Taxonomical Analysis of Genus *Heliotropium* L. and its Phytochemical and Pharmaceutical Properties

Dr. Rakesh kumar verma

Associate Professor, Department of Botany

M D Girls Science P G College, Jhunjhunu (Rajasthan)-333001

Abstract

Heliotropium (Boraginaceae) is a widely spread genus of plants found in the temperate and the tropical regions of both hemispheres and used for the treatment of diseases from ancient times. In folk medicine history, the plants of genus Heliotropium include treatments of inflammations, gout, rheumatism, skin diseases, menstrual disorder, and poisonous bites. Phytochemical reports on genus Heliotropium revealed the presence of many bioactive components especially pyrrolizidine alkaloids, terpenoids and flavonoids. A large number of extracts and bioactive constituents of different species of genus Heliotropium revealed significant biological activities such as antimicrobial, antitumor, antiviral, anti-inflammatory, wound healing, cytotoxicity and phytotoxicity. Different parts of plants of genus Heliotropium are examined for valuable pharmacological and pharmaceutical properties. Although it's medicinal importance is recognized worldwide, this review artifact will thus, comprehensively describe the various medicinal effects of the plants, isolation of a large number of secondary metabolites and important pharmacognostic characteristics of genus Heliotropium of Boraginaceae family.

Keywords - Heliotropium, Boraginaceae, Bioactive constituents, Pharmacological and Pharmaceutical properties.

Introduction

The family Boraginaceae comprised of 100 genera and about 2000 species. The plants of this family are widely distributed in temperate, especially mediterranean and tropical regions. In Pakistan, this family is represented by 32 genera and 135 species. Moreover, some species namely *Cordia*, *Echium*and *Anchusa*are cultivated. *Heliotropium*, *Cordia*, *Arnebia*, *Martensia* and *Trichodesma* are the important genera of the Boraginaceae family. Fruits of the *Cordia* are used as diaphoretic and sometimes as astringent. The leaves and roots of *Trichodesmaindicum*Lehm. are effective against snake bites, urinary diseases and used as diuretic. The roots of this plant is also applied as a paste on swellings, joints and is used in dysentery in children. Today, *Alkanna*(*Alkannatinctoria*L.) root is used almost exclusively as a cosmetic dye. Orally, it has been used for diarrhea and gastric ulcers. Traditionally, *Alkanna* root has been used topically to treat skin wounds and diseases.

*Heliotropium*is a large genus of family Boraginaceae which consists of about 250-300 species in the whole world. These species are widely distributed in temperate and tropical regions of both hemispheres. The name “heliotrope” derives from the fact that these plants turn their leaves to the sun. In India, it is the largest genera of family Boraginaceae with 23 species. Some of the taxa of this genus are *H. bacciferum*Forssk., *H. europium* L., *H. baluchistanicum* K., *H. gillianum* R., *H. biannulatum* B., *H. ovalifolium*Forssk., *H. strigosum*Willd., *H. eichwaldi*Steud., *H. indicum* L., *H.glutinosum* Phil.,*H. sclerocarpum* Phil., *H. sinuatum*Miers., *H. subulatum*Hochst., *H. foertherianum* D. and *H. ovalifolium*Forssk.

Taxonomical studies of Genus Heliotropium

Heliotropium L. Sp. Pl.: 130. 1753

Annual or perennial herbs, or rarely undershrubs; hairy or scabrous; leaves alternate, linear to lanceolate, flowers in terminal monochasial or dichasial scorpioid cymes; bracts small or leafy; calyx 5-partiate, segment linear or lanceolate; corolla tubular, throat glabrous, lobes triangular rarely caudate, imbricate; stamens 5, inserted in corolla tube; ovary 4-celled, style terminal, short or long with ring-shaped stigma, ending in conical stigmatic head; fruit 2–4 lobed, dry nutlets.

Key to the species

1a Plants prostrate, decumbent or semi-erect:
  2a Plants soft velvety; leaves obovate, not succulent; style present.....***H. supinum***
  2b Plants glabrous; leaves lanceolate, succulent; style absent.....***H. curassavicum***
1b Plants erect and woody:
  3a Corolla other than white:
    4a Corolla yellow, lobes caudate- acuminate; anthers bidentate.....***H. zeylanicum***
    4b Corolla purple, lobes obtuse; anthers not bidentate.....***H. indicum***
  3b Corolla white:
    5a Leaves sessile, lanceolate; stigmatic head conical.....***H. bacciferum***
    5b Leaves petiolate, elliptic; stigmatic head bifid.....***H. europaeum***

Heliotropium bacciferum Forssk. Fl. Aegypt.-Arab. Descr. Pl. 38. 1775; Bhandari, Fl. Ind. Desert 208. 1990; Shetty & Singh, Fl. Raj. 2: 507. 1991.

