Isolation, Phytochemical, Pharmacological and Characterization of Ethanolic Extract of Hygrophila Balsamica

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Abstract: The vast majority of people in this planet rely on their traditional material medica (medicinal plants and other materials) for their primary health care. The literature revealed that only fragmentary information has been available on this plant species relating to phytochemical and pharmacological studies by some investigators. This research was made the first time for phytochemical identification, evaluation of pharmacological activities, and characterization of undiscovered phytoconstituents in the ethanol extract of leaves of Hygrophila balsamica in-order to ascertain their traditional claims. The pharmacognostical study of leaves of powder of Hygrophila balsamica have been subjected to determination of organoleptic characteristics, physicochemical constants and fluorescence analysis. The preliminary photochemical study of ethanolic extract of Hygrophila balsamica showed the presence of alkaloids, glycosides, flavonoids, steroids, tannin, carbohydrate, and protein and the absence of gums and mucilage, phenols, sterols, and terpenoids. Ethanol extract of leaves of Hygrophila balsamica showed the In-vitro antioxidant, anti-inflammatory, and anti-diabetic activities. Characterization of ethanol extract was identified by GC-MS which showed presence of various bioactive compounds justifies the use of the leaves for various ailments by traditional practitioners and IR showed the functional groups of various phytoconstituents in this extract.

Key words: In-vitro antioxidant, anti-inflammatory, anti-diabetic, Ethanolic extract, GC-MS.

1.INTRODUCTION

The history of pharmacy was for centuries identical with the history of pharmacognosy or the study of material medica. There have another fact that one quarter of all medical prescriptions are formulations based on the substance derived from plants or plant derived synthetic analogues which are obtained from natural sources, mostly plants, then minerals, animals and fungi. The World Health Organization (WHO) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for hundreds of years, before the development and spread of modem medicine and are still in use today[1]. The project aims to develop and validate analytical methods for *In-vitro* pharmacological activities such as anti-inflammatory, anti-oxidant and anti-diabetic activities[1].

Inflammation is a response of a tissue to injury, often injury caused by invading pathogens. It is characterized by increased blood flow to the tissue causing increased temperature, redness, swelling, and pain.

Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids, which affect the central nervous system. A major portion of the project involves analysis of thiocolchicoside and combined dosage forms of thiocolchicoside with some anti-inflammatory drugs.

An antioxidant, or a free-radical scavenger, is a molecule capable of decreasing or preventing the oxidation of other molecules. Antioxidants often play the role of a reducing agent, e.g., thiols or polyphenols[2].

Diabetes mellitus is a disease characterized by the inability of peripheral tissues to uptake glucose, caused by a deficit in insulin or insulin intolerance. This disease is subdivided into two categories,

- Type 1 diabetes mellitus pertains to autoimmune destruction of pancreatic beta cells

leading to the inability to produce sufficient amounts of insulin.

• Type 2 diabetes mellitus occurs when insulin receptors are unresponsive or less sensitive to insulin.

The treatment of diabetes is dictated by the specific pathophysiology of the diagnosis. Regardless of the specific etiology, it is imperative for a physical therapist to be aware of the intended effects that may be prevalent with certain medications[2].

2. MATERIALS AND METHODS

2.1 Plant material:

Leaves of *Hygrophila balsamica* were collected from the agricultural lands of nearby village of Erode, Tamil Nadu in the month of April 2019. The collected leaves were authenticated by Dr. K. MadhavaChetty, Professor, Department of Botany, Sri Venkateswara University, Tirupati-517502. A voucher specimen of Hygrophila balsamica has been kept in the Herbarium of the Department of Pharmacognosy, JKKMMRF Annai Sampooraniammal College of Pharmacy.

2.2 Preparation of the Extract:

The collected leaves were washed throughly initially with tap water followed by distilled water. The leaves were shade dried completely under room temperature for 2 weeks. The shade dried leaves is powdered by using mechanical grinder and sieved. The sieved material is stored in well closed container.

