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In Vitro Antimicrobial Activity of Holarrifine–24ol Isolated from the Stem Bark of *Holarrhena antidysenterica*

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ABSTRACT

The alkaloid holarrifine–24ol isolated from the stem bark of *Holarrhena antidysenterica* (Wall.) was screened for its antibacterial and antifungal activity against ten pathogenic bacteria viz., *Bacillus subtilis, B. cereus, B. megaterium, Escherichia coli,* INAVA ET*(Vibrio), Shigella dysenteriae, S. sonnei, Salmonella typhi, Staphylococcus aureus, Pseudomonas mutabilis* and six phyto–pathogenic fungi viz., *Alternaria alternata, Botryodiplodia theobromae, Curvularia lunata, Fusarium equiseti, Macrophomina phaseolina, Colletotrichum corchori*. The alkaloid showed good antibacterial and antifungal activity against almost all the test organisms. The largest zone of inhibition (24 mm in diameter) was recorded of *S. dysenteriae* and *S. typhi* at a concentration of 250 µg alkaloid per disc. The alkaloid showed maximum inhibition (53.33%) of mycelial growth of *B. theobromae* at a concentration of 100 µg per mL media. Minimum inhibitory concentration (MIC) values of the alkaloid against all the test bacteria have been determined and the smallest MIC of the alkaloid holarrifine–24ol was 20 µg against *S. dysenteriae.*

Key Words: Antimicrobial activity; Holarrifine–24ol; Stem bark; *Holarrhena antidysenterica*

INTRODUCTION

Various plant species have been serving as the best natural source of drugs and medicines since the beginning of civilization. Most of the plant constituents, particularly the secondary metabolites possess potent antibacterial and antifungal activity. Among the different plant derived secondary metabolites, alkaloids proved to be the most important group of compounds that showed wide range of antimicrobial activity (Sarker *et al.*, 1991; Hossain *et al.*, 1993; Raman *et al*., 1997). So, there is a continuing need to search for new antimicrobial agents since none of the available drugs is free from adverse effects and limitation. Medicinal plants possess various remedial properties along with worthless materials and it is important to separate the worthless materials from the good ones. So intensive antimicrobial and phytochemical investigation is needed in this field. *Holarrhena antidysenterica* is a laticiferous deciduous shrub or small tree with white flower growing in the forests of Bangladesh. The stem bark of this plant, commercially known as kurchi, has been used traditionally for the treatment of dysentery (Bhutani *et al.*, 1984). In addition, the plant has been reported to be strongly antidysenteric, astringent, stomachic, antidiarrhoeal, anthelmintic and also febrifuge (Ghani, 1998). A lot of work has been done on different aspects of this plant and a number of alkaloids have been reported (Siddiqui *et al.*, 1993; Rahman *et al*., 1999). But no systematic work has so far been reported on the antimicrobial properties of stem bark of the plant. The objective of present work was to explore the antibacterial and antifungal potential of the stem bark of plant.

MATERIALS AND METHODS

Extraction and purification of plant material. Fresh stem bark of *Holarrhena antidysenterica* was collected from Chittagong University campus (Bangladesh), during the month of January. Barks were sun–dried and ground to fine powder. The powder was moistened with 10% aqueous ammonia and then macerated with ethyl acetate at room temperature for five days. The extract was filtered and concentrated under reduced pressure at 50^0C, and passed through the column chromatography for separation. For the separation of active fractions three solvent systems such as (a) chloroform: methanol = 2:1, (b) chloroform: methanol = 1:3 and (c) n–hexane: acetone = 7:3 were used and twelve separated fractions were collected in separate conical flask. All the collected individual fractions were tested against a highly sensitive bacterium (*Shigella dysenteriae*) for preliminary selection of active fraction. Among them, a highly antibacterial fraction (fraction No. 6) was finally selected for purification, characterization and antibacterial assay against the test microorganisms. The antimicrobial compound eluted fraction–6 was then dried under reduced pressure at 50^0C and the material was then washed with Petroleum ether several times. After drying the material was subjected to thin layer chromatographic (TLC) technique to confirm its purity.

Characterization of the active ingredient. The active

ingredient was characterized by various spectroscopic techniques like as IR, MNR, GCMS (Gas chromatography linked to Mass spectroscopy).

