Dhaka Univ. J. Sci. 60(1): 1-3, 2012 (January)

Neoandrographolide Isolated from Leaves of *Adhatoda vasica* Nees.

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Received on 02. 11. 2009. Accepted for Publication on 20. 08. 2011.

Abstract

Neoandrographolide was isolated from the ethylacetate soluble fraction of the ethanol extract of the fresh leaves of *Adhatoda vasica* (Family: Acanthaceae). The crude extracts of hexane, ethylacetate and butanol soluble fractions of ethanol extract were subjected to antimicrobial screening and brine shrimp lethality bioassay. The ethylacetate crude extract exhibited moderate antimicrobial activity against most of the test organisms and also showed significant cytotoxicity having LC_{50} 0.61 µg/ml.

Key words: ***Adhatoda vasica***, Acanthaceae, Neoandrographolide, Brine shrimp lethality bioassay, antimicrobial screening.

I. Introduction

Adhatoda vasica, (Bengali name-Basakpata; English name- Malabar nut; Family**-** Acanthaceae) is an evergreen densely growing bushy shrub with long opposite ascending branches,broadly elliptic leaves and small white or purple flowers in dense axilliary pedunculate and bracteate spikes.It grows widely in all districts of Bangladesh and also in tropical and semi-tropical regions like India, Myanmar, Pakistan**.** Leaves of the plant possess expectorant, bronchodilator, respiratory stimulant, antispasmodic, hypotensive, cardiac depressant, uterotonic, antimicrobial and hypoglycemic properties; roots and barks are expectorant, antispasmodic and antiseptic [1]**.**

Previous phytochemical investigations of ***Adhatoda vasica*** led to the isolation of Quinazoline alkaloids, *l*-vasicinone, adhatodine, vasicolinone, vasicoline, vasicolinine, vasicinine, *l*-vasicine (peganine), *l* -vasicol, vasicinol, anisotine, 3- hydroxyanisotine, adhatodic acid, betaine, visicine, essential oil, fats, resins, β-sitosterol, vasicine, vasicionol, essential oil, indole alkaloid, galactoside, D-galactose, deoxyvasicinone, vasicinine, kaempferol, quercetin, α-amyrin, tritriacontane, fatty oil consisting of arachidic,behenic,lignoceric,cerotic,oleic and linoleic acids[1]**.**

II. Materials and Methods

General experimental procedure:

The ^1H and ^{13}C NMR spectra were recorded using a Bruker AMX-400 (400 MHz) instrument. For NMR studies deuterated methanol was used and the δ values for ^1H spectra were referenced to the residual nondeuterated solvent signals.

Plant Material

The plant of *Adhatoda vasica* was collected from Curzon Hall in Dhaka University campus which was identified by Bangladesh National Herbarium, Dhaka**.** A voucher specimen has been deposited in the Bangladesh National Herbarium, Dhaka (DACB Accession no. **32608**), for the collection**.** After removing mud and dust particles, the leaves were first dried at room temperature, then in the oven (40 ^{0}-50 ^{0}C) and ground to powder by a cyclotec grinder (200 mesh) and the powder was stored for extraction in an air tight bottle.

Extraction and Isolation

The dried and powdered plant material (507.60 gm) was soaked in ethanol for 10 days. The ethanolic solution was filtered through fresh cotton bed and finally Whatman No.1 filter paper. The solvent of the solution was evaporated to a gummy mass in a rotary evaporator under vacuum at a maximum temperature of 40°C. The gummy mass (62.60 g) was partitioned by the modified Kupchan partitioning method [2] into n-hexane, ethyl acetate and butanol soluble fractions. Evaporation of solvents afforded n-hexane (9.8g), ethyl acetate (16.7g) and butanol (13.4g) extracts. The ethylacetate extract was concentrated to dry mass (16.7g) using rotary evaporator. The dry mass of ethylacetate extract (16.7 gm) was mixed with column grade silica gel. The column was first eluted with 100% n-hexane and then eluted with mixtures of n-hexane and ethylacetate increasing amount of ethylacetate and finally with buanol according to increasing polarity.The eluents were collected in an amount of about 20 ml in a series of test tubes. Finally Column Chromatography using 20% butanol in ethylacetate afforded the compound.

