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Anti-Inflammatory Activity and Pharmacognostic Standardization of *Passiflora vitifolia* Leaves - An Endangered Species

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ABSTRACT

Medicinal plants are the nature's gift to human being to have a disease-free healthy life. It plays a vital role to preserve our health. India is one of the most medico-culturally diverse countries in the world where the medicinal plant sector is part of a time-honoured tradition that is respected even today. *Passiflora* is one of the plants used in Ayurveda for several remedies such as sedative, anxiety and hypertension etc. But this plant does not have scientific evidence so far. *Passiflora* are rich in flavonoids having main class, flavonoid glycosides, which produce central action such as sedative and hypnotic, anxiolytic and analgesic. The objective of the present study was to standardize the plant pharmacognostically and photo-chemically and to evaluate for anti-inflammatory activity of *Passiflora vitifolia* leaves (ethanolic extract). Microscopic measurement (fibre length and width), Ash values and extractive values of *Passiflora vitifolia* leaves were determined to set the Pharmacognostic standards. Chemical constituents were evaluated through chemical tests and Thin Layer Chromatography. The ethanolic extract of *Passiflora vitifolia* leaves (PVLE) were subjected to evaluate i*n-vitro* anti-inflammatory activity through HRBC method. PVLE was found to contain alkaloids, flavonoids, tannins and phenolic compounds. PVLE showed significant anti-inflammatory potential in concentration 150 µg/ml.

INTRODUCTION

In India, the traditional health care system is Ayurveda (Ayus – Life, Veda – Knowledge, meaning – science of life) and is also one of the oldest therapeutic systems for treatment of various diseases. India comprises the great biodiversity and has a tremendous potential and advantage in the emerging field of herbal medicines. The total Medicinal plants as a group comprises approximately 7500 species and includes representatives of about 17,000 species of higher flowering plants.

A comprehensive literature survey on *Passiflora* revealed that there are a lot of secondary metabolites present in the different species of Passifloraceae. The work on *Passiflora vitifolia* not yet done to that extent. It has report plant of family Passifloraceae are rich source of flavonoid. These chemical constituents are of great importance in respect to medicinal use. So far the literature review, *Passiflora vitifolia* holds a great potential in phytochemical, Pharmacognostic and pharmacological investigation[5,7,9].

The inflammatory response involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair which are aimed at host defence and usually activated in most disease condition. Now a day's much interest has discovery of new therapeutic agents without too many side effects. Present study was undertaken to establish scientific evidence for anti-inflammatory activity of different extracts leaves of *Passiflora vitifolia.*

The plant has never been subjected to *in-vitro* anti-inflammatory activity. Thus, it was considered worthwhile to evaluate *P. vitifolia* for *in-vitro* anti-inflammatory activity and Pharmacognostic standardization.

Figure No. 1: The plant of *Passiflora vitifolia*

MATERIALS AND METHODS:

Collection, Authentication, Drying and size Reduction of Plant leaves

The leaves of plant *Passiflora vitifolia* were collected from F.R. I (Forest Research Institute), Dehradun. The herbarium of the plant specimen has been deposited to Systemic Botany Discipline, Forest Research Institute, Dehradun and the voucher specimen No. 125/Dis./2019/Syst.Bot./Rev.Gen./4-5.

The freshly collected leaves were washed with running water and cleaned to eliminate the possible contamination before proceeding for extraction. The leaves were dried in sunlight under screen from direct light for 3-4 days until they become perfectly dry. The dried leaves were powered and proceed for further extraction.

Macroscopy

Macroscopic evaluation was done by identifying the odour, colour, taste, shape, texture, and fracture characteristic (WHO, 1998). In present days the macroscopy of leaves were studies by the underlying support of colour, odour, taste and surface characteristics.

Procedure for the study of Powder Microscopy

- Select the clean and dry glass slide. Placed fine powder drug of leaves on the glass slide.
- Tapped the slide for the uniformity of powder on the surface of glass slides.
- Now, observed the glass slides under the microscope by using different reagent, and studies the different characters of leaves of powdered drug (Shah B. N, 2015).

Determination of fibre length and width

- By the help of a stage, micrometer calibrate an eyepiece and calculate the factor.
- Take the little quantity of powdered in attest tube and boil with clearing agent (chloral hydrates solution).
- Transfer clear powder in the watch glass.

- By the staining reagents (phlorogucinol, and concentrated HCl) stained the lignified fibres.
- Mount this treated powder in glycerine water.
- Now, observed the slide under low power.
- Focus on stained fibres and by rotating the scale of eyepiece note the number of divisions which are covered by the length of fibre.
- Again, rotating the eyepiece, now find the number of divisions of eyepiece micrometer covered by the width of same fibre.
- By similar way calculate the length and width of 25 fibres.
- Multiply each value by the factor calculated at the start of procedure to get the values in microns.
- Calculate the average value of length of width of fibre (Shah B. N, 2015).

