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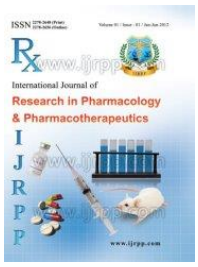

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Pharmacognostical, preliminary phytochemical screening, estimation of phyto constituents and its in-vitro antioxidant activity of *Adenanthera pavonina linn* (leaves)

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ABSTRACT

Adenanthera pavonina L(mimosidae) is a deciduous tree, also known as red sandalwood or coral wood tree. The leaves and bark of this plant are used by Irula tribals for curing various ailments like diarrhoea, asthma, jaundice, tuberculosis, leprosy, rheumatism, hypertension, wound healing, diabetes, inflammation, and cancer. Leaves posseses anti-inflammatory, anti- arthritic, antioxidant, antinociceptive, antiemetic, hypoglycemic, antibacterial, cytotoxicity, radical scavenging activity. Phytochemical review showed the presence of carbohydrates, proteins, flavonoids, phenols, sterols, saponins, glycosides, tannins, alkaloids. The literature review revealed the pharamacognostical preliminary phytochemical evaluation was not investigated. An endeavor study was taken to investigate pharmacognostical, preliminary phytochemicalscreening, quantitative estimation and invitro anti-oxidant activity. Transverse section of petiole, rachis, midrib, the stomata are strictly paracytic type, thick walled uniserate,simple trichomes are present. Powder microscopy of leaf showed presence of thick walled simple trichomes, reticulate vessels, prismatic crystals and scelreids. Thequantitative microscopical parameters such as stomatal number (100-128/mm^2), stomatal index (11-13/mm^2), vein islet number (2-3/mm^2) and vein termination number(51-64/mm^2), palisade ratio(3-5/mm^2). Physicochemical parameters such as loss on drying (6.61±0.39%w/w), total ash(15.83± 0.05%w/w), ethanol extractive value(26.30±0.57),aqueous extractive value(36.73±2.05). Preliminary phytochemical screening of this plant revealed the presence of carbohydrates, proteins, tannins, flavonoid, steroids, saponins, phenols. Quantitative estimation of gallic acid (35gm/gm), tannic acid (86gm/gm) & rutin (252mg/gm) were determined and correlated with standard. In-vitro anti-oxidant by hydrogen peroxide (16.32 µg/mL), nitric oxide (5.58 µg/mL), total antioxidant(24.67 µg/mL) & method were estimated andin comparison with ascorbic acid used as standard.

Keywords: *Adenanthera pavonina*, phytochemical, quantitative estimation, antioxidant

INTRODUCTION

Adenanthera pavonina commonly known as anaikundumani, belongs to (leguminosae) found throughout India, deciduous tree, about 18-24 m tall, erect and upto 2-2.4m in girth depending on location. Decoction of leaves used as aphrodisiac, gout, piles, hemorrhages. Bark and leaves used as astringent, to treat ulcers and pharyngopathy. seeds are used as antiemetic and febrifuge[1-4]. Irula tribals of Nellithurai, Coimbatore district,Tamilnadu used the leaves to treat diarrhoea, asthma, jaundice, tuberculosis, leprosy, rheumatism, cough, fever, vomiting, skin diseases, hypertension, wound healing, diabetes, inflammation, and cancer. Tribals of Srilanka used this plant to treat diabetes mellitus, diarrheoa, dysentery and snake bite[5,6]. Pharmacognostical review revealed characters such as non-glandular trichome, thick walled fibres, paracytic stomata, tracheids, simple vessels elements. Phytochemical review of this plant revealed the presence of carbohydrates, proteins, tannins, glycosides,flavonoid, steroids, saponins, polyphenol [7,8]. Pharmacological review of leaves possesed anti-inflammatory, anti-arthritic, antioxidant, antinociceptive, antiemetic, hypoglycemic, antibacterial, cytotoxity, radical scavenging activity [9-16]. An elaborate study was undertaken to investigate the Pharmacognostical additional findings,preliminary phytochemical analysis, estimation of phytoconstituents and in-vitro antioxidant activity.

Fig 1: Habitat of *Adenanthera pavonina*

MATERIALS AND METHODS

Plant collection and authentication

Leaves collected from local garden, MMC campus Madurai, Tamil Nadu in the monthof July 2021. The species for the proposed study was identified and authenticated by DR.Stephen, Professor, and Department of Botany American College Madurai-625002.The herbarium of this specimen was kept in the department for further reference.

