2. REVIEW OF LITERATURE

2.1 Hybanthus enneaspermus

*Hybanthus enneaspermus* (L) F Muell (Synonym *Ionidicum suffruticosum*) (L) Ging) belonging to family Violaceae is a small, diffuse, perennial herb or under shrub found in the tropical and sub tropical region of the world.119

In India it is found in warmer parts like Bundelkhand, Agra, West Bengal, Madras, Gujarat, and Karnataka.120

**Taxonomical classification of *Hybanthus enneaspermus***121

Taxonomy/Scientific Classification

Class: Eudicots

Subclass: Rosids

Order: Malphighiales

Family: Violaceae

Genus: Hybanthus

Species: *Hybanthus enneaspermus*
Vernacular names: 122

Common name: Spade Flower, Pink ladies slipper

Hindi: Ratan purush

Bengali: Nunbora

Kannada: Purusharathna

Malayalam: Orilathamatai

Telugu: Ratnapurusha

Marathi: Rathanparas

Sanskrit: Rathnapurusha

Figure 2.1: A Twig of Hybanthus enneaspermus Muell.

2.1.1 Description of Hybanthus enneaspermus123

Hybanthus enneaspermus is a perennial herb or small shrub of 40 to 60 cm height with a long slender tap root. The leaves are sub sessile, linear, lanceolate, margin serrate, apex acute and stipules acuminate, 1-4 mm long. Pink-purple spade-shaped flowers solitary. Sepals 3-4 mm long. Lower petal broad spade-shaped, pink-purple with deep purple veins. Upper petals linear-oblong, 3-4 mm long; lateral pair 4.5-5 mm long. The fruits are capsules 4-9
mm long; ribbed seeds 5-12, pitted between ribs. Flowering is from June to November in India. Plants are found along river banks, open grasslands, sandy places and rocky regions. In nature (in vivo) the plants are seasonal and appear for few months. The roots and few basal stem stocks retaining in the soil and are regenerating during rainy season and soon after the rainy season the aerial part dries up and the plants disappear.

2.1.2 Ethanomedicinal and folk medicinal uses of *Hybanthus enneaspermus*

The uses of *Hybanthus enneaspermus* in indigenous system of medicines in India is outlined below:

The flowers of this species are used against jaundice. The plant is used as an aphrodisiac, demulcent, tonic, diuretic, used to treat urinary infections, diarrhoea, leucorrhoea, dysuria, inflammation and male sterility; and to cure diabetes in Orissa by some tribal population. The plant is reported in ayurveda to cure conditions of “kapha” and “pita”, urinary calculi, strangury, painful dysentery, vomiting, burning sensations, wandering of the mind, urethral discharge, blood trouble, asthma, epilepsy, cough and to give tone to the breasts. The decoction of tender leaf stalks are used as demulcent. The plant is mixed with oil and employed in preparing a cooling ointments for headache. The roots are antigonorrhoeic, diuretic, & used in bowel complaints and urinary problems. An infusion of the plant is given in cholera.

2.1.3 Phytochemical aspects

The plant has been reported to contain amino acids, sugars and flavanoids, aurantiamide acetate, isoarborinol, β-sitosterol and triterpenes.

On preliminary screening the methanol extract of plant showed the presence of alkaloids, flavanoids, steroids, terpenoids, phenol, cardioglycosides and anthraquinones like compound on separation by TLC and HPTLC analysis. Further nicotine was confirmed in methanol extract with other alkaloids.
The presence of phenols, alcohols, alkanes, alkyl halides, carboxlic acid, aromatics, nitro compounds and amines in ethanolic extracts of *H. enneaspermus* were confirmed by using UV, FTIR and GCMS. Subsequently GC-MS analysis revealed presence of (5E, 13E)-5,13-Docosadienoic acid (20.90 %) and cedran diol,8S14-(13.02 %).\(^{133}\)

The presence of D-mannitol tetradecandediol, phytol, 2-piperdinone, cedran-diol, 2-mono-linoleo glycerol trimethyl silyl ether and silane in ethanolic extracts of plants were also reported.\(^{134}\)

### 2.1.4 Pharmacological aspects

The extracts from *Hybanthus enneaspermus* showed a wide spectrum of pharmacological activities including anti-diabetic, anti-microbial, hepatoprotective, anti-oxidant, anti-depressant, anti-inflammatory, anti-tussive, anti-convulsant, aphrodisiac etc.

