JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/43899

RESEARCH ARTICLE

Pharmacognostical, Phytochemical Evaluation and Unlocking the Pharmacological Power of *Pyrus communis* L. Leaf Extract on DOCA Salt Induced Hypertension in Wistar Rats

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Abstract

Objectives: Identification of the active ingredient in medication is greatly aided by pharmacognostical and phytochemical research, such as the macroscopic, GC-MS analysis and to study hypertensive treatment with ethanolic extract of *Pyrus communis* L. **Methods:** Transverse sections, powder microscopy, Histochemical analysis also performed preliminary phytochemical analysis and GC-MS of *P. communis*. The different groups of Wistar rats were administered 400mg/kg as a lower dose and 600mg/kg as a higher dose given DOCA salt-induced hypertension. **Results:** Histochemical analysis of this leaf shows the presence of cutin, mucilage cells, tannin, alkaloids, lignin, starch grains, calcium oxalates cluster crystals, and oils. Eleven components in the extract were reported via GC-MS. This report confirms the existence of various phytocomponents which are 4-pyridine methanol; N-Methylmaleimide; 4-acetyl-1h-pyrroline-2-carbaldehyde; Carbamic acid, 2-(Dimethyl amino) Ethyl Ester; 5-Acetyl-4-Amino-3-(2-Dimethylaminoethylthio)Thieno[3,2-C]Isothiazole; Cyclobutaneoctol; 1,3-Propane diamine, N, N'-Bis (3-Aminopropyl)-; Arginine; 9-Methyl-11-Oxo-1,6-Diaza tricyclo [7.2.0.0(6,8)] Undecane; Egtazic Acid; animal study significantly reduced the pressure compared to the control group. *P. communis* ethanolic extract possesses a significant ($p \leq 0.05$) reduction in hypertensive rats from measurements of control group as 157/137 mmHg systolic and diastolic blood pressure to 140/98 mmHg in 600 mg/kg **Conclusions:** These results will also be helpful to add to the advanced knowledge of *P. communis* standardisation and identification, which are essential components in separating real *Pyrus* species from adulterants and imitations in the herbal medicine formulations better for the treatment of hypertension. The empirical and phytochemical study of *P. communis* leaf holds significant potential for the development of novel herbal remedies for hypertensive patients.

Keywords: *Pyrus communis* L., Transversal Section, Quantitative Microscopy, Histochemical Test, DOCA Salts, Hypertensive Rat

1. Introduction

In our Indian medical history, herbal formulations are highly esteemed, and contemporary scientists regularly look for scientific validation of herbal remedies[1]. There is compelling evidence that the worldwide movement towards an enhanced - quality of life drives up demand for medicinal plants[2,3]. Indian tribal and rural communities mostly depend on medicinal plants for possess health of themselves and their cattle. The main sources of chemical compounds with potential medicinal advantages are medicinal plants. Medicinal herbs are used to cure a wide range of illnesses in folk medicine from different cultures[4].

Pharmacognostical analysis of a few of these physical characteristics proved helpful in developing

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Article Received on: 06.05.2024 Revised on: 25.06.2024 Accepted on: 15.07.2024

guidelines for a crude medication because these characteristics are usually constant for all plants. The leaves enormous physic-chemical characteristics were assessed in compliance with WHO guidelines[5,6]. Pear is a rich source in medicinal value as fruit, buds, and bark leaves and as a sedative, spasmolytic, diabetes, cancer, wound healing, and reduced cholesterol level so it will be used for CVD in the role of ROS system[7,8]. The appeal lies not only in the potential efficacy of these remedies but also in their relatively fewer side effects, aligning with a holistic philosophy that addresses overall well-being[9,10].

As scientific research continues to validate the benefits of plant-based interventions, the integration of plant medicine into health care represents a promising avenue for those seeking a comprehensive and natural approach to managing their health. The current investigation involved the extraction process as well as the assessment of the ethanolic extract *vivo* antihypertensive and antioxidant properties of the *P. communis* leaf[11,12].

Despite the many therapeutic uses of this plant, the leaf is adulterated with *Ayurveda* drugs with different pears. Hence, this present study was designed to understand the pharmacognostical importance of establishing quality standards and *in vivo* antihypertensive studies to explain the proper mechanism for the reported hypotensive effect on *P. communis*[13-15].

2. Materials and Methods

2.1 Plant Collection and Authentication

Pyrus communis L. leaf was collected in the surrounding hills of Kodaikanal, Tamil Nadu, India. During October and authenticated via BSI - Botanical Survey of India in the Southern circle, region of Coimbatore Dt, Tamil Nadu. The Certificate Number for authentication is No. BSI/ SRC/5/23/2022/Tech/623. After the collection leaves were washed and put into the shade dried, and pulverised to make a coarse form of powder. It was stored in airtight closures for later use.

2.2 Macroscopy

The leaves were examined in daylight and also by dissecting microscope for various macroscopical characters. The colour, odour, taste, shape, fracture, surface, apex, and external markings of roots, and fruits were observed[16,17].

2.3 Anatomical Studies

The sample spent more than 48 hours in fixative (FAA). The preserved *P. communis* leaf was sliced into thin cut of transverse slices using a sharp knife, then the sections were stained with safranin azo dye. Ultimately, the transverse slices were photographed in bright field illumination with a Zeiss Axiocam-208 colour Digital camera and an Axiolab 5 trinocular microscope. Magnifications were displayed using scale bars[18,19].

2.4 Quantitative Microscopy

Freshly collected leaves were boiled with water; after boiling dip *P. communis* leaves were in chloral hydrate solution, and the samples were stained with safranin dye. Slides are prepared with 50% glycerol drop with focusing of prepared leaf in the trinocular microscope (Olympus BX43) attachment of a drawing tube within the digital camera. The following characteristics of the *P. communis* leaf were finally observed stomatal number, vein islets, stomatal index, epidermal number, vein termination and palisade ratio. The observations were made in a bright area[15,20].

2.5 Powder Microscopy

A tiny amount of the powdered *P. communis* leaf sample was placed on a microscope slide using one drop of 50% glycerol, followed by the addition of saturated chloral hydrate for cleaning and the simultaneous addition of potassium iodide and Jeffrey reagent. Under bright field and polarizer light, characters were seen using an Axiolab 5 trinocular microscope fitted with an Axiocam-208 colour Digital camera. Diagnostic characteristic photomicrographs were taken and recorded[21-23].