Approximately 500 g of powder leaves of Hygrophila balsamica is extracted with 1000 ml ethanol for 48 hours at a temperature between 50-65°C by Soxhlet extractor. The extract is filtered, concentrated and dried in desiccators[3]

2.3 Physicochemical Constants[4]

Physicochemical constants like ash value, extractive value, pH and loss on drying were determined.

2.3.1. Ash Value: About 3 g of the grounded material, previously ignited, cooled and weighed is incinerated with a gradual increase in heating, not exceeding 450 °C, until free from carbon, cooled and weighed. The residue is collected on an ash less filter-paper. Calculate the content in mg of ash per g of air dried material.

2.3.2. Total Ash Value: Total ash is a measure of the mineral oxide content of activated carbon on a weight basis. About 4 gm of the powdered leaves in the silica crucible is ignited at 500-600°C till completely white, indicating the absence of carbon. The crucible is cooled in air, weighed and the total ash value is calculated.

2.3.3. Acid-Insoluble Ash: The ash is boiled for 5 minutes with 25 ml of hydrochloric acid, the insoluble matter is collected in a sintered crucible, or on an ashless filter-paper, washed with hot water, and ignite at about 500 °C to constant weight. The acid insoluble ash per g of air-dried material is calculated.

2.3.4. Water-Soluble Ash:

To the ash obtained as total ash 25 ml water is added and boiled for 5 minutes. The insoluble matter is collected on an ash less filter paper, washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450 ^{0}C. The weight of this residue was subtracted from the weight of total ash. The content of water-soluble ash with reference to dried drug was calculated.

2.3.5. Sulphated Ash: About 2 gm of coarse powder of plant leaves powder weighed into silica crucible, ignited gently the silica crucible at first, till the plant material was completely burnt. Cooled the residue and moistened with 1mL of H_2SO_4. Heated the residue upto no white fumes produced. Ignited the residue at 800^{o}C upto all-black residue upto no white fumes produced. Ignited the residue at 800^{o}C up to all-black particles had went away. Cooled crucible, then few drops of H_2SO_4 was added and ignited. Cooled the residue in the crucible in the desiccators and weighed without delay. Calculated the sulphated ash with reference to the air dried plant leaves powder.

2.4. EXTRACTIVE VALUES[5]

The extractive values are useful for the examination of phytoconstituents present in the crude plant materials. Also the extractive values demonstrate the type of the phytoconstituents found in the crude plant materials.

2.4.1. Petroleum Ether Soluble Extractive: Macerate 5 gm of the dried coarse powder of leaves of *Hygrophila balsamica* with 100 ml of petroleum ether into the iodine flask for one day. The iodine

flask was shaken forsix hours continuously and kept aside for eighteen hours without disturbance. The Filtration is done immediately without loss of petroleum ether. 25 ml of extract solution from filtrate was taken into the flat bottom shallow dish, evaporated on the extract solution on water bath at 105°C. The residue was collected and weighed.

2.4.2. Chloroform Soluble Extractive: Macerate 5 gm of the air dried coarse powder of leaves of *Hygrophilabalsamica* in 100 ml of chloroform into the iodine flask for one day. The iodine flask was shaken for six hours continuously and kept aside for eighteen hours without disturbance. Filtration is done immediately without loss of chloroform. 25 ml of extract solution from filtrate was taken into the flat bottom shallow dish, evaporated on water bath at 105°C. The residue was collected and weighed.

2.4.3. Acetone Soluble Extractive: Macerate 5gm of the air dried coarse powder of leaves of *Hygrophilabalsamica* in 100 ml of acetone into the iodine flask for one day. The iodine flask was shaken for six hours continually and kept aside for eighteen hours without disturbance. Filtration is done immediately without loss of methanol. 25 ml of extract solution from filtrate was taken into the flat bottom shallow dish, evaporated on water bath at 105 °C. The residue was collected and weighed.

