International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2020): 7.803

Phytochemical Screening of *Heliotropium indicum* and Extraction, Isolation of Anti - Tumorouspyrrolizidine Alkaloids from *Heliotropium indicum*

Taufiq Shaikh, Dr. Nitin Labhane

Abstract: ***Pyrrolizidine alkaloids (PA) have been receiving increasing attention due to their occurrence in several species relevant for human and animal nutrition, as well as for their toxicological and pharmacological properties. Pyrrolizidine alkaloids have proven to have anti - cancerous activity. Heliotropium indicumis a herbaceous plant which is widely distributed throughout the India has shown to have higher concentration of pyrrolizidine alkaloids. In this research pyrrolizidine alkaloids are extracted and isolated from Heliotropium indicum.***

Keywords: Pyrrolizidine alkaloids, Anti - Cancerous, *Heliotropium indicum.*

1. Introduction

The name "heliotrope" originates from the old idea that the inflorescence of these plants turned their rows of flowers to the sun. The meaning of ‘helios’ in Greek is ‘sun’ and ‘tropein’ from where the word ‘tropium’ is derived means ‘to turn’ (1)

H. indicum is an erect, robust, coarse, succulent annual herb with branched stem and strong taproot. Usually the plant attains a height of 1 m, but sometimes it may grow as tall as 1.5 m. Stem deeply grooved and covered with large, coarse, white hairs. Leaves opposite or alternate, 3 - 15 cm long, 2 - 10 cm wide, ovate to oblong - ovate, with dense, long white hairs on both surfaces (2). The lower surface of the leaf is pubescent, acute or acuminate, margin with shallow undulating teeth, the base narrowing and extending down along the petiole to form wings on both sides. The lower surface of the leaves prominently veined, the upper surface coarsely rough and grooved (3). Inflorescence internodal, an unbranched and very rarely dichotomous helicoid cyme, the peduncle portion 2 - 3 cm long, pubescent, the fertile portion 9 - 16 cm long. Flowers bisexual with five sepals, lanceolate, 2 - 3 mm long; Corolla lilac to occasionally white. Fruits angular with an apical beak, 2 - 3 mm long, glabrous with two lobes which spread apart and separate to give two nutlets at maturity (4).

Figure 1: Heliotropium Indicum Linn.

Figure 2: HELIOTROPIUM INDICUM LINN (Leaves and inflorescences).

Medicinal uses:

The plant has been widely used for centuries to treat warts, inflammations and tumours. Throughout tropical Africa it is used as an analgesic to ease rheumatic pain, as a diuretic and to treat numerous skin problems including yaws, urticaria, scabies, ulcers, eczema and impetigo. The Continent, there is a wide variation in the plant parts that are used, and also in methods of preparation and administration (5).

A decoction of the whole plant is used to treat thrush, diarrhoea, diabetes, venereal diseases and frequent excretion of urine. The whole plant is boiled and the beverage used as a remedy for heat rash. It is boiled with Desmodium sp. (ironweed) in a decoction that is used as a purgative of the reproductive system to function as a 'cleanout' for men and women (6).

An infusion of the plant is used as an eye - lotion and to clean ulcers. The leaves are haemostatic, stomachic. An infusion is used as a remedy for asthma, ulcers, dysentery, bronchitis, red eyes, boils etc. The leaf juice is used to treat and soothe the pain of conjunctivitis (7). Mixed with coconut oil and a small amount of salt, the leaves are administered to children as a remedy for colds, grippe and coughing (8). The leaves are boiled with Mikania micrantha for treating upset stomachs. The powdered leaves are used to treat infected gums. A poultice made from the leaves is applied to rheumatic limbs, to wounds and insect bites (9). The flowers are emmenagogue in small doses and abortifacient in large. They are used to control menstrual blood loss; yaws; skin ulcers. The plant contains the hepatotoxic pyrrolizidine alkaloids heliotrine and lasiocarpine. The major alkaloid, indicine, shows antitumor activity. The whole plant is buried and, after the fleshy tissue has rotted away, the remaining fibre is used to make false hair for women (10).

2. Material and Methodology

Collection of plant material: The plant was collected from Powai Lake which is located at Mumbai Suburbs from GPS coordinates Latitude: 19.1160 Longitude: 72.9045. The plant was authenticated using Flora of Bombay Presidency by T. Cook. The plant was collected during July (2020) and was cleaned, dried at room temperature and pulverized by mechanical milled and weighed.

Preparation of Plant Extract:

10 grams of Dried plant material was taken into a sterilised Conical Flask of 250 ml and add 100ml of sterilised distal water. Cover the conical flask with aluminium foil put the conical flask on Sonicator for 30 mins. Remove the conical flask and allow it to cool. With help of funnel and Filterol filter paper filter the extract into another sterilised conical flask and cover it with aluminium foil.