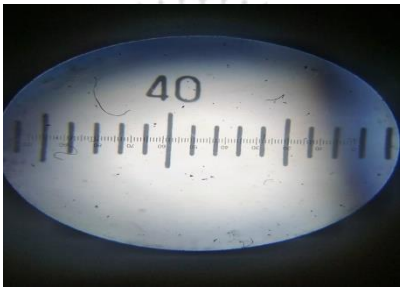


Figure No. 2: Calibration of eyepiece

Determination of ash value

The main aim of determining different ash value of vegetable drugs is to remove complete traces of organic matter that can intervene in an analytical determination. The different ash values are helpful in establishing the quality and purity of crude drugs mainly in powder form. When crude drugs are incinerated, it may consist of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium (Khandelwal 2008).

Total ash value

Weigh accurately 2 or 3gm of drug in a tarred platinum or silica dish previously ignited and weighed. Scatter the ground drug in a fine even layer on the bottom of the dish (Khandelwal 2008). Incinerate the drug by gradually increasing the temperature to 500-600^{0}C until it is white, which indicate about the absence of Carbon. Now cool and weighed on ashless filter paper (V.B. Kadam, 2012) calculate the total ash value.

Acid-insoluble ash value

Wash the ash from the dish after repeating the same procedure of total ash, using 25ml of dil. Hydrochloric acid. Boil for 5min and filter by using ashless filter paper. Now again Incinerate for 4 hours or more, cool and calculate acid insoluble ash value[(2)].

Water soluble ash value

Wash the ash from the dish after repeating the same procedure of total ash, using25ml of water. Boil for 5min and filter with the help of ashless filter paper. Now again incinerate, cool and calculate water insoluble ash value [(1,2)].

Determination of moisture content (Loss on drying)

- Weighed about 2gm of powdered leaves drug.
- Put into porcelain dish and dried the powdered drug in oven at 100^{0}C or 150^{0}C.
- Cool porcelain in desiccator and watch.
- Moisture is recorded as loss in weight.

Determination of foreign organic matter

- Weighed about 100g of powder drug of dried leaves.
- Now, spread the sample of the white tile without overlapping.
- Examine the drug closely with naked eye or by lens (10x or above).
- Now separate the organic matter.

- Weighing is done after complete separation.
- Calculate the % w/w present in the drug [2].

Process of Extraction

Maceration

The dried leaves of plant *Passiflora vitifolia* are crumbled with the help of a mechanical grinder. The powder material was allowed to dry. The plant material was defeated with petroleum ether and now, macerates with different solvents (Ethanol, Acetone, Ethyl acetate and Chloroform) [2].

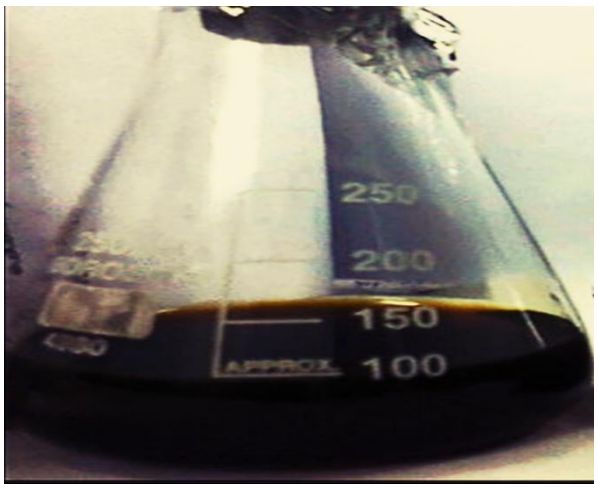


Figure No. 3: Cold extraction (Maceration) of *Passiflora vitifolia* leavesSoxhlet extraction

The dried and coarsely powdered leaves of *Passiflora vitifolia* were packed in a Soxhlet apparatus separately for each solvent (pet ether, ethanol). Each extract was evaporated to dryness and constant extractive values were recorded.

Determination of extractive value

Extractive values are use full for the evaluation, to determine the nature of chemical and estimation of specific constituents soluble in particular solvent used for the extraction. The filter of the various extract of leaves of *Passiflora vitifolia* (maceration and Soxhlet extraction) were evaporate to dryness in the traced flat bottom dish at 150^0 C and record the weight of each. Determine the extractive value for each solvent [1].