A summary of the findings of these studies performed is presented as below-

**Antidiabetic activity**

Patel et al evaluated antidiabetic and antioxidant potential of *Hybanthus enneaspermus* in different models. The oral glucose tolerance test (OGTT) and normoglycemic effect of alcoholic extract of plant were evaluated for 125, 250 and 500 mg/kg p.o. per day for 21 days in streptozotocin (STZ) induced diabetic rats. Further the total polyphenolic and flavanoid content were also determined. The results showed high level of phenolic content in extract. Furthermore there was a significant increase in the body weight and decrease in blood glucose level in STZ induced diabetic model on treatment with the plant extract.\(^{135}\)
Hepatoprotective activity

Rao M. et al investigated hepatoprotective activity of aqueous extract of plant against CCl₄ induced liver injury in rats at dose of either 200 or 400 mg/kg p.o, pretreatment (once daily for 14 days before CCl₄ intoxication) and post treatment (2, 6, 24 and 48h after CCl₄ intoxication) showed significant hepatoprotection by reducing the aspartatetransaminase, alaninetransaminase, and alkaline phosphatase enzymatic activities and total bilirubin levels which had been raised by CCl₄. Pre-and post- treatment with aqueous extract significantly decreased hepatic lipid peroxidation as well as produced a corresponding increase n tissue total thiols revealed it significant antioxidant antioxidant properties.¹³⁶

Aphrodisiac activity

Narayanswamy V. B et al reported aphrodisiac activity of ethanolic activity of ethanolic and aqueous extracts of plant in sexually inactive male rats in a single dose regimen at 300mg/kg, p.o. and chronic regimen as daily dose for 28 days. The aqueous extract produced a decrease in the mounting and intromission latency, with an increase in ejaculatory and intromission frequency in single dose administration. In the chronic model, both the alcohol and aqueous extracts increased the number of mounts, ejaculations and intromissions with decrease in the mounting and intromission latency. Treatment with aqueous extract also elevated the testosterone levels in sexually inactive male rats.¹³⁷

CNS activity

Kar et al reported CNS activity of ethanolic & aqueous aerial parts of plant in mice using different model of general behavioral. The extract was administered at single doses of 250 and 500 mg/kg per orally, while diazepam (1mg/kg), chlorpromazine (5 mg/kg), imipramine (30 mg/kg) were used intraperitoneally as standard drug. The result showed that both extracts at both dose levels significantly exhibited anxiolytic activity. In another study both extracts
significantly decreased the sleeping latency and increased the sleeping time. Tail suspension, head dip and despair test showed that both extracts (250 and 500 mg/kg) was able to induced a significant increase in the immobility time, like that of imipramine, a recognized antidepressant drug.¹³⁸

**Anti-microbial activity**

Anand & Gokulkrishnan investigated anti-microbial activity of ethanolic and aqueous extracts of *H. enneaspermus* against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Shigella shigae, Salmonella typhi, Proteus vulgaris, Candida albicans, and Aspargillus niger* by using agar disc diffusion method. Ethanolic extract was more active against microbes than aqueous extracts.¹³⁹

**Anti-inflammatory activity**

The anti-inflammatory activity of *Ionidium suffruticosam* syn. *H. enneaspermus* methanol extract was evaluated on carrageenin, histamine and serotonin-induced rat hind paw oedema acute models by Boominathan et al. The extract at doses of 200 and 400 mg/kg was found to possess significant anti-inflammatory activity on the tested experimental models. In a chronic test, the extract (400 mg/kg) showed 42.78% reduction in granuloma weight. The effect produced by the extract was comparable to that of phenylbutazone, a prototype of a non-steroidal anti-inflammatory agent.¹⁴⁰