Heliotropium undulatum Vahl, Symb. Bot. 1: 13. 1790; Clarke in Hook.f, Fl. Brit. India 4: 150. 1883;

Blat. & Hall., Fl. Ind. Desert 26 (2): 542. 1919

Erect, bushy perennial herb. Stem hairs stiff, white, bulbous-based; older shoots blackish brown. Leaves subsessile or sessile, 10–45 × 4–12 mm, lanceolate, acute, attenuate at base, stiff hairy on both sides, undulate, slightly revolute. Flowers on forked terminal spikes, ebracteate, 1.5 mm across. Calyx campanulate, 5-lobed to the base, acute, stiff hairy outside, glabrous inside. Corolla tubular, 2.5–4 mm long, tube hairy outside. Stamens 5, inserted on corolla tube, anthers creamy white. Ovary 4-celled, 0.5–0.7 mm; disc toothed; style 0.5 mm long, stigma green, discoid, ringed around the base 1 mm long, stigmatic head conical. Fruits subglobose; style persistent. Seed 2 × 1 mm, white corky cell on dorsal side; becomes brown and flaccid at age.

Flowering and fruiting: Mainly July–September or throughout the year.

Heliotropium curassavicum L. Sp. Pl. 1: 130. 1753; Mahesh. & Sharma, Bull. Bot. Surv. India 11: 455. 1972; Pandey *et al*., Bull. Bot. Surv. India 24: 134. 1982; Bhandari, Fl. Ind. Desert 209. 1990; Shetty & Singh, Fl. Raj.: 508. 1991

Type: Heliotropium curassavicum L. Linnean herbarium (S-LINN), Microfiche no. IDC 67.1

Prostrate, or sub-erect, succulent, grayish green, perennial herb. Stem fragile, glaucous, glabrous. Leaves sub-sessile, oblanceolate, or linear-lanceolate, entire, obtuse or sub-acute. Flowers in extra axillary scorpioid spikes, dichasial, 2.5 mm across, ebracteate, sessile. Calyx campanulate, lobes lanceolate, acute. Corolla tube

2.5 mm long, throat yellow becomes violet, lobes obtuse, glabrous. Stamens 5, anthers ovate-lanceolate, acuminate, 1 × 0.5 mm, epipetalous, creamy white. Ovary 0.2–0.3 mm long, 4-celled, stigma discoid and ringed around the base, stigmatic head micro-papillate. Fruits sub-globose with persistent stigma, 2.5 mm diam., separated into 4 at maturity. Seeds convex with revolute margin, lateral faces slightly rugose, 1.5 × 1 mm, light brown.

Flowering and fruiting: Throughout the year.

Heliotropium europaeum L. Sp. Pl.: 130. 1753. *Heliotropium europaeum* var. *europaeum* Kazmi, J. Arnold Arb. 51(2): 176. 1970

Type: Heliotropium europaeum L. Linnean herbarium (S-LINN), Microfiche no. IDC 66.16.

Erect, sparsely branched up to 1 m height, annual. Stem green woody, densely pilose. Leaves petiolate, elliptical, 3–8 × 2–4.5 cm, adpressed strigose on both sides, sub-acute or obtuse. Flowers in extra axillary binate to trinate, rarely tetranate spikes, ebracteate, sessile. Calyx campanulate, lobes 5, all divide almost to base, lanceolate, acute, enlarge in fruit. Corolla tube 2.5–3 mm long, narrow toward apex, throat yellow, lobes 1 × 1 mm, sub-orbicular, white. Stamen 5, epipetalous, anthers ovoid, acuminate, 1.2 × 0.3 long, creamy white. Ovary 0.5 mm long, 4-celled, disc present, style 0.2 mm long, stigma discoid green and ringed around the base of 1 mm conical stigmatic head. Fruits globose, 2 mm diam., separated into 4 nutlets at maturity. Seeds greenish brown, 2 × 1 mm, dorsal face densely pubescent with yellow hairs, ventral face rugose and blackish.

Flowering and fruiting: December – February.

Heliotropium indicum L. Sp. Pl. 1: 130. 1753; Clarke in Hook.f, Fl. Brit. India 4: 152. 1883; Duthie. Fl. Gangetic Plain 2: 90. 1911. *Tiaridium indicum* Lehm. in Wight, Illustr. Indian Bot. 2. t. 171. 1841

Type: Heliotropium indicum L. Linnean herbarium (S-LINN), Microfiche nos. IDC 66.11 and IDC 66. 12.

Perennial rigid herbs, up to 1–1.5 m height. Stem much branched, green and woody; indumentum of long hispid hairs mixed with short adpressed hairs. Leaves alternate, petiolate, ovate, undulate, slightly crispy, acute, rugose. Flowers on terminal extra axillary helicoid spikes up to 15 cm long, often coiled at tip, 2 mm across, ebracteate. Calyx campanulate, lobes 5, cleft almost up to base, acute, 1.5 × 0.5 mm, puberulent at margins, persistent. Corolla tube 2.5–3 mm, pubescent outside, lobes 5, obtuse, not distinctly separate, purple or bluish purple, glabrous. Stamens 5, epipetalous, 1 mm long, oblong, creamy white. Ovary dome-shaped, 0.5 mm long, style 0.5 mm long, a green disc at base of ovary, stigma discoid, green, ringed around the base, stigmatic head 0.3 mm long, glabrous. Fruits sub-globose, 1.5-2 mm diam., segmented into 4 at maturity. Seeds beaked, dorsal surface ridged and grooved, 3 × 2 mm.

