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Research Article

Isolation, Identification and quantitative analysis of Ellagic acid: a tannin compound from *Helicteres isora*

Ramraj Meena, Ridhi Joshi*, Rishikesh Meena,Vidya Patni

Plant Pathology, Tissue Culture and Biochemistry Laboratory, Department of Botany, University of Rajasthan, Jaipur, India

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ABSTRACT

Plant derived secondary metabolites have widely attracted humans with great interest due to their immense medicinal and pharmacological properties. Ellagic acid, a natural phenolic compound found in many fruits exhibits both antimutagenic and anticarcinogenic activity. Qualitative analysis of the plant samples of *Helicteres isora* showed the presence of ellagic acid in *in vivo* (stem bark) and *in vitro* (callus) samples. Presence of isolated ellagic acid was confirmed by superimposable IR spectra of isolated and authentic samples of ellagic acid. The ellagic acid was further identified and confirmed by using different techniques such as TLC (R_f 0.41), and HPLC (R_t =5.546 min) studies.

Introduction

Medicinal plants have been decisive for sustenance in health and happiness of mankind since time immemorial.

The pharmacological activities of any plant is because of the presence of primary metabolites, secondary metabolites and its secretary products, comprising the phenolic compounds, alkaloids, tannins, saponins, carbohydrates, glycosides, flavonoids, steroids, etc.[1-2] Phenolic compounds are the most ubiquitous and most useful of plant metabolites and represent the most studied phytochemicals in different areas of plant research.[3] They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, anti-inflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities.[4] Extraction and characterization of several active phytocompounds from these green factories have given birth to high activity profile drugs. Tannins are natural compounds widely distributed in the plant kingdom. The function of tannins is the defense system of plants against microbial and animal attacks due to their astringent capacity and the ability to form complexes with proteins and polysaccharides . [5] Ellagic acid was first discovered by chemist Michel Eugene Chevreul in oak galls. It is a phenolic compound that exhibits both antimutagenic and anticarcinogenic activity.[6] It can act as antioxidant, and has been found to cause cell death (apoptosis) in cancer cells. Ellagic acid is also said to reduce heart disease, birth defects, liver problems and promote wound healing[7]. There are also reports that it may help the liver to break down or remove some cancer causing substances from blood.[8] It can also inhibit the growth of skin, oesophagus, lung tumors caused by carcinogens.[9]

Description of the plant

Helicteres isora L. commonly known as Indian screw tree or Spiral bush, belongs to family Sterculiaceae . It is a sub-deciduous large shrub or tree, 4-5m tall and occurs throughout India, Nepal and Sri Lanka and comprises of about 60 species .[10] It is commonly known as "Marorphali". It is generally found on hill slopes in India. In Rajasthan, it is found in dense forests of Aravalli hills upto a height of 200-400 meters.

Medicinal Importance of the Plant

Helicteres isora L. is used as an astringent, in acidity treatment, diarrhoea, yellowspot in the white of the eye. The plant is used in the treatment of

***Corresponding Author: Ridhi Joshi,** Plant Pathology, Tissue Culture and Biochemistry Laboratory, Department of Botany, University of Rajasthan, Jaipur, India **E-Mail:** ridhi.joshi316@gmail.com

gastric ailments and possess antibacterial activity and hypoglycemic activity.[11] The plant also shows inhibitory activity against avian myeloblastosis virus[12] and human immunodeficiency virus .[13] The fruits of this plant are astringent, stomachic, vermifugal, vulnerary, used in griping of bowels and flatulence in children[14] as well as have antispasmodic effect.[15] The root juice is claimed to be useful in treating cough, asthma, diabetes, emphysema, intestinal infection, snake bites and a cure for scabies when applied topically. Phytochemical analysis of the plant revealed the presence of phenols, flavonoids, alkaloids, glycosides, phytosterols, carotenoids, tannins, neolignans, rosmarinic acid derivatives, betulic acid, daucosterol, anthoquinones, sterols, lupeol, β-sitosterol, α and β amyrin, taraxerone and volatile oil in varying concentrations.[16-18] Flavones such as methyl ether, 7,41-di-o-methyleisoscutellarein i.e. (5,8-dihydroxy-7,41-flavones) along with kaempferol-3-ogalactoside (trifolin) and herbacetin-8-oglucoronide(hibifolin) from the leaves were isolated and characterized .[19]

Material and Methods

Plant Material: The stem bark of *Helicteres isora* was collected cut dried and powdered. Similarly, mature callus tissues were harvested at their maximum growth indices (six weeks). Each of the dried and powdered samples were soxhlet extracted in methanol (100ml/gm dry weight) on a water bath for 24 hours.