2.4.5. Ethyl Acetate Soluble Extractive: Macerate 5gms of the air dried coarse powder of leaves of *Hygrophilabalsamica* in 100 ml of ethyl acetate into the iodine flask for one day. The iodine flask was shaken for six hours continuously and kept aside for eighteen hours without disturbance. Filtration is done immediately without loss of methanol. 25 mL of extract solution from filtrate was taken into the flat bottom shallow dish, evaporated on water bath at 105 °C. The residue was collected and weighed.

2.4.6. Water Soluble Extractive: Macerate 5 gm of the air dried coarse powder of leaves of *Hygrophilabalsamica* in 100 ml of distilled water into the iodine flask for one day. The iodine flask was shaken for six hours continuously and kept aside for eighteen hours without disturbance. Filtration is done immediately without loss of distilled water. 25 ml of extract solution from filtrate was taken into the flat bottom shallow dish, evaporated on water bath at 105°C. The residue was collected and weighed.

2.4.7. Ethanol Soluble Extractive: Macerate 5 gm of the air dried coarse powder of leaves of *Hygrophilabalsamica* in 100 ml of ethanol into the iodine flask for one day. The iodine flask was shaken for six hours continually and kept aside for eighteen hours without disturbance. Filtration is done immediately without loss of ethanol. 25 ml of extract solution from filtrate was taken into the flat bottom shallow dish, evaporated on water bath at 105°C. The residue was collected and weighed.

2.4.8. Water Soluble Extractive: Macerate 5 gm of the air dried coarse powder of leaves of *Hygrophilabalsamica* in 100 ml of distilled water into the iodine flask for one day. The iodine flask was shaken for six hours continuously and kept aside for eighteen hours without disturbance. Filtration is done immediately without loss of distilled water. 25 ml of extract solution from filtrate was taken into the flat bottom shallow dish, evaporated on water bath at 105°C. The residue was collected and weighed.

2.5. Determination of pH: The pH of freshly prepared 1% w/v and 10% w/v suspension of leaves powder of *Hygrophilabalsamica* in distilled water was determined by using calibrated pH meter.

2.6. Determination of Loss on Drying: 1 gm of the powdered leaves is weighed and transferred to the previously weighed glass stoppered bottle and placed in a drying chamber. The powdered leaves is dried to constant weight and cooled. The bottle along with its content is weighed. The process is repeated up to a constant weight with difference, not more than 0.5 mg (drying to constant weight) is attained. The percent weight loss is calculated with reference to air dried plant material.

2.7. Thin Layer Chromatographic Profile (TLC)[6]
Stationary phase:
Readymade pre-coated silica gel on aluminium plates were used as a stationary phase
Solvent system:
Ethyl acetate: methanol: water in the ratio of 5:3:1 were selected as solvent system.
Procedure: The ethanolic solution of the extract is taken in a capillary tube and then spotted on TLC plates, 2cm above its bottom end. The plates were then placed in chromatographic tank which is pre saturated by using a mobile phase. The plates were allowed to develop upto 3/4th of the length and then removed. The solvent front is immediately marked

and the plates were allowed to dry. The plates were then examined under UV light or sprayed with different spraying reagents. The spots were identified and R_f values were recorded.

2.8. Quantitative Analysis of Crude Extract Fluorescence Analysis[7]

The powdered leaves is treated with 5% HCl, 5% NaOH, alcoholic 5% NaOH and 5% H_2SO_4 and it is observed in daylight and short UV light (254 nm) and long UV light (365 nm).

