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In vitro Antimicrobial Activity of the Compound Isolated from Chloroform Extract of *Moringa oleifera* Lam.

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Abstract: The research work was conducted to investigate the *in vitro* antimicrobial activity of the compound Aglycon of Deoxy-Niazimicine (N-benzyl,S-ethyl thioformate) isolated from *Moringa oleifera* Lam. in comparison with crude chloroform extract. Antibacterial and antifungal activities of the compound-1 and crude extract were observed against fourteen pathogenic bacteria and six pathogenic fungi. Compound-1 isolated from the chloroform ($CHCl_3$) fraction of ethanol extract showed comparatively more *in vitro* antibacterial activity against *Shigella boydii, Shigella dysenteriae* and *Staphylococcus aureus* than the crude chloroform ($CHCl_3$) extract and produced zone of inhibition in between 09 and 13 mm. The MIC values of the compound-1 and crude extract were also determined against *B. megaterium, St. aureus,, Sh. dysenteriae and Sh. boydii*. The values were found to be between 32 to 128 μg ml^{-1}. The compound-1 also showed moderate antifungal activity against *Candida albicans* and *Aspergillus flavus* than the crude chloroform extract although the crude extract showed moderate activity against *Trichoderma species*. Thus compound-1 has antibacterial and antifungal activity and suggested its therapeutic use as an antimicrobial agent.

Key words: *Moringa oleifera*, moringaceae, *a*ntibacterial and antifungal activity

INTRODUCTION

The plant *Moringa oleifera* Lam. locally known as shajna (English-drumstick) belongs to the family Moringaceae and is widely distributed in the Indo-Bangla subcontinent and cultivated through out the tropical belt (Sastri, 1962). Different parts of this plant are used in the indigenous systems of medicine for the treatment of a variety of human ailments and are also eaten as vegetable (Sastri, 1962; Chopra *et al.,* 1956; Nadkarni and Nadkharni, 1976).

The targeted plant *M. oleifera* Lam. has many uses in traditional medicines. For example, root-barks are used as astringent, anthelmintic, analgesic, useful in heart complains, eye diseases, dyspepsia, enlargement of the spleen, tuberculous glands in the neck, tumors, earache, stuttering and in ulcers. The plant is also considered to be useful by tribals (Santals) in burns, sores, epilepsy, scabies, cholera, dysentery and retention of urine (Kirtikar and Basu, 1984).

The pharmacological activities of *M. oleifera* have extensively been studied, particularly the hypotensive action of the ethanolic extract of leaves (Dashputra *et al.,* 1977), the antimicrobial activity of the seeds (Eilert *et al.*, 1981; Caceres *et al.*, 1991), the antifertility activity of roots (Prakash, 1988; Shukla *et al.*, 1988) and the spasmolytic, anti-inflammatory and diuretic properties of the leaves, fruits, barks and roots (Caceres *et al.*, 1992). The present investigation reports on the isolation of Aglycone of Deoxy-Niazimicine (N-benzyl, S-ethyl thiocarbamate) from the chloroform fraction of ethanol extract of the root barks of *M. oleifera* Lam. and discuss about the antibacterial and antifungal activity of the compounds.

MATERIALS AND METHODS

The plant material: Fresh root barks of *Moringa oleifera* Lam were collected from the adjoining areas of Rajshahi University campus, Bangladesh in March, 2001 and identified by Professor A.T.M. Nadiruzzaman, Department of Botany, University of Rajshahi and a vaucher specimen (no. A Islam 3, collection date 02.05.1982) is deposited in the Department of Botany, Rajshahi University.

Extraction and isolation of compounds: The root barks were cut into small pieces, dried in the sun for 7 days and finally in an oven below 60°C. The dried plant material (1.5 kg) was ground into fine powder and exhaustively extracted with ethanol. The extract was concentrated to a dark brownish syrupy residue (50g) and then successively extracted with pet-ether (40-60°C) and chloroform. The organic layers were washed with water, dried over Na_2SO_4 and concentrated in *vaccuo* to afford a

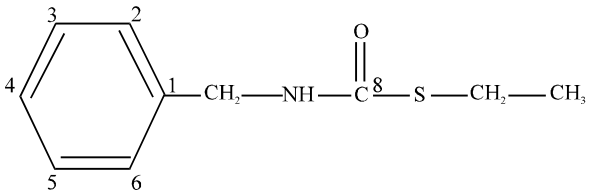

Fig. 1: Aglycon of Deoxy-Niazimicine (N-benzyl, S-ethyl thioformate)

pet-ether soluble fraction (2g) and chloroform soluble fraction (6.9g). The chloroform soluble fraction (6.9g) was subjected to a column of silica gel-60 and eluted with ethyl acetate and n-hexane with increasing portions of ethyl acetate and finally with ethyl acetate, yielding several fractions. The fractions obtained with n-hexane-ethyl acetate (50:50) was rechromatographed eluting with n-hexane-$CHCl_3$ with increasing portions of chloroform and finally with methanol. Fractions obtained with n-hexane-$CHCl_3$ 75:25 showed similar TLC profiles and were pooled. This fraction showed positive test for secondary amine and carbonyl function. Crystallization from pet-ether (40-60°C) affords a new compound-1 (31.4 mg) as color less needles (m.p. 58-60°C). Structure of this compound-1 was confirmed by analysis of its IR, ^1H and ^{13}C-NMR spectral data. (Fig. 1). The isolated compound-1 and the crude chloroform extract were subjected to antibacterial and antifungal screening.

