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Antimicrobial activity of Naphthyl Iso-quinoline alkaloids of *Ancistrocladus heyneanus:* I Extracted from Leaves

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ABSTRACT

Ancistrocladus heyneanus is a tropical liana plant. It is rich in Naphthyl Isoquinolin alkaloids. Antimicrobial activity of three Gram negative and one gram positive bacteria were tested against crude aqueous and organic solvent extracts of Ancistrocladus heyneanus stem; using agar well method. Later the alkaloids from crude organic solvent extract were isolated by HPTLC. Four distinct bands of alkaloids were observed. Each of them was tested for its antimicrobial activity. It was found that crude extracts showed activity against both gram negative and gram positive bacteria. Fraction 1 showed activity against only gram positive bacteria and not against any gram negative bacteria. Whereas, inhibition of growth of gram positive bacteria S. aureus was obviously more when H2 and H4 fractions of alkaloid were tested. Both crude extracts as well as isolated alkaloid fractions showed considerable activity against gram positive bacteria but not against gram negative bacteria.

Keywords: *Ancistrocladus heyneanus*, Antimicrobial, Alkaloids,

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INTRODUCTION

Alkaloids are secondary metabolites, specific to selected plants and the plant parts. The “Secondary Metabolites” are present in plants only incidentally and are not of paramount significance for plant life [1]. As cited by Verpoorte and Alfermann (2000) [2] Bennet and Bentley have defined a secondary metabolite as follows: “A metabolic intermediate or a product, found as a differentiation product in restricted taxonomic groups, not essential to the growth and life of the producing organism, and biosynthesized from one or more general metabolites by a wider variety of pathways that is available in general metabolism”

In plants secondary metabolites function as defense tools, for detoxification, communication, signaling, safeguarding and other biological processes. The large diversity of chemical types and interactions displayed by the secondary metabolites can underlie the impressive multiplicity of protective functions ranging from toxicity and light/UV shielding to signal transduction [3, 4, 5, 6, 7]. In plants there are many secondary metabolites, like alkaloids, isoprenoids, phenylpropanoids etc that act as natural pesticides and protect plants against herbivores and pathogenic microorganisms [8]. Plant secondary metabolites represent an enormous value from the economic point of view. Many secondary metabolites from plants have been reported to have antimicrobial activity [9.10] .

Ancistrocladus is one such genus which is known to contain many different alkaloids and recently, efforts of WHO programme have found that it has various medicinal applications for diseases like malaria, HIV, leishmaniasis, chagas disease etc. As reported by Verpoorte [11], who has assayed 300 different alkaloids, they are antibacterial and antiparasitic compounds.

Ancistrocladus heyneanus is the species that is exclusively found in India, endemic to the Western Ghats. *Ancistrocladus heyneanus* is known for its biologically active Naphthyl iso-quinoline alkaloids. It produces a number of Naphthyl iso-quinoline alkaloids such as secondary Ancistrocladine, Ancistrocladinine, Acistrocladisine, Ancistrocladidine, Ancisheynine and Ancistroheynine [12, 13, 14, 15].

In the present work crude extracts from the stem of *Ancistrocladus heyneanus* in both aqueous and organic solvent; as well as the isolated fractions eluted after HPTLC separation were tested for their antibacterial activity.

MATERIALS AND METHODS

Collection of Plant Material - Stems of *Ancistrocladus heyneanus* were collected from Khandala hills of Maharashtra and sun dried and grinded to fine powder.

Test Pathogens -Three gram negative bacteria *(Escherichia coli, Salmonella typhi, Klebsiella pneumoniae)* and one gram positive organism (*Staphylococcus aureus)* were procured from NCCS, Pune and used for antibacterial testing of plant extracts.

Aqueous Extraction of alkaloids from stem was done in the acidic medium.10g of stem powder + 500 ml distilled + 50 ml of 0.05 N Sulfuric acid was taken in a flask and stirred on a magnetic stirrer for 3 hrs and then boiled for 20 min. To the boiled solution 25 g of heavy magnesium oxide it was added and again boiled for 20 min. Solution was allowed to cool and then filtered through Whatman filter paper no.41. The filtered solution was transferred to Petri plates and the filtrate was evaporated to dryness on a hotplate. The dried residue was collected.