2.9. GC-MS Analysis Characterization

The GC-MS analysis of ethanolic extract of Hygrophilabalsamica was performed using a Perkin Elmer GC-MS (Model Perkin Elmer Clarus 600, USA)

IR Spectroscopy

The infra red spectrum was recorded on FTIR Perkin Elmer at SAIF, IIT Madras, Chennai, Tamil Nadu, India

3. PHARMACOLOGICAL STUDIES

3.1. *IN-VITRO* ANTI-OXIDANT ACTIVITY[8]:

ASSAY DPPH RADICAL SCAVENGING ACTIVITY

DPPH radical scavenging activity of the extract is carried out according to method developed by Alhakmani et al with some modifications[9]. About 0.2 mm solution of DPPH in methanol is prepared, and 1 ml of this solution is added to 1 ml of different concentrations of ethanolic extract and standard. Ascorbic acid 100-400 µg/ml is used as standard. The mixture is shaken and allowed to stand at room temperature for 30 minutes, and the absorbance is measured at 517 nm by using UV-Visible spectrophotometer. Each experiment is done in triplicate, and the average is taken.

3.2. IN-VITRO ANTI-INFLAMMATORY ACTIVITY[10]:

INHIBITION OF ALBUMIN DENATURATION

Inhibition of albumin denaturation by ethanolic extract of *Hygrophilabalsamica*is carried out according to the method developed by Alhakmani et al with some modifications[11].The test solution consist of 1.5 ml of different concentrations of the ethanolic extract ranging from 100-400 µg/ml and the standard Diclofenac sodium (150 and 200 µg/ml) solution is separately mixed with 1.0 ml of aqueous egg albumin solution (1% w/v) and incubated at 37°C for 20 minutes and then the reaction mixture is heated in a water bath at 51°C for 20 minutes. After cooling, the turbidity is measured at 660nm in a UV-Visible spectrophotometer against the reagent blank. Each experiment is done in triplicate and the average is taken.

3.3. IN-VITRO ANTI-DIABETIC ACTIVITY[12]

α-AMYLASE INHIBITION ASSAY

The α-amylase inhibition assay is carried out according to the method developed by Ramachandran et al with slight modification.[13] 1.5ml of the various concentrations of the ethanolic extract and standard acarbose (100-400 µg/ml) were separately mixed with 1.5ml of α-amylase enzyme (1%) and 1.5 ml of 0.1M of sodium acetate buffer (pH-7.2). The mixture was incubated at room temperature for 20 minutes and 2 ml of 1% starch solution was added. The above mixture is incubated for 30 minutes at 37°C. Then 1.5ml of 3,5-dinitrosalicylic acid reagent is added to the mixture. The mixture is kept in a boiling-water bath for 5 minutes. The absorbance is recorded at 540 nm by UV-Visible spectrophotometer. Each experiment is carried out in triplicate and the average was taken.

Calculation of Percentage Inhibition

The percentage inhibition of different concentration of ethanolic extract of *Hygrophilabalsamica* leaves by various *in-vitro* methods such as assay of DPPH free radical scavenging activity, inhibition of albumin denaturation and α-amylase inhibition assay were calculated by using the following formula.

$$\% \text{ inhibition} = (A_0 - A_1 / A_0) \times 100]$$

Where, A_0 is the absorbance of control and $A_1$is the absorbance of test or standard.

4. RESULTS

Physicochemical constituents such as ash value, extractive values, loss on drying and pH of leaves of *Hygrophilabalsamica* were shown in Table 1.

S. No	Parameters	Results
1	Total ash	8.18% ± 0.027
2	Water soluble ash	10.19% ± 0.022

3	Acid insoluble ash	4.17% ± 0.024
4	Sulfated ash	11.45% ± 0.023
6	Petroleum soluble extractive	3.67% ± 0.020
7	Chloroform soluble extractive	4.09% ± 0.059
8	Acetone soluble extractive	10.72% ± 0.057
9	Ethyl acetate soluble extractive	9.17% ± 0.038
9	Ethanol soluble extractive	19.21% ± 0.042
10	Water soluble extractive	7.14% ± 0.049
11	Loss on drying	7.56 % ± 0.062
12	pH (1 and 10% w/v)	7.61±0.023 & 5.19±0.067

Table 1: Physiochemical values of Ethanolic extract of Hygrophilabalsamica leaves

The TLC study of ethanolic extract of *Hygrophilabalsamica* leaves was done and shown in table 2 and Figure 1. Qualitative chromatographic analysis of ethanol extract by TLC was performed to separate and identify the single or mixture of phytoconstituents in the extract. The ethanolic extract showed three spots (R_f values 0.2, 0.4, and 0.7) which indicates the ethanolic extract of leaves of *Hygrophilabalsamica* contain the three types phytoconstiuents.

