

Evaluation of Histochemical and Phytochemical analysis of *Passiflora vitifolia* kunth.

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ABSTRACT

The genus Passiflora vitifolia belongs to the family Passifloraceae which includes the passion fruit is the largest and the most widespread genus of tropical flora. Medicinal plants have been a valuable source of natural active phyto constituents that play an important role of many human diseases. Histochemistry is a bordered line field between histology and analytical chemistry or biochemistry. Its subject matter is the identification and localization of chemical substances in the tissue on cytological scale. P. vitifolia, Plant used in Ayurveda for Sedative, anxiety and hypertension. Plant from this genus passiflora has been used in traditional medicine by many cultures. In the present study, about nine qualitative phytochemical screening was performed. It summarizes that the selected plant is a good source of various metabolites like sterols, flavonoids, triterpenoids, glycosides, tannins, saponins, resin alkaloids, phenols. Methanol extract of leaf and stem show highest metabolites. Plants generally produce many secondary metabolites which are biosynthetically derived from primary metabolites and constitute an important source of microbicide, pesticide and many pharmaceutical drugs. Several studies confirmed the presence of these phytochemicals contribute medicinal as well as physiological properties in the treatment of different ailments.

Keywords: *Passiflora vitifolia, Histochemical analysis, Phytochemical analysis, microbicides and pesticides*

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INTRODUCTION

Plants are the backbone of life on the earth and are essential nutrients for human beings and animals. A medicinal plant is that can be used for therapeutic purposes, such plants are designated as medicinal plants. Since from very long time, man has used all parts of the plant in the treatment and prevention of many ailments. [1]. According to the World Health Organization [2], a medicinal plant means any plant which contain substances that can be used for the treatment of diseases. A plant is considered as a medicinal plant only it's biologically activity has been ethno-botanically reported or scientifically validated [3].

In Nigeria, according to WHO, it is estimated about 75% of population preferred traditional medicine than English Medicine [4]. Plants are considered as the main sources of medicines for human before the advancement of science and technology [5]. India is considered as biodiversity hotspots of the richest and highly endangered eco-regions of the world [6]. Plants generally produce many secondary metabolites which are biosynthetically derived from primary metabolites and constitute important sources of microbicide, pesticide and many pharmaceutical drugs. Medicinal plant play important role in the human society to combat diseases [7]. Histochemical methods have been developed for qualitative and quantitative analysis of virtually all cellular components, including proteins, carbohydrates, lipids, nucleic acids and range of ionic element occurring in cell solutions. Tannin is the heterogeneous group of phenol derivatives, usually related to glycosides. Tannins are particularly abundant in the leaves (xylem) of many plants. Saponins are of rare occurrence. Fats are reserve material in seeds, spore and embryos in meristematic cells glucosides are the degradation product of carbohydrates. Alkaloids are degradation product of protein [8].

Here we have to compare in vitro and in vivo histochemical analysis with Invitro & in vivo phytochemical analysis of stem, leaves, flower etc. Phytochemical are chemical compounds which are produce during the plants normal metabolically process [9]. The secondary metabolites are of medicinal value to man and these are derived from various structures of plants [10].

Plant description:

Passiflora vitifolia belongs to the family Passifloraceae. This family consists of about 400 species. It is a vine with cylindrical stems covered with red-brown hairs during young stage. The leaves are serrate, three-lobed, up to 15cm long and 18cm broad. The fruit is a berry 5cm long and 3cm broad (Fig-1). With green flesh speckled with white, slight downy hairs, containing numerous seeds. Plant from this genus passiflora has been used in traditional medicine by many cultures.



Fig 1. Habit of *Passiflora vitifolia* kunth

MATERIAL AND METHODS

I) Test for carbohydrates. Picric acid-Schiff's reaction: The Schiff reagent is the reaction product of a dye formulation such as fuchsine and sodium bisulphate; Pararosanilin and new fuchsine are dye alternatives with comparable detection chemistry.

II) Test for Starch: Iodine-Potassium-Iodide test (IKI): The iodine -Potassium iodide (IKI) stain is specific for starch. Apparently, the basis of the reaction is the accumulation of iodine in the center of the helical starch molecule. The length of the starch molecule determines the colour of the reaction- the shorter the molecule, the more red the colour; the longer the molecule, the more blue the color [11].

III) Test for cellulose: Methylene blue is a heterocyclic aromatic chemical compound with molecular formula $C_{16}H_{18}N_3SCl$. At room temperature it appears as solid, odour less, dark green powder that yields a blue solution when dissolved in water.

IV) Test for Tannin, Lignin and pectin- Toluidine Blue O stain:

The stain, Toluidine blue is an excellent stain for free hand sections. TBO is a cationic dye that binds to negatively charged groups. An aqueous solution of this dye is blue, but different colours are generated when the dye binds with different anionic group in the cell.

V) Test for Alkaloid:

Wagner's reagent [8]: 1gm iodine and 2gPotassium iodide were dissolved in 50 ml of distilled water. Section were kept in stain for 1-2min and then observed under the microscope.

