Research Article

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PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF *ADHATODA ZEYLANICA*

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Received: 14-07-2010; Revised: 07-08-2010; Accepted: 05-09-2010

ABSTRACT:
Chemical constituents of *Adhatoda zeylanica* (MP-1 to MP-4) were isolated successfully with higher yield. The structures of isolated compounds were confirmed by the use of spectral data UV, FTIR, ^{1}H NMR, Mass and HPLC. The compound MP-1 to MP-3 of *Adhatoda zeylanica* was evaluated for antimicrobial activity.

KEYWORDS: *Adhatoda zeylanica,* antimicrobial, disc diffusion method

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INTRODUCTION

The potential of higher plants as a source of new drugs is still largely unexplored. Hence, last decade witnessed an increase in the investigation on plants as a source of new biomolecules for human disease management[3]. Plant derived drugs serves as a prototype to develop more effective and less toxic medicines. A survey of literature showed that no systematic approach has been made to study antimicrobial activity in this plant[1, 2].

Adhatoda zeylanica is indigenous to India, where it is found in sub-Himalayan track up to an altitude of 1000 m and in Maharashtra especially, in Kankan region. Besides India, it is found in Myanmar, Srilanka and Malaya. The leaves, flowers, fruits and roots are extensively used for treating cold, cough, whooping cough, chronic bronchitis and asthma[4]. It has also aroused considerable interest for its beneficial effects in malaria, dysentery, diarrhea, anthelmintic, expectorant and antiperiodic. Owing to their immense importance and varied bioactivities exhibited by *Adhatoda zeylanica*, efforts have been made from time to time to generate libraries of isolated compounds and screen them for potential activities[5].

MATERIAL AND METHOD

The experimental work was carried out on leaves of Adhatoda *zeylanica*

Collection & Authentication of plant material The leaves and twigs of *Adhatoda zeylanica* were collected from Srinagar Garhwal, district Pauri, Uttrakhand, India in the month of December. The plant was identified by Botanical survey of India, Northern regional centre, Dehradun (BSD) with the accession number BSD-112751.

Successive Solvent Extraction Air-dried and powdered leaves of *Adhatoda zeylanica* 500g were extracted successively with different solvents like hexane, petroleum ether (40-60°), benzene, acetone, chloroform, ethanol and methanol in a soxlet apparatus for 48 hrs. Each time before extracting with the next solvent, the powdered material was dried in the hot air oven below 50°C. The extracts were concentrated by distillating off the solvent.

Column chromatography of compounds Silica gel (60-120 mesh) was used as absorbent for column chromatography. The column was taken and packed with cotton at the bottom of the column. The slurry was prepared by silica gel and chloroform was used as solvent for free flowing consistency. It was poured slowly from the top of the column of the apparatus in a little quantity allowing for the even and uniform packing. The 2/3 of column was packed by using above procedure. The extract was dissolved in the minimum quantity of ethanol and chromatographed over silica gel. It was then eluted with different solvents in increasing order of polarity eg. hexane, petroleum ether, benzene, acetone, chloroform, alcohol and water. The fractions were collected and marked. The marked fractions were subjected to thin layer chromatography to check homogeneity of various fractions[6].

Isolation of compound MP-1 from F_4 Elution of column of methanolic extract with hexane: petroleum ether (40-60°) (8:2) fraction (F4) furnished as yellowish amorphous powder of MP-1.Yield was 120 mg (0.024 %).

Isolation of compound MP-2 from F_6 Elution of column of methanolic extract with petroleum ether (40-60°) fraction (F_6). furnished as pale yellow amorphous powder of MP-2, Yield was 110 mg (0.022 %).

Isolation of compound MP-3 from F_{20} Elution of column of ethanolic extract with petroleum ether (40-60°): benzene (8:2) fraction (F20) furnished colourless amorphous powder of MP-3.Yield was 102 mg (0.020 %)

Isolation of compound MP-4 from F_{23} Elution of column of ethanolic extract with benzene: acetone (9:1) fraction (F_{23}) furnished as pale yellow amorphous powder of MP-4. Yield was 108 mg (0.021 %).