Test organisms. The pure compound was tested for its antimicrobial activity against ten human pathogenic bacteria such as *Shigella dysenteriae* AE 14396, *S. sonnei* CRL.(ICDDR,B), *Salmonella typhi* AE 14612, *S. paratyphi* (No. unknown) , *Bacillus subtilis* BTCC 17, *B. cereus* BTCC 19, *B. megaterium* BTCC 18, *Staphylococcus aureus* ATCC 6538, *Pseudomonas mutabilis* CRL(ICDDR,B), *Escherichia coli* ATCC 25922 and INABA ET *(Vibrio)* AE 14748 and six phytopathogenic fungi such as *Alternaria alternata* (Fr.) Kedissler, *Botryodiplodia theobromae* Pat., *Curvularia lunata* (Wakker) Boedijin, *Colletotrichum corcori* Ikata (Yoshida), *Fusarium equiseti* (Corda) Sacc. and *Macrophomina phaseolina* (Tassi) Goid.

Determination of antimicrobial activity. *In vitro* antibacterial activity of column separated fractions and pure compound of the plant was determined by disc diffusion method (Bauer *et. al*., 1966). Nutrient agar (NA) medium was used to culture bacteria. The test bacterial culture seeded NA plates with paper disc having active ingredient (250µg/disc) were kept at low temperature (4^0C) for 6 h for diffusion of test compounds from the disc to the surrounding medium. The plates were then incubated at 35 ± 2^0C for the growth of bacteria and the data were recorded after 24 h. The activity was expressed in terms of diameter of inhibition zone (mm). Each experiment was replicated thrice and control plates were also carried out with the respective solvent. All the results were compared with the standard antibiotic Ampicillin [20µg/disc, BEXIMCO Pharma. (Bangladesh)].

The *in vitro* antifungal activity of the pure alkaloid was done by the poisoned food technique (Miah *et al*., 1990). Potato dextrose agar (PDA) medium was used for the culture of fungi. Pure alkaloid (ethanolic solution) was mixed with sterilized melted PDA medium to have 100 µg (dry weight)/mL PDA and this was poured (about 20 mL/plate) in sterilized petridishes. In the center of each petridish 4 mm. in diameter agar block of 4 days old culture of the test fungi was inoculated and incubated at 25 ± 2^0C. A control set was maintained in each experiment using only PDA as growth medium. The diameter of each fungus was measured after 3–5 days of incubation. All the antifungal results were compared with the standard antifungal antibiotic Nystatin (100 µg/mL PDA). The percent inhibition of mycelial growth of the test fungus was calculated as follows:

$$I = \frac{C - T}{C} \times 100$$

Where,

I= Percent of inhibition.

C= Diameter of the fungal colony in the control petridish.

T= Diameter of the fungal colony in the treated petridish.

Determination of MIC. The MIC values of pure alkaloid against ten test bacteria were determined following the method described by Bauer *et al.* (1966) using different disc concentration.

RESULTS AND DISCUSSION

After spectroscopic analysis the compound was found closely related to the compound holarrifine which was previously reported by Siddiqui and Shamsuddin (1989).

The sensitivity of ten pathogenic bacteria to the alkaloid holarrifine–24ol isolated from the stem bark of *Holarrhena antidysenterica* was tested and compared to that of antibacterial antibiotic ampicillin. The results of the sensitivity test are presented in Table I.

It was found that the alkaloid (250 µg/disc) gave promising inhibitory activity against all the bacterial strain tested herein. The largest zone of inhibition (24mm in diameter) was recorded against *Salmonella typhi* and *Shigella dysenteriae*. Standard antibiotic Ampicillin (20µg/disc) showed activity against all the bacteria except *Pseudomonas mutabilis*. Similar antibacterial activity of alkaloid has been reported previously (Sokomba *et al*., 1986; Hossain *et al*., 1993; Rahman *et al*., 1997). The result of statistical analysis showed that both the sources of variation in our experiment is significant at 1% level, because the calculated values of F (183.5 & 6.95) are greater than the tabulated values.