Mayer's reagent: Potassium mercuric iodide solution; 13.55g of $HgCl_2$ and 50g of KI, were dissolved in one liter of distilled water. Presence of gray colour in the section reveals the presence of alkaloids.

Hager's test: To a few ml of filtrate, 1-2 ml of reagent were added. A prominent yellow precipitate indicated the test as positive.

VI) Test for Flavonoids: In higher Plants, flavonoids are involved in UV filtration, symbiotic nitrogen fixation and floral pigmentation. Flavonoids secreted by the root of their host plant help Rhizobia in the infection stage of their symbiotic relationship with legumes.

VII) Test for cellulose: Dissolve 0.5g Congo red in100ml of distilled water to prepare a 0.5% Congo red solution appearance of red colour.

RESULTS AND DISCUSSION

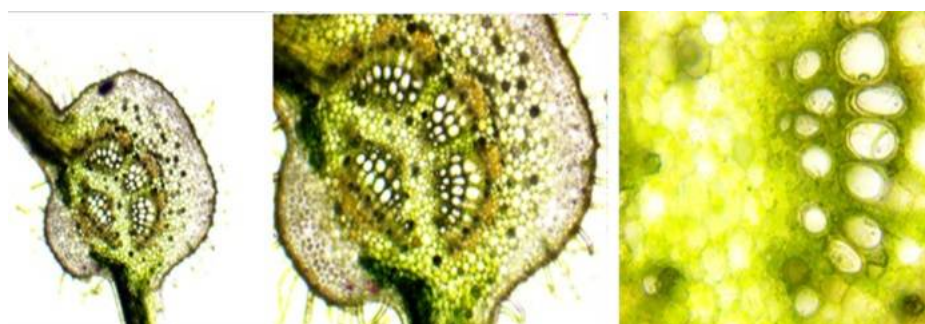
In vivo and *in vitro* histochemical colour reaction of *P. vitifolia* leaf and stem were carried out by taking free hand section of leaf and stem followed by treatment with various reagent and observation under microscope. The results revealed that the, presence of cellulose, pectin, Alkaloids, Saponins, The results are presented in the table 1,2,3 & 4. The presence of Cellulose in leaf sections was confirmed by the presence of

blue-coloured cells after treating the sections with Methylene blue stain and also appearance of red coloured cells after treating with Congo red stain. The presence of pectin in leaf sections was confirmed by the presence of pink to purple-coloured cells after treating the sections with Toluidine blue stain. The occurrence of alkaloids in leaf sections was revealed by the presence of golden yellow coloured cells after treating the sections with Wagner's reagent and Hager's reagent and also Gray coloured in the pith parenchyma treating with Mayer's reagent. The presence of flavonoids in the leaf, free hand section was confirmed by the presence of yellow coloured cells after treating with 10% NaOH. (Fig. 2-7)

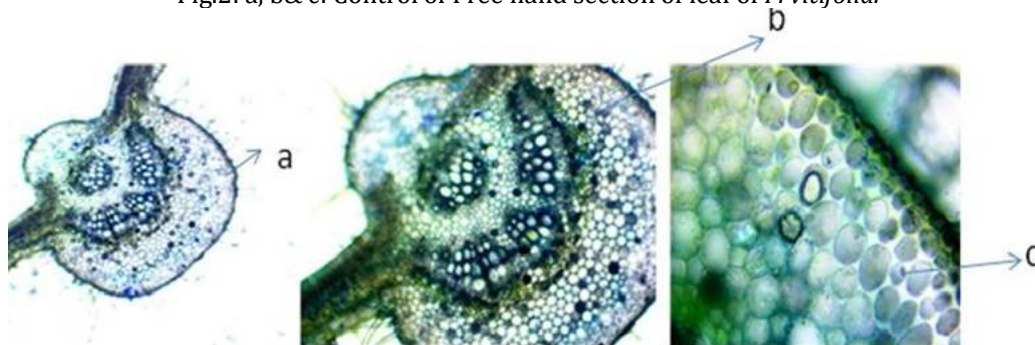
The presence of Cellulose in the free hand section of stem was confirmed by the presence of blue coloured cells after treating with Methylene blue stain and also appearance of red coloured cells after treating with Congo red solution. The cellulose was located in the epidermis, cortex and parenchyma. The presence of pectin in the free hand section of stem was confirmed by the presence of pink/ purple coloured cells after treating with Toluidine blue stain. The presence of lignin in the free hand section of stem was confirmed by the presence of green coloured cells after treating with Toluidine blue stain. The presence of Alkaloid in the section of stem was confirmed by the presence of golden yellow coloured cells after treating with Wagner's and Hager's reagent. The presence of flavonoids in the free hand section of stem, was confirmed by the presence of yellow coloured cells after treating the sections with 10% NaOH. The presence of Alkaloids in the microtome section of microtome section of leaf callus was confirmed by the presence of yellow coloured cells after treating the section with Wagner's and Hager's reagent (Fig.8-16).