Procedure for Phytochemical investigations

Test for carbohydrates

- **Fehling's test:** 1ml of Fehling's solution A and B was mixed and boil for 1minutes. Equal volume of extract was added then it was heated in boiling water bath for 5-10 minutes, first a yellow, brick red ppt was observed.

- **Benedict's test:** 2ml of Benedict's reagent and test solution was mixed in test tube. It was heated in boiling water bath for 5 minutes. The solution was appeared to be green in colour which indicates the presence of reducing sugar in test solution.

Test for monosaccharide

- **Barford's test:** Take equal volume of test solution and Barford's reagent. Kept for 1-2 min in boiling water bath and allowed to cool. Red ppt was obtained.

Test for hexose sugars

- **Tollen'sphloroglucinol test:** take 1-2 ml of test solution and add 3 ml of conc. HCl and 4 ml of 0.5% phloroglucinol, heat the mixture. Yellow to red colour appears.

Test for reducing polysaccharides (STARCH)

- **Iodine test:** To the 3ml of test solution add few drops of dil. Iodine solution appears which will disappears on boiling and reappears on cooling.

- **Tannic acid test:** With test, solution add 20% of tannic acid solution, gives yellow ppt[(2)].

Tests for alkaloids

Each extract was dissolved in HCl and filtrates were obtained. They were used for testing.

- **Mayer's test:** Mayer's reagent (Potassium Mercuric Iodide) was treated with filtrates. The yellow colour ppt are appeared which give indication ok presence of alkaloids.

- **Wagner's Test:** Filtrates were Taken and treated with Wagener's reagent (solution of Potassium Bismuth Iodide). Brown/reddish ppt was formed that indicates the presence of alkaloids.

- **Dragendroff's Test:** Filtrates were Taken and treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Reddish ppt was obtained which indicates the detection on alkaloids.

- **Hager's Test:** Filtrates were Taken and treated with Hager's reagent (saturated picric acid solution). Detection of alkaloids was confirmed by the formation of ppt of yellow colour.

Test for flavonoids

- **Alkaline Reagent Test:** The Extracts was taken and treated with few drops of sodium hydroxide solution. Indication for flavonoids was detected by formation of yellow colour and on addition of dil. acid it turns colourless.

- **Lead acetate Test:** Extracts were treated with few drops of lead acetate solution. Indicates the presence of flavonoids by the formations of yellow colour precipitate **(**Prashant Tiwari *et al*, 2010**)**.

- **Shinoda test:** To the extract, 95% of ethanol was added with few drops of conc. HCl and 0.5 g of magnesium turnings were also added. The presence of flavonoids was confirmed by observation of pink colour.

Test for cardiac glycoside

- **Legal's test (cardenoloids):** Take extract, add pyridine and 1ml of nitroprusside. The confirmation of cardiac glycoside was indicating by appearance of pink to red colour.

Test for tannins and phenols

- **Ferric chloride test:** To the extract solution few drops of 5% of $FeCl_3$ solution were added the presence of phenolic compounds was determined by dark green colouration.

- **Lead acetate test:** To the extract solutions add few drops of 10% of lead acetate solution formation of white ppt indicate the presence of phenolic compounds.

- **Gelatin test:** To the extract solution few drops of 10% gelatine were added which results the formation of white ppt, indication for the presence of phenolic compounds.

- **Bromine water test:** To 2-3 ml of the extract solution add few drops of bromine water. The decolouration of water indicates the presence of phenolic compounds.

- **Acetic acid test:** To 2-3 ml of the extract solutions add few drops of acetic acid; solution becomes red in colour which indicates the presence of phenolic compounds.

Test for proteins

- **Biuret test (general's test):** To 3ml of the extract solution add few drops of 4% NaOH and few drops of 1% $CuSO_4$ solution. The appearance of pink or violet colour indicates the presence of proteins.

- **Xanthoprotein test (for protein containing tyrosine or tryptophan):** To 2-3 ml of the extract solution add 1ml of conc. H_2SO_4, white ppt appears which turns yellow on boiling. Now add NH_4OH ppt turns orange.

- **Test for proteins containing sulphur:** To 2-3 ml of the extract solution add few drops of 40% NaOH and few drops of lead acetate solution (10%). Black or brownish colour appears due to PbS formation which confirms protein[2].

Thin layer chromatography

The thin layer chromatography (TLC) plates are washed with water and they are allowed to dry in hot air oven. The plates for TLC were prepared by using pouring method. The silica gel G IS used to make slurry with sufficient distilled water. The slurry is uniformly poured on the glass slide. Now, these TLC plates were allowed to dry in the oven at temperature 110^{o}C for 30 min. TLC plates get activated. After, the activation of TLC plates, they were used to examine for different compound in pre-formed activated solvent system[2].