CHARACTERIZATION OF ISOLATED COMPOUNDS

Characterization of compound MP-1 The compound MP-1 obtained as yellow crystals having m.p.197-198°C and gave positive test for alkaloid. TLC Methanol: water (6:4 v/v)**,** R_f 0.7, UV λmax 270 (0.857)nm. Yield 120 mg (0.024 %)**,** HPLC Solvent system, methanol: water (4:6 v/v) RT (min) 3.12, IR (KBr)υ: 3416.2 (O-H stretching), 3065.3 (Ar C-H stretching), 2930.2 (C-H stretching), 1613.4 (C=N stretching), 1584.1 (C=C stretching), 1299.6 (C-N stretching), 1164.7 cm^{-1} (C-O stretching).MS showed $(M)^+$ peak at m/z 188. ^{1}HNMR (DMSO): δ 2.66 (s, 2H, CH_2), 3.23 (s, 2H, CH_2), 4.23 (s, 2H, CH_2), 5.23 (s, 1H, OH), 7.42-7.83 ppm (m, 4H, Ar-H).

Characterization of compound MP-2 The Compound MP-2 obtained as pale yellow crystals having m.p. 201-204°C and gave positive test for alkaloid.TLC Toulene: methanol: dioxane: ammonia (1:1:25:0.5v/v), R_f 0.6, UV λmax 282 (0.047) nm, Yield110 mg (0.022 %), HPLC Solvent system, methanol: water (4:6 v/v), RT (min) 3.13, IR (KBr)υ: 3216.2 (O-H stretching), 3020.1 (Ar-C-H stretching), 2926.5 (C-H stretching), 1726.5 (C=O stretching), 1603.9 (C=C stretching), 1603.9 (C=N stretching), 1215.8 (C-N stretching), 1144.5 cm^{-1} (C-O stretching).MS showed $(M)^+$ peak at m/z 202. ^{1}HNMR (DMSO): δ 2.57 (s, 2H, CH_2), 4.45 (s, 2H, CH_2), 5.40 (s, 1H, OH), 7.13-7.40 ppm (m, 4H, Ar-H).

Characterization of compound MP-3 The compound MP-3 obtained as colourless crystals having m.p. 138-140°C and gave positive test for alkaloid.TLC Benzene: methanol (6:4 v/v), R_f 0.9, UV λmax222(0.117) nm.Yield 102 mg (0.020 %)HPLC Solvent system, methanol: water (4:6 v/v), RT (min) 3.19, IR (KBr)υ: 3066.3 (Ar C-H), 2933.2 (C-H stretching), 1690.8 (C=O stretching), 1613.9 (C=N stretching), 1541.8 (C=C stretching), 1300 cm^{-1} (C-N stretching).MS showed $(M)^+$ peak at m/z 200, ^{1}HNMR (DMSO): δ 2.57 (s, 2H, CH_2), 4.45 (s, 2H, CH_2), 5.40 (s, 2H, CH_2), 7.13-7.40 ppm (m, 4H, Ar-H).

Characterization of compound MP-4 The compound MP-4 obtained as pale yellow crystals having m.p. 278-280°C and gave positive test for alkaloid. TLC Methanol: water (6:4 v/v), R_f 0.9, UV λmax 270 (0.832) nm, Yield 108 mg (0.021 %), HPLC Solvent system, methanol: water (4:6 v/v), RT (min) 3.12, IR (KBr)υ: 3221.0(O-H stretching), 3021.0 (Ar C-H stretching), 2926.1 (C-H stretching), 1680.8 (C=O stretching), 1590.5 (C=N stretching), 1541.3 (C-C stretching), 1216.2 cm^{-1} (C-N stretching). MS showed $(M)^+$ peak at m/z 218.^{1}HNMR (DMSO)**:** δ 2.48 (s, 2H, CH_2), 4.31 (s, 2H, CH_2), 5.29 (s, 1H, OH), 7.21 (s, 1H, Ar-OH), 7.22-7.67 ppm (m, 3H, Ar-H).