The presence of Carbohydrates in the microtome section of leaf callus was confirmed by the presence of magenta coloured cells after treating with PAS. The presence of Cellulose in the microtome section of leaf callus was confirmed by the presence of blue coloured cells after treating with Methylene blue & also red coloured cells, after treating with Congo red stain. The presence of pectin in the microtome section of leaf callus, was confirmed by the presence of pink/ purple coloured cells after treating with Toluidine blue stain (Fig. 17-21).

Histochemical colour reaction of leaf and stem free hand section of leaf



a: 4X b:10X c:40X
Fig.2: a, b& c: Control of Free hand section of leaf of *P. vitifolia*.

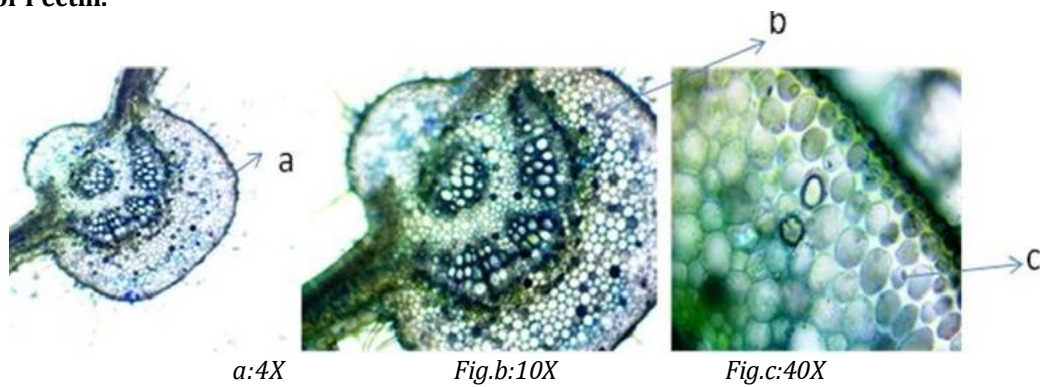


Test for Cellulose:

a : 4X b:10X c:40X
a-epidermis, b-cortex and c-parenchymatous region

Fig.3: a , b & c: Blue coloured cells indicates the presence of cellulose in the epidermis, cortex and parenchymatous region of leaf of *Passiflora vitifolia*.

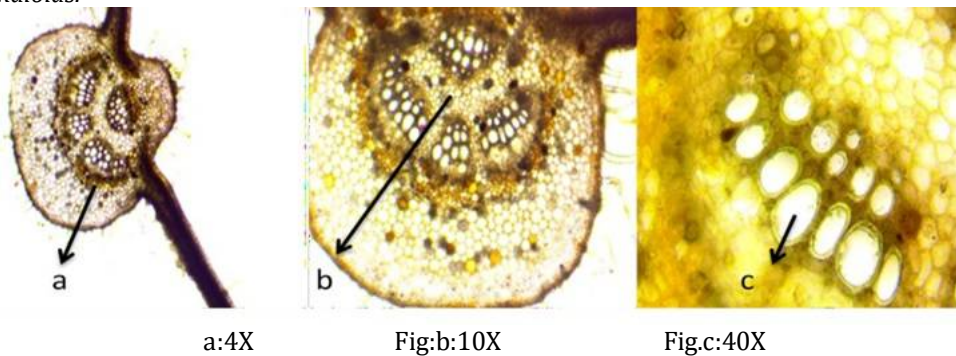
Test for Pectin:



a:4X Fig.b:10X Fig.c:40X
a-epidermis, b-cortex and c-parenchyma tissue

Fig.4 a, b & c: Purple coloured cells indicates the presence of Pectin substances in the epidermis, cortex, endodermis and parenchymatous region of leaf of *P. vitifolia*.

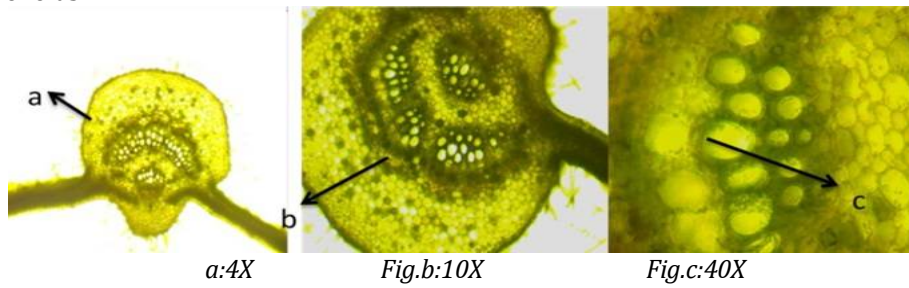
Test for Alkaloids:



a:4X Fig.b:10X Fig.c:40X
A-cortex, b-pith and c-xylem

Fig.5a ,b &c: Golden yellow colour indicate the presence of Alkaloid in the Cortex, pith and xylem of leaf of *P. vitifolia*, after treating with Wagner's reagent.