Fluorescence Analysis

It helps to evaluate the selected plant material's quality, strength, and purity. The powdered form of drug is mixed with solvent system to make paste like material. This paste is putted on the glass slides and now these slides are examined under the region of light (visible light, long U.V light, Short U.V light). The florescence produce by the extract is examined after treatment of various inorganic/organic reagents [1,2].

Pharmacological Studies

The method used to determine the *in-vitro* anti-inflammatory activity of ethanol extract of leaves of *Passiflora vitifola.*

Methods to Determining the *In-Vitro* Anti-inflammatory Activity

1. HRBC (Hypotonicity Induced Human Red Blood Cell) Membrane Stabilisation Method.

2. Inhibition of Protein Denaturation Method.

Membrane Stabilisation Method

Preparation of suspension of blood for HRBC Method.

5 ml of blood was collected from healthy human volunteer who had not taken any NSAIDS for 2 weeks prior to the experiment with equal volume of Alsever solution (2% dextrose, 0.8% Sodium citrate, 0.5% citric acid and 0.45% sodium chloride). All the blood samples were stored at 4^0C for 24 hours. After completion of 24 hours, it was centrifuged at 2500rpm for 5 min and the supernant was removed. The cell suspension was washed with sterile saline solution (0.9% w/v NaCl). This solution was again centrifuge for 5 min at 2500rpm. The same procedure was repeated at least 3 times till supernant was become clear and colourless. The packed volume was reconstituted to 40% suspension (v/v) with phosphate buffer saline (pH 7.4). This suspension was used for assay (R. Sarveswaran *et al*, 2017).

HRBC (Hypotonicity Induced Human Red Blood Cell)

1.0ml of test sample of different concentrations (0.05, 0.1, 0.15, 0.2) in phosphate buffer and 0.5ml of 10% HRBC suspension, 0.5ml of 25% hyposaline were incubated at 37^0C for 30 min and centrifuge at 3000 rpm for 20-25 min. The supernatant solution was estimated on UV spectrophotometer at 560 nm. The % haemolysis was estimated by assuming the haemolysis produced in the control as 100%. Diclofenac sodium was used as reference standard and a control was prepared by omitting the extracts (Kota Karunakar *et al*, 2018). The % of haemolysis and membrane stabilisation or protection was calculated by using formula give below: -

% of Haemolysis= Optical density of test sample / Optical density of control ×100

% Protection = 1- [Optical density of test sample / Optical density of control ×100]

Inhibition of Protein Denaturation Method

The reaction mixture of 0.5ml was prepared containing 0.45ml of bovine serum albumin (5% aqueous solution) and different concentration of leaves extract of *P. vitifolia* was taken. The pH was maintained by using phosphate buffer (pH 7.4) (R. Sarveswaran *et al*, 2017). The % of protection from denaturation was calculated by using formula given below: -

% inhibition=100- [Optical density of test sample- Optical density of control / Optical density of control] ×100

RESULTS

Determination of width and length of fibres

The determination of fibre length and width of *Passiflora vitifolia* are listed below in table 1.

Table No. 1: Determination of width and length of fibres

Sr. No.	No of fiber covering eyepiece	Division of eyepiece covering width of fibre (x)	Least count in micron (Y)	(X&Y)
1.	3	4	52.72	158.16
2.	24	2	26.36	316.32
3.	25	3	39.54	329.50
4.	3	5	65.90	197.70
5.	2	6	79.08	158.16
6.	1	7	92.26	92.26
8.	8	8	105.44	105.44

Total size of 60 particles= 1357.54µm

Average size of 1 particle= 22.62µm

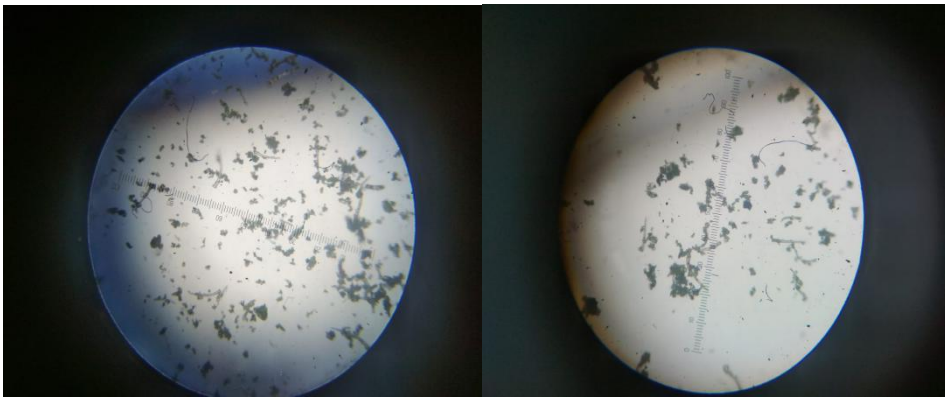


Figure No. 4: Determination of fibre size of *Passiflora vitifolia* leaves

Ash value of leaves of *Passiflora vitifolia*

The results of ash values for the leaves of *Passiflora vitifolia* are given in table 2.