Thin Layer Chromatography

The glass plates (20 × 20 cm) coated with silica gel 'G' (0.2-0.3 mm thick and 30gm/60 ml distilled water) were dried at room temperature. The dried plates were activated at 100°C for 30 minutes in an oven and cooled at room temperature. Each of the extracts was applied 1 cm above the edge of the chromatographic plates along with the marker (standard ellagic acid) and developed in an air tight chromatographic chamber which was already saturated with a solvent system of Toluene-Ethyl acetate-Formic acid-Methanol (3:3:0.8:0.2 v/v). Other solvent system such as Toluene- Ethyl Formate- Formic Acid (5:5:2) were also used but Toluene-Ethyl acetate-Formic acid-Methanol (3:3:0.8:0.2) gave better separation of the compounds. Such chromatograms were air-dried, visualized under UV light at 254 nm and the fluorescence or the colours were noted. Bands were detected after spraying with 5% methanolic ferric chloride solution.[20] Plates were also placed in a chamber saturated with I2 vapours to observe the colour of spots and to locate the spots in unsprayed developed chromatograms, exposure to I2 vapours also proved useful. The spots corresponding to the respective marker (Ellagic acid) were scraped separately eluted with methanol and the process was repeated until sufficient crystallizable amount of each of the substances was obtained. Each was retested by co-TLC, revealing their homogenous nature and subjected for further identification.

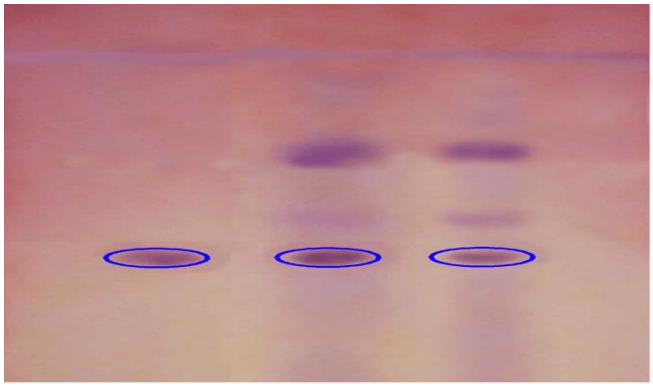

Fig 1 : TLC chromatogram of standard and isolated Ellagic acid of *Helicteres isora*

Detection and quantification of Ellagic acid

Detection was done using reverse-phase HPLC (High Performance Liquid Chromatography). Waters HPLC (Model 2487) instrument. The column used was a 15 cm hypersil C18 reverse phase column (150 *4.6 mm) with 5µ particle packing and while the mobile phase (Composition: 495 V Methanol, 495V Water, 10 V orthophosphoric acid) passed through the column (injection volume =20 µl)at the rate of 1ml/min. System configuration was LC 2010 AHT Auto Sampler (UV-Vis detector)Shimadzu and LC Solution(Shimadzu).Peaks of standard ellagic acid (rt-5.549)were compared with the peaks of *in vivo* plant parts(stem bark) (rt 5.546) of *Helicteres isora*.