Pharmacognostical studies

Fresh leaves were subjected to pharmacognostical studies includes organoleptic andmorphological studies.

Morphological studies of *Adenanthera pavonina Linn*

Leaves were studied separately for its morphological characters and the results aredisplayed in the table 1 and fig 2.

Microscopical studies of *Adenanthera pavonina Linn*

The method was adopted as per Wallis (1965). Thin sections were taken and was stained with routine methods and was observedunder microscope. Results were displayed in Fig 3 -5.

Quantitative microscopy of *Adenanthera pavonina Linn*

Determination of phyto –constants

vein islet and vein termination number, stomatal index, stomatal number, petiole, palisade ratio of fresh leaves were determined as per standard procedure [16]. Results were displayed in table 2 and Fig 6.

Preparation of leaf powder

The leaves were collected and shade dried coarsely powdered and stored in a well closed container.

Powder microscopy

The coarse powder was stained with routine reagent to identify the diagnostic features of the plant. Results were displayed in Fig 7**.**

Determination of physio-chemical parameters

The powder was subjected to physiochemical

parameters such as foreign organic matter, loss on drying, ash value, and extractive value with different solvents in increasing order of polarity and results were displayed in the table 3[17].

Preparation of 70% hydroalcoholic extract of *Adenanthera pavonina Linn* leaf(HAEAP)

The leaves were collected, shade dried and coarsely powdered, passed through sieve no 40,was extracted with 70% hydro-alcohol by maceration technique, was concentrated to dryness and stored in a closed container or further use.

Preliminary phytochemical screening

Hydro-alcoholic extract of *Adenanthera pavonina Linn* (Leaf) is subjected to qualitative chemical analysis and was determined as per Harbone method[18]. Results were displayed in table 4.

Estimation of phyto-constituents Determination of gallic acid content

About 1 mL (1mg/ml) of hydroalcoholic extract of *Adenanthera pavonina*(HAEAP), 0.5 mL of Folin-ciocalteu reagent (1N) were added and allowed to stand for 15 minutes. Then 1 mL of 10% sodium carbonate solution was added to the above solution. Finally the mixtures were made up to 10 mL with distilled water and allowed to stand for 30 minutes at room temperature and total phenolic content was determined spectro-photometrically at 760nm wavelength. The calibration curve was generated by preparing Gallic acid at different concentration (2, 4, 6, 8 µg/mL). The reaction mixture without sample was used as blank. Total phenolic content of HAEAP extract is expressed in terms of mg of Gallic acid equivalent per gm of extract (mg GAE/g) as per Singleton etal.,1999 and results were displayed in table 5 and fig 8 [19].

Determination of tannin acid content

0.2 mL of (1 mg/mL) hydroethanolic extract of *Adenanthera pavonina*, was made up to 1 mL with distilled water. Then add 0.5 mL of Folin Denis reagent and allowed to stand for 15 mins, then 1 mL of sodium carbonate solution was added to the mixture and it was made up to 10 mL with distilled water. The mixture was allowed to stand for 30 mins at roomtemperature and the tannin content was determined spectrophotometrically at 760 nm. The calibration curve was generated by preparing tannic acid at different concentration (2,4,6,8µg/mL). The reaction mixture without sample was used as blank. The total tannin content in the leaf extract was expressed as milligrams of tannic acid equivalent per gm of extract as per Rabianaz and AsghariBano, 2013 and results were displayed in table 6 and fig 9[20].

Determination of rutin (flavonoid) content

1mL of hydro-ethanolic extract of *Adenanthera pavonina*, 0.1 mL of aluminium chloride 0.1 mL of potassium acetate solution and 2.8 mL of ethanol were added and the final volume was then made up to 5 mL with distilled water. After 20 min the absorbance wasmeasured at 415 nm. A calibration curve was constructed by plotting absorbance reading of Rutin at different concentrations (10,15,20µg/mL). The sample without aluminium chloride was used as a blank. The total flavonoid content in the extract was expressed as milligrams ofrutin equivalent per gram of extract as per Zhishen etal., 1999 and results were displayed in table 7 and fig 10[21].