2.6 Histochemical Tests

Plant sections for identification of fats, fatty oils volatile oils and resins, starch, tannin, lignified cell walls, suberized or cuticular cell walls, alkaloids, and crystals were treated with reagents using standard procedures[24-26].

2.7 Preparation of the Leaf Extract

The leaves of *P. communis* are coarsely powdered and then defatted with petroleum ether (60–80°C)

followed by adding ethanol as a solvent for Soxhlet extraction. Continuously run for 72 h at 40°C. After that sediment was drained off using Whatman No.1 filter paper (Whatman Ltd., England). Store the final volume of ethanolic extract was collected and noted its weight. Finally stored at 4°C for the later procedures[27-29].

2.8 Preliminary Phytochemical Analysis

The extracts of *P. communis* were screened as per the procedure given by (Kokate CK.) for the identification of various secondary active components such as tannin, glycosides alkaloids, flavonoids, and saponin performing suitable chemical test[30,31].

2.9 GC–MS Spectral Elucidation

Gas chromatography along with mass spectrometry (Shimadzu's GC-MS) was used to separate and identify phytocompounds in given samples. The ethanolic extract was analyzed using a capillary column. The starting temperature was kept at 60°C for 1 min, and then the oven temperature steadily increased at 4°C/min until it reached 250°C. Helium is employed as a carrier gas and the flow rate is fixed at 1 mL/min. A 0.1 μL sample was employed and manually injected into the split/splitless injector at 260°C addition with the split ratio of 1:50. The scan was observed at a range from 30-450 m/z to acquire Mass spectra at 70 eV (EI). The final data were processed using the computer programme (AMDIS version 2.62) and used the NIST-library version 2.0 for compound detection. Using retention indices with resultant spectra data were produced[32-35].

2.10 Acute Toxicity Study of *P. communis* Ethanolic Extract

Rather than providing complete toxicity results, a simple toxicity study of the ethanolic extract of *P. communis* was conducted to highlight the appropriate and safe dose range that could be utilized for subsequent investigations. Acute toxicity testing verified that *P. communis* ethanolic extract was administered at a dose of 2000 mg/kg. The ethanolic extract of *P. communis* did not significantly change the behaviour of the animals. No deaths were reported up to a dose of 4000 mg/kg body weight[36-38].

2.11 Determination of Antihypertensive Activity by DOCA Salt Model of Hypertension

Twelve-week-old male Wistar rats were selected randomly into five groups (n = 6). Group 1 received only standard vehicle (1mL of 1% methylcellulose). Group 2 was given 10mg DOCA s.c. and 1% NaCl on alternate days for four weeks. Group 3 got a hydrochlorothiazide dose of 10mg/kg for four weeks. Group 4 received 400 mg/kg of Test I, and Group 5 received 600 mg/kg of Test II according to acute toxicity studies. SBP and heart rate were measured weekly once in the morning, after 18-20 h of drug administration, in awake to warm, restrained rats using tail-cuff for measurement of plethysmography. In each session, at least seven determinations were made, and the SBP level was calculated as the average of the three lowest assessments[39-42].

2.12 Statistical Analysis

Statistical evaluation was done using one-way Analysis Of Variance (ANOVA) followed and then performed by Tukey's Test n=6.The significance level was set at $p \leq 0.05$[43,44].

3. Results and Discussion

The petiole's TS is urn-shaped, with two small protuberances on either side; the epidermis shows a single layer covered in a distinct cuticle; the cortex contains four-five layers of collenchyma cells on the outside, then by six to eight layers of chlorenchyma cells on the inside, and finally, seven to eight rows of thick-walled parenchyma cells; there are two small trace bundles visible on the wings; in the centre, the collateral vascular bundle is arranged with xylemradially arranged vessels surrounded by tracheids and fibres and phloem with normal elements and the central bundle is surrounded by discontinuous patches of four to five cell thick pericycle, while the trace bundle is covered by an arc-shaped patch; Several cluster crystal groups can be detected scattered throughout the parenchymatous cortex (Figure 1).

3.1 Leaf

3.1.1 Midrib

A single single-layered epidermis with a unique cuticle covered is visible in the TS of the leaf via the midrib.