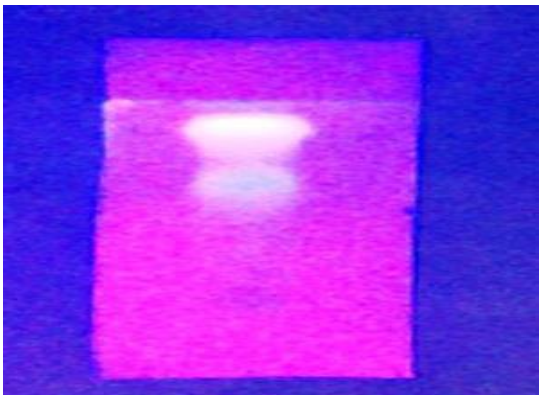

Figure 1: TLC profile of ethanol extract of Hygrophilabalsamica

Solvents system	Distance traveled by solute	Distance traveled by solvent	Detecting agent	Rvalue
Ethyl acetate: methanol: water (5:3:1)	0.8	3.9	Iodine chamber	0.2
	1.7			0.4
	2.6			0.7

Table 2: Rf values of the ethanolic extract of Hygrophilabalsamica

The fluorescence analysis of leaf powder of *Hygrophilabalsamica* was viewed under the day light and UV (Short-254 nm and long 365 nm) light. The results were shown in Table 3. The leaf powder of *Hygrophilabalsamica* produced the green fluorescence which shows the existence of chromophore containing phytoconstituents in the *Hygrophilabalsamica*.

Parts used	Treatment	Day Light	Short UV Light (254 nm)	Long UV Light (365 nm)
Leaves powder	Powder as such	Light green	Dark green	Green
	Powder + 5% HCl	Light brown	Brown	Dark green
	Powder + aqueous 5% NaOH	Brown	Reddish brown	Light green
	Powder + alcoholic 5% NaOH	Chocolate brown	Dark green	Light green
	Powder + 5% H_2SO_4	Bluish green	Brown	Dark green

Table 3: Fluorescence analysis of powder of leaf and stem of *Hygrophilabalsamica*

Chrmatogram of ethanolic extract was performed and shown in Figure 2 and structure of phytoconstituents present in the ethanolic extract of leaves of *Hygrophilabalsamica* were shown in Figure 3 to Figure 14. Ethanolic extract of leaves of *Hygrophilabalsamica* majorly contain 13 compounds which is responsible for various pharmacological activities such as Antifungal, Antioxidant, Anti cancer, nematicide, Anticholesterolemic.