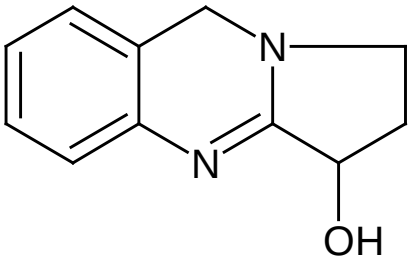
ANTIMICROBIAL ACTIVITY

The pathogenic bacteria used were *Staphylococcus aureus* , *B. subtilis*, *E. coli* and *K. pneumonae* and fungal strains were *A. niger* and *C. albicans* They were obtained from the Department of pharmacy, SGRRITS, Dehradun, Uttrakhand. The Compounds MP-1 - MP-3 were isolated evaluated for their *in vitro* growth inhibitory activity against a variety of strains of bacteria and fungi by disc diffusion method[7]. Ciprofloxacin and fluconazole were used as standard drugs against bacterial and fungal strains at concentration of 50 μg/ml along with DMF as solvents (**figure-1&2**).

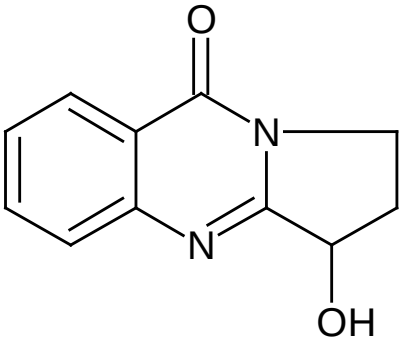
RESULTS AND DISCUSSION

The work carried out concluded that *Adhatoda zeylanica* contains: vasicine (MP-1), Vasicinone (MP-2), Deoxyvasicinone (MP-3) and Vasicinolone (MP-4).

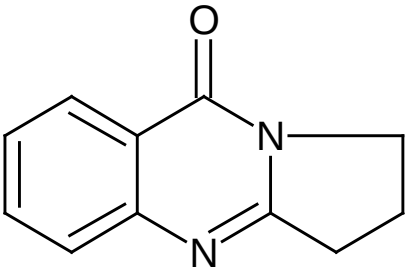
Vasicine (MP-1)



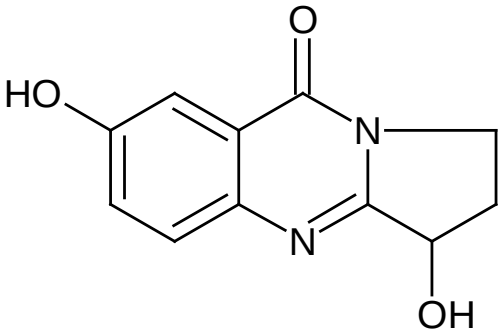
Vasicinone (MP-2)



Deoxyvasicinone (MP-3)



Vasicinolone (MP-4)



ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

The results revealed that isolated compounds MP-1, MP-2, MP-3 were found to be potent against *B. subtilis*, *S. aureus*, and *E. coli*, showed zone of inhibition at concentration of 50 µg/ml. From the analysis of three bacterial strains on isolated compounds, F = 29.245 and P = 0.0008 showed significant difference, thus zone of inhibition in all bacterial strains, against the isolated compounds are comparable. Similarly, compounds

MP-2 and MP-3 were found to be potent against *C. albicans* and MP-1 against *A. niger* at concentration of 50 μg/ml (**table-1,figure-3**).