Flowering and fruiting: September – December.

Heliotropium supinum L. Sp. Pl. 1: 130. 1753; Clarke in Hook.f., Fl. Brit. India 4: 149. 1883; Wight, Icon. t. 1387. 1850; Duthie, Fl. Gangetic Plain 2: 91. 1911; Blat. & Hall., Fl. Ind. Desert 26(2): 541. 1919. *Heliotropium malabaricum* Retz., Observ. Bot. 4: 24. 1786

Type: Europe: Spain: in Salamanticae juxta agros Monspelii in littore. Herb. LINN-179.8

A procumbent annual herb; branches spreading erect or procumbent, terete, densely pilose. Leaves opposite with each branch and then alternate, petiolate, obovate, 2–3 × 1–1.5 cm, wavy, grayish green. Flowers in extra axillary scorpioid spike up to 5 cm long, ebracteate, sessile. Calyx campanulate, 3-5 × 1 mm long, lobes oblong, divided to the base, obtuse. Corolla tube 3 mm long, lobes 1 mm long, obtuse, glabrous inside, white. Stamen 5, epipetalous, 1 mm long, acuminate, creamy white. Ovary 4-celled, dome-shaped, 0.3 mm long, green, glabrous, slightly constricted between carpels, disc toothed; style 0.5 mm, retrosely hairy, stigma discoid green, ringed, cone-shaped stigmatic head bears characteristic 5 hairs on top. Fruits sub-globose, 3 mm diam., covered in persistent calyx light brown. Nutlets ovoid, 3 × 3 mm, middle part dark brown to blackish, minutely tuberculate with lateral faces light in colour, wings 3 mm wide.

Heliotropium zeylanicum (Burm.f.) Lam., Encycl. 3(1): 94. 1789; Clarke in Hook.f., Fl. Brit. India 4: 148. 1883; Wight, Icones Pl. Indae Orient. 3: 892. 1846.

Tournefortia zeylanicum Wight, Illustr. Indian Bot. 2: t. 170b. 1850. *Heliotropium subulatum* (Hochst. ex DC.) Vatke, Oesterr. Bot. Zeitschr. 25: 166. 1875

Holoype: Southern India, Madras, Tuticorin, *Garcin* s.n. (G-BRUM)
Icones: Burmann, N.L., Flora Indica: 40. t. 16. f. 2. 1768; Wight, Illustration of Indian Botany 2: 170 f. B. 1841; Wight, Plantarum Indiae Orientalis 3: t. 892. 1849

Erect perennial herb, up to 0.5 m height, looks bushy. Young stem green older woody, with brown fissured bark. Leaves alternate, 4–8 × 1.5–2 cm long, sessile or subsessile, broadly lanceolate in monsoon, undulate, slightly revolute, acute, base attenuate, clothed with white bulbous-based strigose hairs intermixed with glandular ones. Inflorescence on a 2–4 cm long common peduncle**.** Flowers in bifurcate

scorpioid up to 40 cm long terminal spike, 5 mm across, sessile, ebracteate. Calyx cup-shaped, 5-lobed acute, glandular hairy outside, glabrous inside. Corolla tube 4 mm long, lobes 2.5 × 1 mm, caudate or acuminate, inflexed just above from middle, a minute deltoid teeth present at sinuses between the lobes. Stamen 5, apex bidentate, 1 mm long. Ovary 0.5 mm, disc thick, style 1.5 mm, stigma discoid, green and ringed around the base of 1 mm long conical stigmatic head. Fruits sub-globose, 3 mm in diam., separated into 4 nutlets at maturity. Seed dark or light brown, 3-faced, dorsal face convex, reticulate rugose; lateral faces white, pointed at ends.

Flowering and fruiting: Throughout the year.

