

Neoandrographolide Isolated from Leaves of *Adhatoda vasica* Nees.

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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 LC₅₀ 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 axillary 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, vasicine, essential oil, fats, resins, β-sitosterol, vasicine, vasicinol, 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 ¹³C 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^o-50^oC) 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 butanol 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 µg/disc. Kanamycin disc (30µg/disc) was used as standard in each study. For cytotoxicity screening DMSO solutions of the

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				
<i>Bacillus cereus</i>	36	8	8	7
<i>Bacillus megaterium</i>	36	8	8	7
<i>Bacillus subtilis</i>	36	8	7	7
<i>Staphylococcus aureus</i>	35	9	8	9
<i>Sarcina lutea</i>	37	7	NA	NA
Gram Negative				
<i>Escherichia coli</i>	35	7	8	9
<i>Pseudomonas aureus</i>	35	7	8	7
<i>Salmonella paratyphi</i>	36	8	8	7
<i>Salmonella typhi</i>	35	8	9	7
<i>Vibrio mimicus</i>	34	NA	NA	NA
<i>Vibrio parahemolyticus</i>	35	7	6	9
<i>Shigella dysenteriae</i>	36	8	8	7
<i>Shigella boydii</i>	36	7	7	7
Fungi				
<i>Candida albicans</i>	35	7	7	7
<i>Aspergillus niger</i>	35	7	8	7
<i>Sacharomyces cerevacae</i>	35	7	7	7

“NA” Indicates ‘No Activity’.

Table. 3. LC₅₀ 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

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