Bioassays

The antimicrobial activity of the crude extracts was determined by the disc diffusion method [3, 4] The extracts were dissolved separately in chloroform and applied to sterile filter paper discs at a concentration of 500 ug/ disc. Kanamycin disc (30ug/disc) was used as standard in each study. For cytotoxicity screening DMSO solutions of the

compound were applied against *Artemia salina* for 24 hours in a simplified in vivo simplified assay.[5, 6] In this experiment, the extracts were dissolved in DMSO and by serial dilution technique, solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563, 0.781 µg/ml were obtained. Then each of these test solutions was added to test tubes containing 10 shrimps in simulated brine water (5 ml). After 24hrs, the median lethal concentration (LC_{50}) of the test samples was obtained by a plot of percentage of the shrimps killed against the logarithm of the sample concentration. Vincristine sulphate was used as positive control in this assay to compare the cytotoxicity of the extracts.

III. Results and Discussion

Neoandrographolide was isolated from the ethylacetate soluble fraction of an ethanol extract of the fresh leaves of *Adhatoda vasica* by repeated chromatographic separation and purification over silica gel. The structure of the isolated compound was determined by ^1H and ^{13}C NMR data analysis as well as by comparison with previously reported values.[7]

The IR spectrum of the compound showed and absorption band at 3421.5 cm^{-1} which indicated the presence of OH group, a peak at 1747.4 cm^{-1} indicated C = O group and a peak at 910.3cm^{-1} indicated the presence of $–CH_2$- in cyclic structure. The band at 2929 cm^{-1} and 2868 cm^{-1} were due to the presence of aliphatic C-H stretching of – CH_3, - CH_2 - , = CH- groups .

The UV spectrum showed λ_{max}^{MeOH} 240nm, suggesting an α, β-unsaturated lactone ring.

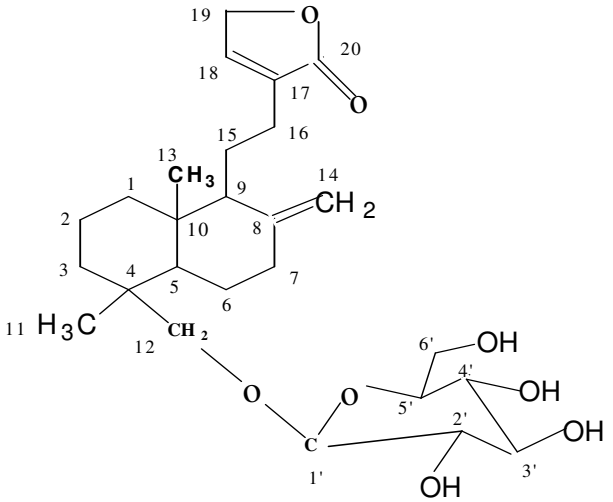

Fig. 1. Structure of the Compound (Neoandrographolide).

The ^1H NMR (400Hz,in CD_3OD)spectrum and ^{13}C NMR spectrum of this compound have shown general features of substituted andrographolide system. The ^1H NMR spectrum has shown the signals of the olefinic β-proton of the α,β - unsaturated lactone system resonating at δ 7.33(s).The C-14 H resonated as two singlet's at δ 4.86 and δ 4.52. Two tertiary methyl groups showed signals at δ 0.71(13-CH_3) & δ 1.03(11-CH_3).

