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# Isolation and characterisation of bioactive terpene from the fruits of *Helicteres isora* Linn.

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**ABSTRACT**

*Helicteres isora* Linn is an important plant described in Indian Materia Medica; the fruits of which are ground and used in colic pain, gripping bowels and flatulence. A fraction rich in terpenoids and phenolic compounds was extracted from the pericarp of the fruits of *Helicteres isora*. This fraction was studied phytochemically and a pure terpene derivative was isolated by using column, thin layer and flash chromatography techniques. It was subjected to several spectroscopic analysis such as IR, NMR and Mass spectroscopy and was determined to be 10-methyl, 4-isopropenyl, dodecahydro- ethanophenanthrene. This terpene derivative exhibited considerable antimicrobial and antispasmodic activities. As far as our knowledge goes this terpene derivative is novel and is not reported in this plant.

**KEY WORDS :** *Helicteres isora*, Terpene, Chromatography, Bioactive compounds

## INTRODUCTION

*Helicteres isora* Linn (Sterculiaceae) is a plant growing gregariously throughout India. The fruits of this plant are commonly called as Mrigashringa in Sanskrit (1).The fruit is an aeterio of 5 follicles twisted together like a corkscrew borne at the tip of a long gynophore. It is an important article of Indian Materia Medica and widely prescribed in the indigenous system of medicine for colic pain, griping bowels and flatulence (2). The fruits have a very good antispasmodic activity. The dichloromethane extract of the fruits when tested on isolated guinea pig ileum has shown $IC_{50}$ value equivalent to 200μg (3). Hence detailed phytochemical investigation on the fruits of *H.isora.* was undertaken. This included the complete phytochemical profile of the powdered pericarp as per the scheme suggested by Harborne (4). Four fractions namely F- I, II, III and IV were collected and they were studied for their phytoconstituents and bioactivity. The yield of F-II was 0.553% and it contained moderately polar constituents like terpenes and phenolics. It was found to be active against the organisms *Bacillus subtilis, Escherichia coli, Shigella dysentrica and Staphylococcus aureus.* The $IC_{50}$ value for F-II was found to be 576.76μg/ml (5). Since F-II had the most potent antimicrobial and antispasmodic activity, it was chosen for further studies. This research paper describes extensive phytochemical investigation on F-II which resulted in isolation & identification of a bioactive terpene.

## MATERIALS & METHODS

### *Plant Material*

The dried fruits of *H.iso*ra Linn were procured from the crude drug market of Mumbai and their identity was confirmed by comparing the morphological characters with those given in the literature (1,2). The voucher specimen has been deposited in the Pharmacognosy laboratory of C.U. Shah College of Pharmacy, Mumbai. The pericarp was separated, powdered and passed through sieve no. 45.

All chemicals and solvents used were of A.R grade purchased from S.D. Fine Chemicals, Mumbai. The TLC and HPTLC plates were obtained from E Merck. Separation by flash chromatography was carried out in ISCO SFX system (Model 260D) using carbon dioxide as a supercritical fluid. The U.V. spectrum was recorded on JASCO V -530 UV/Visible Spectrophotometer using chloroform as the solvent. The Fluorescence spectrum was recorded on RF-5000/ Spectroflorophotometer using methanol as solvent. Infra Red spectrum was recorded using NaCl cell on JASCO FTIR/410/ Spectrometer. NMR spectra were recorded in $CDCl_3$ using TMS as internal standard at 300 mHz. The model used was Variance Mercury Plus. For GCMS the sample was dissolved in methanol and injected at 75° C in a Shimadzu GCMS model with helium as carrier gas. An EIMS spectrum was recorded.

### *Extraction of Fraction F-II*

The Fraction F-II was separated from the pericarp of the fruits based on the method of Harborne (4).

Fraction F-II was subjected to various chromatographic techniques to isolate and purify its various components

***Column chromatography of F-II***

Column chromatography of Fraction F-II was done using Silica Gel G (AR Grade) as the stationary phase and the solvent system with various proportions of dichloromethane (DCM) : Ethylacetate (EA). A total of 21 fractions were collected. Each of these eluted fractions was checked for the number of phytoconstituents by TLC and HPTLC using the solvent system Hexane: E.A. (85:15). Two fractions labeled E-1 and E-II showed a compound with Rf 0.61 in maximum amount. Therefore these two fractions were pooled and subjected to preparative TLC.

***Preparative TLC of Fractions E-I and E-II***

Fractions E-I and E-II were pooled together and subjected to preparative TLC using solvent system Hexane: EA =85:15. The band of Rf 0.61 could not be scrapped out exclusively hence the entire region from the range of Rf 0.55 to Rf 0.7 was scrapped out and the compound present in this region was recovered from silica using EA as solvent. The solvent was evaporated at low temperature and pressure and a partially purified compound was obtained. This was labeled as compound A and was subjected to flash chromatography for further purification.