Heliotropium bacciferum

Heliotropium curassavicum

Heliotropium europaeum

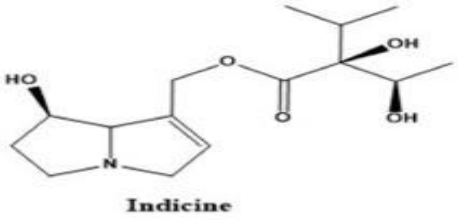
Heliotropium indicum

Heliotropium supinum

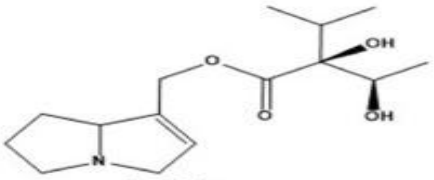
Heliotropium zeylanicum

Phytochemical Analysis of Genus Heliotropium

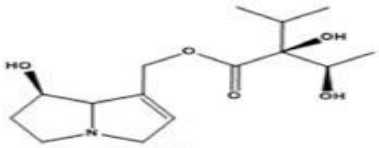
A variety of constituents are identified and isolated from different species of genus *Heliotropium* which are phytochemically active and have significant therapeutic effects. Many classes of organic compounds such as pyrrolizidine alkaloids (PAs), phenolic compounds, terpenoids and quinones are very abundantly present in *Heliotropium*. Pyrrolizidine alkaloids (PAs) are mainly occur as esters being accompanying with characteristic mono or dibasic acids known as necic acids. Triterpenoids are the compounds which contain almost 30 carbon atoms and occur as esters or glycosides. Flavonoids are the largely occurring phenols formed of three acetate units and a phenylpropane unit.



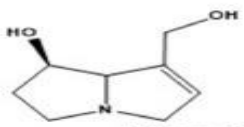
Indicine



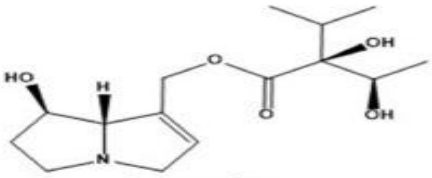
Supinine



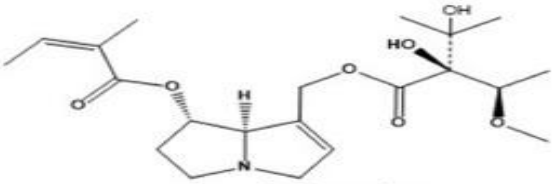
Rinderine



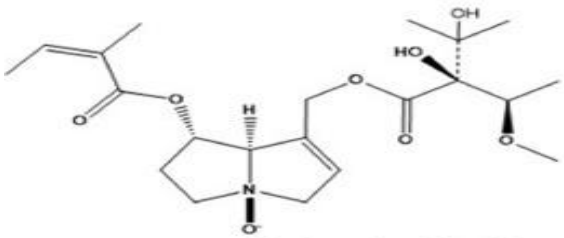
Retronecine



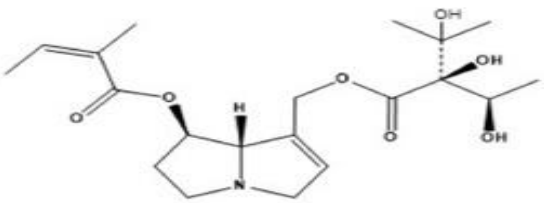
Lycosamine



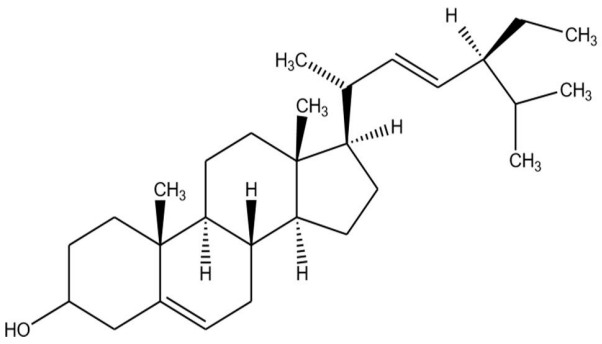
Lasiocarpine



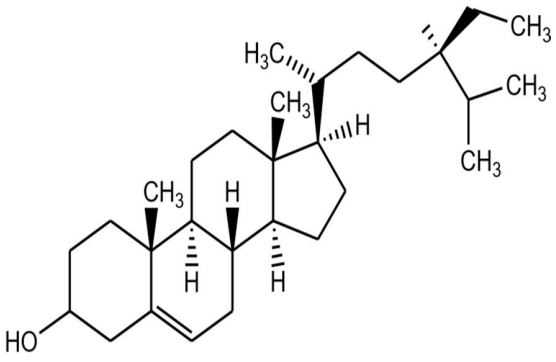
Lasiocarpine- *N*- oxide



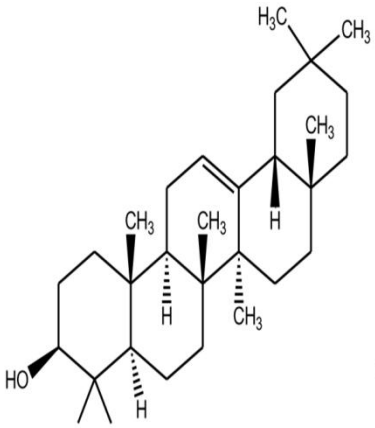
Heliosupine



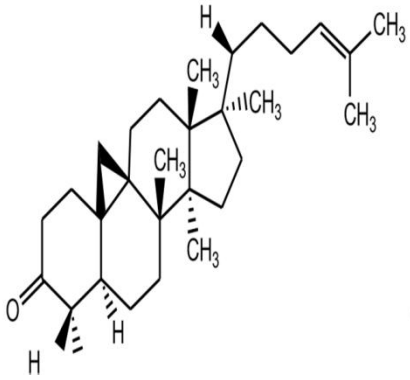
(Stigmasterol)