Moisture content:

A small container was weigh, weigh 5g of the material into the container. The same was dried for 24 hrs - 48 hrs in the hot air oven. Reweigh the sample, substrate the weight of container and determine the moisture content using following equation.
Mn = [(Ww. Wd /Ww)] x 100
In which Mn= Moisture content (%)
Ww= Wet weight of sample.
Wd= Wet weight of sample.

Calculation: - Ww= 5g.

Wd= 0.502g.
Mn = [(5x.0.502 /5)] x 100 = 50%
Percentage of moisture in the plant= 50%

Total Ash content: -

1g of air - dried powdered was accurately weight in a Gooch crucible and incinerate at a temperature not exceeding 450 degree Celsius until free from Carbon, Cool & weight.

Empty Crucible weight= 28.20g
Weight of Sample= 1g
Crucible containing Ash = 28.45g
Total ash = Crucible containing Ash - Empty Crucible weight
= 28.20 – 28.45
= 0.25.
Total ash = 0.25.

Acid Insoluble Ash:

Boil the total Ash obtained from the procedure mentioned above with 25ml of 2M HCl for 5 min, collect the insoluble matter in a crucible or on an ash less filter paper. Wash with hot water then cool in desiccator and weight. Calculate the percentage of acid insoluble ash with respect to the air - dried drug.

Calculation: -

Filter paper weight: - 2.020g
Weight of filter paper after filtration: - 2.090g
Acid Insoluble = Weight of filter paper after filtration - Filter paper weight
= 2.090 - 2.020
= 0.07g

Phytochemical Analysis

Table 1: Phytochemical Analysis of *Heliotropium indicum*

Sr. No	Test for Constituents	Results	Conclusion
1.	Carbohydrates: - 2ml extract + 1 ml Benedict's reagents	Reddish colour	Present
2.	Tannins: - 1ml extract + 2ml 5% $FeCl_2$	Dark blue or Greenish colour	Absent
3.	Saponins: - 2ml extract + 2ml distilled water shake in graduated cylinder for 15min	No layer of foam	Absent
4.	Flavonoids: - 2 ml of 2.0% NaOH mixture + 2ml extract	Yellow colour	Present
5.	Alkaloids: - 2ml extract + few drops of Conc. HCl + few drop of Mayer's reagent	Yellow colour	Present
6.	Glycosides: - 2ml extract + 3ml of Chloroform + 10% ammonia	No colour change	Absent
7.	Quinones: - 1ml extract + 1ml Conc. H_2So_4	Red colour	Present
8.	Phenols: - 1ml extract + 2ml distilled water + few drops of 10% $FeCl_2$	No colour change	Absent
9.	Anthracyanine: - 1ml extract + 1ml 2N NaOH + heat for 5 mins	No colour change	Absent
10.	Terpenoids: - 0.5ml extract + 2M chloroform + Conc. H_2So_4	Red Brown Colour	Present
11.	Cardiac Glycoside: - 0.5 ml extract + 2ml glacial acetic acid + few drops of Ferric Chloride + 1ml H_2So_4	No brown ring at interface	Absent
12.	Ninhydrin Test: - 2ml extract + 0.2% of Ninhydrin reagent + heat for 5 mins	Blue colour formation	Present
13.	Steroids: - 1ml extract + 1ml chloroform + Conc. H_2So_4	Bluish Brown Colour	Present
14.	Anthraquinones: - 1ml extract + 10% ammonia	No pink colour precipitation	Absent
15.	Coumarins: - 1ml extract + 10% NaOH	No colour change	Absent
16.	Chalcone: - 1ml NH_4OH + 2ml extract	No Colour Change	Absent

Extraction of Pyrrolizidine alkaloids: -

The extraction of the pyrrolizidine alkaloids was done by using the continuous extraction method using the Soxhlet apparatus. Ten grams (10 g) of grounded whole parts of *Heliotropium indicum*. plant was weighed and packed in a Filterol's Filter paper bag which considered as an extraction thimble. The thimble, then placed in the Soxhlet extractor.100ml of 85% ethanol was placed in the solvent flask (500 ml). The sample was extracted for 6 hours. The ethanol extract was filtered and placed in 150 ml beaker in hot air oven at 50 degree Celsius to get dark coloured residue crude fraction. To find the presence of pyrrolizidine alkaloid N –Oxide, the crude fraction was divided into two equal fractions: Fraction (A) and fraction (B), each fraction weight 50 gm. The fraction (A) extract treated with 5% hydrochloric acid until PH reach 2, then partitioned with equal volume of chloroform in a separatory funnel (three times) and allow to separate into two layers. Separate the upper layer which contains the salt of a free tertiary base while the lower chloroform layer contains the fat and other non - alkaloidal substances. The upper aqueous acidic layer of fraction (A) was separated and basified with ammonia

25% to PH 10. After basification process, the solution become warm and allowed to stand for 2 hours. Then extracted with an equal volume of chloroform in separatory funnel (three times) to get two layers (the upper aqueous basic layers and the lower chloroform layer). The chloroform layer which was separated and evaporated under reduced pressure at a temperature not exceeding 50 0C to get yellowish colour residue designated as (F1 - F) which contain free tertiary alkaloid and give a positive test with Dragendorff reagent.