The ^{13}C- NMR spectrum revealed the presence of 26 carbons. Among them 20 signals were accounted for a terpenoid skeleton and 6 signal for a sugar moiety. A signal at δ_c =105.07 ppm in the ^{13}C spectrum was for the anomeric carbon of the sugar residue in the compound which was linked to the terpenoid aglycone part by β- linkage. Finally the signals at δ_c = 40.66 (C-4), δ_c = 149.25ppm (C-8) and δ_c = 39.37 ppm (C-10), δ_c = 134.83ppm at (C-17), δ_c = 176.89ppm (C-20) were assigned to five quaternary carbons respectively. The ^{13}C data of the sugar residue was identical with those of the reported signals of glucose residue. [7]

The structure of the compound has been finally established by the combined IR, ^1H, ^{13}C and DEPT.-NMR spectral data with the reported NMR spectral data of terpinoidal compounds and all these data were in well agreement with those of neoandrographolide[7].

Table. 1. ^1H (400 MHz, CD_3OD) and ^{13}C (100 MHz, CD_3OD) NMR data for the compound

SL no.	δ_H (mult., *J* in Hz)	δ_C(ppm)
1	2.10 (1H, m)	40.23
2	1.89(1H, m)	20.26
3	1.96(1H, m)	37.21
4		40.66
5	1.27 (1H , dd, *J*=2,13.2Hz)	57.71
6	1.77 (1H, m)	25.44
7	2.40 (1H, m)	39.68
8		127.2
9	1.67(t, *J* = 11.6Hz)	57.86
10		39.37
11	1.03 (1H, s)	28.31
12	4.08 (d, *J*=9.6Hz)	73.46
13	0.71(s)	15.84
14	4.86(s), 4.52 (s)	107.25
15	1.08(1H ,dd,*J*=3.6,13.2Hz)	22.97
16	1.27(1H , dd, *J*=2,13.2Hz)	25.64
17		134.83
18	7.33(s)	147.59
19	4.86(1H,s)	72.06
20		176.89
1´	4.17(t, *J*=7.6Hz	105.07
2´	3.16(t, *J*=8.8Hz)	75.28
3´	3.33(t, *J*=8.4Hz)	78.25
4´	3.28(t, *J*=8.8Hz)	71.72
5´	3.23(m)	77.74
6´	3.86(1H ,dd,*J*=2.4,11.6Hz) 3.68(1H ,dd,*J*=5.2,12.0Hz)	62.77

Table-2. Antimicrobial activity of *Adhatoda vasica,* extracts (500 µg/disc) and Kanamycin (30 µg/disc)

Test microorganisms	Kanamycin	Ethanol extract	Ethyl acetate extract	n-Hexane extract
Gram Positive				
Bacillus cereus	36	8	8	7
Bacillus megaterium	36	8	8	7
Bacillus subtilis	36	8	7	7
Staphylococcus aureus	35	9	8	9
Sarcina lutea	37	7	NA	NA
Gram Negative				
Escherichia coli	35	7	8	9
Pseudomonas aureus	35	7	8	7
Salmonella paratyphi	36	8	8	7
Salmonella typhi	35	8	9	7
Vibrio mimicus	34	NA	NA	NA
Vibrio parahemolyticus	35	7	6	9
Shigella dysenteriae	36	8	8	7
Shigella boydii	36	7	7	7
Fungi				
Candida albicans	35	7	7	7
Aspergillus niger	35	7	8	7
Sacharomyces cerevacae	35	7	7	7

"NA" Indicates `No Activity'.

Table. 3. LC_{50} data of *Adhatoda vasica* extracts and vincristine sulfate.

Samples	**LC_{50} (µg/ml)**
vincristine sulphate (Std.)	0.33
n-Hexane extract	1.2
Ethanol extract	0.6
Ethylacetate extract	10.3
Pure Compound(neoandrographolide)	4.08

Acknowledgements

The authors wish to thank the Bangladesh Council of Scientific and Industrial Research under Ministry of Science and Information & Communication Technology, Government of the Peoples' Republic of Bangladesh.

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Dhaka Univ. J. Sci. 60(1): 1-3, 2012 (January)