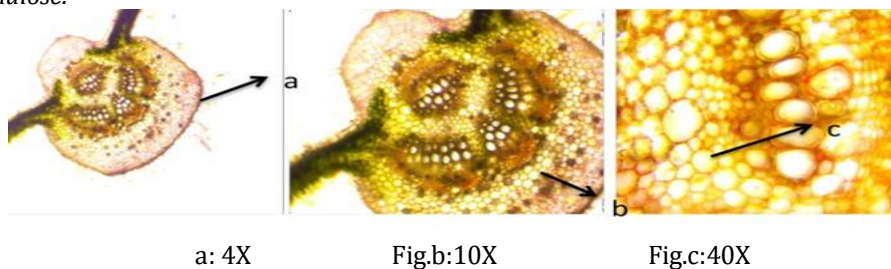
Test for flavonoids:



a:4X Fig.b:10X Fig.c:40X
a-epidermis, b-parenchyma and c-xylem

Fig.6 a,b & c: Yellow colour indicate the presence of flavonoids in the epidermis, parenchyma and xylem of leaf of *P. vitifolia*.

Test for Cellulose:



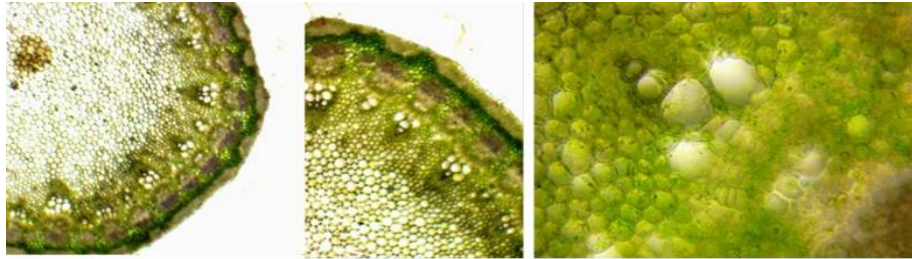
a: 4X Fig.b:10X Fig.c:40X
a-epidermis, b-cortex and c-xylem

Fig.7a,b & c: Red colour indicates the presence of cellulose in the epidermis, cortex & xylem of leaf of *Passiflora vitifolia*, after treating with Congo red reagent.

Table 1: Histochemical color reaction and their respective constituents and confirmation of leaf

Sl. No	Reagents	Colour	Constituent	Localization
1	Methylene blue	Blue	Cellulose	Epidermis, cortex & parenchymatous region
2	Toludine blue	Purple	Pectin	Epidermis, cortex & parenchymatous region
3	Wagner's	Golden yellow	Alkaloids	Parenchyma region, epidermis & xylem
4	10%NaoH	Yellow	Flavonoides	Parenchyma region, epidermis & xylem
5	Congo red	Red	Cellulose	Epidermis, cortex & pith region

Histochemical color reaction of Stem.



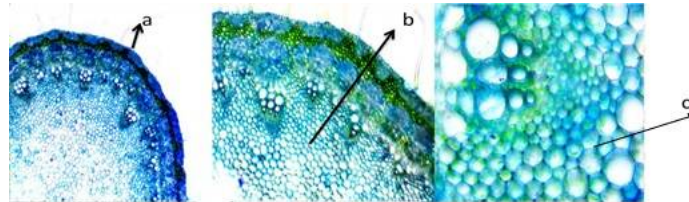
a: 4X

Fig.b:10X

Fig.c:40X

Fig.8 a,b&c: Control of Freehand section of stem of *Passiflora vitifolia*.

Test for Cellulose:



a:4X

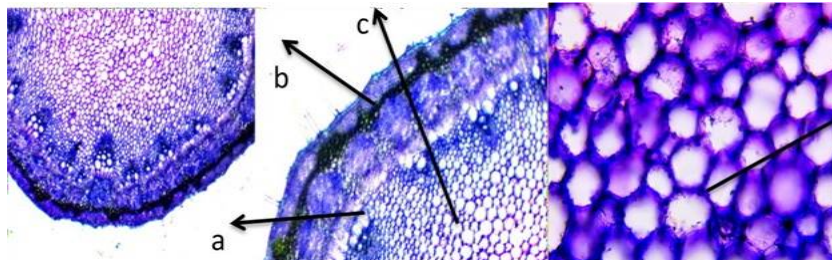
Fig.b:10X

Fig.c:40X

a-epidermis, b-cortex and c-parenchymatous region

Fig.9a,b &c-Blue coloured cells indicates the presence of cellulose in the epidermis, cortex and parenchymatous region of stem of *Passiflora vitifolia*.

Test for Pectin:



a:4X

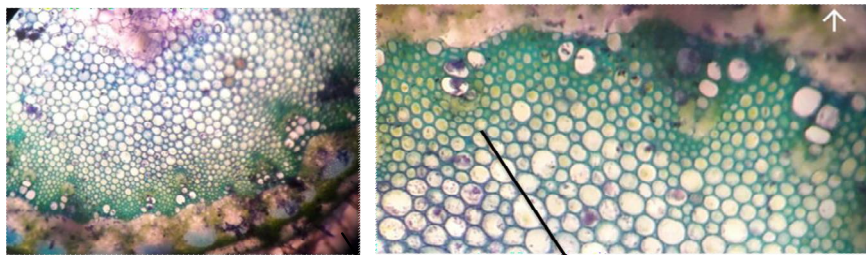
Fig.b:10X

Fig.c:40X

a-cortex, b-epidermis, c-cortex and d- parenchymatous region.

