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ANTI BACTERIAL ACTIVITY OF ALLIUMONOATE, A NEW CYCLOPENTANE DERIVATIVE ISOLATED FROM *ALLIUM VICTORIALIS* L.

Sadia Khan[1], Mehdi Hassan Kazmi[1], Darshna Kumari[2] and Shakeel A. Khan[2]

[1]*Department of Applied Chemistry and Chemical Technology, University of Karachi, Karachi-75270, Pakistan*
[2]*Department of Microbiology, University of Karachi, Karachi-75270, Pakistan*

ABSTRACT

Alliumonoate, a new cyclopentane derivative, was isolated from the chloroform soluble fraction of *Allium victorialis* L. Spectroscopic techniques were used to determine the structure of the compound. Antimicrobial activity tests were performed for the pure and crude sample extract, which showed some retardation activity against bacteria.

Key-words: Alliumonoate, *Allium victorialis* L., antibacterial activity, cyclopentane.

INTRODUCTION

Plants of genus *Allium* are famous for the production of sulfur containing biological active natural compounds (Kang *et al.,* 2007; Inoue *et al.,* 1995). In this genus garlic (*A. sativum*) and onion (*A. Cepa*) are among the oldest cultivated indigenous herb with their origin dating back to ancient Egypt and used for the curing of opthalmia, earache, jaundice, dysentery and cholera (Krishna, 2003). In fact *Allium* species are a rich source of phyto nutrients useful for the treatment and prevention of number of disease, including cancer, coronary heart disease, obesity, hypertension and disturbance of gastrointestinal tracts (e.g. Colic pain, Dyspepsia) (Barile *et al.,* 2004).

One of the species of the genus *Allium* 'A. *Victorialis L'* is a small shrub with pink flowers commonly found in moist and shady places in the temperate Himalayas from Kashmir eastward to Sikkim. The young leaves and bulbs of A. Victorialis L are used as pot herb. Medicinally it is used as antithrombotic, anti Scorbutic and carminative in western Garhwali and to treat profuse menstruation and cold (Krishna, 2003; Nasir *et al.,* 1974; Perry and Metzger, 1980; Nishimura *et al.,* 1988). The chemotaxonomic and ethanopharmacological importance of the genus *Allium* impelled us to carry out phytochemical studies on *A. Victorialis*. We report here a new Cyclopentane derivative named as Alliumonoate, which has restrained activity against various bacteria.

MATERIALS AND METHODS

The whole plant material of *Allium victorialis* L. was collected from Northern areas of Pakistan in 2004 and identified by Dr. Surriya Khatoon, Plant Taxonomist, Department of Botany, University of Karachi, Karachi, Pakistan, where a voucher specimen has been deposited in the herbarium (Voucher specimen No. 202/KUH). The freshly collected whole plant material of *A. victorialis* (20 kg) was shade dried, ground and extracted with ethanol (3 X 40 L). The combined ethanolic extract was evaporated under reduced pressure to yield a residue (800 g) which was suspended in water (1.0 L) and successively extracted with *n*-hexane (80 g), $CHCl_3$ (170 g), ethyl acetate (220 g), and *n*-butanol (150 g) soluble fractions. The $CHCl_3$ soluble fraction (80 g) was subjected to column chromatography over silica gel and eluted with *n*-hexane, *n*-hexane-$CHCl_3$, $CHCl_3$, and $CHCl_3$-MeOH in increasing order of polarity to obtain 20 sub-fractions. The sub-fraction which was obtained with *n*-hexane-$CHCl_3$ (4.0:6.0) (35 mg) was re-chromatographed over silica gel and eluted with *n*-hexane-$CHCl_3$ (4.5:5.5) to afford unknown compound Alliumonoate.

Microbial cultures and maintenance

During the present work four fungal cultures i.e. *Aspergillus niger*, *Curvularia* sp., *M. gypseum*, *Penicillium* sp. and nine bacterial cultures, namely, *Bacillus cereus*, *Bacillus subtilis*, *Corynebacterium diphtheria*, *Micrococcu luteus* (local isolate), *Micrococcus luteus* (Typed strain), *Staphylococcu aureus*, *Streptococcus faecalis*, *Shigella dysenteriae* and *Escherichia coli* were used. Fungal cultures were routinely inoculated on Suboraud's dextrose agar (SDA) slants and incubated at room temp for 5 days and maintained in a refrigerator, until needed. Bacterial cultures were maintained as glycerol stocks in a freezer and working cultures were grown on nutrient agar slants at 37°C and maintained in a refrigerator.

Determination of antimicrobial activity

Antimicrobial activity of compounds was screened on SDA against fungi and on nutrient agar against bacteria disc diffusion method (Bauer *et al*., 1966).

Preparation of discs:

Sterile filter paper discs (6mm) containing test compounds (200 µg/disc) were prepared by applying 10 µl of stock solution (20 mg/ml).

Screening for antibacterial activity

Sterile Petri plates were poured with nutrient agar and overnight grown bacterial culture evenly spread on nutrient agar, air dried and filter paper discs loaded with compounds were placed at different positions on the plate. Plates were then incubated at 37ºC for 18-24 hours and zone of inhibition around each disc was measured.

Screening for antifungal activity

Antifungal activity was determined by pour plate method (Tabata *et al*., 1982). Briefly, a small amount of 5-6 days old fungal culture was transferred to a screw capped tube containing 5 ml of sterile saline a few glass beads. Tubes were vortexed to obtain a homogenous suspension, 0.5 ml of fungal suspension was transferred to a tube containing 18-20 ml of molten SDA, maintained at 45ºC. After thorough mixing, the contents were poured into a sterile Petri plates. After solidification of SDA, discs soaked in compound was placed at different positions and plates incubated at room temperature for about 2-3 days. Results were recorded as zone of inhibition.

Table 1. Antimicrobial results for compound expressed as zone of inhibition in mm.

Microorganisms	Compound **1**
Bacteria	
Gram positive	
Corynebacterium diphtheria	8
Micrococcu luteus	9
M. luteus ATCC	9
Staphylococcu aureus	7
Bacillus cereus	8
B. subtilis	-
Streptococcus faecalis	9
Gram negative	
Shigella dysenteriae	9
Escherichia coli	9
Fungi	
M. gypseum	-
Aspergillus niger	-
Penicillium sp.	-
Curvularia sp.	-

RESULTS AND DISCUSSION

The ethanolic extract of the whole plant was divided into n-hexane, chloroform, ethyl acetate, n-butanol and water soluble fractions. Column chromatography of the chloroform soluble fraction gives an unknown compound Alliumonoate (Kang *et al.,* 2007).

The antibacterial activity was determined by using disc diffusion method and fungal activity was determined by using pour plate method. The results indicated that compound 1 was active against *M. luteus*, *M. luteus ATCC, S.*

fecalis, *S. dysenteriae*, *E. coli* with zone of inhibition of 9mm while *C. diphtheria*, *B. cereus* showed 8mm zone of inhibition. No activity was, however, noted against any of the test fungi (Table 1).

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