Determination of In-vitro Anti-oxidant activity

Determination of scavenging activity against hydrogen peroxide

The method was as per MG. Rana *etal.*,1996, To 1 mL of test solutions of different concentrations, 3.8 mL of 0.1 M phosphate buffer solution (pH 7.4) and then 0.2 mL of hydrogen peroxide solution were added. The absorbance of the reaction mixture was measured at230 nm after 10 min. The reaction mixture without sample was used as blank. Sample blank was also prepared without reagents. Ascorbic acid was used as standard. The percentage inhibition of hydrogen peroxide was calculated using the formula,

%inhibition = [(Control–Test) /Control]×100

The concentration of the sample required for 50% reduction in absorbance (IC_{50}) was calculated using linear regression analysis. Results were displayed in Table 8 and fig 11[22].

Determination of radical scavenging activity against nitric oxide

The method was as per Marocci etal., 1995, Nitric oxide scavenging activity was determined according to the method reported byMarocci et al. HAEAP extract, Ascorbic acid as standards in the range of 2 –10 µg were taken in respective tubes containing phosphate buffer saline, so that the volume in each tube was made up to 1ml. For controls, volume was made up to 3ml with phosphate buffer saline. Then 2ml of 10mM sodium nitroprusside added to all the tubes except the

controls. Nitric oxide radicals were generated from the samples spontaneously during the incubation period of 150 min. 0.5ml of the solution taken from each tube to their respective tubes. To this 1ml of 0.33% sulphanilamide added and allowed to stand for 5 min for completing diazotization, followed by the addition of 1ml of NED (0.1%) to each tube. Then incubate for 30 min at room temperature. The nitrite ions released were measured at 516nm and % Nitric Oxide radical scavenging activity was calculated using the following formula and results were displayed in table 9 and fig 12[23].

$$\% \text{ inhibition of nitric oxide radical activity} = \frac{(\text{Abs of control} - \text{Abs of sample})}{\text{Abs of control}} * 100$$

Determination of total antioxidant activity

The method was as per Prieto *et al*., 1999), Hydro-alcoholic extract of *Adenanthera pavonina* in different concentration ranging from 2µg to 10 µg were added to each test tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. These tubes were kept incubated at 95 □C for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm. Mean values from three independent samples were calculated for each extract. Ascorbic acid was used as positive reference standard. Results were displayed in Table 10 and fig 13**[**24].

RESULTS AND DISCUSSION

Macroscopy

Leaf

Leaves green coloured with a characteristic smell and taste; bipinnate, alternate, with minute stipules; rachis 14 to 50 cm long, pulvinate, with a gland at the tip; pinnae 2 to 3 pairs,2to40 cm, opposite or rarely sub opposite; leaflets 6 to 18, alternate, estipellate;petiolule up to 3mm long; lamina 1.2 to 8×0.8 to 4 cm, -elliptic, slightly oblique base, apex round, margin entire; lateral nerves9-10pairs, parallel, slender, obscure, intercostae reticulate, faint.

Dorsal view **Ventral view**

Fig 2: Macroscopy of *Adenanthera pavonina* leaf

Table 1: Determination of macroscopical characters of *Adenanthera pavonina Linn*

Characteristics	Report
Color	Green
Odour	Characteristic
Taste	Characteristic
Shape	Ovate
Leaf	Compound leaf
Apex	Round
Base	Elliptic slightly oblique
Margin	Entire
Venation	Pinnate/foliar
Texture	Smooth
Petiole	3mm long
Rachis	14 to50cm long,pulvinate with gland at the tip
Leaf type	Bipinnate, alternate with minute Stipules

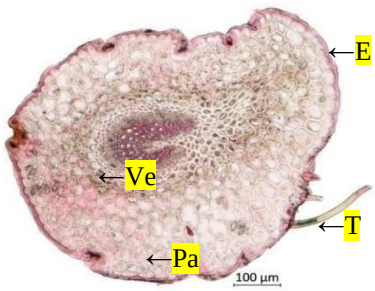
Lamina	Thin1.2cm
Arrangement	Leaflet 6to18,alternate,exstipulate

