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Antimicrobial and Cytotoxic Activity from *Lasia spinosa* and Isolated Lignan

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SUMMARY. Studies were carried out on the rhizome of *Lasia spinosa* (L.). The petroleum ether, ethyl acetate and the methanolic extract revealed moderate activities against *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Candida albicans, Aspergillus niger* and *Vibrio parahemolyticus* test organisms. The crude extracts and purified compound, meridinol (**1**) were screened for antimicrobial activity against a wide range of gram-positive and gram-negative bacteria and fungi by the disc diffusion method. The cytotoxic potential of the extractives and meridinol was also determined by using brine shrimp lethality bioassay, where the extractives demonstrated significant cytotoxic activities. The petroleum ether, ethyl acetate, methanol extracts, one column fraction and compound 1 demonstrated LC_{90} of 11.22 µg/ml, 12.3 µg/ml, 13.49 µg/ml, 11.57 µg/ml and 15.85 µg/ml, respectively.

INTRODUCTION

The interest in medicinal plants has been increased during the last decades as they are potential sources of therapeutic substances. In particular, the emergence of microbial resistance to the available antibiotics [1-3] and the unexpected side effects of synthetic compounds have increased the need for new substances with antimicrobial properties. Studies of the antimicrobial activity of plant extracts and phytochemicals may lead to the discovery of new antibiotics useful to treat infectious diseases caused by resistant microorganisms.

On the other hand, hundred of plants are known to be used in the treatment of cancer and tumor in different parts of the world. According to Fransworth [4] more than 150 plant species could be added to a folklore list of cancer and tumor substitutes. Ricin, a toxin produced by the beans of *Ricinus communis,* has been found to effectively couple to tumor targeted monoclonal antibodies and has proved to be a very potent antitumor drug [5,6]. Taxol, the world's first billion-dollar anticancer drug, is found in each of the world's yew *(Taxus)* species, but was originally isolated from *Taxus brevifolia* [7-9] .

Lasia spinosa (L.) Thw. syn. *Lasia aculeata* Lour. (Araceae, subfamily Lasioideae) is a herb with an underground rhizome, native in Bangladesh is commonly known as ''Kanta kachu'' and used in traditional medicine as an antirheumatic and anti-inflammatory remedy [10,11].

The Araceae family includes many plants distributed in the tropic and subtropic regions. Many species are used as traditional remedies or food. Previous work on flavonoid content in the family showed that the Araceae have a simple profile with flavone C-glycosides, flavonols, flavones, and proanthocyanidins as the main classes [12]. Previous Phytochemical and Biological investigation on the whole plant of *Lasia spinosa* (L.) Thw. anticestodal efficacy [13], oxidase inhibitory activity [14] and revealed the occurrences of a number of lyoniresinol, vitexin

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2''-O-b-D-glucopyranoside, *p*-hydroxy benzoic acid, 4-methoxyphenethyl alcohol, *p*-hydroxy benzaldehyde, isovanillic acid, 3'-methyl quercetin-3-O-α-L-rhamnopyranosyl-(1/6)β-D-glucopyranoside, vitexin, meridinol [15] and triglochinin [16], while β-sitosterol acetate, stigmasterol, and its acetate were isolated from the rhizome of the plant [17]. So the main object of this study was to investigate the antimicrobial and cytotoxic activity of plant extracts.

MATERIAL AND METHODS

General experimental procedures

^{1}H and ^{13}C NMR spectra were obtained using the Ultra Shield Bruker DPX 400 NMR instrument, and the chemical shifts are reported in ppm with respect to TMS or residual non deuterated solvent signals.

Plant material

The rhizomes of *Lasia spinosa* were collected from Manikgonj in Bangladesh, April 2006. The plant was identified by Sardar Nasir Uddin, Scientific officer, Bangladesh National Herbarium, Dhaka, where a voucher specimen has been deposited (DACB Accession no. 31,579). The rhizomes were first sun dried and then ground into a coarse powder using a grinding machine.

Extraction and isolation

The air-dried and powdered plant material (530 g) was separately extracted to exhaustion in a Soxhlet apparatus with petroleum ether, ethyl acetate and finally with methanol (MeOH). The individual extractive was filtered through fresh cotton bed and finally with Whatman no.1 filter paper. The filtrates were concentrated with a rotary evaporator at low temperature (40-50 °C) and reduced pressure to provide petroleum ether (2.25 g), ethyl acetate (2.0 g) and methanol (3.0 g) soluble materials.

The ethyl acetate soluble partitionate was fractionated by column chromatography over silicagel (Kiesegel 60, mesh 70-230) using petroleum ether-dichloromethane and dichloromethane-MeOH mixtures of increasing polarity to afford a total of 23 fractions. The eluates were combined together on the basis of TLC analysis. Concentration of the column fractions eluted with 0.5-5 % MeOH in dichloromethane was subjected to column chromatography for further fractionation, using petroleum ether-EtOAc and EtOAc-MeOH mixtures of increasing polarity to afford a total of 18 fractions. Preparative TLC (toluene-EtOAc-AcOH = 90:10:1) of the column fraction eluted with 15-25 % EtOAc in petroleum ether yielded the compound **1** (12.0 mg).