Table No. 2: Ash value of leaves of *Passiflora vitifolia*

Sr. No.	Ash value	Yield (%w/w)
1.	Total ash value	6.5±0.3
2.	Acid-insoluble ash value	1.0±0.1
3.	Water soluble ash value	4.1±0.26

Foreign matter determination value

The result of foreign matter for the leaves of *Passiflora vitifolia* is given in Table 3.

Table No. 3: Foreign matter value of leaves of *Passiflora vitifolia*

Sr. No.	Weight of crude drug(g)	Weight of the drug after removal of foreign material (g)	Weight of Foreign matter (g)	Foreign matter (%)
1.	10.0	9.75	0.25	2.50
2.	10.0	9.78	0.22	2.20
3.	10.0	9.80	0.20	2.00
Mean		9.77	0.22	2.23

Extractive value of leaves of *Passiflora vitifolia* by hot extraction (maceration process)

The result for the different extractive value was given the table 4 below: -

Table No. 4: Extractive value of leaves of *Passiflora vitifolia*

Sr. No.	Solvents	Weight of plant material (g)	Colours of extract	Weight of extract	Extractive value (%w/w)
1.	Ethanol	10	Green	1.08	10.8
2.	Ethyl acetate	10	Dark green	0.55	5.5
3.	Chloroform	10	Light green	2.9	2.9
4.	Petroleum ether	10	Yellow green	0.44	4.4
5.	Acetone	10	Dark green	0.85	8.5

Extractive value of leaves of *Passiflora vitifolia* by cold extraction (Soxhlet extraction)

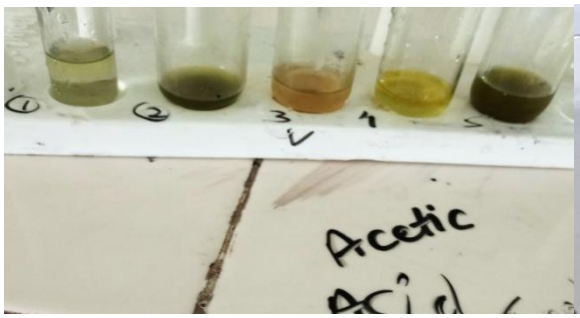
The extractive value of *Passiflora vitifolia* leaves by Soxhlet extraction (hot extraction) with ethanol and petroleum ether were determine and shown in table 5.

Table No. 5: Extractive value of leaves of *Passiflora vitifolia*

Sr. No.	Solvents	Weight of plant material (g)	Colours of extract	Weight of extract	Extractive value(%w/w)
1.	Ethanol	20	Green	1.69	16.9
2.	Petroleum ether	20	Yellow green	0.63	6.3

Phytochemical investigation of *Passiflora vitifolia*

(a) **(b)**



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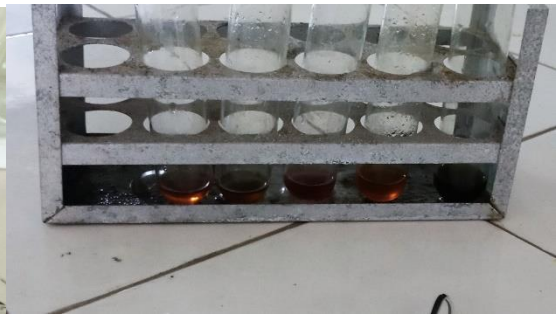

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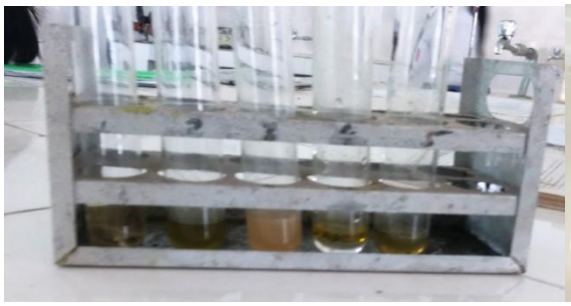