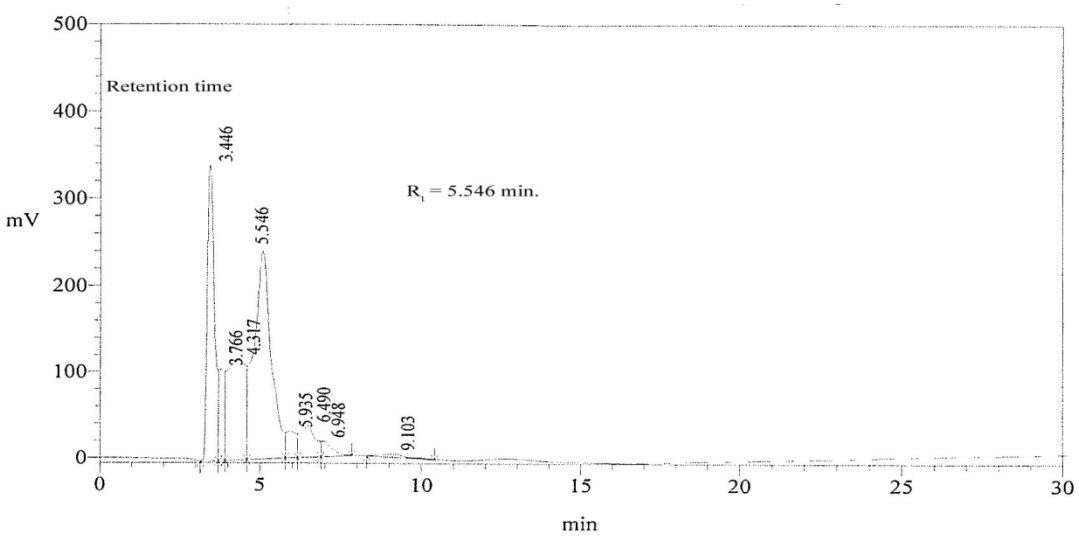

Fig 2: HPLC chromatogram of Ellagic acid isolated from stem bark

IR studies

Crystallised isolates from stem bark sample were subjected to infra-red spectropotometric(Perkin-Elmer 337 Grating, Infra-red spectrophotometer using nujol or Kbr pellets)studies along with respective standard compound of ellagic acid.

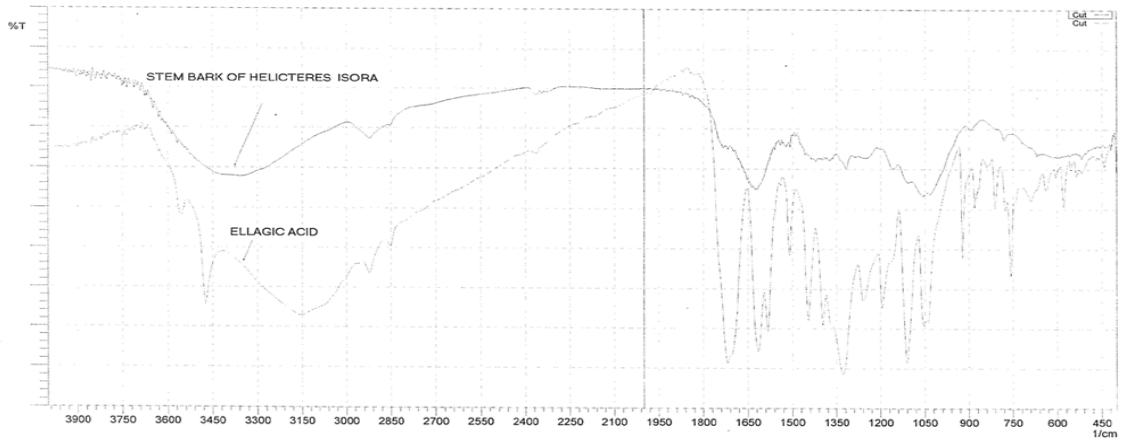

Fig 3: Superimposed IR spectra of standard Ellagic acid and isolated Ellagic acid

Result and Discussion

Ellagic acid from plant part (stem bark) and callus tissue samples were confirmed by TLC, IR spectra and HPLC chromatogram. When the developed plates were sprayed with 50% sulphuric acid these showed dark coloured spots which coincided with that of the reference ellagic acid. Rf value (0.41) of ellagic acid isolated from the samples coincided with the Rf value of standard ellagic acid. The characteristic IR spectral peaks were found to be superimposible with those of their respective standard reference compounds of ellagic acid. *In vivo* (stem bark) tissue of *Helicteres isora* have shown almost similar type of chromatogram with peak at 5.546 and standard ellagic acid has shown peak at 5.549.