Antimicrobial assay

The disc diffusion method [18] was used to test antimicrobial activity against thirteen bacteria and three fungi (Table 1). The bacterial strains

Test bacteria and fungi	PE	EtOAc	MeOH	Column fraction	Compound 1	Kanamycin
Bacteria						
Bacillus cereus	15	12	16	12	7	39
Bacillus megaterium	12	13	12	9	8	32
Bacillus subtilis	12	10	9	14	7	20
Staphylococcus aureus	16	15	18	13	–	22
Micrococus luteus	–	10	8	–	8	20
Escherichia coli	17	8	16	8	7	23
Pseudomonas aeruginosa	12	13	9	10	8	26
S. enterica ser. *Paratyphi*	25	10	20	15	9	30
S. enterica ser. *Typhi*	14	18	15	13	8	20
Shigella boydii	13	8	9	8	7	26
Shigella dysenteriae	14	8	14	10	8	24
Vibrio mimicus	21	12	21	17	ND	24
Vibrio parahemolyticus	14	9	17	14	8	38
Fungi						
Candida albicans	16	13	11	12	9	24
Aspergillus niger	15	8	–	9	7	32
Sacharomyces cerevaciae	14	9	8	11	ND	30

Table 1. Antimicrobial activity of extractives of *L. spinosa* and kanamycin. "–" Indicates 'no activity' and "ND" indicates 'not done'; PE: Petroleum ether; EtOAc: Ethyl acetate; MeOH: Methanol.

listed in Table 1 were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. Solutions of known concentration (µg/ml) of the test samples were made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) were then impregnated with known amounts of the test substances using micropipette. Discs containing the test material were placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs (impregnated with solvents) were used as positive and negative control. These plates were then kept at low temperature (4 °C) for 24 h to allow maximum diffusion. The plates were then incubated at 37 °C for 24 h to allow maximum growth of the organisms. The test material having antimicrobial activity, inhibited the growth of the microorganisms and a clear, distinct zone of inhibition was visualized surrounding the medium. The antimicrobial activity of the test agent was determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment was carried out three times and the mean of the readings was recorded [6]. Standard disc of kanamycin (30 µg/disc) was used for comparison purpose.

Cytotoxicity study

Brine shrimp lethality bioassay [19,20] technique was applied for the determination of cytotoxic property of crude extracts, column fraction and compound **1**.

Preparation of positive control group

Vincristine sulphate was used as the positive control. Measured amount of the vincristine sulphate was dissolved in DMSO to get an initial concentration of 20 µg/ml from which serial dilutions were made using DMSO to get 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625, 0.078125, and 0.0390 µg/ml. Then the positive control solutions were added to the premarked vials containing ten living brine shrimp nauplii in 5 ml simulated sea water to get the positive control groups.

Preparation of negative control group

100 µl of DMSO was added to each of three pre-marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii to use as control groups. If the brine shrimps in these vials showed a rapid mortality rate, then the test was considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the test samples.

Counting of nauplii

After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration.

RESULTS AND DISCUSSION

The petroleum ether (PE), ethyl acetate (EtOAc), methanol (MeOH) crude extracts (500 µg/disc), one column fraction (300 µg/disc) and compound **1** (100 µg/disc) were subjected to microbiological investigation against 13 test bacteria and 3 fungi and exhibited mild to moderate antimicrobial activity against most of the test organisms whereas, petroleum ether (PE) & methanol (MeOH) extracts showed significant activity in some cases and the compound **1** (100 µg/disc) showed poor antimicrobial activity in most cases (Table 1). The zone of inhibition produced by petroleum ether (PE), ethyl acetate (EtOAc), methanol (MeOH) extracts were found to be 12–25 mm, 08–18 mm and 08–21 mm, respectively at a concentration of 500 µg/disc. Similarly, the column fraction and compound **1** yielded zones of inhibition 08–17 mm and 07–08 mm respectively (Table 1). The petroleum ether (PE) soluble fraction showed moderate activity against *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Candida albicans* and *Aspergillus niger* and significant activity against *Salmonella enterica* serovar *Paratyphi* and *Vibrio mimicus*. The methanolic crude extract showed moderate activity against *B. cereus , S. aureus, E. coli* and *Vibrio parahemolyticus* and significant activity against *S. enterica* serovar *Paratyphi* and *V. mimicus*. The ethyl acetate (EtOAc) extract and column fraction showed mild to moderate antimicrobial activity in most cases (Table 1).

In the cytotoxicity study, all samples were found to be highly lethal to brine shrimp nauplii. As a result, LC_{50} values could not be determined. The petroleum ether (PE), ethyl acetate (EtOAc), methanol (MeOH) crude extracts, one column fraction and compound **1** demonstrated LC_{90} of 11.22 µg/ml, 12.3 µg/ml, 13.49 µg/ml, 11.57 µg/ml and 15.85 µg/ml, respectively, against brine shrimp nauplii. This results suggest that the presence of potent bioactive priciples in these extractives which might be very useful as antiproliferative, antitumor, pesticidal and other bioactive agents [19].

Compound **1** (Fig. 1) was isolated from ethyl acetate fraction of *Lasia spinosa* (L.). This is the

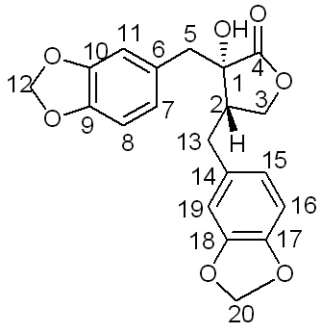

Figure 1. Structure of compound **1** (meridinol).

second report of isolation of compound **1** from this plant species. The compound was identified as meridinol by comparing the ^{1}H and ^{13}C-NMR data with those published for this compound [21].

CONCLUSION

The extractives and the isolated compund (meridinol) showed strong cytotoxicity against brine shrimp nauplii. None of them demonstrated significant inhibition of growth of the test microorganisms. These substances may be potentially useful mainly as antitumor drugs.

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