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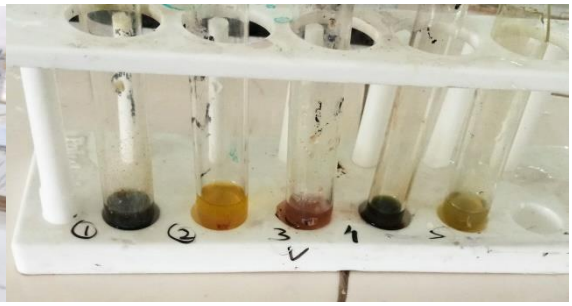


(f)

Figure No. 5: Result of various phytochemical present in the leaves of *Passiflora vitifolia* (a) Tollen's test, (b) Barfoed's test, (c) Acetic acid solution test, (d) Legal test, (e) Shinoda Test, (f) Tollen's test

The preliminary phytochemical screening of various extract viz., ethanol, ethyl acetate, chloroform, petroleum ether and acetone was carried out and results obtained the leaves of *Passiflora vitifolia* is tabulated in the table 6 below: -

Table No. 6: Phytochemical investigation of *Passiflora vitifolia*

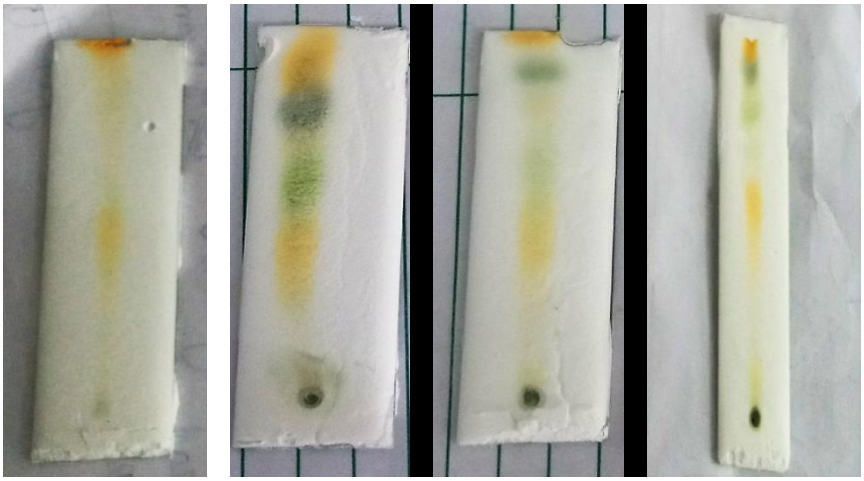
Phytoconstituent	Method	Ethanol extract	Ethyl acetate extract	Chloroform extract	Petroleum ether extract	Acetone extract
Flavonoids	Shinoda Test	+ve	-ve	+ve	+ve	+ve
	Lead acetate Test	+ve	-ve	-ve	-ve	-ve
Alkaloids	Wagner Test	+ve	+ve	+ve	+ve	-ve
	Hager's Test	+ve	-ve	+ve	+ve	+ve
	Dragendroff's test	-ve	-ve	+ve	+ve	+ve
	Mayer's test	+ve	-ve	+ve	_ve	-ve
Tannins and Phenols	Lead acetate test	+ve	-ve	+ve	+ve	+ve
	Bromine water test	+ve	+ve	+ve	+ve	-ve
	Acetic acid solution test	+ve	+ve	-ve	+ve	-ve
	Dil. Potassium permanganate test	+ve	+ve	-ve	+ve	-ve
	Dil. Iodine solution test	+ve	+ve	+ve	+ve	-e
	Dil. HNO_3 test	-ve	+ve	+ve	+ve	+ve
	Potassium dichromate test	+ve	-ve	+ve	-ve	+ve
Carbohydrates	Fehling's test	+ve	-ve	+ve	-ve	-ve
	Benedict's test	+ve	+ve	+ve	-ve	-ve
	Barfoed's test	+ve	+ve	+ve	-ve	-ve
	Tannic acid test	+ve	-ve	-ve	-ve	+ve
	Iodine test	-ve	+ve	+ve	+ve	-ve
	Tollen's test	-ve	+ve	+ve	+ve	+ve
Cardiac glycoside	Legal test	+ve	+ve	+ve	-ve	-ve
Proteins	Biuret test	+ve	-ve	+ve	+ve	-ve
	Xanthoprotein test	-ve	-ve	-ve	-ve	-ve
	Test for protein containing sulphur	+ve	-ve	-ve	-ve	+ve

T.L.C profile

The result of T.L.C profile of different extractives of the leaves of *Passiflora vitifolia* is given in Table 7.

