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Antibacterial and Cytotoxic Activities of *Hedychium coronarium* J. Koenig

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Abstract: In this study, *In vitro* antibacterial activity of methanol, ethyl acetate and dichloromethane extracts prepared successively from rhizomes of *Hedychium coronarium* J. Koenig, was carried out. Methanol and dichloromethane extracts exhibited antibacterial activity against both Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium* and *Sarcina lutea*) and Gram negative (*Escherichia coli*, *Shigella sonnei*, *Shigella shiga*, *Pseudomonas aeruginosa* and *Salmonella typhi*) bacteria. Dichloromethane extract showed comparatively better activity than the other two extracts. The minimum inhibitory concentrations (MICs) of the extracts were found to be in the range of 08 ~ 128 µg/ml. The cytotoxicity (LC_{50}) against brine shrimp nauplii (*Artemia salina*) were also evaluated and found to be 34.85 µg/ml for methanol extract, 62.59 µg/ml for ethyl acetate extract and 55.59 µg/ml for dichloromethane extract.

Key words: *Hedychium coronarium*, Rhizomes, Antibacterial activity

INTRODUCTION

For the last few years many natural antimicrobial products have been isolated from a wide range of animal, plant and bacterial species. Natural product have therapeutic potential in the treatment of infections in humans and are now considered to be an alternative way for and future therapy[1,2]. Hence, the plant kingdom is being screened for newer and effective chemotherapeutic agents. Higher plants can serve both as potential antimicrobial crude drugs as well as a source of new anti-infective agents[3].

Hedychium coronarium J. Koenig (Family: Zingiberaceae) is a perennial plant and widely cultivated in Japan, India, South China and South Asian countries including Bangladesh[4]. Its rhizomes have been used for the treatment of headache, contusion inflammation and sharp pain due to rheumatism[5]. Various cytotoxic diterpenes, farnesane-type sesquiterpenes and labdane-type diterpenes were isolated and characterized from the rhizomes of *Hedychium coronarium*[6, 7, 8, 9 & 10]. Anti-inflammatory, analgesic, antihypertensive, diuretic, leishmanicidal and antimalarial activities of rhizomes of this plant, have also been reported by several researchers [11, 12, 13 & 14]. In present study, we report the antibacterial and cytotoxic effects of methanol, ethyl acetate and dichloromethane extracts prepared successively from rhizomes of *Hedychium coronarium*.

MATERIAL AND METHODS

Plant Source and Identification: Fresh rhizomes of *Hedychium coronarium* were collected from Bangladesh National Herbarium and taxonomically indentified by Professor A. T. M. Naderuzzaman, Department of Botany, Rajshahi University, Rajshahi, Bangladesh. A voucher specimen was deposited under the accession number DACB-34334 at the Bangladesh National Herbarium.

Extraction: The collected rhizomes were cleaned and shade-dried. The dried rhizomes were then pulverized into a coarse powder by a grinding machine (FFC-15, China). The powdered rhizome (250.0 gm) was extracted successively with methanol, ethyl acetate and dichloromethane at room temperature to yield 5.4 g methanol, 3.9 g ethyl acetate and 2.5 g dichloromethane extracts. All extracts were run on pre-coated silica gel plate using n-hexane and ethyl acetate (9:1) as the mobile phase and vanillin-H_2SO_4 reagent was used as spray reagent. All the extracts gave positive test for terpenoids[15].

Microorganisms: Four Gram positive (*Staphylococcus aureus* ATCC25923, *Bacillus subtilis* QL40, *Bacillus megaterium* QL38 and *Sarcina lutea* QL166), five Gram negative (*Escherichia coli* ATCC27853, *Shigella sonnei* C182, *Shigella shiga* C180, *Pseudomonas*

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aeruginosa ATCC14228 and *Salmonella typhi* ATCC20889) pathogenic bacterial strains were collected from the Institute of Biological Science (IBS), University of Rajshahi, Bangladesh.

Antibacterial and Antifungal Study: The Methanol, ethyl acetate and dichloromethane extracts were tested separately for antibacterial activity by disc diffusion assay technique[16]. *Kanamycin* disc (30 µg/disc) was used as positive antibacterial control. Blank disc impregnated with the respective solvent was used as negative control. The antibacterial activity of each sample was tested against each bacterium at concentrations of 30 µg/disc, 60 µg/disc and 90 µg/disc. Antibacterial assay plates were incubated at 37±1°C for 24h and each experiment was carried out in triplicates. Diameter of the zone of inhibition surrounding each disc was recorded. The minimum inhibitory concentration (MIC) for the samples having antimicrobial activity, were also determined by serial dilution technique[17].

Brine Shrimp Lethality Bioassay: The experiment was carried out using the method described by Meyer[18]. In brief, *Artemia salina* Leach (brine shrimp eggs) was allowed to hatch and mature as nauplii (Larvae) in seawater for 48h at 25°C. Serially diluted test solutions (80 µl in DMSO from a stock solution of 5 mg/ml DMSO) were added to the seawater (5 ml), containing 10 nauplii. After incubation for 24h at 25°C, the number of survivors was counted. The LC_{50} (50% lethal concentration, µg/ml) was determined from triplicate experiments using Probit analysis as described by Finney[19]. Ampicillin trihydrate was used as positive control.