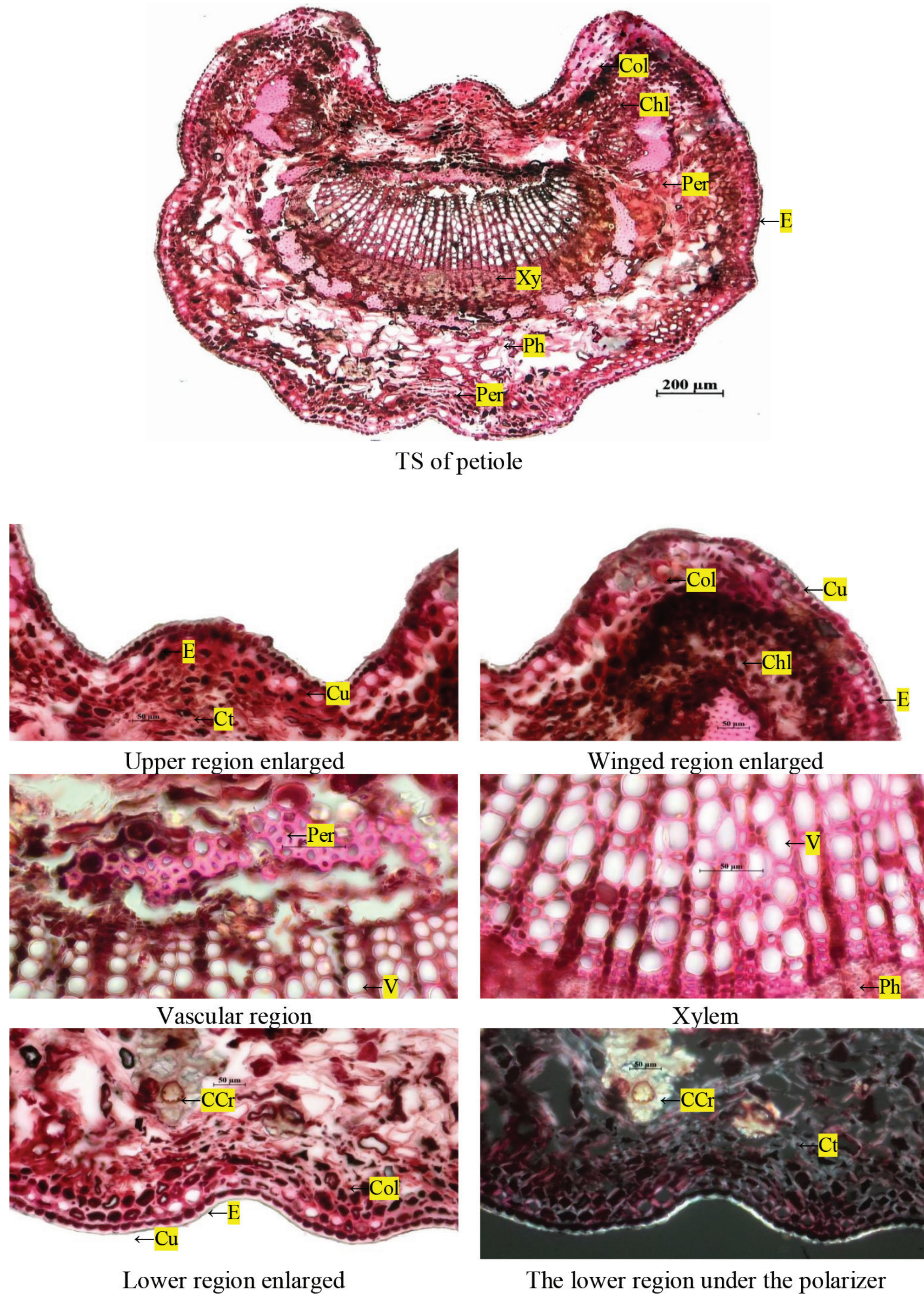


Figure 1. Illustrates Transvers Section of *P. communis* L. Petiole **(CCr** - cluster crystal, **Chl–**chlorenchyma, **Col –** Collenchymal cells, **Ct–** cortex, **Cu** – cuticle, **E–** Epidermal cells, **Pa–** Parenchymal cells, **Per–**Pericycle, **Ph–** Phloem fibres, **V –** Vessel, **Xy–** Xylem.)

This makes six to eight layers of Collenchyma cells. The Ground tissues were presented with 6-7 layers of parenchyma cells, with a thick pericycle layer and a collateral and closed vascular bundle visible at the centre. The xylem is arranged radially and is made up of parenchyma, fibres, and xylem vessels. The phloem is found arranged below the xylem and is composed of sieved tubes and the phloem parenchymal cells. Some rhomboidal crystals are found scattered within the cortical parenchyma region (Figure 2).

3.1.2 Lamina

The Lamina's TS is dorsiventral, with a single-layer of epidermis were composed of round to oval-shaped cells which are then covered with thick cuticles; the mesophyll tissue is composed of three-four layers arranged loosely spongy parenchyma cells, which are layered after the outer, double-layered palisade parenchyma cells. Veins can be seen passing through the mesophyll (Figure 2).