Table No. 7: T.L.C profile of different extractives of the leaves of *Passiflora vitifolia*

S. No.	Extract	Solvent system	Ratio	Rf value
1.	Ethanol	Toluene : Ethyl acetate	7:3	0.46, 0.57, 0.69, 0.780.80
		Toluene : Ethyl acetate	8:3	0.34, 0.41, 0.76, 0.80
		n-Hexane : Ethyl acetate	7:3	0.16, 0.25, 0.66, 0.75
		n-Hexane : Ethyl acetate	8:3	0.25, 0.26, 0.58, 0.63
2.	Ethyl acetate	Toluene : Ethyl acetate	7:3	0.24, 0.37, 0.51, 0.71, 0.81, 0.93
		Toluene : Ethyl acetate	8:3	0.20, 0.54, 0.81, 0.96
		n-Hexane : Ethyl acetate	7:3	0.25,0.35,0.56
		n-Hexane : Ethyl acetate	8:3	0.34, 0.56, 0.67, 0.87
3.	Chloroform	Toluene : Ethyl acetate	7:3	0.23, 0.45, 0.78
		Toluene : Ethyl acetate	8:3	0.56, 0.76
		n-Hexane: Ethyl acetate	7:3	0.34
		n-Hexane : Ethyl acetate	8:3	0.30
4.	Pet. ether	Toluene : Ethyl acetate	7:3	0.36, 0.61, 0.76, 0.90
		Toluene : Ethyl acetate	8:2	0.21, 0.31, 0.36, 0.40, 0.50, 0.60, 0.71, 0.83
		n-Hexane : Ethyl acetate	7:3	0.13, 0.25, 0.33, 0.81, 0.96
		n-Hexane : Ethyl acetate	8:2	0.08, 0.28, 0.41, 0.69, 0.87
5.	Acetone	Toluene : Ethyl acetate	7:3	0.10, 0.17, 0.34, 0.53, 0.61, 0.71, 0.80, 0.88
		Toluene : Ethyl acetate	8:2	0.18, 0.27, 0.45, 0.57, 0.72, 0.83, 0.98
		n-Hexane : Ethyl acetate	7:3	0.24, 0.34, 0.36, 0.39,0.48, 0.78,
		n-Hexane : Ethyl acetate	8:2	0.45,0.57, 0.67,



(a) (b) (c) (d)

Figure No. 6: T.L.C of (a) ethanol, (b) petroleum ether, (c) acetone, (d) ethyl acetate of *Passiflora vitifolia* leaves

HRBC membrane stabilization method of extract of leaves of *Passiflora vitifolia*

The percentage inhibition of proteinase action of various concentrations of ethanol extract, the leaves of *Passiflora vitifolia* is given below in table 9.

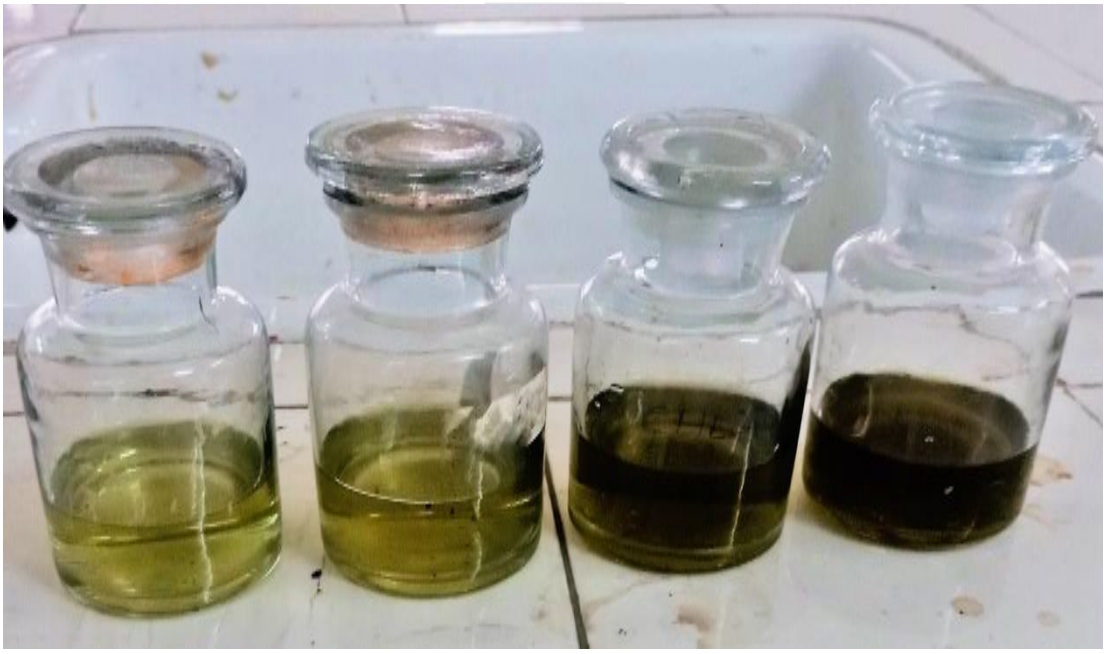


Figure No. 7: Different ethanolic concentration in µg/ml (50, 100, 150, and 200) of *Passiflora vitifolia* leaves