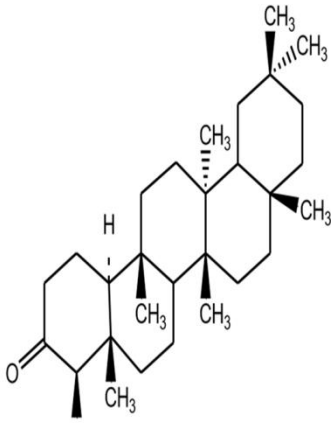
(β-sitosterol)



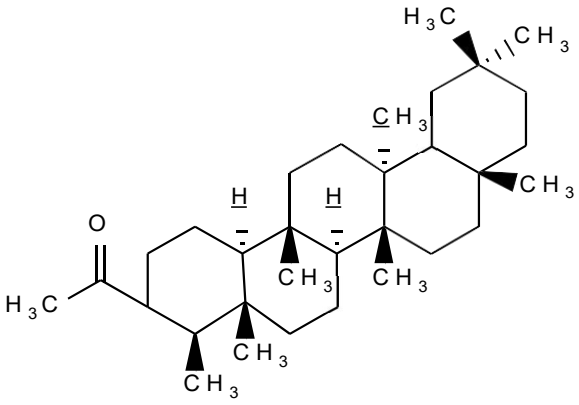
(β-amyrin)



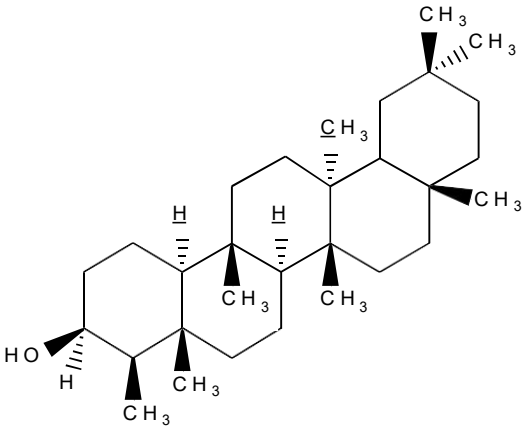
(Cycloartenone)



(Friedelin)



Epifriendenyl acetate



Friedelan-3 beta-ol

Pharmaceutical properties of Genus Heliotropium

Plants of the genus *Heliotropium* display a wide range of pharmacological activities. Different biological activities of extracts and their bioactive constituents provide a basis for better understanding of the underlying mechanisms involved.

Antibacterial activity

Antibacterial activity of the methanolic extract of whole plant of *H. strigosum* showed different zones of inhibition which are formed by crude extract, ethyl acetate fraction, chloroform fraction, aqueous fraction, n-hexane fraction and standard doxycycline (30µg). All these fractions are active against *Staphylococcus epidermidis* with the minimum inhibitory concentrations (MICs) of 8, 6, 8, 8, 6 mg/ml but no fraction showed any activity against *Escherichia coli.* The activity against methicillin resistant *Staphylococcus aureus* was only shown by ethyl acetate fraction with the zone of inhibition recorded is 8mm. Other fractions and crude extract did not demonstrate any antibacterial activity against methicillin resistant *S. aureus.* The standard doxycycline fraction showed activity against all bacteria used in the bioassay. From the ethanolic extraction of aerial parts of *H. subulatum* two fractions such as petroleum ether and chloroform experienced the significant activity against bacteria such as *E. coli*, *Streptococcus pneumoniae*, *Bacillus subtilis*, *B. anthracis* and *S. aureus*. Among these two fractions, the chloroform fraction retains maximum activity against *E. coli* with the zone of inhibition logged is 12.61±0.361.

The methaolic extract of aerial parts of *H. indicum* has broad spectrum of antibacterial activity against *S. aureus*, *Streptococcus pyogenes*, *S. pneumonia*, *Salmonella typhi*, *Corynebacterium ulcerans*, *E. coli* and *Klebsiella pneumonia* with the zones of inhibition 32, 35, 30, 0, 0, 28, 27 mm verified for these bacteria. Methanol extract of the leaf of *H. indicum* was evaluated for its antibacterial activity against five bacterial isolates comprising of four gram-negative bacteria including

E. coli, *Pseudomonas aeruginosa*, *Klebsiella species* and *Proteus mirabilis* and one gram positive, *S. aureus* at the concentrations of 6.25, 12.5, 25, 50, 100 and 200 mg/ml of plant extract respectively. Both *S. aureus* and *Klebsiella spp*. were inhibited at 50, 100 and 200 mg/ml with MIC of 3 mg/ml while *P. aeruginosa* and *P. mirabilis* with MIC of 10 mg/ml were inhibited at 100 mg/ml and 200 mg/ml and *E. coli* with MIC of 20 mg/ml was inhibited only at 200 mg/ml concentration of the extract respectively. The essential oil of *H. europaeum* obtained from the process of hydrodistillation were tested on *B. subtilis*, *S. aureus*, *E. coli* and *S. typhi*.