**Anti-tussive activity**

The study was carried out to evaluate the antitussive potential of methanol extract of *Ionidium suffruticosam* which was investigated for its effect on a cough model induced by sulfur dioxide gas in albino mice by Boominathan et al. 250 and 500 mg/kg (p.o.) of the extract showed 28.37% and 54.16% inhibition of the cough respectively with respect to control group which was comparable to that of codeine phosphate, a prototype antitussive agent.¹⁴¹
Antiplasmocidal activity

Weniger et al evaluated twenty extracts from nine medicinal plants including *H. enneaspermus* traditionally used to treat malaria, were screened for *in vitro* antiplasmodial activity towards *Plasmodium falciparum* K1 chloroquine resistant and 3D7 chloroquine sensitive strains. All plants showed antiplasmodial activity below 10 microg/ml. Nine extracts exhibited IC50 values below 5 microg/ml towards one or both of the two strains. The best inhibition of the growth of *Plasmodium falciparum* resistant K1 strain was observed with the methylene chloride extract of *Hybanthus enneaspermus* and with the methanolic extract of *Croton lobatus* roots (IC50=2.57 and 2.80 microg/ml, respectively).  

Anticonvulsant activity

Hemlatha et al reported anti-convulsant and free radical scavenging activity of *Hybanthus enneaspermus*. Anticonvulsant activity of aqueous (AQHE) and ethanolic (ETHE) extract of plant was studied using maximal electric shock (MES) and strychnine induced convulsions models. AQHE at the doses of 200 mg/kg and 400 mg/kg orally showed significant protection in both models. The activity was equipotent to phenobarbitone sodium (30 mg/kg; i.p.). The AQHE exhibited free radical scavenging activity in an *in vitro* system using DPPH.  

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2.2 *Cardiospermum halicacabum*

*Cardiospermum halicacabum* Linn., commonly known as Ballon vine, is an important medicinal herb belonging to family Sapindaceae. It is an annual or sometimes perennial climber, found as a weed throughout India, ascending up to 1200 m in the hills.\textsuperscript{144}

**Taxonomical classification of *Cardiospermum halicacabum*\textsuperscript{145}**

Taxonomy/Scientific Classification

Class: Eudicots

Subclass: Rosids

Order: Sapindales

Family: Sapindaceae

Genus: Cardiospermum

Species: *Cardiospermum halicacabum*

**Vernacular names:\textsuperscript{146}**

Bengali: Lataphatkari, Sibjhul

English: Balloon Vine, Blister creeper, Heart pea, Heartseed, Wintercherry

Hindi: Kanphuti, Kanphata

Kan: Agniballe

Mal.: Jyotishmati, Katabhi

Marathi: Kanphuti, Kapala-phodi
2.2.1 Description of *Cardiospermum halicacabum*\(^{147}\)

Stem-slim, cylindrical, furrowed, ridges yellow, glabrous, internodes 6 to 7 cm in length and 2-4 mm in diameter, fracture outer short, inner fibrous. Taste bitter, odour indistinct.

Leaf-alternate, deltoid, 2-3 ternate compound, margin inciso-serrate, acute, rachis 5 to 7 cm long, longitudinally grooved, petiole 1.5 to 3 cm long, winged, somewhat pubescent on both surfaces and margin of the leaf, upper surface dark green, lower pale. Taste mucilaginous.

Inflorescence- umbellate cyme, peduncle, stiff, green, 4 to 5 angled, up to 8 cm long bearing 1.5 to 2 cm long circinate tendrils one on either side, towards the apex.