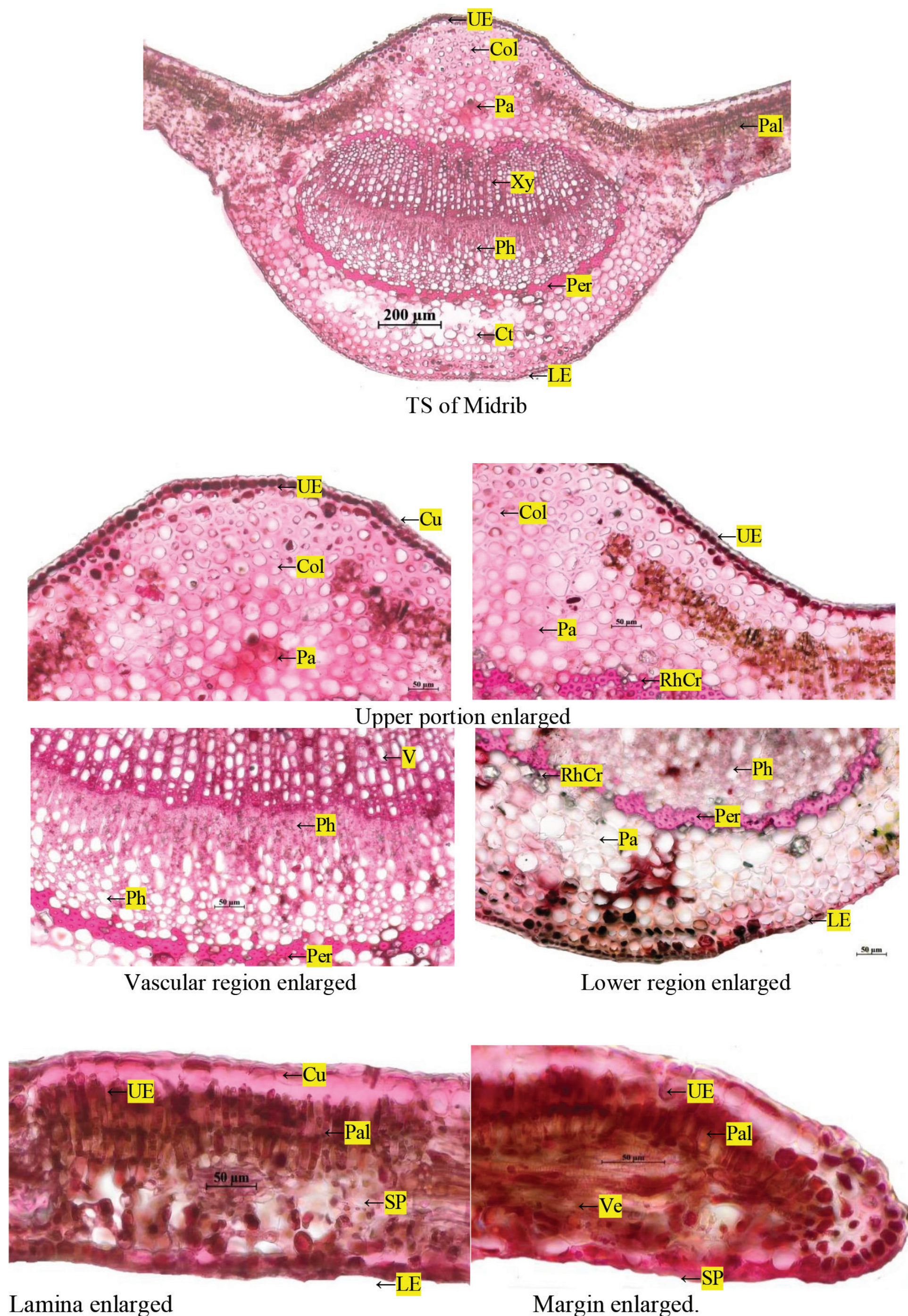


Figure 2. Illustrates Transvers Section of *P. communis*; midrib and lamina **(Chl –** Chlorenchyma, **Col –** Collenchymal cells, **Ct–** Cortex, **Cu** – Cuticle, **E–** Epidermis, **LE** - Lower Epidermal cells, **Pa–** Parenchymal cells, **Pal** – Palisade, **Per–** Pericycle, **Ph–** Phloem, **Rhcr -** Rhomboidal Crystal, **SP -** Spongy Parenchymal cells, **UE -** Upper Epidermis, **V –** Vessel, **Ve** – Vein, **Xy-** Xylem).

3.2 Quantitative Microscopy

Table 1 lists the quantitative measurements taken during microscopic examination of leaf epidermal peelings. The bottom surface of the leaves has anisocytic stomata, and they are hypostomatic (Figure 3).

3.3 Powder Microscopy

Powder is dark green with an agreeable odour and there is no characteristic taste was found. The surface view on the upper epidermis and lower epidermis shows the presence of anisocytic stomata, palisade tissue, mesophyll tissue, crystal fibre, fibre bundle, vascular tissue and fibrosclereid (Figure 4).

3.4 Histochemistry

3.4.1 Petiole

Cell walls contain cutin; mucilage cells can be found in the hypodermis; ground tissue and the cortex contain tannin; phloem contains alkaloids; xylem arteries and fibres are lignified, but pericyclic fibres are not; the cortical parenchyma contains starch grains and calcium oxalate cluster crystals that are dispersed throughout the tissue (Figure 5).

Table 1. The quantitative microscopy of *P. communis* leaf

Parameters	Upper-epidermis region (/mm^2)	Lower-epidermis region (/mm^2)
No of Epidermis	170-180	200-220
Stomatal number	-	60-80
Stomatal index	-	23 - 27
Palisade ratio	6-9	
Vein islets number	10-12	
Vein termination number	18-20	

3.4.2 Midrib and Lamina

Oil is present in the midrib's ground tissue, but the lamina has relatively little cutin deposition. The cutin deposition in the midrib is thick. Phloem contains alkaloids, while ground tissue and the collenchymatous cortex contain tannins. Both the midrib and lamina contain starch grains, and the pericyclic fibres and xylem arteries are lignified. There are tannins in the phloem area; the midrib area contains calcium oxalates in cluster crystal form; Absence of mucilage (Figure 6).

3.5 Phytochemical Results

The extracts of ethanol extract of *P. communis* leaf were evaluated for the presence of various phytoconstituents like Alkaloids, Tannins, Glycosides, Flavonoids, and Saponins using chemical tests. The report obtained from GC-MS (Figure 7) states that there may be probably the presence of 11 compounds (Table 2) in *P. communis* leaf ethanolic extract with the structure of the compounds (Figure 8).

3.6 *In Vivo* Hypertensive Rat Model Effect

10mg DOCA s.c. and 1% NaCl on alternate days for four weeks injected into rats produced moderate hypertension. When all rats were put in a tail-cuff apparatus with an AD instrument, they displayed Systolic Blood Pressure (SBP) and Diastolic Blood pressure (DBP) according to respective treatment in normal control, disease control, standard and test groups (400 mg, 600mg *P. communis*) respectively,

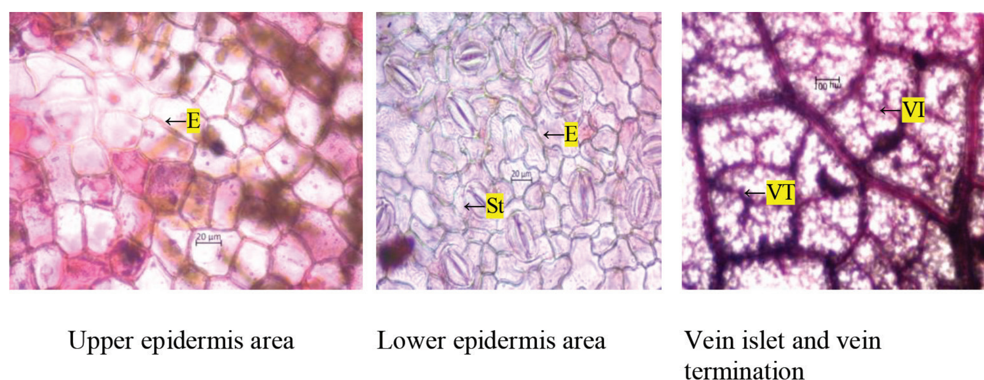
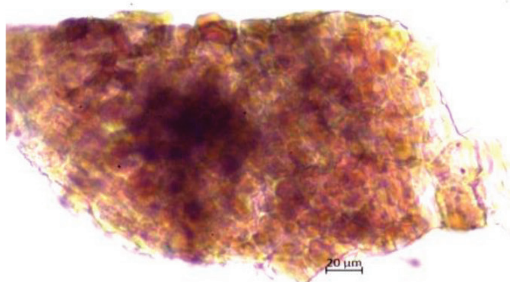
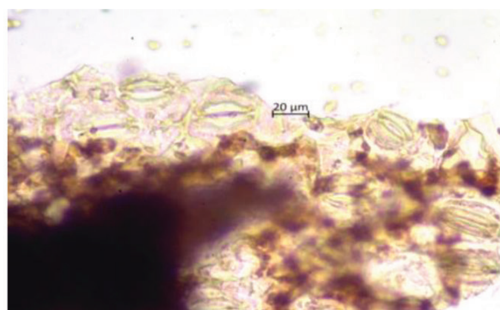


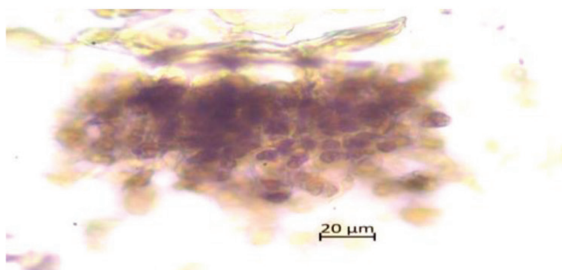
Figure 3. The Quantitative microscopy of *P. communis*; leaf (**E**-Epidermis; **St** –Stomatal No; **VI** -Vein islet; **VT** -Vein termination number).