The consequences showed the major antibacterial activity against *B. subtilis* and *S. typh i*respectively. Different fractions of methanolic extract such as chloroform, petroleum ether, ethyl acetate and aqueous fraction of aerial parts of *H. bacciferum* showed significant antibacterial activity against *S. aureus*, *B. cereus*, *E. coli*, *Salmonella enteritidis* and *P. aeruginosa.* The chloroform and petroleum ether fraction showed that it inhibits the growth of *S. aureus*, *B. cereus* and *P. aeruginosa* with MIC of 15.625 µg/ml, *S. enteritidis* with 62.5 µg/ml and *E. coli* with 125 µg/ml respectively. The aqueous extract exhibited that it prevents the growth of *S. aureus*, *B. cereus* and *S. enteritidis* with MIC of 7.8125 µg/ml and *E. coli*, *P. aeruginosa* with 15.625 µg/ml correspondingly.

Antifungal activity

Different fractions of methanolic extract of whole part of *H. strigosum* revealed prominent antifungal activity. The chloroform and n-hexane fractions exposed antifungal activity against *Aspergillus niger*, *A. fumigatus*, *Fusarium solani and A. flavus* with the MIC of 2.5 mg/ml. Crude extract was inactive against *A. flavus*but showed activity against *A. niger*, *A. fumigatus* and *F. solani* with MIC of 2.5 and 3.5 mg/ml. Ethyl acetate and aqueous fractions did not show activity against any fungal strain.

The ethanolic, chloroform, petroleum ether, aqueous and residue extracts of stem and leaves of *H. curassavicum* exhibited significant in vitro antifungal activity. The diffusable metabolites of *H. curassavicum* demonstrated noticeable inhibitory effects against *Penicilliumcitrinum* followed by *Candida albicans*.The alcoholic extract of whole plant including roots of *H. indicum* was tested against certain fungi named as *A. niger*, *A. wentii*and *Rhizopusoryzae*. The extract exhibited significant activity at the concentration of 100 µg/ml with the inhibition area logged against *A. niger*, *A. wentii*and *R. oryzae* is 8.00, 9.00, 8.00 mm respectively as compared with the standard fluconazole.

Antioxidant activity

The crude extract and subsequent sub-fractions of whole plant of *H. strigosum* were screened for antioxidant activity by using 1,1-diphenyl-2-picrylhydrazyl scavenging assay (DPPH). The n-hexane fraction of methanolic extract displayed strong antioxidant activity with an EC_{50} value of 35.53 µg/ml while ethyl acetate fraction also showed significant antioxidant activity with an EC_{50} value of 30.34 µg/ml. The aqueous fraction also revealed good antioxidant activity and had an EC_{50} value of 20.51 µg/ml. The crude extract did not show any antioxidant activity, same was true about the chloroform subfraction.The flavonoids isolated from the resinous exudate of *H. sinuatum* revealed significant antioxidant activity. The chloroform and methanolic extract of whole plant material of *H. zeylanicum* hold substantial antioxidant activity along with itsantidiabetic andantihyperlipidemic effects.

Anti-inflammatory activity

The crude extract of the whole plant of *H. strigosum* and its subsequent solvent fractions showed antiinflammatory activity in carrageenan-induced edema and xylene-induced ear edema. In carrageenaninduced edema, the ethyl acetate fraction was most dominant with 73.33% inhibition followed by hexane fraction (70.66%).

When the extracts were tested against xylene-induced ear edema, ethyl acetate and hexane fractions were found active with 38.21% and 35.77% inhibition, respectively. The chloroform extract of dried leaves of *H. indicum* demonstrates significant anti-inflammatory activity in carrageenan-induced edema and cotton pellet granuloma models of inflammation. The extract of *H. indicum* with a concentration of 150mg/kg body weight showed maximum 80.0% inhibition on carrageenan-induced raw paw edema compared with the positive control drug, diclofenac sodium.

Antinociceptiveand anticonvulsant activity

The crude extracts and subsequent solvent fractions of *H. strigosum* were tested for antinociceptive and anticonvulsant activity in animal models. In acetic acid-induced writhing test, crude extract, n-hexane, ethyl acetate and aqueous fractions established marked reduction of nociception at test doses 50, 100 and 200 mg/kg intraperitoneally. When challenged against thermally induced pain model, pretreatment of extracts demonstrated prominent enhancement at test doses 50, 100 and 200 mg/kg intraperitoneally. In both tests, inhibition of noxious stimulation was in a dose-dependent manner, and the ethyl acetate fraction was most dominant.