The MIC values of the alkaloid were 80,160, 160, 80, 160, 20, 80, 40 g, 80, and 160 µg against *Bacillus subtilis*, *B. cereus*, *B. megaterium*, *E. coli*, INABAET*(Vibrio*), *Shigella dysenteriae*, *S. sonnei*, *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas mutabilis* respectively (Table II).

From the results it appears that the alkaloid holarrifine–24ol had broad spectrum antibacterial properties against almost all the test bacteria including ampicillin resistant *Pseudomonas mutabilis.*

The antifungal activity of the alkaloid holarrifine–24ol from *H. antidysenterica* stem bark was tested against six phytopathogenic fungi and compared to that of antifungal antibiotic Nystatin. The results of the inhibition of fungal mycelial growth are given in Table III.

The alkaloid [100 µg (dry weight)/mL of PDA medium] showed prominent inhibition against all the fungi tested herein. The alkaloid showed marked (more than 50%) inhibition of mycelial growth of *B. theobromae, C. corchori, C. lunata* and *A. alternata* while *F. equiseti* and *M. phaseolina* showed mild sensitivity towards the alkaloid. The maximum inhibition of mycelial growth (53.33%) was recorded against *B. theobromae*. Antifungal antibiotic Nystatin (100 µg/mL PDA) was also found to be active against all the six fungi but it was much less active against *C. corchori* compared to the alkaloid holarrifine–24ol.

In conclusion, the alkaloid holorrifine–24ol from the stem bark of *H. antidysenterica* has a wide spectrum of antibacterial and antifungal activity. So the plant can be

Table I. Antibacterial activity of the alkaloid holarrifine-24ol isolated from the stem bark of *Holarrhena antidysenterica* against ten pathogenic bacteria

Name of bacteria	Diameter of zone of inhibition in millimeter		% increase or decrease over ampicillin*
	Holarrifine-24ol (250 µg/disc)	Ampicillin* (20 µg/disc)	
Bacillus subtilis	18	19	(-)5.26
B. cereus	12	18	(-)33.33
B. megaterium	12	16	(-)25.00
E. coli	14	10	(+)40.00
INABA ET(Vibrio)	13	15	(-)20.00
Shigella dysenteriae	24	22	(+)9.00
S. sonnei	10	20	(-)50.00
Salmonella typhi	24	20	(+)20.00
Staphylococcus aureus	13	22	(-)40.90
Pseudomonas mutabilis	14	00	(+)100.00

Statistical analysis (RBD) at 1% level : organisms significant (F value 183.5) , replica significant (F value 6.95); Note: * Standard antibacterial antibiotic; (+)/(-) = increase/decrease

Table II. Minimum inhibitory concentration (MIC) values of the alkaloid holarrifine-24ol of *H. antidysenterica* against ten pathogenic bacteria

Name of bacteria	Diameter of inhibition zone in mm. (Alkaloid concentration µg/disc.)					
	320	160	80	40	20	10
Bacillus subtilis	20	12	07	00	00	00
B. cereus	14	09	00	00	00	00
B. megaterium	14	08	00	00	00	00
E. coli	17	12	08	00	00	00
INABA ET(Vibrio)	14	08	00	00	00	00
Shigella dysenteriae	26	18	14	10	06	00
S. sonnei	12	08	06	00	00	00
Salmonella typhi	25	17	12	08	00	00
Staphylococcus aureus	15	10	07	00	00	00
Pseudomonas mutabilis	14	08	00	00	00	00

Table III. Antifungal activity of the alkaloid holarrifine-24ol of *H. antidysenterica* stems bark

Name of fungi	Percentage inhibition of fungal mycelial growth		
	Holarrifine-24ol (100 µg/ml PDA)	Nystatin* (100 µg/ml PDA)	Increase or decrease over nystatin*
Alternaria alternata	50.50	51.55	(-)01.05
Botryodiplodia theobromae	53.33	70.05	(-)16.72
Curvularia lunata	52.50	75.00	(-)22.50
Colletotrichum corchori	52.33	40.51	(+)11.82
Fusarium equiseti	37.50	44.70	(-)07.20
Macrophomina phaseolina	38.50	71.78	(-)33.28

Note: (+)/(-)=increase/decrease; Growth measured- radial growth in cm; * Standard antifungal antibiotic

taken as a potential source for developing new and better antimicrobial agent(s) against a number of pathogenic organisms.

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