References

1. Naik GH., Priyadarsini KI., Hari M., Free radical scavenging reactions and phytochemical analysis of Triphala, an ayurvedic formulation, Curr. sci 2006; 90: 1100-1105.
2. Sati SC., Sati N., Rawat U., Sati OP., Medicinal plants as a source of antioxidants, Res. J. Phytochem 2010; 4: 213-224

3. Boudet A.M., Evolution and current status of research in phenolic compounds, Phytochemistry 2007; 68 :22-24: 2722-2735.

4. Han X., Shen T and Lou H., Dietry polyphenols and their biological significance, Int.J. Mol. Sci 2007; 8:950-988

5. Swain T. and Bate-Smith EC., Flavonoid Compounds in Comparative biochemistry, M. A. M. Florkin. New-York, Academic Press. 1962; 3: 755-809.

6. Festa F., Aglitti T., Duranti G., Ricordy R., Perticone P and Cozzi R., Strong antioxidant activity of ellagic acid in mammalian cells *in vitro* revealed by Comet assay. Anticancer Res 2001; 21:6A: 3903-3908.

7. Ahn D., Putt D., Kresty L., Stoner GD., Fromm D and Hollenberg PF., The effect of dietary ellagic acid on rat hepatic and esophageal mucosal cytochrome p450 and phase II enzymes. Carcinogenesis 1996; 17: 821-828.

8. Labrecque L., Lamy S., Chapus A., Mihoubi S., Durocher Y., Cass B *et al*. Combined inhibition of PDGF and VEGF receptors by ellagic acid, a dietary-derived phenolic compound. Carcinogenesis 2005; 26:4: 821-826.

9. Mertens-Talcott SU., Talcot ST and Percival SS., Low concentration of quercetin and ellagic acid synergistically influence proliferation, cytotoxicity and apoptosis in MOLT-4 leukemia cells. J. Nutr. 2003; 33:8:2669-2674.

10. Kubitzki K., and Bayer C: Flowering plants – Dicotyledons: Malvales,Capparales, and non-betalain Caryophyllales. In: The families and genera of vascular plants. Berlin: Springer-Verlag; 2003; 5.259–60.

11. Jagadheshan H.**,** Charkrabati R., Sharma VM., Vikramadithyan RK., Mullangi R., Rao YN., Euglycemic and hypolipidemic activity of *Helicteres isora* extract. J Ethnopharmacol 2002; 81:343-349.

12. Kusumoto IT., Shimada I., Kalkiuchi N., Hattori M., Namba T., Supriyatna S., Inhibitory effects of Indonesian plant extracts on reverse transcriptase of an RNA tumor virus (I). Phytother Res 1992; 6:241- 244.

13. Otake T., Mori H., Morimoto M., Ueba N., Sutardjo S., Kusomoto I., *et al*. Screening of Indonesian plant extracts for anti human immuno deficiency virus type1 (HIV-1) activity, Phytother Res 1995; 9:6–10.

14. Kumar G., Banu GS., Murugesen AG., Influence of *Helicteres isora* L. bark extracts on glycemic control and renoprotective activity in streptozotocin- induced diabetic rats, Int Jour Pharma Sci Nanotech 2008; 1:275-280.

15. Pohocha N and Grampurohit ND., Antispasmodic activity of the fruits of *Helicteres isora* L., Phytotherapy R 2001; 15:49-52.

16. Gayathri P., Gayathri D., Srinivasan S and Saroja S., Screening and quantitation of phytochemicals and nutritional components of the fruit and bark of *Helicteres isora.*, Hygeia Journal for Drugs and Medicines 2010; 2:1:57-62.

17. Loganayaki N., Siddhuraju P and Manian S., Antioxidant activity and free radical scavenging capacity of phenolic extracts from *Helicteres isora* L. and *Ceiba pentandra* L., Journal of Food Science and Technology 2013; 50:4:687-95

18. Satake T ., Kamiya K., Saiki Y., Hama T., Fujimoto Y and Kitanaka S., Studies on the constituents of fruits of *Helicteres isora* L., Chemical and Pharmaceutical Bulletin 1999; 47:10: 1444-1447.

19. Ramesh P and Yuvarajan C., A new flavones methyl ether from *H. isora* , Journal of Natural Products 1995; 58:8:1242-1243.

20. Wagner H and Bladt S: Plant Drug Analysis: A thin layer chromatography Atlas. Springer-Verlag Berlin Heidelberg New York , second edition 1996.

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