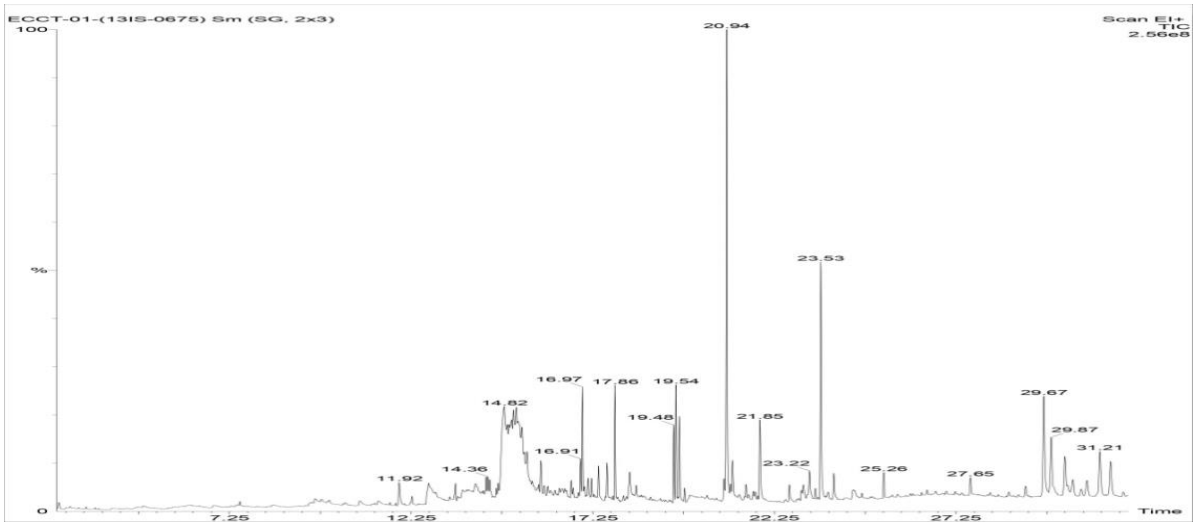

Figure 2: Chromatogram of ethanol extract of leaves of *Hygrophilabalsamica*

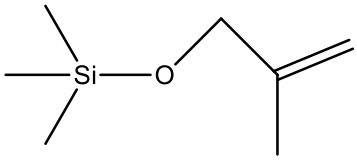

Figure 3: Structure of (2-methyl-prop-2-enyloxy)-trimethylsilane(RT 11.92)

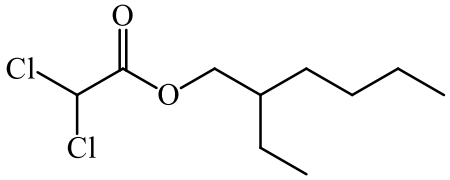

Figure 4: Structure of dichloroacetic acid, 2-ethylhexyl ester (RT 14.82)

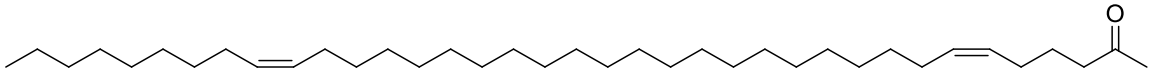

Figure 5: Structure of Z,Z-6,28-heptatriactontadien-2-one (RT 16.97)

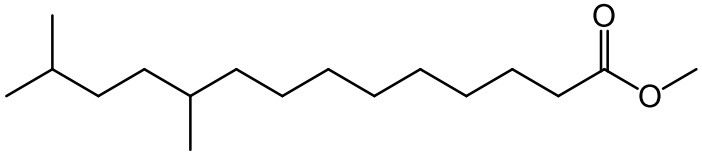

Figure 6: Structure of tetradecanoic acid, 10,13-dimethyl-, methyl ester(RT 17.86)

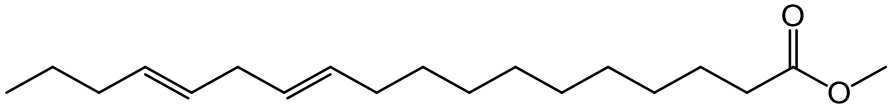

Figure 7: Structure of methyl 11,14-octadecadienoate(RT 19.48)

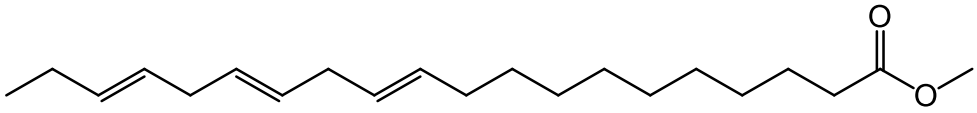

Figure 8: Structure of Methyl 11,14,17-eicosatrienoate
(RT 19.54)

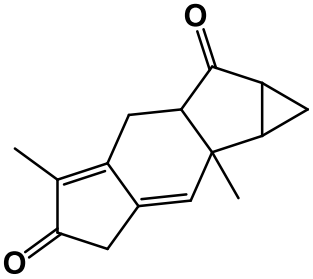

Figure 9: Structure of Chloranthalactone A (RT 20.94)