Extraction of alkaloids in Organic Solvent Methanol – was done by adding 10g of the dried stem powder to 90 ml Methanol and 25% Ammonia. Solution was left uncovered overnight. Next day, it was filtered through Whatmann filter paper no. 12

Separation of Alkaloids was done by HPTLC. Sample was loaded onto the Silica Gel 60 F_{254} HPTLC plates (MERCK) of dimensions 20x10 cm, in the form of uniform bands with the help of a Linomat 5 Applicator. Prior to use plates were marked with a pencil indicating a solvent front and the direction of run in the form of arrows at the upper edge of the plate. Bands were applied maintaining a distance of 10mm from each other and a distance of 15 mm from the edge of the plate. After sample application, the plates were air dried with an air drier. After that they were placed very carefully in the twin trough chamber. Four combinations of mobile phases were used i.e.

1. Toluene : Ethyl acetate: Di-ethylamine (7:2:1)
2. Ethyl acetate: Methanol: Water (10:1.35:1)
3. Toluene : Chloroform: Ethanol (4:4:1)
4. Ethyl acetate: Methanol: 17%Ammonia (8: 1.75:0.75)

Out of the four above solvent systems, only systems 2, 3 and 4 were used for HPTLC of aqueous extract.

The twin trough chambers were saturated with the mobile phase by placing a filter paper soaked in the solvent system present in the trough. After the solvent system covered a stipulated distance, the plates were removed and air dried. These developed plates were then visualized under the UV chamber at 254nm and at 366 nm. The plates were then derivatized with Dragendorrf reagent and again visualized at 254 nm and 366 nm.

After detection of bands, they were carefully cut into strips and then into very small pieces and were transferred to test tubes containing minimum amount of methanol. The tubes were further sonicated for 30-45 minutes. The strips were then separated from methanol and this methanol was collected in separate tubes. Excess of methanol was then dried at 70^0 C using a water bath.

For ***Anti Bacterial Assay*** – Antibacterial activity of aqueous extract, solvent extract and isolated alkaloids was determined by Agar Well Diffusion method .. All the six bacterial cultures were incubated on NA slants for 24 hrs, and then three dilutions of their suspension was prepared in saline and adjusted to an OD of 0.08 taken at 540nm. 0.1 ml of the diluted cultures was spread on the plate using a sterilized glass spreader. Using 5mm cork borer wells were bored into the NA plates. 100 µl of crude sample and separated fractions of alkaloids (conc. 0.5 mg/ml) was poured into each well up to the brim. Methanol, methanol-ammonia and distilled water controls were also prepared. Plates were incubated for 24 hrs at 37^0 C. All the tests were performed in triplicates for anti-microbial study.

RESULTS AND DISCUSSION

Results of Bactericidal assay

Impact of Crude Extract - As mentioned above crude extracts using both aqueous as well as organic solvent were tested against gram negative and gram positive bacteria. After 24hrs of incubation, the plates were checked for their zones of inhibition.

Gram negative organisms -The antibacterial effect of **aqueous extract** was tested on four commonly found non pathogenic microorganisms. The results are tabulated below (Table 1). Aqueous extract was not very inhibitory to gram negative bacteria but could slightly inhibit the growth of gram positive bacteria.