Results further revealed that the compounds MP-2 and MP-3 possessed comparable antibacterial activity with that of standard drug ciprofloxacin against *B. subtilis* MP-2 and *S. aureus*. Similarly, compounds MP-3 possessed comparable antifungal activity against *A. niger* with that of standard drug Fluconazole. The statistical analysis was done by applying t- test. From the analysis of fungal strains on isolated compounds t=1.601 at 4 degree of freedom and p = 0.1845 thus zone of inhibition in all fungal strains, against the isolated compounds are comparable (**table-2, figure-4)).**

CONCLUSION

The Isolated compounds of *Adhatoda zeylanica* were identified on the basis of melting point range, R_f values, solubility in different solvents, elemental analysis, UV absorbance, IR and ^{1}H NMR spectral analysis. IR, ^{1}H NMR, Mass and HPLC spectral data confirmed the identity of the isolated compounds.

The newly isolated compounds were screened for various pharmacological activities. The isolated compounds were screened for their antimicrobial and anthelmintic activities. Antimicrobial activity was screened against various Gram positive and Gram negative bacteria's and against various dimorphic species of fungi by disc diffusion method, using nutrient agar medium. The results revealed that isolated compounds MP-1, MP-2, MP-3 were found to be potent against *B. subtilis*, *S. aureus* and *E. coli.* Compounds MP-2 and MP-3 were found to be potent against *C. albicans* and MP-1 A. *niger.*

In the present study all the isolated compounds showed activity against Gram positive bacteria like *B. subtilis; S. aureus* and Gram negative bacteria *E. coli.* Thus the compounds isolated could be used as broad spectrum antibacterial agents.

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Table 1: Antibacterial activity of compounds against *B. subtilis, E. coli* and *S. aureus*

Code of compounds	Diameter of zone of inhibition (mm)								
	B. subtilis			*S. aureus*			*E. coli*		
	25 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}	25 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}	25 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}
MP-1	8.43 ±0.43	17.43 ±0.33	21.02 ±1.00	5.67 ±0.43	10.18 ±0.32	14.17 ±0.14	7.85 ±0.38	11.78 ±0.42	12.17 ±0.23
MP-2	7.85 ±0.35	18.48 ±0.56	20.78 ±1.25	6.42 ±0.33	13.28 ±0.12	15.28 ±0.15	7.43 ±0.32	10.32 ±0.62	15.68 ±1.23
MP-3	7.96 ±0.68	18.34 ±0.95	22.78 ±0.48	5.78 ±1.02	12.28 ±0.16	14.28 ±0.11	8.37 ±0.43	12.58 ±0.78	21.33 ±0.42
Ciprofloxacin	.	20.33 ±0.84			16.12 ±0.12			16.28 ±0.64	

Data are given as mean ± S.D. (n=3); S.D. = Standard deviation

Table 2: Antifungal activity of compounds

Code of compounds	Diameter of zone of inhibition in mm [mean ± S.D. (n=3)]					
	C. albicans			*A. niger*		
	25 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}	25 µg ml^{-1}	50 µg ml^{-1}	100 µg ml^{-1}
MP-1	6.76 ±0.28	8.13 ±1.00	12.67 ±0.31	4.67±0.34	8.33±1.00	13.13 ±0.78
MP-2	7.32±0.01	12.69 ±1.00	15.45 ±0.67	2.33±0.11	6.29 ±0.15	15.87 ±0.10
MP-3	5.23±0.16	10.43±0.45	18.73 ±0.65	2.33±1.15	9.12±0.12	17.25 ±0.17
Fluconazole		15.83±0.12			14.67±0.16	