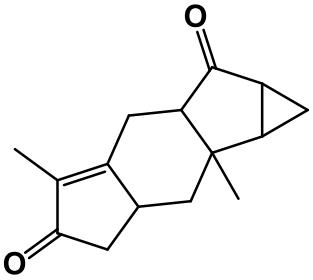

Figure 10: Structure of shizukanolide(RT 21.85)

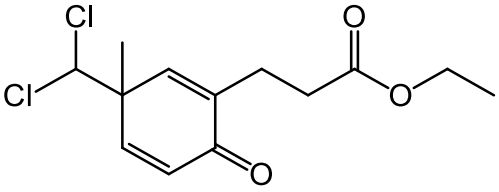

Figure 11: Structure of 1,4-cyclohexadiene-1-propanoic acid, 3-(dichloromethyl)-3-methyl-6-oxo-, ethyl ester (RT 23.22)

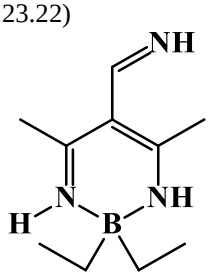

Figure 12: Structure of Boron,Diethyl[3-imino-2-(1-iminoethyl)butanenitrilato-n2,n3]-, (t-4)-(RT 23.53)

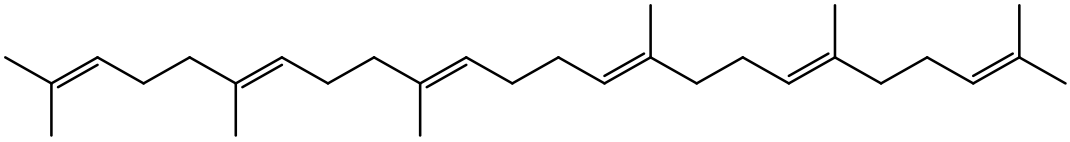

Figure 13: Structure of2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-e)-(RT 25.26)

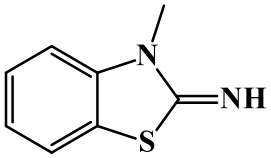

Figure 14: Structure of 2 (3H)-benzothiazolimine, 3-methyl (RT 27.65)

4.1 IN-VITRO ANTI-OXIDANT ACTIVITY OF ETHANOL EXTRACT

DPPH ASSAY OF FREE RADICAL SCAVENGING ACTIVITY

The assay of DPPH radical scavenging activity of ethanol extract of *Hygrophilabalsamica* was performed and tabulated in Table 4 and Figure 38. As a result, there was a reduction of DPPH free radical concentration, which inturn decreases the absorbance at 517nm. The Ethanolic extract of *Hygrophilabalsamica* produced the DPPH radical scavenging activity in a concentration-dependent manner. The percentage of DPPH radical scavenging activity was increased with an increase in the concentration of the ethanolic extract and ascorbic acid from 100 to 400µg/ml. The extract, in all the concentrations, shows a significant DPPH radical scavenging activity.

S. No	Concentration(µg/ml)	% of activity (±SEM)	
		Ethanolic extract	Ascorbic acid
1	100	24.95±0.318	29.22±0.652
2	150	31.15±0.556	38.79±0.482
3	200	35.15±0.398	43.27±0.558
4	250	43.88±0.469	52.99±0.854

5	300	53.64±0.703	63.28±0.869
6	350	64.98±0.659	71.79±0.965
7	400	72.15±0.667	76.56±0.951

Table 4: Percentage of DPPH radical scavenging activity of ethanol extract of leaves of *Hygrophilabalsamica*
*All values are expressed as mean ± SEM for three determinations

4.2. IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF ETHANOL EXTRACT
INHIBITION OF ALBUMIN DENATURATION

Inhibition of albumin denaturation of ethanol extract of *Hygrophilabalsamica* was performed and shown in Table 5. The inhibitory effect was increased with an increase in the concentration of the ethanol extract of *Hygrophilabalsamica* and diclofenc from 100 to 400 µg/ml. The extract, in all concentrations, showed a significant inhibition of albumin denaturation. The result showed that the percentage inhibition values of diclofenc were high when compared to ethanol extract of *Hygrophilabalsamica*.