Fig.10a,b&c:Pink or purple colour indicates the presence of Pectin in the epidermis, cortex, endodermis and parenchymatous region of stem of *Passiflora vitifolia*.

Test for lignin:



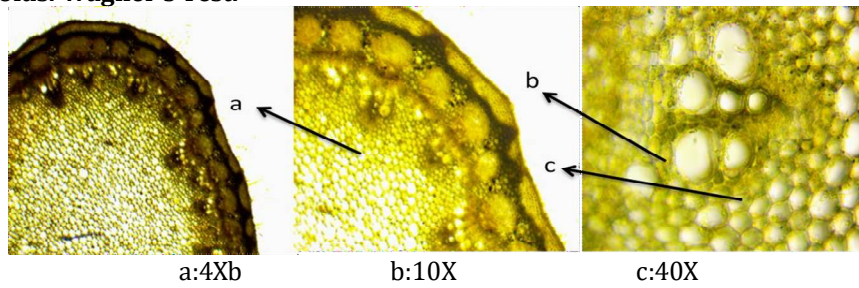
a:4x

b: 10x

a-epidermis b-cortex

Fig.11a & b: Green colour indicates the presences of Lignin in the epidermal region and cortex of stem of *Passiflora vitifolia*, after treating with Toludine blue reagent.

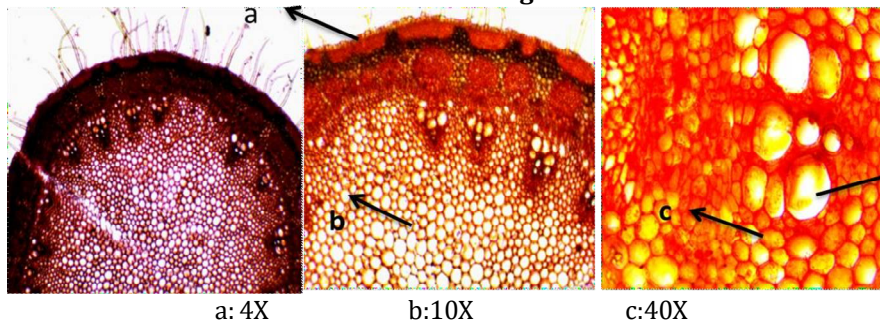
Test for Alkaloids: Wagner's Test:



a-cortex, b-xylem and c-pith

Fig.12a,b & c: Golden yellow colour indicate the presence of Alkaloid in the cortex, pith, xylem of stem of *Passiflora vitifolia*, after treating with Wagner's reagent.

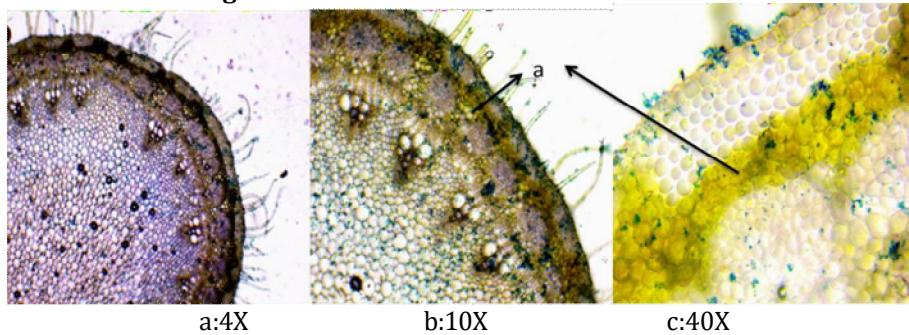
Test for Cellulose: Congo red test:



a-epidermis, b-cortex, c-pith and-xylem

Fig.13a, b & c-Red color indicate the presence of cellulose in the epidermis, cortex, xylem and parenchymatous region of stem of *Passiflora vitifolia*.

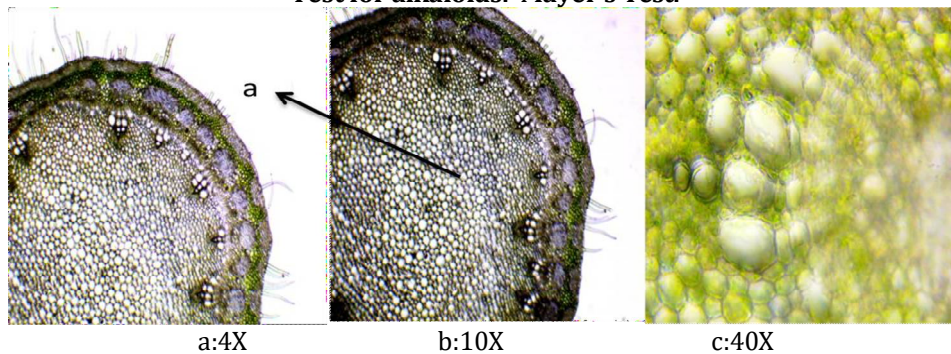
Test for Alkaloids: Test for Hager's:



a-epidermis

Fig.14a,b& c -Yellow coloured indicate the presence of alkaloids in the epidermal region of stem of *passiflora vitifolia*.