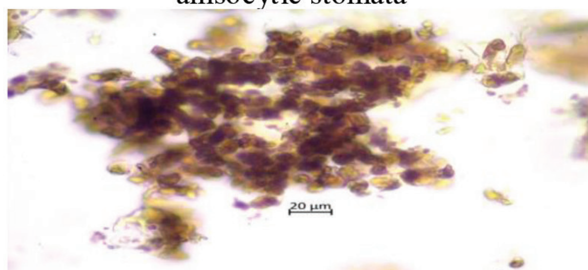
Surface view of the upper epidermis



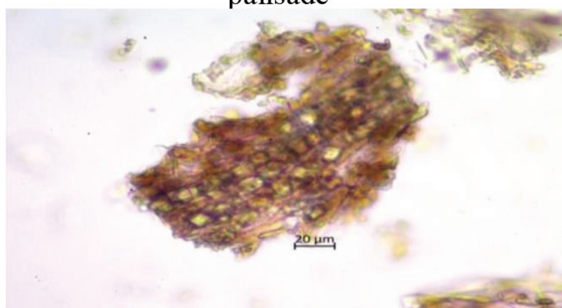
Surface view of the lower epidermis with anisocytic stomata



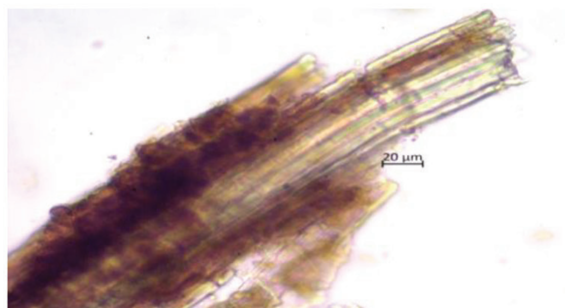
Surface view of upper epidermis and palisade



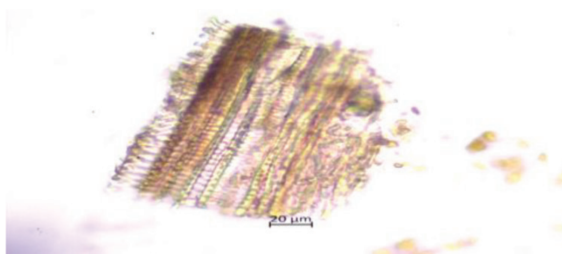
Mesophyll tissue



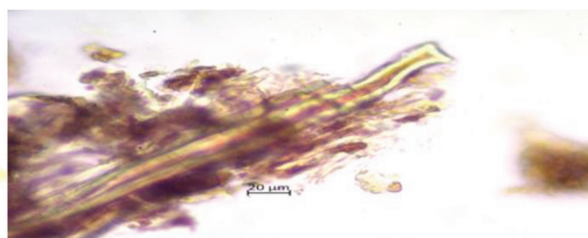
Crystal fibre



Fibre



Vascular tissue



Firosclereid

Figure 4. Powder microscopy of *P. communis* leaf.

which were observed (Tables 3 and 4). SBP in normal control, disease control, standard and test groups were measured. There were significant reductions in SBP were found in standard and test groups as compared to the disease control group. The systolic and diastolic blood pressures were considerably considerably P<0.05 increased in DOCA salt-induced hypertensive rats (Figures 9 and 10).