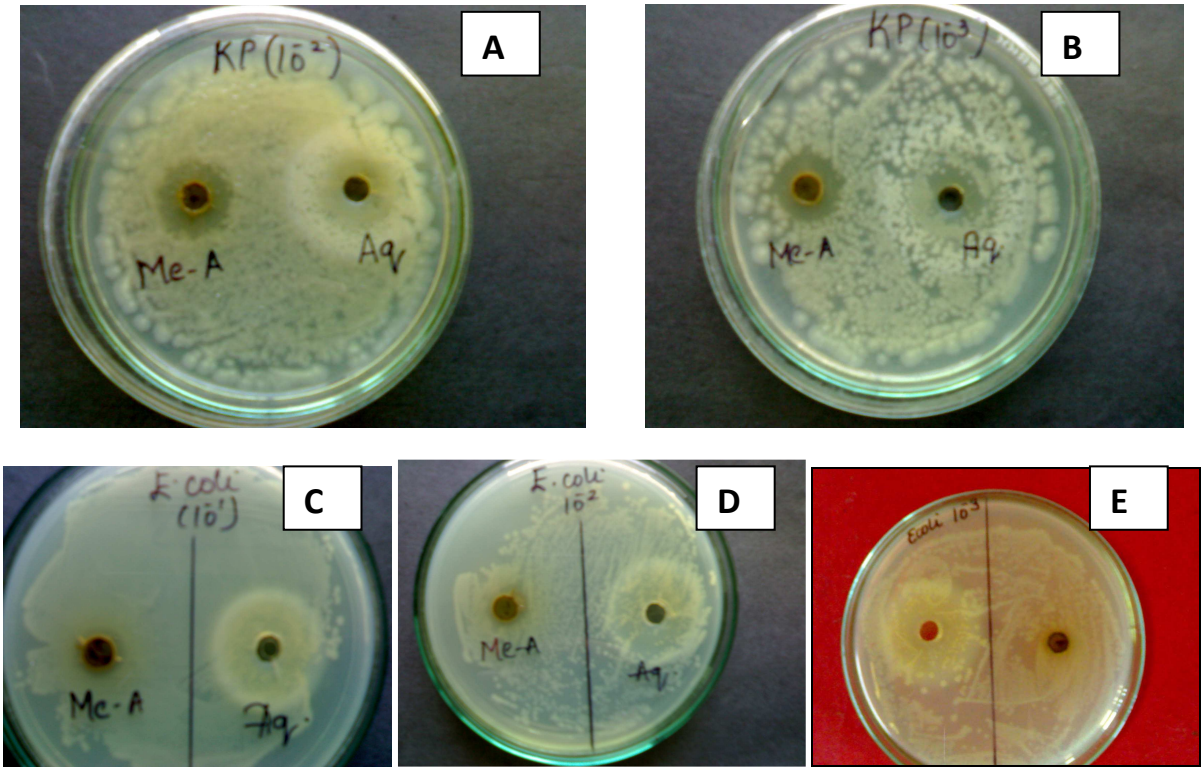
Table 1: Effects of Aqueous extract on microorganisms

Bacterial Dilutions	Gram negative organisms			Gram +ve organism
	K. pneumoniae	*E. coli*	*S. typhi*	*S. aureus*
10^{-1}	No proper inhibition, but reduction of colonies around the well	Irregular inhibition	Irregular inhibition	Zone of inhibition of 0.3cm
10^{-2}	No proper inhibition, but reduction of colonies around the well	Irregular inhibition	No effect	Zone of inhibition of 0.4cm
10^{-3}	Very prominent but irregular inhibition.	No effect	No effect	Zone of inhibition of 0.5cm

The results of the antimicrobial activity of **organic solvent extract** are presented in Table 2 and Figure 1.

Table 2: Effects of Organic solvent extract on microorganisms

Bacterial Dilutions	Inhibition zone of Gram negative organisms			Inhibition zone of Gram +ve organism
	K. pneumoniae	*E. coli*	*S. typhi*	*S. aureus*
10^{-1}	0.5cm	Irregular inhibition	Irregular inhibition	0.4cm
10^{-2}	0.4cm	Irregular inhibition	No inhibition	0.4cm
10^{-3}	0.32cm	No inhibition	No inhibition	0.8cm
Solvent (control)	_	_	_	_