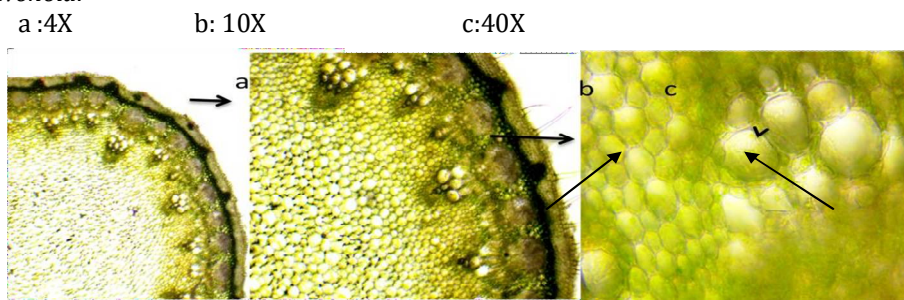
Test for alkaloids: Mayer's Test:



a-epidermis

Fig. 15a, b & c-grey coloured cells indicates the presence of alkaloid in the pith parenchyma region of stem of *Passiflora vitifolia*, after treating with Mayer's reagent.

Test for Flavonoid:



a-epidermis, b-endodermis and c-xylem

Fig.16a,b&c:yellow coloured cells indicate the presence of flavonoids in the parenchymatous region of stem of *Passiflora vitifolia*

Table: 2- Histochemical color reactions and their respective constituent confirmation of stem

Sl. No	Reagents	Colour	Constituent	Localization
1	Methylene blue	Blue	Cellulose	Epidermis, cortex & parenchyma
2	Toluidine blue	Pink/purple	Pectin	Epidermis, cortex & parenchymatous region
3	Toluidine blue	Green	Lignin	Epidermal region & cortex
4	Wagner's	Golden yellow	Alkaloids	Parenchymatous Region , cortex and xylem
5	Congo red	Red	Cellulose	Epidermis, cortex, parenchymatous region
6	Hager's	Yellow	Alkaloids	Epidermis, cortex & parenchyma
7	Mayer's	Grey colour	Alkaloids	Epidermis, cortex & parenchyma
8	10%NaOH	Yellow	Flavonoids	Epidermis,

Histochemical analysis of leaf callus *Passiflora vitifolia* (Microtome section)

Test for Alkaloids: Test for Wagner's :

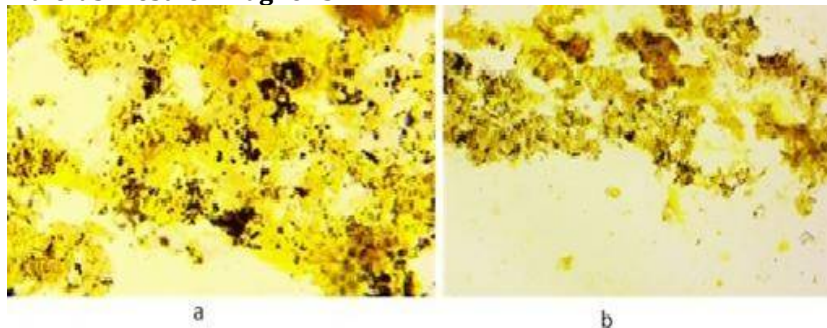


Fig.17 a and b – golden yellow colour indicates the presence of alkaloids in the leaf callus of *Passiflora vitifolia*.

Test for Pectin:

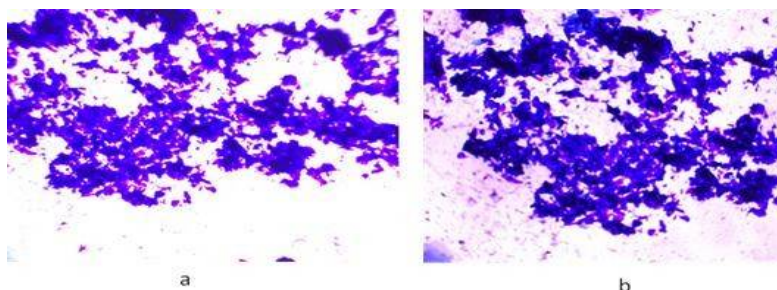


Fig.18: a & b– Purple / pink coloured cell indicates the presence of pectin in the leaf callus of *Passiflora vitifolia*.