Thus, the extracts of *H. strigosum*showed significant antinociceptive effect in both centrally and peripherally acting pain models. The chloroform extract ofdried leaves of *H. indicum* was examined for antinociceptive activity in hot plate model in male swiss albino mices. The extract of *H. indicum* with a concentration of 150 mg/kg body weight showed maximum 82.79% antinociception in the hot-plate test as compared to a control drug, pentazocine. The methanol extract of the dried roots of *H. indicum* was observed for substantial antinociceptive activity in acetic acidinduced writhing mices. The extract produced significant inhibition in acetic acid-induced writhing mices at the oral doses of 250 and 500 mg/kg body weight

comparable to the standard drug diclofenac sodium at the dose of 25 mg/kg of body weight.

Antineoplastic and antiviral Activity

The n-hexane, dichloromethane fractions of ethanolic extract of aerial parts of *H. subulatum* and its subsequent crude extract was examined forsignificant antineoplastic and antiviral activities. For antineoplastic activity, it was found that ethanol extract, n-hexane and dichloromethane fractions revealed significant activity with the inhibition of 19.3 &32.2 %, 22.5 & 16.1 %, 09.6 & 06.4 % at the dose of 50 and 100 µg/kg/day. For antiviral activity, it was revealed that theethanol and hexane crude extracts showed significant activity to *Coxsackie, Poliomyelitis* and *Measles* at concentrations of 500 & 100 µg/ml respectively.

Cytotoxicity and phytotoxicity

The crude extract of *H. strigosum* and its resultant fractions possessed strong cytotoxic and phytotoxic activity. In brine shrimp toxicology assays, the fractions of ethyl acetate and chloroform showed strong cytotoxic actions with LD_{50} 8.3µg/ml and LD_{50} 8.8µg/ml respectively, followed by relatively weak crude methanolic extractwith LD_{50} 909µg/ml and n-hexane fraction with LD_{50} 1000µg/ml while in the case of phytotoxic activity against *Lemnaacquinoctialis*, strong phytotoxic effect was showed by ethyl acetate fraction with LD_{50} 91.0µg/ml respectively while chloroform fraction, plant crude extract and n-hexane fraction caused 50%, 30.76 ± 1.1% and 30.7 ± 1.1% inhibitory action respectively at maximum concentration that is 1000 µg/ml. From the ethanolic extract of aerial parts of *H. subulatum*, nhexane, dichloromethane fractions of extract and crude extract were examined for cytotoxic activity. It was revealed that that n-hexane fraction showed potent cytotoxic activity at a concentration of 3mg/ml.

The aqueous extract of senescent leaves of *H. foertherianum* and one of its isolated compounds rosmarinic acid were assessed for its effects against a pacific ciguatoxin (P-CTX-1B) in the neuroblastoma cytotoxicity assay and the receptor-binding assay. The cytotoxicity elicited by P-CTX-1B was inhibited by the aqueous extract of *H. foertherianum* at concentrations up to 2734µg/ml and by rosmarinic acid up to 607 µg/ml, the concentrations at which they began to be cytotoxic. The methanolic extract of dried plant material of aerial parts of *H. zeylanicum* was examined for cytotoxicity *in vitro* against MRC5 human cell line. The extract demonstrated significant cytotoxic activity with an IC_{50} of 13.00 µg/ml.The methanolic extract of the dried roots of *H. indicum* was studied for considerable cytotoxic activity by using the brine shrimp lethality bioassay. The extract showed different mortality rate at different concentrations with the LC_{50} of 47.86 µg/ml and LC_{90} of 75.85 µg/ml respectively.

Antiproliferative and antitumor activity

Ethanolic extract of whole plant of *H. indicum* revealed substantial anti-proliferative activity against SK-BR-3 human breast adenocarcinoma cell line using MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide]assay. The IC_{50} value of extract is 34 ± 9.09 µg/ml as compared to the standard drug used, paclitaxel with IC_{50} value 22.20 ± 2.30 µg/ml. The petroleum ether extract of aerial parts of *H. ovalifolium* were tested to identify its ability to inhibit specific cytokines, interleukin-6 (IL-6) at the dose of 7.6 µg/ml respectively.The methanolic extracts of stem and leaf of *H. indicum*possessed a significant antitumor activity andIC_{50} for both the extracts found to be 200µg/ml, whereas stemextracts exhibited excellent activity up to 64.5% at 200µg/mlfollowed by leaf extract up to 49.67% at 200µg/ml respectively.

Antituberculosisactivity

The volatile oil isolated from the aerial parts of *H. indicum* was tested for antituberculosis activity and the outcomes exhibited significant activity against *Mycobacterium tuberculosis* attenuated strain with the MIC of 20.8 µg/ml as compared to the standard drugs used that were isoniazide and kanamycin.