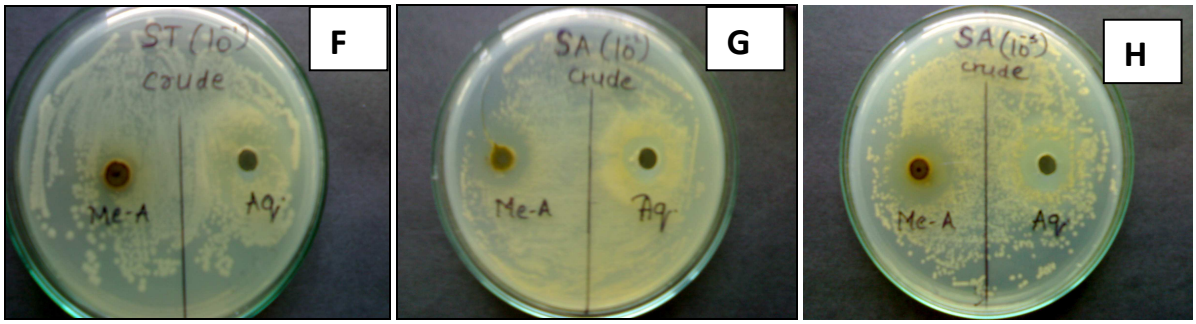


Fig 1: Effect of crude organic (Me-A) & Aqueous (Aq) extracts on gram -ve bacteria *Klebsiella pneumoniae* having 2 dilutions (A) 10^{-2} and (B) 10^{-3}
***Escherichia coli* having 3 dilutions (C) 10^{-1} (D) 10^{-2} and (E) 10^{-3}**
***Salmonella typhi* having 3 dilutions (F) 10^{-1} (G) 10^{-2} and (H) 10^{-3}**

From the above figures it can be concluded that the organic solvent extract exhibited more antibacterial effect on all the 3 tested gram negative microbes i.e. *Klebsiella pneumoniae* , *Escherichia coli* and *Salmonella typhi;* than the aqueous extract.

Gram positive organisms - The antibacterial effect of **aqueous extract** as well as **organic solvent extract** was tested on *Staphylococcus aureus.* The results are presented in Figure – 2 and Table – 1 & 2. *Staphylococcus aureus* showed better response to both aqueous as well as solvent extract as compared to gram negative microbes. Figure 2 shows Clear zone of inhibition in *S. aureus* growth, measuring 0.4cm (at the dilutions10^{-1} and 10^{-2}) and 0.8cm (at the dilutions 10^{-3}).

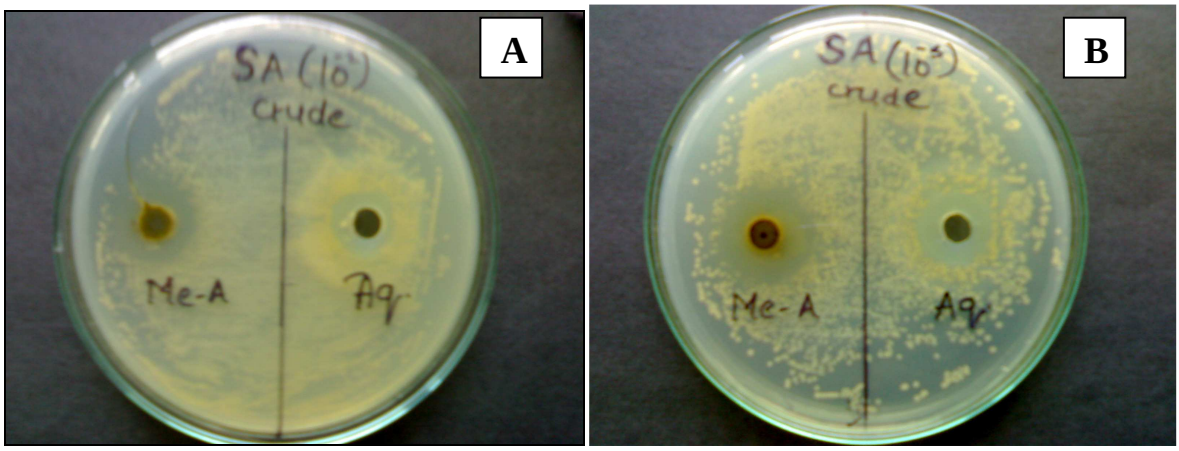


Fig 2: Effect of crude organic (Me-A) & Aqueous (Aq) extracts on gram + ve bacteria *Staphylococcus aureus having* 2 dilutions (A) 10^{-2} and (B) 10^{-3}

Impact of isolated fractions (by HPTLC) of extract

Since the organic solvent extracts showed better bactericidal activity it was fractionated by HPTLC, and their antimicrobial assay was done. Four fractions of alkaloids were isolated by HPTLC. Alkaloids from aqueous extract were not used because they were showing less antimicrobial activity and their separation was not proper and bands were not distinct. The amount of isolated fractions was very less hence the agar wells could not be filled to their brim. The amount of isolated fractions was very less hence only one dilution was tested.