Flower- irregular, complete, bisexual, sepals 4, green, gamosepalous, outer two small, obovate upto 2 mm long, with few hairs on the outer surface, inner longer upto 2 mm long membranous. Petals 4, white, gamopetalous. Stamens 8, creamy white, free, excentric, filaments up to 2 mm long, unequal. Ovary superior, trilocular, style very short, stigma trifid. Capsule sub-globose or depressed pyriform, trigonous, membranous, bladdery, truncate at top, winged at angles, yellowish green to purple, inflated, three chambered each with a single seed. Seeds globose 4 to 5 mm in diameter, smooth, black with a heart shaped white aril along one side.
2.2.2 Ethanomedicinal and folk medicinal uses of *Cardiospermum halicacabum*

The herb is used as diuretic, stomachic, rubefacient; in rheumatism, lumbago, nervous disorders and in preparing hair oil for curing dandruff and alopecia. The juice of the herb is used to cure eaeache and asthma. The decoction is given in piles. The pungent vapours from the crushed leaves are inhaled to relieve headache. The root is mucilaginous and considered emetic, laxative and antirheumatic. It is effective in didymitis and early stages of hydrocele and is used in asthma and colic. Aerial root is used to induce abortion. It is useful in amenorrhoea, gonorrhea, nervous diseases, haemorrhoids and erysipelas.\(^{148}\)

Plant is used as diaphoretic, diuretic, laxative, emmenagogue, rubefacient, in rheumatism, flatulence, fever, diseases of the nerves, in stiffness of limbs, pain, in gonorrhea, snake bite.\(^{149}\)

Fruits are applied on boils. Seeds are used in dysentery, as tonic.\(^{150}\)

Leaves are rubefacient, used in weakness, to cure skin diseases, earache, as purgative, in rheumatism, asthma, fever, headache, muscular spasm, joint pain, arthritis, itching, analgesic, diarrhea and as diuretic.\(^{151}\)

Leaves are also used for weight reduction, to relieve pain in joint and at the time of delivery. Leaves, root and seeds are used in lung diseases and rheumatism, stiffened limbs, ear pains and eye sores.\(^{152}\)

Stem is used in fever. Leaves and root are used as diuretic, stomachic.\(^{153}\)

Leaves, stem and root are used in chronic rheumatism, nervous diseases and earache.\(^{154}\)

Root is diaphoretic and used as diuretic.\(^{155}\)

2.2.3 Phytochemical aspects

In a preliminary screening, saponins, alkaloids and flavonoids were found to be absent in the plant. In a subsequent study, the plant showed presence of saponins and absence of alkaloids, steroids, glycosides, flavonoids, tannins, essential oil and gum-resin-mucilage.\(^{156}\)
The plant yielded stigmasterol, stigmasterol-β-D-glucoside, β-sterol-β-D-glucoside, apigenin and luteolin. The ethanolic extract of seeds showed presence of arachidic acid and β-sitostrol-beta-D-galactoside.\textsuperscript{157}

The aqueous extract fractions of seeds showed presence of alkaloids, sugars, amino acids, proteins, saponins and tannins in traces. The ethanolic extract of the seeds showed presence of alkaloids, glycosides, sugars, amino acids, proteins, steroids, saponins, tannins in traces while from the petroleum ether extract sterols and tannins were estimated in good quantity.\textsuperscript{158}

The seeds were analyzed for their protein content and amino acid composition. The amino acids detected were aspartic acid, threonine, serine, glutamine, proline, glycine, alanine, valine, methionine, isoleucie, leucine, tyrosine, phenylalanine, histidine, lysine and arginine. The chemical composition of fixed oil from seed was capric, lauric, myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, archidic, 11-ecoscanoic, boheric, lignoceric acids.\textsuperscript{159}

The major constituents of the oil are Erucic acid (43%) Oleic Acid (30%) Eicosanic acid (12%), Octanoic acid (4.57%) and n-Hexa decanoic acid (4.15%). The minor constituents of the oil are arachidic acid, Oleic acid, Palmitic acid (Hexadecanoid acid), stearic acid (Octadecanoid acid) and lauric acid (Dodecanoid acid).\textsuperscript{160}

The chemical composition of leaves was reported as: protein, fat, minerals and carbohydrates.\textsuperscript{161}