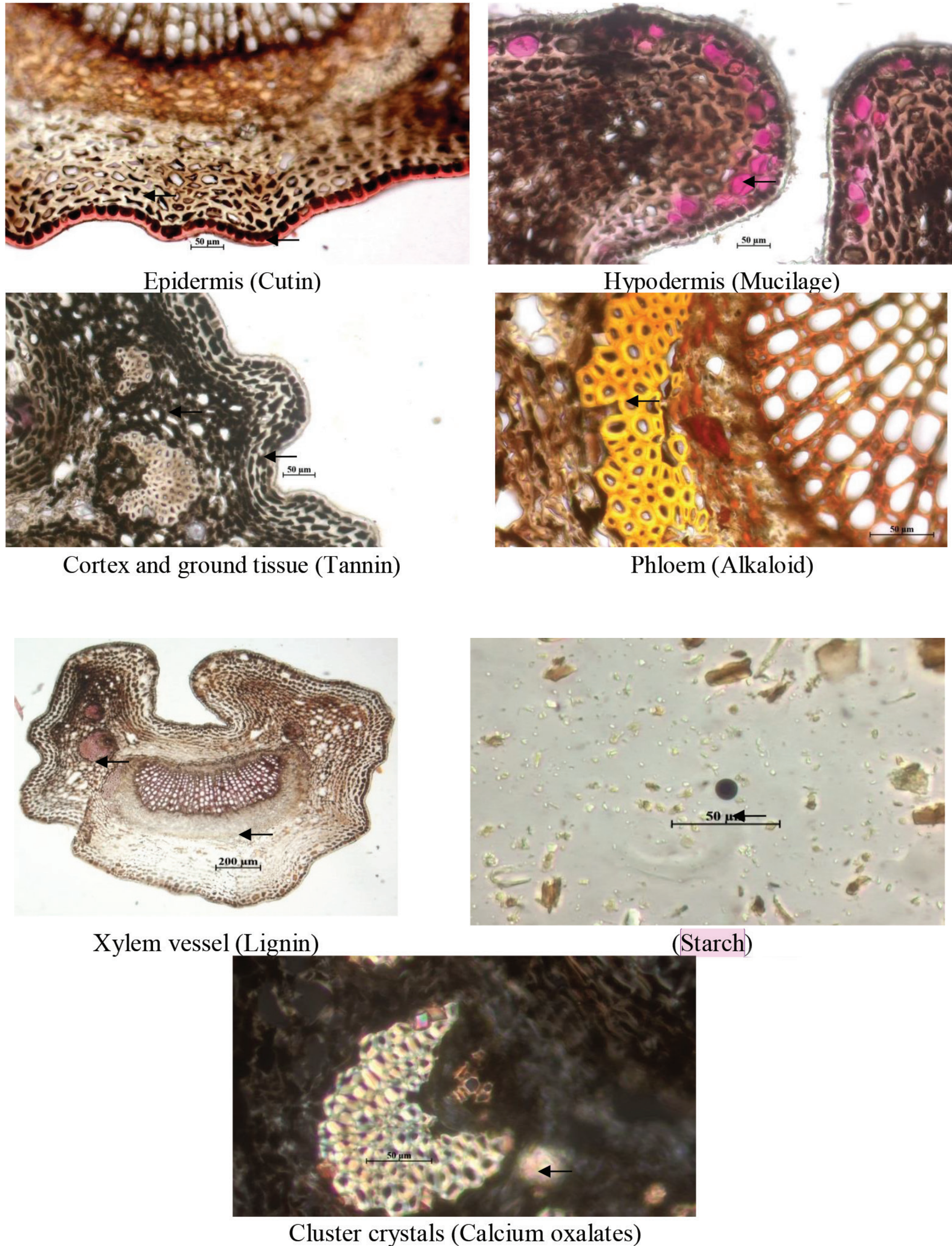


Figure 5. Histo-chemical test of *P. communis* petiole.

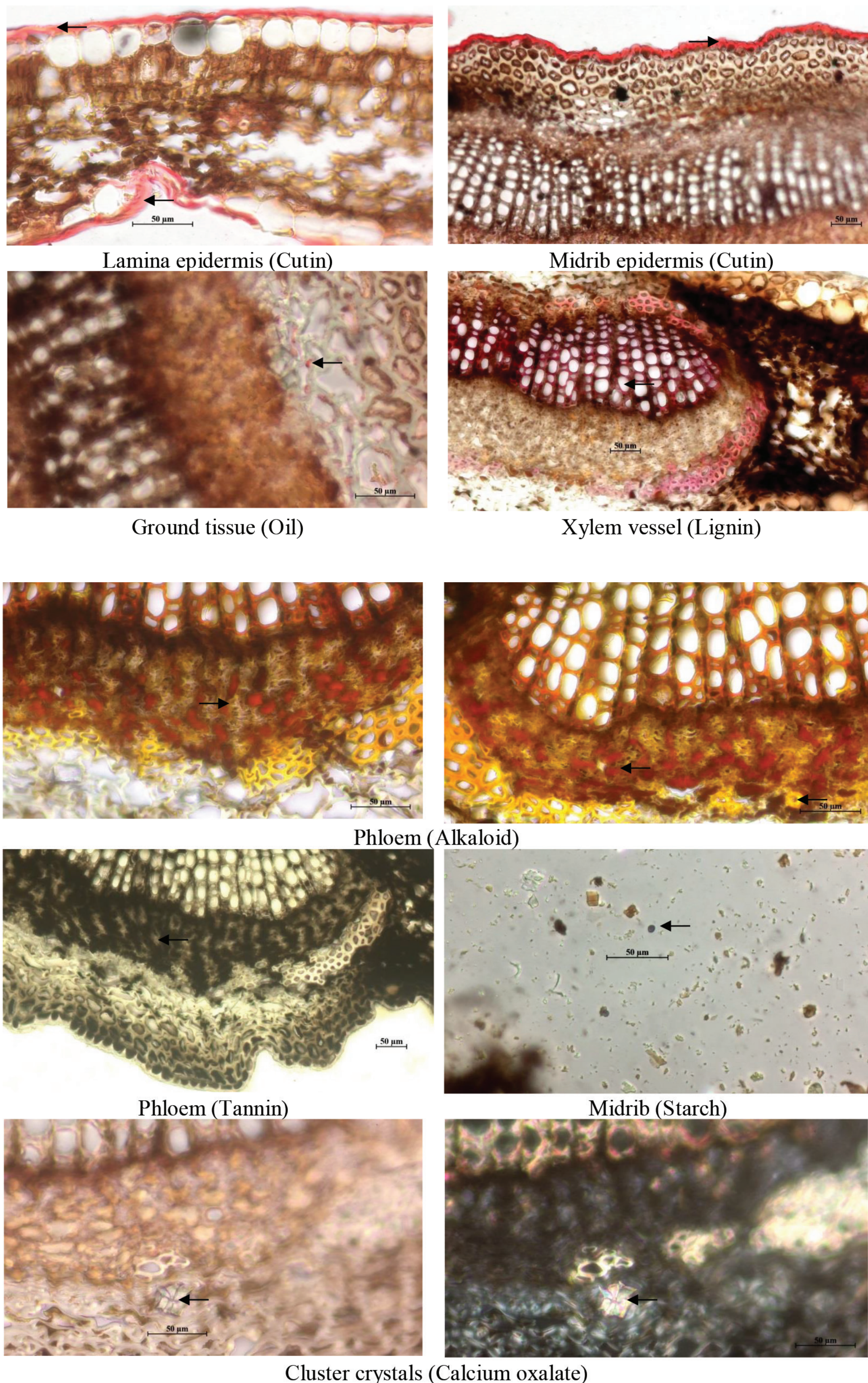


Figure 6. Histo-chemical test of *P. communis* midrib and lamina.

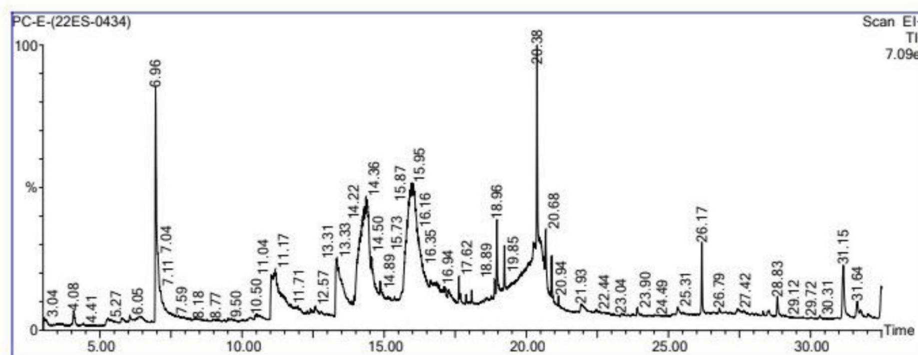


Figure 7. GC-MS report of chromatogram spectrum of *P. communis* leaf of ethanolic extract.