S. No	Concentration(µg/ml)	% of activity (±SEM)	
		Ethanolic extract	Diclofenac
1	100	21.80±0.812	59.53±0.614
2	150	30.60±0.805	72.27±0.611
3	200	37.80±0.856	
4	250	44.33±0.812	
5	300	54.33±1.004	
6	350	61.80±0.803	
7	400	64.54±0.822	

Table 5: Percentage inhibition of albumin denaturation by ethanol extract of leaves of *Hygrophilabalsamica*
*All values are expressed as mean ± SEM for three determinations

4.3. IN-VITRO ANTI-DIABETIC ACTIVITYOF ETHANOL EXTRACT
α-AMYLASE INHIBITION ASSAY

Percentage inhibition of α-amylase by ethanol extract of leaves of *Hygrophilabalsamica* was performed and results are shown in Table 6 and Figure 40. The inhibitory effect was increased with the increasing the concentration of ethanolic extract and acarbose from 100 to 400 µg/mL. The experimental data showed that percentage inhibition values of standard acarbose were high when compared to ethanol extract.

S. No	Concentration(µg/mL)	% of activity (±SEM)	
		Ethanol extract	Acarbose
1	100	21.16±0.516	18.12±0.858
2	150	28.59±0.850	32.42±0.674
3	200	34.56±0.516	38.61±0.709
4	250	46.94±0.676	49.31±0.656
5	300	55.31±1.086	60.80±0.645
6	350	64.67±1.032	69.47±0.512
7	400	72.16±1.014	78.17±0.502

Table 6: Percentage inhibition of α-amylase by ethanol extract of leaves of *Hygrophilabalsamica*
*All values are expressed as mean ± SEM for three determinations

5. SUMMARY AND CONCLUSIONS

A study of the literature revealed that only fragmentary information has been available on this plant species relating to phytochemical and pharmacological studies by some investigators. Hence this research was made the first time for phytochemical identification, evaluation of pharmacological activities, and characterization of undiscovered phytoconstituents in the ethanol extract of leaves of *Hygrophilabalsamica* in-order to ascertain their traditional claims. The pharmacognostical study of leaves of powder of *Hygrophilabalsamica* have been subjected to determination of organoleptic characteristics, physicochemical constants and fluorescence analysis. These studies used to find out the quantity of soluble phytoconstituents on the given quantity of plant material, used to judge the authenticity of the plants as well as to distinguish the plant material

from the adulterants or allied species. The preliminary phytochemical study of ethanolic extract of *Hygrophilabalsamica* showed the presence of alkaloids, glycosides, flavonoids, steroids, tannin, carbohydrate, and protein and the absence of gums and mucilage, phenols, sterols, and terpenoids. Qualitative chromatographic analysis of ethanol extract using thin layer chromatography was performed to separate and identify the single or mixture of phytoconstituents in the extract and for the identification of different components in the extract qualitatively. Ethanolic extract of leaves of *Hygrophilabalsamica* showed the *In-vitro* antioxidant, anti-inflammatory, and anti-diabetic activities. Characterization of ethanol extract by GC-MS showed presence of various bioactive compounds justifies the use of the leaves for various ailments by traditional practitioners and characterization of ethanol extract by IR showed the functional groups of various phytoconstituents in extract. This scientific study revealed the efficacy of the plant and it would definitely have wide scope in future. Hence, the leaf can be recommended therapeutically for the investigated medicinal claims. These observations will stimulate further research in the field of phytochemistry and also in the clinical application of phytoconstituents of *Hygrophilabalsamica*.

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