* inhibition of the rice fungal pathogens by extracts from higher plants. *Int. Rice Res. Newsl.* **6**(5)**:** 12-14.

PEREZ, C. AND ANESIN, C. 1994. *In vitro* antibacterial activity of Argentine folk medicinal plants against *Salmonella typhi*. *J. Ethnopharmacol*. **44**: 41-46.

RAHMAN, M. S., ANWAR, M. N. AND CHOWDHURY, A. Z. M. S. 1999. Antibacterial activity of secondary metabolites from *Holarrhena antidysenterica* stem bark. *Bangladesh J. Microbiol*. 1**6**(2) : 101-105.

RAHMAN, M. S., SULTANA, S., AND ANWAR, M. N**.** 2004. *In vitro* antimicrobial activity of halarrifine-24ol isolated from the stem bark of *Holarrhena antidysenterica. International J. of Agri. and Biol*. **6** (4) : 698-700.

RENU, R. O. 1983. Fungitoxicity of leaf extracts of some higher plants *against Rhizoctonia solani.* National Academy of Science, *Science letters*. **6**(8)**:** 245-246.

ROJAS, A., HERNANDEZ, L., PEREDA-MIRANDA, R., AND MATA, R. 1992. Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants. *J. Ethnopharmacol*. **35:**275-283.

SAKANAKA, S., KIM, M., TANIGUCHI, M. AND YAMAMOTO, T. 1989. Antibacterial substances in Japanese green tea extract against *Streptococcus mutans*, a cariogenic bacterium. *Agric. Biol. Chem*. **53:**2307-2311.

SOHRAB, M. H., MAZID, M. A., RAHMAN, E., HASAN, C. M. AND RASHID, M. A. 2001. Antibacterial activity of *Clausena heptaphyllla*. *Fitoterapia***. 72**: 547-549.

SOHRAB, M. H., MAZID, M. A., RAHMAN, E., HASAN, C. M. AND RASHID, M. A. 2001. Antibacterial activity of *Clousena heptaphylla*. *Fitoterapia*. **72:**547-549.

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