Table 2. GC-MS reported Phytochemicals in ethanolic extract of leaves of *P. communis*

S. No.	RT	Height	Area	Area %	Norm %	Molecular formula and Mol.weight	Name of the Phytocomponents
1	6.960	582,927,616	45,371,480.0	10.573	36.43	C_6H_7ON /109	4-Pyridinemethanol
2	11.167	115,435,184	37,753,656.0	8.798	30.31	$C_5H_5O_2N$ /111	N-Methylmaleimide
3	13.333	140,607,824	33,646,812.0	7.841	27.02	$C_7H_7O_2N$ /137	4-Acetyl-1H-Pyrroline-2-Carbaldehyde
4	14.358	243,279,472	83,193,464.0	19.386	66.80	$C_5H_{12}O_2N_2$ /132	Carbamic Acid
5	14.553	94,257,560	9,061,743.0	2.112	7.28	$C_5H_{12}O_5$ /152	Xylitol
6	16.009	276,731,808	124,545,664.0	29.023	100.00	$C_{11}H_{15}ON_3S_3$/301	5-Acetyl-4-Amino-3-(2-Dimethylaminoethylthio) Thieno[3,2-C]Isothiazole
7	18.965	182,684,016	6,767,176.0	1.577	5.43	$C_4H_8O_8$ /184	Cyclobutaneoctol
8	20.375	594,577,792	64,882,644.0	15.119	52.10	$C_9H_{24}N_4$ /188	1,3-Propanediamine,N,N′-Bis (3-Aminopropyl)
9	20.681	147,081,344	7,194,657.0	1.677	5.78	$C_6H_{14}O_2N_4$ 174	Arginine
10	26.173	178,489,024	6,447,749.0	1.503	5.18	$C_{10}H_{16}ON_2$ /180	9-Methyl-11-Oxo-1,6-Diazatricyclo [7.2.0.0(6,8)] Undecane
11	31.150	130,546,832	10,267,718.0	2.393	8.24	$C_{14}H_{24}O_{10}N_2$/380	Egtazic Acid

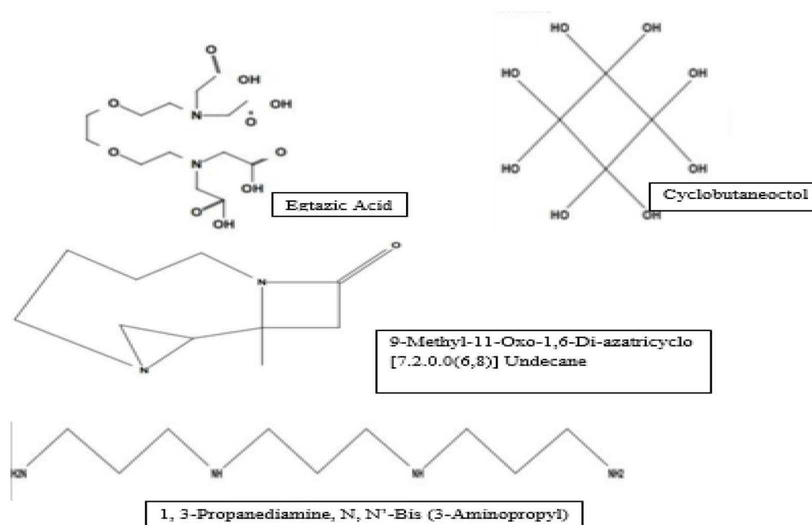


Figure 8. Images of individual structural compounds predicted by GC-MS chromatogram spectrum of ethanolic extract of *P. communis* leaf.

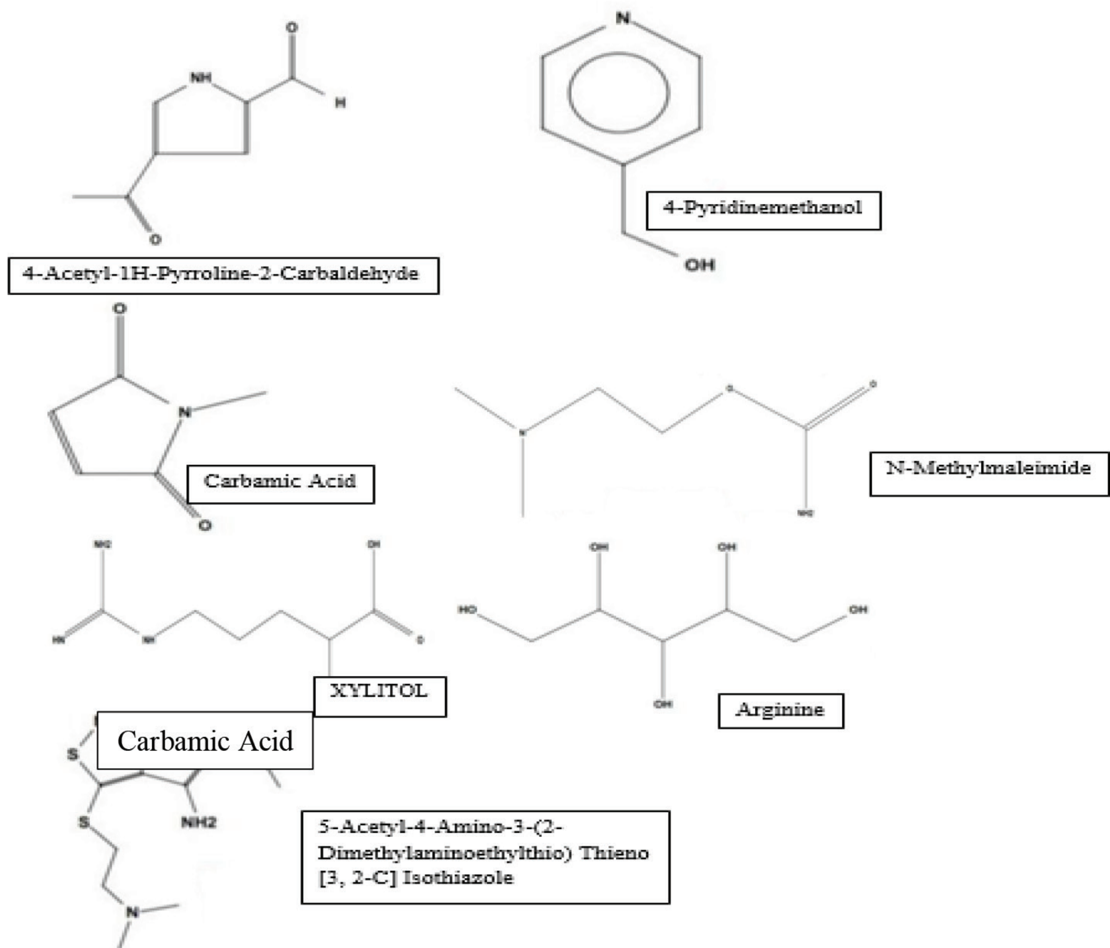
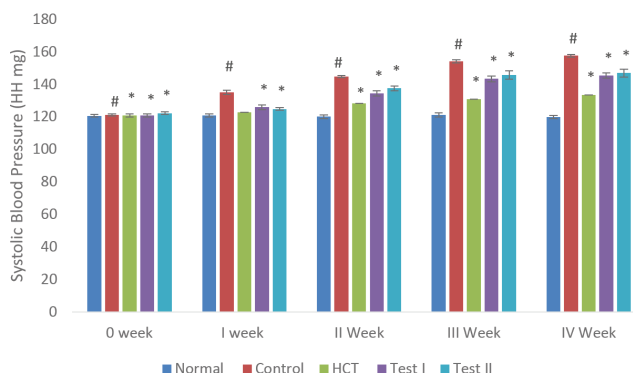