Antimicrobial screening: *In vitro* antimicrobial screening is generally performed by disc diffusion method (Vander and Vlietnck, 1999) for primary selection of the compounds as therapeutic agent. Disc diffusion method is highly effective for rapidly growing microorganisms and the activities of the test drugs are expressed by measuring the diameter of the zone of inhibition. The standard test microorganisms were available in the microbiological research laboratory of Biochemistry and Molecular Biology Department, University of Rajshahi, Bangladesh. The pure cultures of these were collected from the Microbiological Laboratory of the Institute of Nutrition and Food Science (INFS) and Department of Microbiology, University of Dhaka, Bangladesh. For antibacterial activity Fourteen pathogenic bacteria (Five Gram positive and Nine Gram negative) were selected. The compound-1 and crude extracts were dissolved separately in CH_3OH to get a concentration of 30 μg disc^{-1} and 100μg disc^{-1}, respectively. The diameter of zone of inhibition produced by both crude extract and compound-1 were then compared by the standard antibiotic Kanamycin 30 μg disc^{-1}.

Minimum inhibitory concentration (MIC): The MIC values of the compounds were determined against three Gram positive and three Gram negative bacteria. The test was carried out by a serial tube dilution technique (Reiner, 1982). Nutrient agar was used as bacteriological media.

Antifungal activity : The antifungal activities of the compound-1 and crude extract were determined at a concentration of 50 μg disc^{-1} and 100μg disc^{-1} against six pathogenic fungi. Nystatin (50 μg disc^{-1}) was used as standard.

RESULTS AND DISCUSSION

Compound -1 isolated from the chloroform fraction of ethanol extract from the root barks of the plants *Moringa oleifera* Lam., as colorless needles, melting point 58-60°C from pet-ether.

The antibacterial activity of the isolated compound-1 and crude extract against all tested organisms is shown in Table 1. Compound-1 showed strong antibacterial activity against gram positive *Staphylococcus aureus* and gram negative *Shigella dysenteriae, Shigella boydii,Salmonella typhi* and *Pseudomonas aeruginosa* and produced zone of inhibition between 09 to 13 mm. While the crude extract showed comparatively

Table 1: *In vitro* antibacterial activity of compound-1 and crude extract Zone of inhibition (Diameter in mm)

	Compound-1		Crude extract		
Name of bacterial strains	30μg disc^{-1}	100μg disc^{-1}	30μg disc^{-1}	100μg disc^{-1}	Kanamycin 30μg disc^{-1} Standard
Gram positive					
Bacillus subtilis	00	08	00	08	20
Bacillus megaterium	08	12	08	10	18
Sarcina lutea	00	09	00	09	20
Staphylococcus aureus	10	14	09	13	30
Staphylococcus β- hæmoliticus	00	10	00	10	24
Gram negative					
Escherichia coli	00	10	00	09	21
Klebsiella species	08	11	00	10	17
Pseudomonas æruginosa	09	12	00	11	25
Shigella dysenteriae	13	16	10	14	20
Shigella flexneri	00	09	08	14	22
Shigella shiga	00	10	09	14	25
Shigella sonnei	00	09	00	00	19
Shigella boydii	12	15	11	14	30
Salmonella typhi	10	13	00	08	20

Table 2: The MIC values of the compound-1 and crude extract against test organisms Minimum inhibitory concentration in μg ml^{-1}

Sample	*Shigella dysenteriae*	*Shigella boydii*	*Bacillus megaterium*	*Staphylococcus aureus*
Compound-1	32	32	64	32
Crude Extract	64	-	128	-

Table 3: *In vitro* antifungal activity of the compound-1, crude extract and standard Nystatin. Zone of inhibition (diameter in mm.)

Name of fungas	Compound-1 50μg disc^{-1}	Compound-1 100μg disc^{-1}	Crude extract 50μg disc^{-1}	Crude extract 100μg disc^{-1}	Nystatin 50μg disc^{-1}
Candida albicans	10	12	00	00	20
Aspergillus niger	00	10	00	00	22
Aspergillus flavus	10	13	09	14	19
Aspergillus fumigatus	09	11	00	00	22
Trichoderma species	00	09	12	15	25
Fusarium speciess	08	10	00	00	25

lower activity against gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Shigella dysenteriae, Shigella boydii* and produced zone of inhibition between 09 to 11mm. The MIC values of the isolated compound-1 and crude extract were determined against *B. megaterium*, *St. aureus*, *Sh. dysenteriae* and *Sh.boydii* shown in Table 2. Compound-1 showed moderate activity against *Shigella dysenteriae, Shigella boydii, Staphylococcus aureus* and MIC values were 32 μg ml^{-1}. On the other hand, crude extract showed little activity against *Shigella dysenteriae* and the MIC value was 64 μg ml^{-1}.

The antifungal activity of the compound-1 and crude extract were tested against six pathogenic fungi and the obtained result are cited in Table 3. Compound-1 showed moderate activity against *Candida albicans* and *Aspergillus flavus* in comparison with the standard Nystatin. But the crude extract showed only little activity against *Trichoderma species.*

Rao and George (1949) reported that the oil extracted from the root of *Moringa oleifera* showed pronounced antibacterial activity. Kurup and Rao (1952) also found the antibacterial activity of the alcoholic extract of the root of *Moringa pterygosperma* against *Micrococcus pyogenes* (*aureus*). Moreover, Sen Gupta *et al.* (1956) reported the root bark of the plant *Moringa pterygosperma* has highly bactericidal effect against *Vibrio cholerae*.

In conclusion, this investigation reports that compound-1 isolated from the chloroform extract possesses antibacterial and antifungal activity and could be used in medicine after toxicity test.

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