The Same thing was done for a fraction (B) but the only difference is that when the acidic layer separated after the portioning with the chloroform in the first step, the zinc powder was added to this portion with continuous stirring on magnetic stirrer for 24 hours at room temperature in order to reduce pyrrolizidine N - Oxid to free tertiary base. The mixture was filtered to remove Zinc and basified the filtrate to pH 10 by using 25% ammonia, then extracted three times with equal volume of chloroform. The chloroform layer was dried under vacuum using a rotary evaporator to obtain the total alkaloids (tertiary base and N - oxides) in the form of tertiary pyrrolizidine alkaloids and this fraction was designated as (F1 - T) which gave a positive test with Dragendorff reagent. The amount of N - oxide, then calculated by subtracting of free tertiary base, obtaining from fraction (F1 - F) from the total alkaloids (free and N - oxide base) obtained from fraction (F1 - T).

Percentage yield of crude extract and alkaloids fraction from *Heliotropium indicum*

Table 2: Fractionation process results of ***Heliotropium indicum***

Dried plant weight	Aqueous fraction weight	Percent
10 g	100g	10 %
Pyrrolizidine alkaloids		
Free pyrrolizidine alkaloids (F1 - F)	150 mg	0.0015%
N - Oxide pyrrolizidine alkaloids	40 mg	0.0004%
Total pyrrolizidine alkaloids (F1 - T)	110 mg	0.0011%

Identification of the isolated pyrrolizidine alkaloids by TLC

For Isolation of pyrrolizidine alkaloids special solvent system is used which used dichloromethane, Methanol, ammonia 25% in proper proportion i. e.84: 14: 1 (System 1) and also solvent system of Toluene, ethyl acetate, diethyl amine in ratio of 70: 20: 1. (System 2)

The solvent system used was prepared in separate flask and appropriate quantity was poured into the TLC chamber. Silica gel precoated plates was used for TLC. The Sample with pyrrolizidine alkaloids is loaded 1cm apart from one end of the plate with help of capillary tube and was allowed to dry. The TLC plate was then placed in the pre saturated chamber till the solvent was reached ¾ of the TLC plate. Then, the TLC plate was viewed under the ultra violet (UV) at 254nm. The plates were sprayed from the side with Dragendorff, s reagent and then observed. Orange spots indicated the presence of pyrrolizidine alkaloids.

3. Results

Figure 3: TLC plates (System 1) with isolated pyrrolizidine alkaloids

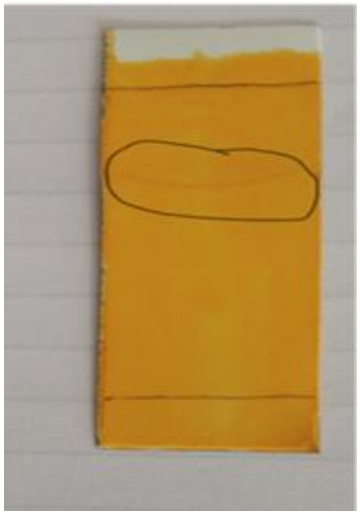

Figure 4: TLC plates (System 2) with isolated pyrrolizidine alkaloids

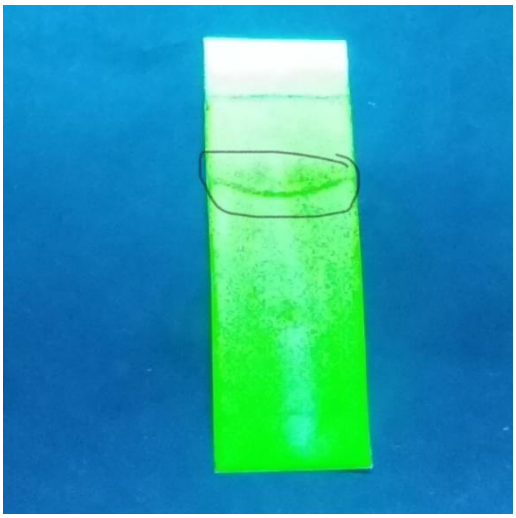

Figure 5: Plate with pyrrolizidine alkaloids in (UV) at 254nm (System 1)

4. Conclusion

The result of the present study established the presence of many biologically important phytochemicals in the Aqueous extract obtained from the whole plant of ***Heliotropium indicum.*** The plant contains a large number of pyrrolizidine alkaloids proved to be potent anti - tumorous.