Microscopy TS of Petiole

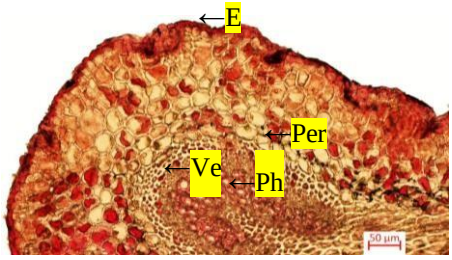
TS of petiole is nearly circular shaped in outline with wavy ridges and furrows;outer layer is covered by single layered epidermis covered by thick cuticle; epidermis gives out few unicellular trichomes; cortex is broad and formed of 8 to 10 layers of thickwalled parenchyma cells; some of the parenchyma cells shows pigment contents; starchgrains are found distributed throughout the cortex; central portion of section is occupied by horse-shoe shaped vascular bundle which is surrounded by pericycle; xylem isarranged towards inner side and phloem towards outside encircling xylem on lower side.

TS of Rachis

TS of rachis shows nearly circular shape with wavy outline; epidermis is single layered and covered by thick cuticle; cortex is made up of 5 to 6 layers of chlorenchyma cells followed by a ring of conjoint, collateral vascular bundles surrounded by thick layers of pericycle; xylem is arranged facing towards inner region and phloem towards outer side; central ground tissue is occupied by a wide pith formed of parenchymatous cells; upper elevated portion of section contains two trace bundles in the outer corticalregion.



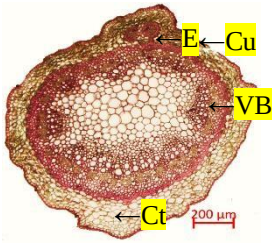
TS of Petiole

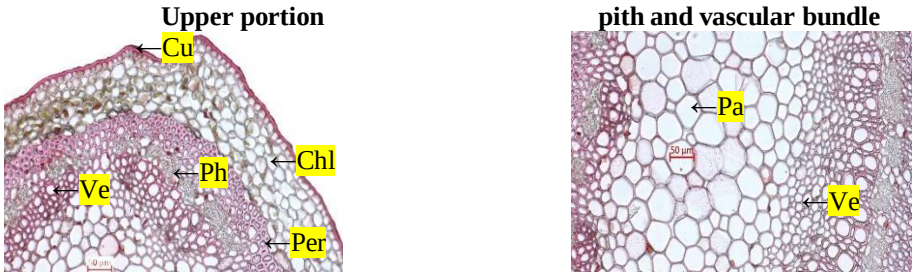


A portion enlarged

Ct-Cortex;**E**-epidermis;**Pi**-Pith;Pa-parenchyma;**Pcr**-prismaticcrystals;**Per**-Pericycle;**Ph**-phloem;**T**–Trichome;**V**-vessel;**VB**-Vascular bundle;**Xy**-Xylem

Fig 3: TS of Petiole





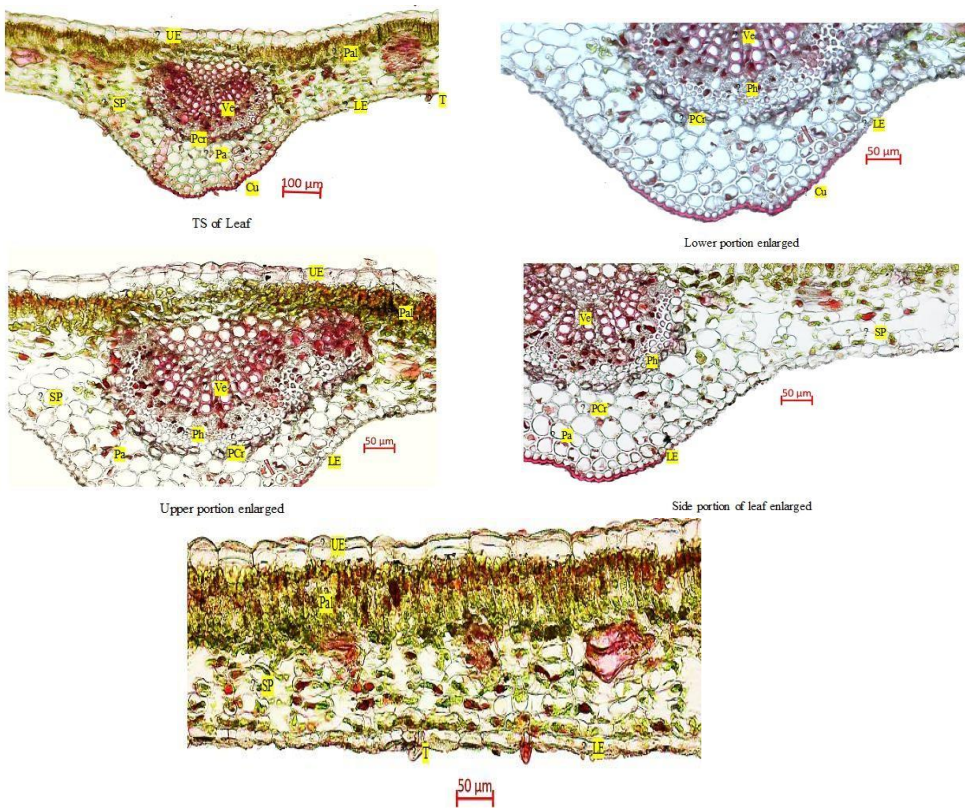
Chl-Chlorenchyma;**Ct**-Cortex;**Cu**-Cuticle;**E**-epidermis;**Pi**-Pith;**Pa**-parenchyma;**Per**-Pericycle; **Ph**-phloem; **TB**-Tracebundle;**Ve**-vessel;**VB**-Vascularbundle