Antidiuretic activity

The methanol extract of the dried roots of *H. indicum* was examined for antidiuretic activity by observing different urination parameters of mices. The study revealed that the extract has a marked diuretic effect by the electrolyte loss ratio (Na^+/K^+ excretion ratio was 1.38 and 1.45 at the doses of 200 and 400 mg/kg respectively) as that of the standard diuretic furosemide whose ratio was 1.37.

Histo-Gastroprotective activity

The histo-gastroprotective activity of the aqueous extract of the dried leaves of *H. indicum*was evaluated in wistar rats, where ulceration of the gastric mucosa was induced by the oral administration of 80mg/kg/body weight of Indomethacin. The aqueous extract exhibited histo-gastroprotective effect at the dose of 100, 200 and 400mg/kg/body weight respectively in a dose dependent manner. This effect of the aqueous extract might be due to the presence of its tannins, alkaloids and saponins.

Nephroprotective effect

The nephroprotective effect of methanolic extract of dried roots of *H. eichwaldi* was estimated in male swiss albino mices against cisplatin-induced acute renal damage. The results revealed that methanolic extract can be reflected as a potential contestant for protection of nephrotoxicity induced by cisplatin at the dose of 200 mg/kg and 400 mg/kg.

Wound healing activity

The petroleum ether, chloroform, methanol, and aqueous extracts of leaves of *H. indicum*were separately evaluated for their wound healing activity in rats using excision (normal and infected), incision, and dead space wound models. In the incision wound infection model, group of animals treated with methanolic extract demonstrated significant healing activity with the period of epithelialization that was 16.23 ± 0.98 days as compared to the group of animals treated with standard drug nitrofurazonewith the period of epithelialization 13.5 ± 1.54days. It is also observed in this model that the methanol and aqueous extract treated animals showed significant increase in the wound breaking strength up to 478.55 ± 12.63g and 378.63 ±18.02gwhereas the other extracts are unsuccessful to produce significant effects.

Anti-plasmodial activity

The ethanolic extract of flowers, roots and stems of *H. europaeum var. lasiocarpum* revealed significant anti-plasmodial properties against *Plasmodium falciparum*. At the concentration of 100, 50, 25 µg/ml, the ethanolic extract of flowers demonstrated 33, 10, 6 % of inhibition while the extract of roots revealed 91, 59, 19 % of inhibition and the extract of stems shown 80, 72, 37 % of inhibition at the same concentration. The dichloromethane, methanol and aqueous extracts of fresh plant material of *H. indicum* were tested for significant anti-plasmodial activity against *P. falciparum*. The dichloromethane extract was generally more active than other extracts but among these extracts, no one exposed the substantial antiplasmodial activity. *H.indicum*revealed some anti-plasmodial activity because of its only use in the treatment of few malarial symptoms named as hyperthermias or colics. The methanolic extract of dried plant material of aerial parts of *H. zeylanicum* was examined for anti-plasmodial activity *in vitro* against chloroquine-resistant strain

(KI) and sensitive strain (FCR3). The extract demonstrated significant anti-plasmodial activity with an IC_{50} of 8.41 µg/ml.

Antifertility activity

The n-hexane and benzene fractions of the ethanol extract of *H. indicum*were studied for antifertility activity in rats using anti-implantation and abortifacient models. The study revealed that the effect of ethanolic extract and its n-hexane and benzene fractions on percentage pre-implantation lost in pregnant rats as 30% and 35%, 40% and 60%, 30% and 50% at the dose of 200 & 400 mg/kg body weight respectively while the effect of ethanolic extract and its fractions on percentage abortion in pregnant rats as 50% and 60%, 50% and 60%, 30% and 60% respectively at the same dose. Thus, the *H. indicum* study revealed better abortifacient activity and moderate anti-implantation and sperm motility.

Anti-cataract activity

The ethanolic leaf extract of *H. indicum*was found to be having anti-cataract activity in the galactose induced rats. The results revealed that ethanolic extract at the dose of 200 mg/kg along with Vitamin E whose dose was 50 mg/kg and 30% galactose diet leads to the significant increase in the glutathione lens, soluble proteins and water contents as compared to the standard galactose diet given to the rats. Thus, it was concluded that *H. indicum* leaf extract possessed protective action against galactose induced cataract in rats.

Analgesic activity

The aqueous and ethanolic extract of fresh plant material of *H. indicum* demonstrated the significant analgesic activity in formalin-induced pain model in mice. For comparison of analgesic effect, morphine and diclofenac sodium were used as a reference opioid and NSAID, respectively. At the dose of 30-300 mg/kg, the aqueous and ethanolic extracts inhibited both the first and second phases of

formalin-induced nociception in a dose dependent manner. Oral administration of aqueous extract at the dose of 1-5 g/kg in formalin-induced mice were tolerated in acute toxicity studies but oral administration of 1-2 g/kg of the extracts in sprague-dawely rats produced pathologic effects on heart, kidney, liver and lungs. Therefore, instead of the fact that aqueous and ethanolic extracts have analgesic activity, it could have a cumulative toxic effects. Thus, prolonged and continuous use is not recommended.

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