**Flash Chromatography**

Repeated elution of Compound A in flash chromatogram at different flow rates and various proportions of solvent systems i.e. DCM and EA resolved the compound A as single component. The best resolution which eluted Compound A as single component was obtained at flow rate of 10ml/ min and in the solvent system DCM : EA. = 99.5 : 0.5

***Chemical and Spectroscopic analysis of Compound A:***

Compound A was subjected to chemical and spectroscopic analysis. The chemical properties were tested using various spray reagents like Liebermann Burchard, anisaldehyde sulphuric acid and antimony trichloride reagent (6). The number of double bonds was determined using $CCl_4$ – pyridine method (7). The spectroscopic analysis included UV, spectrofluorimetry, IR, $^1$H-NMR, $^{13}$C-NMR & GC-MS. The chemical and spectroscopic data for compound A is described below

Compound A was obtained as a yellow coloured liquid showing blue florescence under UV 366nm. It gave positive Liebermann Burchard test, showed bluish green colour on spraying with anisaldehyde sulphuric acid reagent and dark yellow zones with antimony trichloride. The IR (NaCl cell $cm^{-1}$) spectrum showed major peaks at 2859.4, 2920 1459. $^1$H-NMR ($CDCl_3$) gave peaks from 0.8ppm to 1.6 δ ppm. $^{13}$ C-NMR ($CDCl_3$) showed signals at 29.6, 22.66, 29.6, 42.28, 21.01, 25.59, 29.32, 37.22, 19.84, 29.17, 31.85, 41, 33.86, 31.52, 33.86, 27.4, 127.8 and 130.2 δ ppm.. Major peaks observed in EIMS were at m/e values 271 ($M\text{-}1^+$), 241, 227, 205, 185, 171, 157, 143, 129, 110, 101, 87, 74, 68, 55. Base peak was found at 74 m/e.

***Biological activity of Compound A***

Compound A was evaluated for two biological properties viz., antispasmodic (8) and antimicrobial activity (9). The permission of ethics committee at institutional level was obtained before carrying out any animal studies. The antispasmodic activity of the Compound A was tested on isolated guinea pig ileum by its ability to reduce the tone of muscle contractions induced by the agonist acetylcholine. An isolated piece of ileum dissected from 18hr fasted guinea pig was placed in an organ bath containing Tyrode solution at 35° C. The air was bubbled through organ bath and a basal tension of 0.5gm was applied. The preparation was allowed to equilibrate for 30min.

The sub maximal dose of Acetylcholine (100 μg/ml) was selected by following a 3 min time cycle and a contact period of 30 sec. The contractile response of the spasmogen was recorded on a kymograph paper using a frontal writing lever. Compound A was tested in doses of 100, 200,400 & 800μg/ml and was allowed to act on the tissue for one minute. The direct effect on the tissue, if any, was observed. After 1 min., the sub maximal dose of spasmogen was added. The % inhibition of contraction induced by each dose of the drug extract was calculated (Table 1). The concentration, which inhibited 50% response, $IC_{50}$ value was determined by plotting a graph of % inhibition against log dose (Fig.1).

The antimicrobial activity was determined by agar cup plate method against four organisms namely *Bacillus subtilis, Escherichia coli, Shigella dysentrica and Staphylococcus aureus*. Standard inoculum of the organism was prepared and seeded on the Nutrient agar plates. Cups of 9mm diameter were bored on the plates. Compound A was tested for its antimicrobial activity in the concentration of 0.5%w/v in DMF. The standard used was chloramphenicol and a blank run of DMF was done to ensure that DMF does not show any antimicrobial activity. The plates were incubated after addition of the test solution at 37 °C for 15-18hrs. The zone of inhibition was measured in mm (Table 2).

**RESULTS AND DISCUSSION -** Fraction F-II obtained from the pericarp of *H.isora* Linn when examined

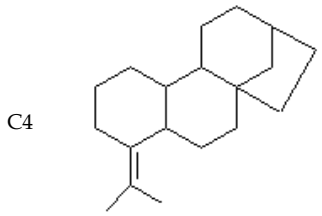
chemically contained terpenes and phenolic compounds. Column chromatography of Fraction F-II with solvent systems DCM : EA in the ratio 95:5 and 90:10 eluted most of the compounds. These fractions labeled as E-I and E-II had a compound with Rf 0.61 in maximum amount. Preparative TLC was done on pooled sample of E-I and E-II to isolate this compound using solvent system Hexane: EA =85:15. The recovered compound labeled as Compound A was partially purified. It was injected in a flash chromatograph and on elution with the mobile phase comprising of DCM: E.A = 99.5: 0.5 resolved as a single component.