Table 3: Effects of HPTLC isolated fractions of alkaloids from organic solvent extract on bacteria (+ = inhibition observed; - = inhibition was observed)

Alkaloid fractions	Inhibition of Gram negative organisms			Inhibition of Gram positive organism
	K. pneumoniae	*E. coli*	*S. typhi*	*S. aureus*
H1	+	+	+	_
H2	+	+	+	+
H3	_	_	+	_
H4	_	+	+	+

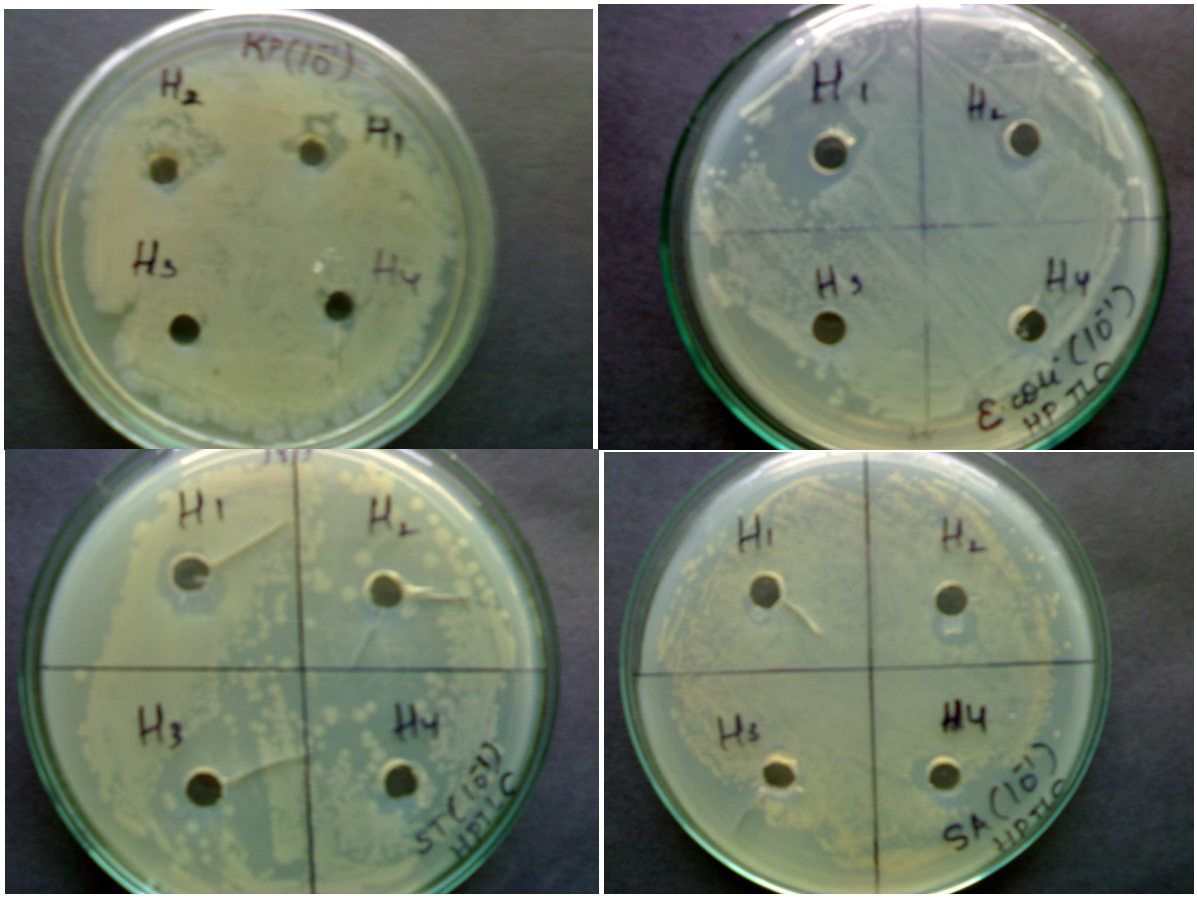


Figure - 3: Effects of HPTLC isolated four fractions of alkaloids from organic solvent extract (A) *K. pneumonia* (B) *E. coli* (C) *S. typhi* and (D*) S. aureus*

Figure 3 show that the first two alkaloid fractions H1 and H2, showed highly irregular inhibition of growth of *K. pneumoniae*. *E. coli* exhibited more inhibition when exposed to H1 and less to H2. *S. typhi* showed susceptibility to all the four isolated alkaloids. However, H1 was more inhibitory. Susceptibility of *E. coli* to many plant extracts have been reported [9],.