Table No. 8: HRBC membrane stabilization method of extract of leaves of *Passiflora vitifolia*

Treatment	Concentration (µg/ml)	Absorbance at 660nm	% stabilisation of hemolysis
Control	----------	0.65	----------
Ethanol extract	50	0.40	38
	100	0.15	77
	150	0.099	85
	200	0.095	85
Aspirin	100	0.099	85

Effect of *Passiflora vitifolia* leaves extract on inhibition of protein denaturation

The effect of various Ethanolic concentrations of *Passiflora vitifolia* leaves is tabulated below in table 9.

Table No. 9: Effect of *Passiflora vitifolia* leaves extract on inhibition of protein denaturation

Treatment	Concentration (µg/ml)	Absorbance at 660nm	% Inhibition of proteinase action
Control	----------	0.67	----------
Ethanol extract	50	0.42	37
	100	0.12	82
	150	0.091	86
	200	0.090	87
Aspirin	100	0.100	85

SUMMARY & CONCLUSIONS

SUMMARY

The current research entitled “The Pharmacognostic standardization and evaluation of *In-vitro anti-inflammatory* activity of *Passiflora vitifolia* leaves. The plant was chosen and authenticated with the objective of standardizing the pharmacognostical characters, extraction

of phytoconstituents from the leaves using ethanol, ethyl acetate, chloroform, pet. ether and acetone. These studies help to understand the major phytoconstituents present in the different extract of *vitifolia* leaves. Further, the evaluation of *In-vitro anti-inflammatory* activity was carried out by the two different methodologies i.e. HRBC method and inhibition of protein denaturation using different concentrations of ethanol extract. To full fill the above objective, a systematic approach was adopted and executed.

CONCLUSION

The plants play an important role and using since ancient time as human health care. Traditional plants are very important, and they have significance role in discovery of new drugs. And the first step towards the development of plant is standardization.

Now present days, majority of human population is getting affected by inflammation related disorders like rheumatoid arthritis, osteoarthritis, inflammatory bowel diseases, multiple sclerosis, psoriasis, retinitis and atherosclerosis. It is believed that there are several drugs available in market like NSAIDs and opiates, but they are not use full in all cases because of their side effects such as liver dysfunction, GIT irritation and many more. There are many immunosuppressing agents are also available, but they cause severe side effect on long term administration. Therefore to overcome this problem new drugs requisite and plants contains many phytoconstituents which are helpful in inflammation and have very few side effects as compared to already available drugs. So, this research gives some satisfactory result on the method used as *anti-inflammatory.*

Thus, the present study opens the quest further evaluate the *anti-inflammatory* action of *Passiflora vitifolia* leaves. Anti-inflammatory activity supposed to be due to the presence of flavonoids in the plant. Anti-inflammatory activity has been recognised long back in rodents and reviewed exhaustively. Some example includes quercetin, silymarinapigenin, daidzein, genistein etc.

Hence proper isolation of active constituent might help in finding of new lead compounds in the field of ant-inflammatory drug research.

FUTURE ASPECT OF *PASSIFLORA VITIFOLIA*

The study unfolds the phytoconstituents present in *P. vitifolia*, evaluation of *In-vitro* anti-inflammatory activity resulted in comparable result with aspirin as standard. Therefore, plant can be subjected to further investigation on inflammation responses.

- The plant can be subject for the preparation of analgesic formulation like ointment, sprays, lotions, creams etc.
- The plant can be subjected for the development of ayurvedic preparations.
- The plant can be subjected to *In-vivo* assay for anti-inflammatory responses.
- The other extracts can also be subjected of the anti-inflammatory.
- The others evaluation can be determining as the plant contains large number of phytoconstituents.
- The plant can be evaluated by selecting other NSAIDS as standards.

The future aspect also defines the further study on vitifolia as the plant contains large number of Phytoconstituent, but the plant is never subjected for evaluation of much study. The study in future can be extended to the other parts also as the also need to be standardised and evaluated for the pharmacological responses.

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CONFLICT OF INTEREST

No conflict of interest is declared.

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