Figure 8. Continued...

Table 3. Measurement of systolic blood pressure by DOCA salt model of hypertension

SBP level (mm of Hg)					
Group	**0 week**	**I week**	**II Week**	**III Week**	**IV Week**
Normal	120.5	120.7	120	121.1	119.8
Control	121	135	144.8	154	157.5
HCT	120.7	122.7	128.3	130.8	133.5
Test I	120.8	125.8	134.3	143.3	145.3
Test II	122.2	124.7	137.5	145.7	140.8

Values are denoted as Mean ± SEM. Statistical reports were obtained after being performed via one-way Analysis of Variation (ANOVA) and then performed by Tukey's test. *n*=6; ns – non-significant; #* $p<0.05$ value was considered to be significant. The control group was compared against the normal, treated groups compared against the control.



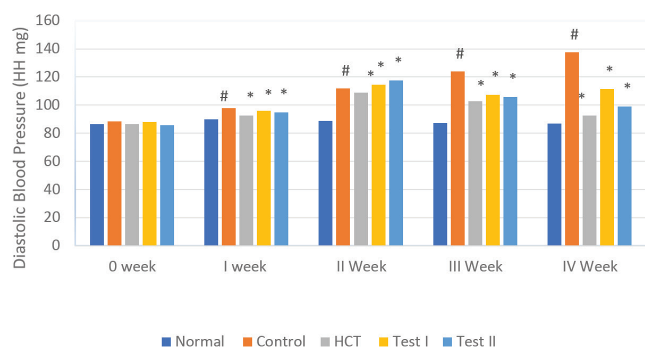
Mean±SEM data were analysed by one-way ANOVA, then performed by Tukey's test. *n*=6; #Control vs Normal; *Treated vs Control; p<0.05 was considered to be significant).

Figure 9. Systolic blood pressure levels in normal and hypertensive experimental rats (mmHg).

Table 4. Measurement of diastolic blood pressure by DOCA salt model of hypertension

	DBP level (mm of Hg)				
Group	**0 week**	**I week**	**II Week**	**III Week**	**IV Week**
Normal	86.4	89.7	88.6	87.1	86.8
Control	88.4	97.8	111.8	124	137.5
HCT	86.3	92.7	108.8	102.8	92.5
Test I	87.8	95.8	114.3	107.3	111.3
Test II	85.9	94.7	117.5	105.7	98.8

Values are denoted as Mean ± SEM. Statistical reports were obtained after being performed via one-way Analysis Of Variation (ANOVA) and then performed by Tukey's test. *n*=6; ns – non-significant; #* $p<0.05$ value was considered to be significant. The control group was compared against the normal, treated groups compared against the control.



Mean±SEM data were analysed by one-way ANOVA, then performed by Tukey's test. *n*=6; #Control vs Normal; *Treated vs Control; p<0.05 was considered to be significant).

Figure 10. Diastolic blood pressure levels in normal and hypertensive experimental rats (mmHg).

4. Conclusion

The World Health Organisation highlights the significance of investigating herbal monographs and pharmacopoeia standards to ascertain the authenticity and purity of a specimen. The uniqueness of a *P. communis.* leaf can be predicted and microscopically evaluated. Many studies revealed, however, a lack of ability to detect cell features and standardize the profiles of herbal medications. The presence of cutin, mucilage cells, tannin, alkaloids, lignin, starch grains, calcium oxalates cluster crystals, and oils is shown by histochemical analyses of *P. communis* leaves. This pharmacognosy focus might aid in assessing the acceptability and quality of the plant. In addition, a preliminary study on the phytocompounds of an ethanolic extract of *P. communis* leaf verifies the plant's relevance by indicating the possibility of a variety of bioactive chemicals. Measurements of control group as 157 mmHg reduced systolic blood pressure levels goes 140 mmHg and 145 mmHg measurements of rats after for 4 weeks, were compared against control group with treatment of 400 mg/kg most effectively reduce the higher pressure than that of 600 mg/kg alike same in diastolic pressure due to the presence of active components present in the extract. It is proposed that more research be conducted to separate to find structure of novel bioactive element in order to lead compound for the evident to development of plant-derived medications after safe and effective results in clinical trial.

5. Acknowledgements

The authors acknowledged the Department of Pharmacognosy, Siddha Central Research Institute (CCRS), Ministry of Ayush, Govt. of India, Chennai-600106 and Department of Pharmacy, Annamalai University, Chidambaram, Tamil Nadu, India for their support.

6. Animal Ehical Approval

JSSCP/OT/IAEC/48/2022–23.

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