References

[1] Chittenden FJ: , The Royal Horticultural Society Dictionary of Gardening, Vol. II, Oxford at Clarendon Press, Great Britain, 1951: 513 - 1088.
[2] Shalini S, Kaza R, Shaik F: Study on the anti - inflammatory activity of Heliotropium indicum. Journal of Innovative Trends in Pharmaceutical Sciences 2010; 1 (1): 43.
[3] Rahman MA, Mia MA and Shahid IZ: Pharmacological and phytochemical screen activities of roots of Heliotropium indicum Linn. Pharmacologyonline 2011; 1: 185 - 192.
[4] Boye A, Koffuor GA, Ameyaw EO and Abaitey AK: Analgesic activity and safety assessment of Heliotropium indicum Linn. (Boraginaceae) in rodents. International Journal of Pharmacology 2012; 8 (2): 91 - 100.
[5] Mattocks AR, Schoental, R, Crowley, HC and Culvenor, CCJ: Indicine: The Major alkaloid of Heliotropium indicum L. J Chem Soc.1961: 5400.
[6] Mehta R, Arora OP and Mehta M: Chemical investigation of some Rajasthan desert plants. Indian J Chem.1981; 20B: 834.
[7] Mannan A and Ahmad K: Preliminary study of sex hormone of medicinal importance in Bangladeshi plants. Bangladesh Med Res Counc Bull 1978; 4: 78 - 85.
[8] Rahman MA, Mia MA and Shahid IZ: Pharmacological and phytochemical screen activities of roots of Heliotropium indicum Linn. Pharmacologyonline 2011; 1: 185 - 192.
[9] Anonymous: The Ayurvedic Pharmacopoeia of India, Part - I, Vol. VI, Ministry of Health and Family Welfare, Government of India, AYUSH, New Delhi, 2008: 66.
[10] Wilson E, Rajamanickam GV, Vyas N, Agarwal A and Dubey GP: Herbs used in Siddha medicine for arthritis - A review. Indian Journal of Traditional Knowledge 2007; 6 (4): 678 - 686.
[11] A review of Heliotropium indicum L. (Boraginaceae) Dash and Abdullah, IJPSR, 2013; Vol.4 (4): 1253 - 1258.
[12] Pandey VB, Singh JP, Rao YV and Acharya SB: Isolation and pharmacological action of heliotrine, the major alkaloid of Heliotropium indicum seeds. Planta Med.1982; 45: 229 - 233.
[13] Dattagupta S and Datta PC: Pharmacognostic study of the leaf of Heliotropium indicum Linn. (Boraginaceae). Pharmaceutical Biology 1977; 15 (3): 141 - 151.
[14] Meher A, Panda SK, Agrahari AK, Pradhan AR and Bhoi R: Pharmacognostical and phytochemical evaluation of Heliotropium indicum L. Stem. Indian Journal of Novel Drug Delivery 2011; 3 (4): 303 - 306.
[15] Birecka N, Frohlich MW and Glickman LM: Amino alcohols of pyrolizidine alkaloid in Heliotropium species. Part - 4. Free and esterified necines in Heliotropium species from Mexico and Texas. Phytochemistry 1983; 22 (5): 1167 - 1171.
[16] Hoque MS, Ghani, A and Rashid H: Alkaloids of Heliotropium indicum grown in Bangladesh. Pharm J 1976; 5 (3): 13.29. Willaman JJ and Schubert BG: Alkaloid bearing plants and their contained alkaloids, ARS, USDA, Tech Bull 1234, Supt Documents, Govt Print Off, Washington, 1961.
[17] Pandey VB, Singh JP, Rao YV and Acharya SB: Isolation and pharmacological action of heliotrine, the major alkaloid of Heliotropium indicum seeds. Planta Med.1982; 45: 229 - 233.
[18] Birecka H, Dinolfo TE, Martin WB and Frolich MW: Polyamines and leaf senescence in pyrollizidine alkaloid bearing Heliotropium plants. Phytochemistry 1984; 23 (5): 991 - 997.
[19] Andhiwal CK, Has C and Varshney RP: Chemical and Pharmacological studies of Heliotropium indicum. Indian Drugs 1985; 22 (11): 567 - 569.
[20] Mehta R, Arora OP and Mehta M: Chemical investigation of some Rajasthan desert plants. Indian J Chem.1981; 20B: 834
[21] Hussein Kamil Hamid and Enas J Kadhim: Extraction, isolation and characterization of Pyrrolizidine Alkaloids present in Senecio vulgaris Linn grown in IraqHussein Kamil Hamid and Enas J Kadhim. Journal of Pharmacognosy and Phytochemistry 2016; 5 (6): 28 - 37