Inhibition of growth of gram positive bacteria *S. aureus* was obviously more when alkaloid fractions H2 and H4 were tested.

It can be concluded that Crude Extracts showed activity against both gram negative and gram positive bacteria. Crude extracts from plant seeds have also been reported [10].

Fraction 1 showed activity against only gram positive bacteria and not against any gram negative bacteria. Both crude extracts as well as isolated alkaloid fractions showed considerable activity against gram positive bacteria but not against gram negative bacteria. The reason could be the fact that outer membrane of gram negative bacteria presents a barrier to the penetration of numerous antibiotic molecules and periplasmic space contains enzymes which are able to degrade exogenous molecules[16].Antimicrobial activity of plants have been reported in leaves and areal parts of many plants [17,18]

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REFERENCES

[1] A. Edreva, V. Velikova, T. Tsonev, S. Dagnon, A. Gürel, L. Aktaş, E. Gesheva; *Gen. Appl. Plant Physiology, Special Issue,* **2008,** 34 (1-2), 67-78
[2] R.Verpoorte and A.W. Alfermann (eds): Metabolic Engineering of Plant Secondary Metabolism. Dordrecht, Boston, London: Kluwer Academic Publishers, **2000**.

[3] Y. Yang, J. Shah, D.F. Klessig, *Genes and Development*, **1997,** 11, 1621-1639.
[4] J. Grassmann, S. Hippeli, E.F. Elstner, *Plant Physiolgy and Biochemistry*, **2002,** 40, 471–478.
[5] F. Hadacek, *Crit. Rev. Plant Sci.*, **2002,** 21, 273-322.
[6] A.E. Osbourn, X. Qi, B. Townsend, B. Qin, *New Phytol.*, **2003,** 159, 101-108.
[7] A. Vasconsuelo, R. Boland, *Plant Sci.*, **2007**, 172, 861-875.
[8] Hans-Walter Heldt, Fiona Heldt; Plant Biochemistry, Academic Press, **2005**
[9] K.S. Chukwuka, J.O. Ikheloa, I.O. Okonko, J.O; Moody, T.A. Mankinde, *Advances in Applied Science Research*, **2011**, 2(4) :37-48
[10] M. N. Alo, C. Anyim, and M. Elom; *Advances in Applied Science Research*; **2012**, 3(2) : 887-894
[11] R. Verpoorte, DDT, **1998,** 3, 232 -238.
[12] T.R Govindachari, P.C Parthasarathy, H.K. Desai , *Indian Journal of Chemistry*, **1972,** 10, 1117–1119.
[13] T.R Govindachari, P.C Parthasarathy, *Indian Journal of Chemistr*, **1970,** 8, 567–568
[14] G. Bringmann, C. Gunther, S. Busemann, M. Schaffer, J.D. Olowokudejo, B.I. *Phytochemistry*. **1998**, 47(1): 37-43.
[15] Lay-Kien Yang, P. Robert. K. Glover, J.P. Yoganathan, Sarnaik, Archana J. Godbole, Doel D. Soejarto, Antony D. Buss, Mark S. Butler. *ChemInform* **2003,** 34(46) :46
[16] C. F. Duffy, R. F. Power, *Int. J. Antimicrob. Agent*s, **2001, 17**, 527 – 529
[17] S. R. Sathish Kumar, K. M. Sakthivel, L.Karthik, S.Mythili, A.Sathiavelu , *Asian Journal of Plant Science & Research*, **2011,** 1(1): 48-56
[18] M. Prabakaran, N. Chandrakala and A. Panneerselvam, *Asian Journal of Plant Science & Researc* , **2011,** 1(2): 18 - 25