\subsection*{2.2.4 Pharmacological aspects}

\textbf{Anti-pyretic}

Asha & pushpangadah revealed that ethanol and n-hexane extract of the powdered plant showed potent antipyretic activity against yeast induced pyrexia in rats. However, the water extract was devoid of antipyretic activity.\textsuperscript{162}

\textbf{Anti inflammatory}

Sadique et al reported the ethanolic extract of the aerial parts (100, 250, 500 mg/kg orally) showed dose-related anti-inflammatroy activity against carrageenan induced
raw paw oedema in rats. In cotton pellet granuloma the extract suppressed the transudative, exudative and proliferative components of chronic inflammation. The biochemical studies indicated the inhibition of formation of mediators of inflammation or the stabilization of lysosomes by the extract.\textsuperscript{163}

Pillai et al reported the alcoholic extract of leaves when administered orally produced significant anti-inflammatory effect in granuloma pouch and cotton pellet implantation methods in rats. The aqueous extract of the plant (10 g/kg) also exhibited significant anti-inflammatory activity.\textsuperscript{164}

Antioxidant and anti-inflammatory properties of aqueous and ethanolic extract of \textit{Cardiospermum halicacabum} (ACH & ECH respectively) were investigated by Huang et al. They reported that ECH inhibited the development of paw edema, increased the activities of CAT, SOD and GPx in the liver tissue and decreased the level of NO in serum as well as in oedematous paw tissue.\textsuperscript{165}

Sheeba & Asha evaluated anti-inflammatory action of ethanolic extract of whole plant of CH in mouse macrophage cell line RAW 2647 cells. They found that the ethanol extract dose dependently inhibited m-RNA expression of COX-2, TNF and iNOS protein expression. But the extract did not affect the expression of COX-1 m-RNA expression.\textsuperscript{166}

**Antimicrobial**

Valsaraj et al reported the ethanolic extracts of leaves and stem showed antibacterial activity against \textit{Bacillus subtilis}, \textit{Staphylococcus aureus}, \textit{Escherichia coli} and \textit{Pseudomonas aeruginosa} at 25 mg/ml concentration. The extracts, however, did not show anti-fungal activity against \textit{Aspergillus niger} and \textit{Candida albicans}.\textsuperscript{167}

Crude extracts from the leaf and stem of \textit{Cardiospermum halicacabum} in different solvents were evaluated for their antimicrobial activity by Viji et al. All the extracts showed varying degree of inhibitory potential against all the tested bacteria. Acetone extracts of both leaf and stem showed maximum inhibitory action against \textit{S. typhi}. The chloroform extracts of leaf and benzene extract of stem had higher inhibitory action against \textit{Salmonella typhi} and \textit{Streptococcus subtilis} respectively.\textsuperscript{168}
Antidiarrhoeal activity

Petroleum ether, aqueous & alcoholic extracts of whole plant of CH were investigated by Rao et al for antidiarrhoeal activity using different experimental animal models. All the three extracts at 400mg/kg po significantly (p<0.01) reduced fecal output in castor oil induced diarrhea, intestinal secretions in charcoal meal test, indicating antidiarrhoeal activity.\textsuperscript{169}

Anxiolytic activity

Anti anxiety effects of alcoholic and aqueous root extracts of plant (100 or 300 mg/kg p.o.) was investigated by Malaviya et al. Both the extracts increased the time spent in open arm EPM model, time spent in light compartment in light-dark model and time spent in central compartment in open field test. These results suggest that alcoholic and aqueous extracts of \textit{Cardiospermum halicacabum} possess anti anxiety activity.\textsuperscript{170}

Antihyperglycaemic activity

Veeramani et al investigated the antihyperglycaemic effect of ethanolic extract of plant leaves on normal and streptozotocin (STZ) diabetic rats. The plant leaf extract (50,100&200mg/kg, p.o.) was administered to normal and STZ-diabetic rats for 45 days and significantly decreased plasma glucose and HbA1c, and increased the levels of insulin and Haemoglobin. Thus, the results show that ethanolic extract of \textit{Cardiospermum halicacabum} possess an antihyperglycaemic activity.\textsuperscript{171}