Fig 4: TS of Rachis

TS of Leaf passing through midrib

TS of leaf shows upper and lower epidermis with hypostomata; epidermis is single layered and covered by cuticle; cuticle is found very thick at the lower portion of midrib; at the midrib region 3 to 4 layers of chlorenchyma cells are found beneath the epidermis followed by a central vascular bundle; a layer of pericycle sheath containing prismatic crystals of calcium oxalates surrounds the bundle; vascular bundle is conjoint and collateral; xylem occupies the centre of bundle which is surrounded by phloem towards the lower sides.

TS of lamina shows upper and lower epidermis; mesophyll is differentiated into palisade and spongy parenchyma; double layered palisade cells follows loosely arranged spongy parenchyma cells; small vascular strands of vein-lets traverses the leaf lamina; several trichomesare observed arising from both the epidermis while they distributed in low density in the upper epidermis.



Col - collenchyma; **Cu** – cuticle; **LE** - lower epidermis; **Me** - mesophyll cells; **Pa** - parenchyma; **Pal** - palisade; **Pcr** - prismatic crystals; **Per** - pericycle; **Ph** - phloem; **SP** - spongy parenchyma; **St** - stomata; **UE** - upper epidermis; **V**- vessel; **Ve** - vein.

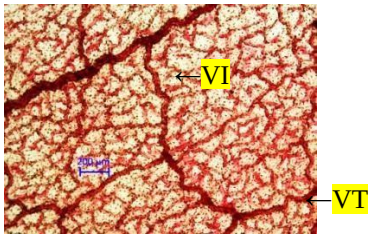
Fig 5: TS of Lamina

Quantitative microscopy

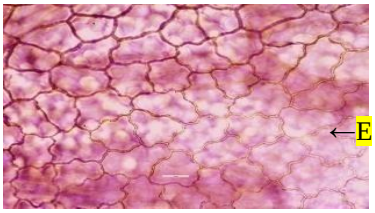
The quantitative parameters obtained during microscopic observation of epidermal peelings of leaves were recorded in Table 2. The leaves showed paracytic stomata onlower surface with presence of dense unicellular covering trichomes in lower side andless number in upper side.

Table 2:Quantitative microscopy of leaf of *Adenanthera pavonina Linn*

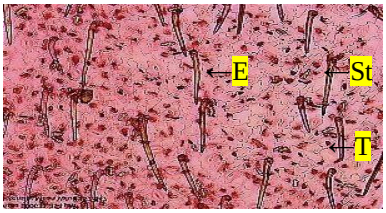
S.no	Parameters	Upper epidermis(/mm^2)	Lowerepidermis(/mm^2)
1	Epidermal number	800–864 /mm^2	784-850 /mm^2
2	Stomatal number	----------	100-128 /mm^2
3	Stomatal index	----------	11-13 /mm^2
4	Palisade ratio	3–5 /mm^2	----------
5	Veinislets number	2-3 /mm^2	----------
6	Vein termination number	51–64 /mm^2	----------



Veinislet and termination



Upper epiderm



Lower epidermis

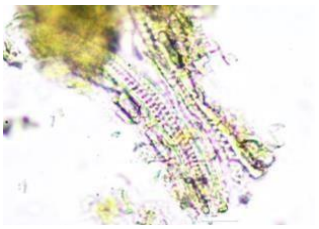
E–Epidermis; **St–**Stomata; **VI–**Veinislet; **VT–**Vein termination.