Compound A was a yellow coloured viscous liquid having an intense blue florescence under UV 366nm. The physicochemical tests revealed the absence of nitrogen in the compound. GC-MS analysis showed the molecular ion peak at m/z 271. As there was no nitrogen present, the molecular weight (M) of the compound cannot be an odd number. The molecular ion peak therefore was M-1 peak and the molecular weight of compound A was confirmed as 272. The IR spectrum showed major peaks at 2859.4, 2920 (CH stretching), 1459 (CH bending). $^1$H-NMR ($CDCl_3$) gave peaks from 0.8ppm to 1.6 δ ppm indicating methyl, methylene and methane groups. Thus both I.R and $^1$H-NMR spectrum of compound A did not give any functional group signals. The $^{13}$C-NMR also gives signals for alkane group ranging from 22.66 to 42.48 δ ppm and for alkene groups at 127.8 and 130.2 δ ppm (10). Therefore using the molecular weight 272, the most probable molecular formula assigned to compound A was $C_{20}H_{32}$ . Using this molecular formula the double bond equivalent was calculated as 5

Compound A showed positive tests for terpenes. Yellow zones with antimony trichloride reagent indicated it to be a diterpene. The presence of $C_{20}$ carbon atoms also supported the diterpene nature of Compound A. The molecular formula $C_{20}$ $H_{32}$ fits in the formula $C_nH_{2n-8}$ which is the general formula for tetracyclic diterpenes. Secondly the number of double bonds as determined by $CCl_4$- Pyridine method was 1. The $^{13}$C-NMR also indicated the presence of a double bond. Thus compound A could be a tetracyclic diterpene with one double bond. This correlated with the DBE =5 i.e. four rings and one double bond. By screening the literature for tetracyclic diterpenes with molecular weight 272, the most probable parent moiety for compound A was determined as dodecahydro ethano phenanthrene (11).

The position of double bond in compound A was determined with the help of $^1$HNMR and $^{13}$C-NMR spectroscopy. The $^1$H-NMR spectrum did not give any signal in the –CH=CH– range i.e. 4-6ppm indicating that the carbon moieties associated with the double bond did not have a hydrogen atom attached to it. No bands were observed at 885 $cm^{-1}$ in I.R. spectroscopy. This also suggested the absence of hydrogen on the alkene group. The signal at 1.6ppm in $^1$HNMR spectrum also indicated the presence of a geminyl dimethyl group. Since the alkene group did not have hydrogen, these geminyl dimethyl groups could be assigned to the alkene carbon. ($(R)_2$ -C=C-) This whole group was positioned at C-4 based on $^{13}$ CNMR study (Fig .2)

Thus the most probable structure for compound A was deduced as




Based on the chemical studies and spectral characteristics the structure of Compound A was deduced as a tetracyclic diterpene with one double bond, having a molecular weight of 272. The IUPAC nomenclature of compound was 10-methyl, 4-isopropenyl, -dodecahydro ethanophenanthrene.

Compound A was then evaluated for antispasmodic and antimicrobial activity. A plot of log dose versus % inhibition was linear and directly proportional to the dose. The contractile response induced by acetylcholine on isolated guinea pig ileum was completely inhibited by the compound at 800μg signifying that compound A had good antispasmodic action.

***Table 1- Antispasmodic activity of Compound A on Guinea pig ileum against acetylcholine***

| Dose ( μg/ml) | Log Dose | Inhibition (cm) | % Inhibition |
|---|---|---|---|
| 100 | 2 | 1.75 | 40.69 |
| 200 | 2.301 | 2.4 | 55.81 |
| 400 | 2.602 | 3.05 | 70.93 |
| 800 | 2.903 | 4.3 | 100 |

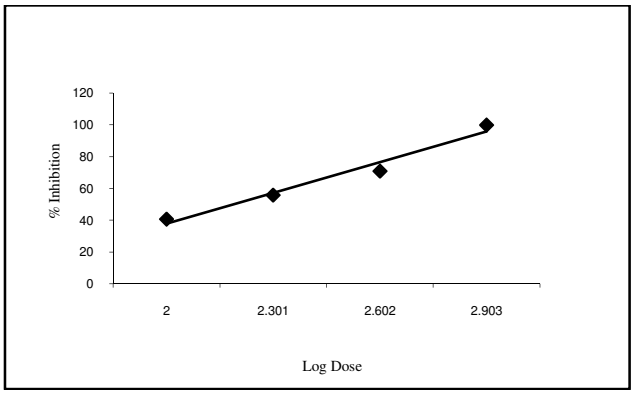



*Fig.1. Antispasmodic activity of Compound A -A plot of log-dose versus % inhibition*