Test for Carbohydrates: Periodic acid-Schiff's reaction (PAS):

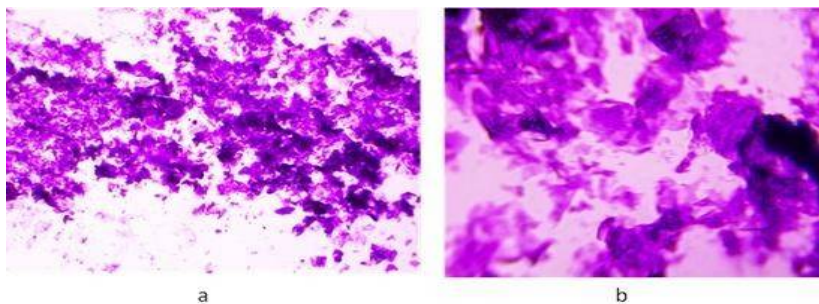


Fig.19 a & b: Magenta coloured cells indicates the presence of Carbohydrates in the leaf callus of *Passiflora vitifolia*.

Test for Cellulose:

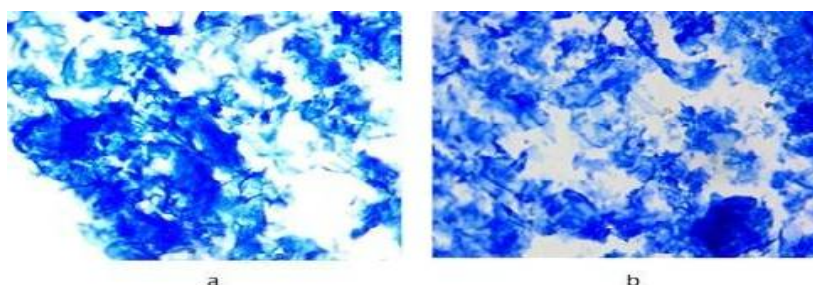


Fig.20a &b: Blue coloured cells indicates the presence of cellulose in the leaf callus of *Passiflora vitifolia*.

Test for Cellulose

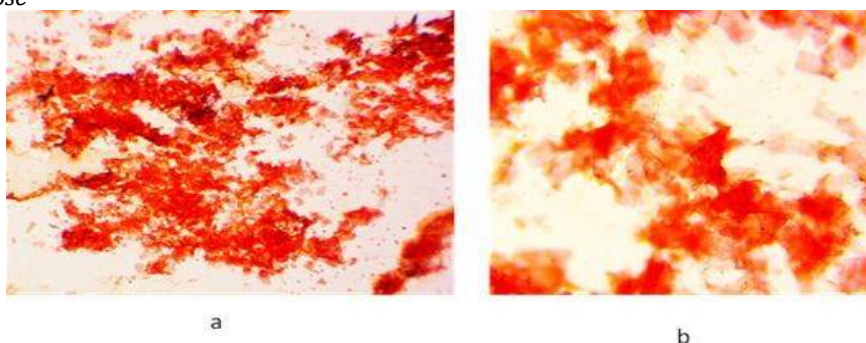


Fig.21a & b: red coloured cells indicate the presence of cellulose in the leaf callus of *Passiflora vitifolia*.

Table-3 Histochemical colour reactions and their respective constituent confirmation of leaf callus.

Sl. No	Reagents	colour	Constituents	Leaf callus
1	Wagner's	Golden yellow	Alkaloids	+
2	Toludine blue	Purple	Pectin	+
3	Methylene blue	Blue	Cellulose	+
4	PAS	Magenta	Carbohydrates	+
5	Congo red	red	Cellulose	+

"+"= Positive

In-vivo and in vitro phytochemical analysis of *Passiflora vitifolia*

Table 4: *In vivo* preliminary phytochemical analysis of leaf and stem of *P. vitifolia*

Tests		Leaf				Stem			
		P.E	CHL	E.A	M	P.E	CHL	E.A	M
Test for Sterols	Salkowski test	-	-	-	-	-	-	-	+
	Liebermann Burchard test	-	-	-	-	-	-	-	+
Test for Triterpenes	Salkowski test	-	-	-	-	-	-	-	+
	Liebermann Burchard test								+
Test for saponins	Foam test	-	-	-	+	+	+	-	+
Test for alkaloids	Mayer's test	-	-	-	-	-	-	-	-
	Dragendroff's test	-	-	-	+	-	-	-	-
	Wagner "test	-	-	-	-	-	-	-	-
Test for Tannins	Ferric chloride test	-	-	-	+	-	-	-	+
	Nacl	-	-	-	-	-	-	-	-
	Lead acetate	-	-	-	+	-	-	-	+
	Gelatin test	-	-	-	-	-	-	-	-
Test for Flavonoids	Shinoda test	-	-	-	+	-	-	-	+
	Ferric chloride test	-	-	-	+	-	-	-	-
	Lead acetate test	-	-	-	+	-	-	-	-
Test for carbohydrates	Fehling's test	+	+	-	+	-	+	-	+
	Benedict's test	+	+	-	-	-	-	-	+
	Iodine	+	+	-	-	-	-	+	-
Test for Resins	Turbidity test	-	-	-	-	-	-	-	-
	Acetic anhydride	-	-	-	-	-	-	-	-
Test for Phenols	Phenol test	-	+	-	+	-	-	-	+
	Ellagic test	-	+	-	-	-	-	-	-
	Gelatin test	-	-	-	-	-	-	-	-
	Ferric chloride test	-	-	-	+	-	-	-	-
	Lead acetate test	-	-	-	+	-	-	-	-
Test for terpenoids	Terpenoids test	-	-	-	-	-	-	-	-
Test for proteins	Biurete test	-	-	-	+	-	-	-	-
	Ninhydrin test	-	-	-	+	-	-	-	-
Test for Glycosides	kellar-kilani test	-	-	-	-	+	-	-	+