Fig 6: Quantitative microscopy of *Adenanthera pavonina*

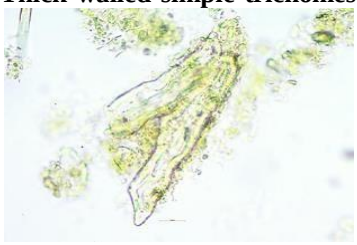
Powder microscopy of Leaf

The powder is slight green coloured with a characteristic smell and taste; it shows the characters such as thick walled uniseriate simple trichomes, epidermal cells in surface view, vessels with reticulate and simple pitted thickenings, thick-walled crystal fibres with narrow lumen, oil globules and prismatic crystals of calcium oxalates.

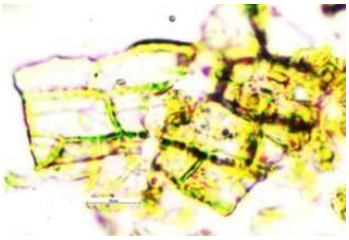
Thick walled simple trichomes



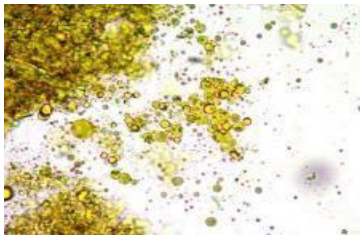
Reticulate vessels



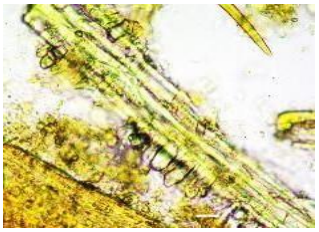
Fragment of epidermis



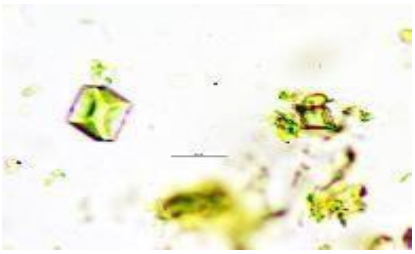
Epidermis in surface view



Sclereids



Crystal fibre



prismatic crystals

Fig 7: Powder microscopy of leaf of *Adenanthera pavonina Linn*

Table 3:Determination of physicochemical parameters of *Adenanthera pavonina Linn*

S.no	Physio-chemical constant	Reports % w/w
1	Foreign matter	Nil
2	Bitterness value	Nil
3	Loss on drying	6.61 ± 0.39
4	Total solids	93.39
5	Petroleum ether extractive	30.31± 1.08
6	Ethyl acetate extractive	12.53 ± 0.73
7	Chloroform extractive	14.34±0.45
8	Ethanol extractive	26.30±0.57
9	Aqueous extractive	36.73±2.05
10	Total ash	15.83± 0.05
11	Water soluble ash	42.43±0.02
12	Acid insoluble ash	81.51± 0.05

Table 4: Preliminary phytochemical screening of hydro alcoholic extract of *Adenanthera pavoninaLinn*

S.no	Analysis	HAEAP
1	Test for Carbohydrates	Present
2	Test for Alkaloids	Absent
3	Test for Glycosides	Present
4	Test for Protein	Present
5	Test for Flavonoid	Present
6	Test for Saponins	Present
7	Test for Sterols	Present
8	Test for Tannins	Present
9	Test for Mucilage	Absent
10	Test for Resins	Absent
11	Test for Gum	Absent

QUANTITATIVE ANALYSIS OF *ADENANTHERA PAVONINA LINN* (HAEAP)

HAEAP was subjected to quantitative estimation of gallic acid, tannin acid, rutin and resultswere displayed in table 5,6&7 and fig 8,9&10 respectively.