*Table 2 – Antimicrobial activity of Compound A by agar cup plate method*

| Microorganism | Zone of Inhibition (mm) |
|---|---|
| *Bacillus subtilis,* | 15 |
| *Escherichia coli,* | 15.5 |
| *Shigella dysentrica* | 15 |
| *Staphylococcus aureus.* | 16 |

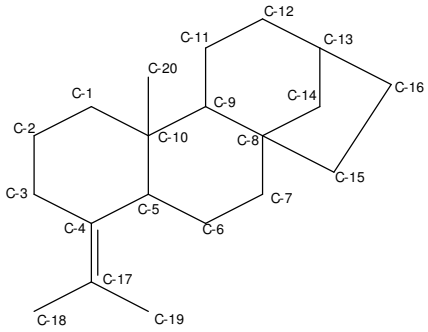



*Fig. 2 - $^{13}$ CNMR assignments for compound A*

| Carbon Atom | Peak Value assigned (ppm) | Carbon Atom | Peak Value assigned (ppm) |
|---|---|---|---|
| C-1 | 29.6 | C-11 | 29.07 |
| C-2 | 22.66 | C-12 | 31.85 |
| C-3 | 29.6 | C-13 | 41.0 |
| C-4 | 127.8 | C-14 | 33.86 |
| C-5 | 42.28 | C-15 | 31.52 |
| C-6 | 21.01 | C-16 | 33.86 |
| C-7 | 25.59 | C-17 | 130.2 |
| C-8 | 29.32 | C-18 | 27.4 |
| C-9 | 37.229 | C-19 | 27.4 |
| C-10 | 19.84 | C-20 | 28.2 |

The IC50 value was found to be 156µg. The antimicrobial activity for compound A when tested in the concentration of 0.5%w/v by agar cup plate method, against the test organisms showed moderate zones of inhibition (15-16mm) indicating good antimicrobial activity.

**CONCLUSION**

From the results it can be concluded that the fruits of *H. isora* has a bioactive compound. This compound labeled as Compound A, isolated from the pericarp was a yellow coloured viscous liquid, chemically identified as a 10-methyl, 4-isopropenyl, -dodecahydro ethanophenanthrene. It was also observed that the compound had good antimicrobial and antispasmodic activity. Hence it could be suggested that the antispasmodic activity of the fruits of *H.isora* could partly be due to Compound A and thus consequently supports the use of these fruits in the indigenous

system of medicine as an antispasmodic agent for colic pain, gripping of bowels and flatulence. As far as our knowledge goes this terpene derivative is novel one, named as helecterene and not yet reported in this plant.

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**REFERENCES**

1. *Wealth of India*, Raw Materials, (Council of Industrial and Scientific Research, New Delhi, 1969 ) 5, p.27.
2. K.R. Kirtikar, B.D. Basu, ***Indian Medicinal Plants***, ( Lalit Mohan Basu publication, 1975) 2nd edn Vol.1, p.371.
3. P. Naznnen, N.D. Grampurohit, Antispasmodic activity of the fruits of *Helicteres isora* Linn, *Phytotherapy Research*, **15 :** 49-52 (2001)
4. J.B. Harborne, ***Phytochemical Methods – A Guide to Modern Techniques of Plant analysis***, Chapman & Hall, 2nd ed, 4-6 (1984)
5. P. Sandhya, N.D. Grampurohit, Bioactivity guided fractionation and Phytochemical investigations on the fruits of *Helicteres isora* Linn, proceedings of World Ayurveda Conference, Kochin (2002 ), p. 25.
6. Farnsworth N.R.; Biological and Phytochemical Screening of Plants, *Journal of Pharmaceutical Sciences*, **55 (3) :** p. 225 – 276, (1966)
7. Muller, ***Fuctional group determination of olefinic and acetylinic unsaturation,*** (United States edition, Academic press INC). p. 6-10
8. Thompson E.B.; ***Drug Bioscreening : Drug evaluation techniques in Pharmacology***, VCH Publisher, p. 193 – 210, (1990).
9. Finegold S.M. and Baron E.J.; ***Bailey and Scott`s Diagnostic Microbiology***, 7th ed., The C.V. Moshby company, p. 173, (1986).
10. Silverstein, Bassler, Morril, ***Spectroscopic identification of organic compounds,*** John Wiley and Sons, 4 th edition. (1991)
11. John S Glasby, ***Encyclopaedia of terpenes*****, Vol. I & II,** ( Wiley – Interscience) (1991).