RESULTS AND DISCUSSIONS

The results of the antibacterial activity of methanol, ethyl acetate and dichloromethane extracts assessed by disc diffusion technique are shown in table 1. All the three extracts showed antibacterial activity against most of the tested bacteria but the dichloromethane extract exhibited comparatively better activity than the other two extracts. The dichloromethane extract of *Hedychium coronarium* rhizomes showed potent antibacterial activity against both Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus megaterium* and *Sarcina lutea*) and Gram negative (*Escherichia coli*, *Shigella sonnei*, *Shigella shiga*, *Pseudomonas aeruginosa* and *Salmonella typhi*) bacteria, with the inhibition zones in the range of 12-31 mm (Table 1). All the tested bacteria were also found to be very sensitive to methanol extract and this extract produced inhibition zone ranging from 11 to 23 mm (Table 1). Ethyl acetate extract showed mild to moderate activity against *Staphylococcus aureus*, *Sarcina lutea*, *Shigella sonnei*, *Shigella shiga*, *Pseudomonas aeruginosa* and *Salmonella typhi* and produced inhibition zone ranging from 06 to 18 mm (Table 1). Ethyl acetate extract was inactive against *Bacillus subtilis* and *Bacillus megaterium*. The data for *Kanamycin* (30 µg/disc) were also included in table 1 for comparison.

Minimum inhibitory concentration (MIC) value for methanol, ethyl acetate and dichloromethane extracts were also determined against those bacteria that were found to be sensitive to the extracts in the disc diffusion method. The methanol extract had the lowest MIC (16 µg/ml) against *Sarcina lutea* and *Shigella sonnei* while the lowest MIC value for ethyl acetate extract was found to be 16 µg/ml against *Shigella shiga* (Table 2). The lowest MIC value (8 µg/ml) was observed for dichloromethane extract against *Bacillus subtilis* (Table 2). MIC values were roughly in agreement with the results of disc diffusion, thereby confirming differential antibacterial effects of the three extracts. The pattern of above results indicated that dichloromethane extract had antibacterial constituents at high concentrations but the concentration of active constituents in methanol and ethyl acetate extracts become low. So dichloromethane solvent can be employed for extraction of potent antimicrobial constituents. The similar type of results has been reported by Hayet *et al.*[20] when they evaluated antimicrobial activity of *Salvia sclarea*.

Methanol, ethyl acetate and dichloromethane extracts showed toxicity against brine shrimp nauplii (*Artemia salina*) (Table 3). Among the samples, methanol extract showed the highest toxicity and LC_{50} value was 34.85 (µg/ml). Ethyl acetate (62.59 µg/ml) and dichloromethane (55.59 µg/ml) extracts exhibited poor activity in comparison with ampicillin trihydrate (11.38 µg/ml).

In literature, it has been found that the presence of diterpenes and sesquiterpenes in crude extract plays an important role for producing antibacterial activity[21 & 22]. Since the rhizomes of H*edychium coronarium* contained diterpenes and sesquiterpenes[7 & 10], the antibacterial activity of methanol, ethyl acetate and dichloromethane extracts may be due to the presence of these compounds. Our future plan will be designed to isolate and characterize these active phytochemicals and determine their activities against microorganisms.

Table 1: *In vitro* antibacterial activity of the rhizomes of *Hedychium coronarium*.

Microorganisms	Zone of Inhibition (diameter in mm)									
	Methanol extract			Ethyl acetate extract			Dichloromethane extract			*Kanamycin*
	Dose (µg/disc)									
	30	60	90	30	60	90	30	60	90	30
Staphylococcus aureus	11	14	18	07	07	09	12	20	23	33
Bacillus subtilis	15	17	18	R	R	R	29	30	31	32
Bacillus megaterium	13	15	18	R	R	R	14	19	22	28
Sarcina lutea	17	19	22	14	15	17	13	14	15	25
Escherchia coli	13	15	17	14	16	17	18	20	23	26
Shigella sonnei	18	19	23	06	08	10	15	18	20	27
Shigella shiga	14	18	20	15	16	18	13	16	18	28
Pseudomonas aeruginosa	14	17	19	06	11	13	18	20	22	32
Salmonella typhi	16	18	20	11	14	17	13	15	18	29

R = Resistance

Table 2: Minimum inhibitory concentrations (MICs) of the extracts of *Hedychium coronarium* rhizomes.

Microorganisms	Methanol extract (µg/ml)	Ethyl acetate extract (µg/ml)	Dichloromethane extract (µg/ml)
Staphylococcus aureus	64	128	16
Bacillus subtilis	32	-	8
Bacillus megaterium	32	-	32
Sarcina lutea	16	32	64
Escherchia coli	64	32	16
Shigella sonnei	16	128	32
Shigella shiga	32	16	64
Pseudomonas aeruginosa	32	128	32
Salmonella typhi	32	32	64

Table 3: Cytotoxicity of the rhizomes of *Hedychium coronarium* against brine shrimp nauplii.

Sample	LC_{50} (µg/ml)
Amphicillin trihydrate	11.38
Methanol extract	34.85
Ethyl acetate extract	62.59
Dichloromethane extract	55.59

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