Table 5: Determination of gallic acid equivalent in hydroalcoholic extract of *Adenantherapavonina(*HAEAP)

S.No	Concentration of Gallic acid	Absorbance of Gallic acid	Concentration of HAEAP	Absorbance of HAEAP
1	2	0.204 ± 0.00115	10	0.0363± 0.00088
2	4	0.403 ±0.00088	20	0.0446 ± 0.00145
3	6	0.546 ±0.00185	30	0.0613± 0.00085
4	8	0.704 ±0.00057	40	0.836 ± 0.00290
5	10	0.775 ±0.00115	50	0.093 ± 0.00145
			GAE	**35 mg/gm.**

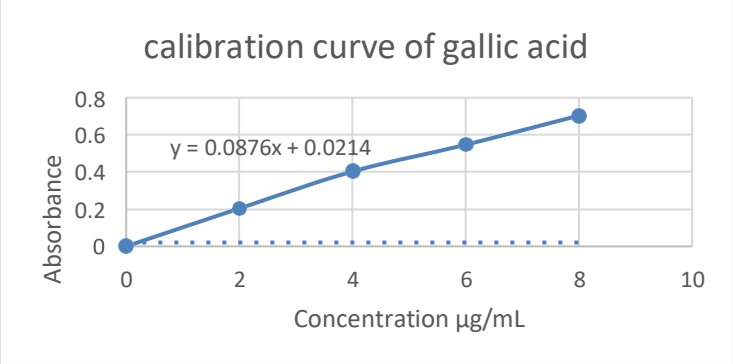


Fig 8: Calibration curve of gallic acid

It is noticed that hydroalcoholic extract of *Adenanthera pavonina* showed total gallic acid equivalent 35mg/gm.

Table 6: Determination of tannic acid equivalent in hydroalcoholic extract of *Adenantherapavonina*(HAEAP)

S.No	Concentration of tannicacid	Absorbanceof tannic acid	Concentration ofHAEAP	Absorbance of HAEAP
1	2	0.076± 0.00176	10	0.019 ± 0.00115
2	4	0.124 ±0.00088	20	0.0583± 0.00033
3	6	0.175 ±0.00057	30	0.104± 0.00057
4	8	0.214 ±0.00066	40	0.162 ± 0.00057

5	10	0.355 ±0.0991	50	0.213± 0.00057
			TAE	**86mg/gm**

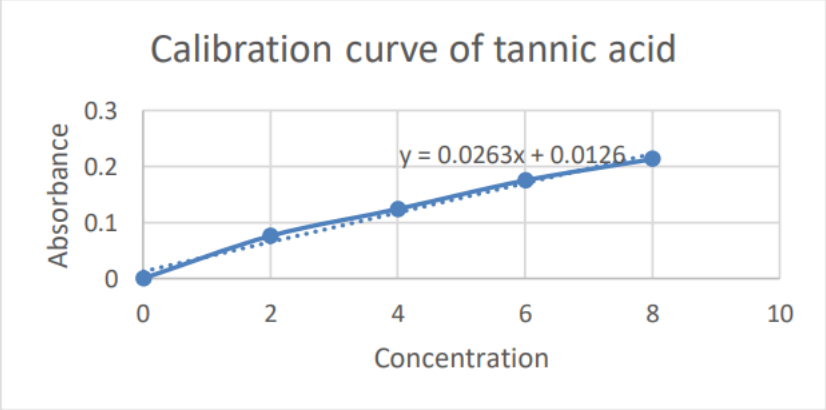


Fig 9: calibration curve of tannic acid

It is estimated that hydroalcoholic extract of *Adenanthera pavonin*a showed total tannic acid equivalent 86 mg/gm.

Table 7: Determination of rutin equivalent in hydroalcoholic extract of Adenanthera pavonina (HAEAP)

S.No	Concentration of rutin	Absorbance of rutin	Concentration of HAEAP	Absorbance of HAEAP
1	8	0.084± 0.00088	10	0.0693 ± 0.00088
2	10	0.092 ±0.00066	20	0.0743± 0.00088
3	15	0.104± 0.0017	30	0.078 ±0.00152
4	20	0.109 ±0.0014	40	0.083 ±0.00318
5			50	0.089 ±0.00251
			RAE	**252mg/gm**