Data are given as mean ± S.D. (n=3); S.D. = Standard deviation

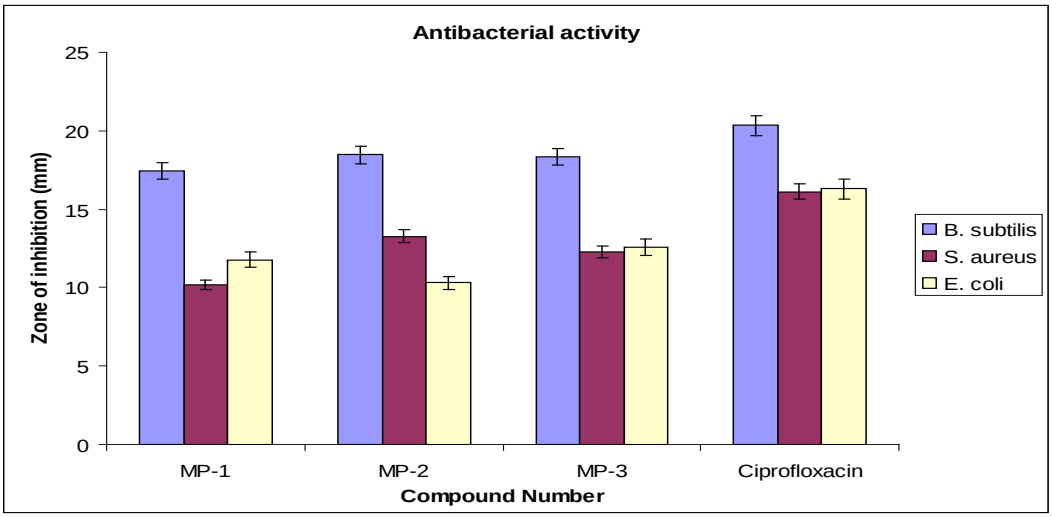

Figure1: Graph showing the antibacterial activity of isolated compounds (potent) in comparison with the standard at 50µg/ml.

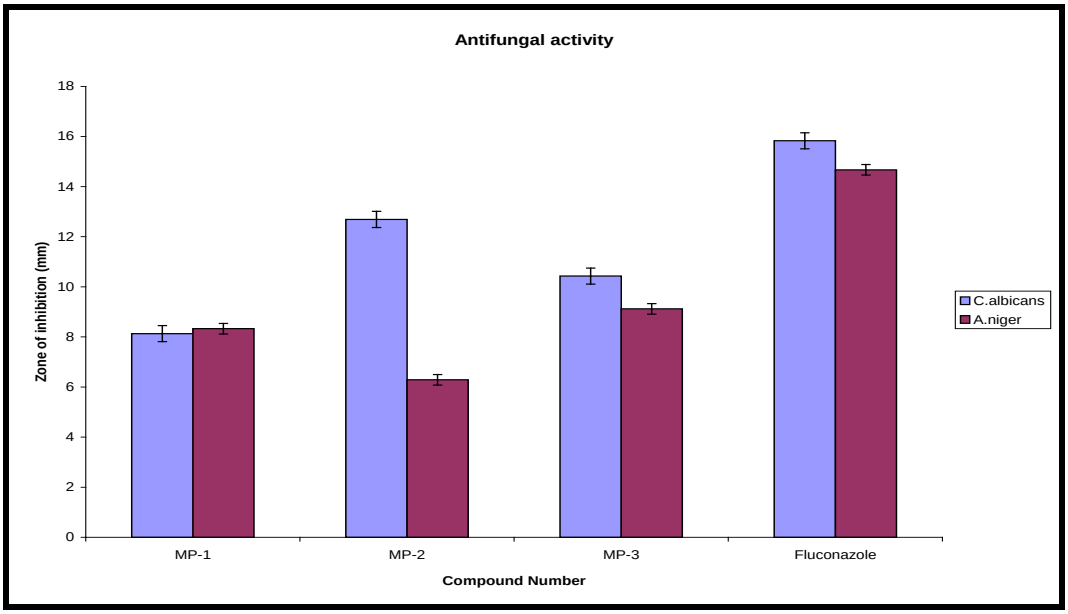

Figure2: Graph showing the antifungal activity of isolated compounds (potent) in comparision with the standard at 50µg/ml.

Figure 3: Zone of inhibition in antibacterial activity

Figure 4: Zone of inhibition in antifungal activity

Source of support: Nil, Conflict of interest: None Declared