+= Positive.- =Absent. P.E=Petroleum ether, CHL=Chloroform. E.A= Ethyl acetate, M=Methanol

Table5: Preliminary Invitro Phytochemical analysis of callus of stem of *P. vitifolia*.

Tests		P.E	CHL	E.A	M
Test for Sterols	Salkowski test	-	-	-	-
	Liebermann Burchard test	-	-	-	-
Test for Triterpenes	Salkowski test	-	-	-	-
	Liebermann Burchard test				
Test for saponins	Foam test	-	-	-	+
Test for alkaloids	Mayers test	-	-	-	-
	Dragendr off's test	-	-	-	+
	Wagner's test	-	-	-	-
Test for Tannins	Ferric chloride test	-	-	-	+
	NaCl	-	-	-	-
	Lead acetate	-	-	-	+
	Gelatin test	-	-	-	-
Test for Flavonoids	Shinoda test	-	-	-	+
	Ferricchloride test	-	-	-	-
	Leadacetate test	-	-	-	-
Test for carbohydrates	Fehling's test	+	+	-	+
	Benedict's test	+	+	-	-
	Iodine	+	+	-	-
Test for Resins	Turbidity test	-	-	-	-
	Aceticanhydride	-	-	-	-

Test for Phenols	Phenoltest	-	+	-	+
	Ellagic test	-	+	-	-
	Gelatin test	-	-	-	-
	Ferric chloride test	-	-	-	-
	Lead acetate test	-	-	-	-
Test for terpenoids	Terpenoids test	-	-	-	-
Test for proteins	Bi urete test	-	-	-	+
	Ninhydrin test	-	-	-	+
Test for Glycosides	kellar-kilani test	-	-	-	-

'+'- Possitive. '-' - Absent. P.E -Petroleum ether, CHL-Chloroform. E.A-Ethyl acetate, M- Methanol

The present study Invivo and Invitro phytochemical analysis of leaf and stem of *P. vitifolia* in different solvents like petroleum ether, chloroform, ethyl acetate and methanol was carried out using standard protocols. Qualitative analysis carried out on leaf and stem s extract of the plant confirm the presence of various phytochemical constituents and results are summarized in Table 4&5. It shows the presence of sterols, triterpenes, saponins, alkaloids, Tannins, Flavonoids, carbohydrates, phenols, terpenoids, protein, Glycosides. Methanol extract of leaf and stem showed the maximum results where as petroleum ether, chloroform and ethyl acetate showed minimum results. Petroleum extract of the leaf showed the positive test for carbohydrates and stem showed the presence of saponins test. Chloroform extract of leaf showed the positive test for carbohydrates and phenols where as stem showed saponins and carbohydrates. Ethyl acetate of leaf showed the negative results but in stem showed the presence of carbohydrates. Methanol of leaf and stem showed the presence of Saponins, alkaloids, Tannins, carbohydrates, resin, phenols, proteins, Glycosides, sterols. In almost all the extract of both leaf and stem we obtained positive results for carbohydrates and saponins.

CONCLUSION

Medicinal plants have been valuable sources of natural active phytoconstituents that play an important role of much human disease. Histochemistry is a bordered line field between histology and analytical chemistry or biochemistry. Its subject matter is the identification and localization of chemical substances the in the tissue on cytological scale. In the present investigation, *P. vitifolia*, Plant used in Ayurveda for several such as Sedative, anxiety and hypertension. Plant from this genus passiflora have been used in traditional medicine by many cultures. Passion flower is said to be use full for back pain due to action on the nerves. This plant was selected to conduct histochemical investigation of leaf, stem and leaf calli, which showed the presence of Secondary metabolites and different compound such as carbohydrates, starch, tannin, Lignin, pectin, alkaloid, cellulose etc. The overall results of the present study revealed the presence of several medicinally important constituents in *P. vitifolia*. Several studies confirmed the presence of these phytochemicals contribute medicinal as well as physiological properties in the treatment of different ailments. Therefore extracts of this plant could be seen as good sources for useful drug. It is concluded that this study would lead to the establishment of some valuable compounds that has to be used to formulate new, different and more potent drugs of natural origin, with the availability of Primary information, further studies can be carried out like Psychopharmacology of different extracts, standardization of the extracts, identification and isolation of active principles and pharmacological studies of isolated compounds.

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