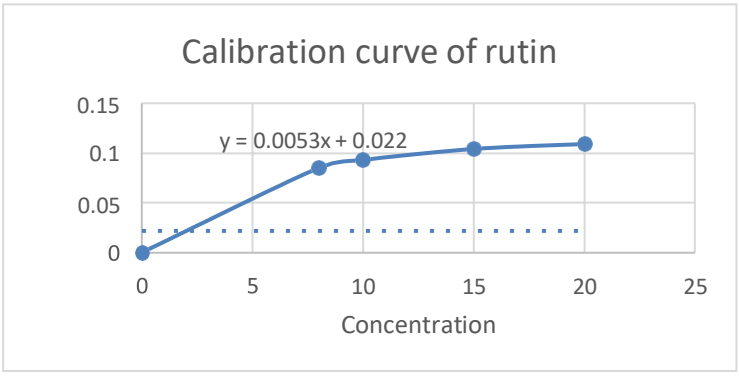


Fig 10: calibration curve of rutin

It is observed that hydroalcoholic extract of *Adenanthera pavonin*a showed total rutin equivalent 252 mg/gm.

In-vitro anti-oxidant activity

HAEAP was screened for in vitro anti- oxidant activity. It was carried out by hydrogen peroxide, nitric oxide method and total antioxidant capacity. Results were displayed in table 8, 9&10 and figure 11,12&13 respectively.

Table 8: Determination of hydrogen peroxide scavenging method

S.NO	Concentration Of ascorbic acidand HAEAP	% inhibition of ascorbicacid	% inhibition of HAEAP
1	10	93.66±0.00088	32.14± 0.001579
2	20	94.06±0.00761	31.50± 0.00068
3	30	94.46±0.00348	31.90± 0.001246
4	40	94.72±0.00066	30.96± 0.00085
5	50	95.01±0.00088	30.17± 0.00176
	IC50	**0.81µg/mL**	**16.32µg/ML**

It is intimated that hydrogen peroxide scavenging effect was found to be *Adenantherapavonina* HAEAP 16.32µg/mL against % inhibition of ascorbic acid 0.81µg/mL

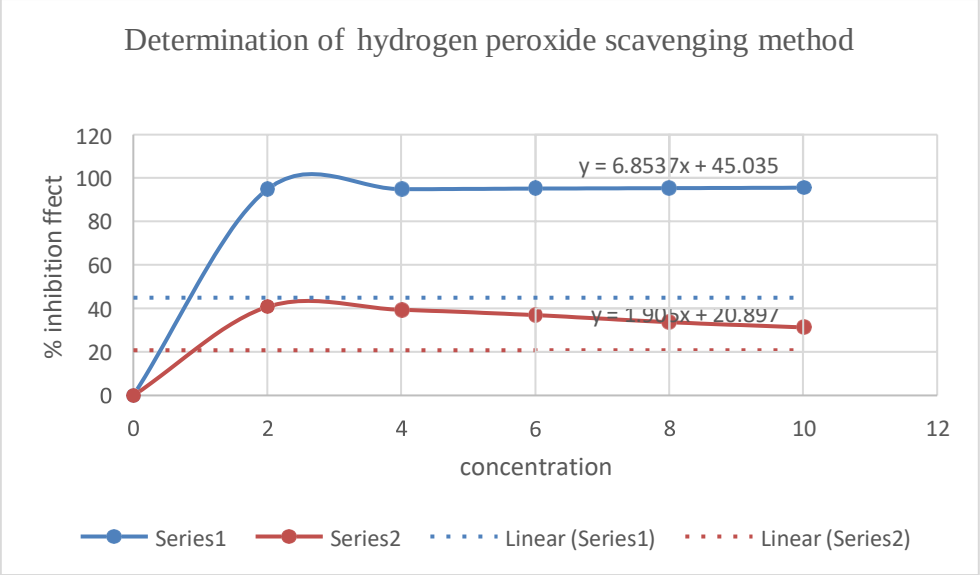


Fig 11: Determination of hydrogen peroxide scavenging method

Table 9: Determination of nitric oxide scavenging method

S.NO	Concentration of ascorbic acidand HAEAP	% inhibitionof ascorbic acid	% inhibition of HAEAP
1	10	67.71± 0.1058	63.42± 0.004166
2	20	76.96± 0.0200	59.51± 0.003195
3	30	81.41± 0.0480	56.46± 0.001104
4	40	84.47± 0.0240	55.74± 0.001699
5	50	86.38± 0.00666	53.01±0.00079
	IC50	**2.67µg/mL**	**5.58µg/mL**

It is determined that nitric oxide scavenging effect was found to be a *Adenanthera pavonina*, HAEAP 5.58µg/mL against % inhibition of ascorbic acid 2.67µg/mL

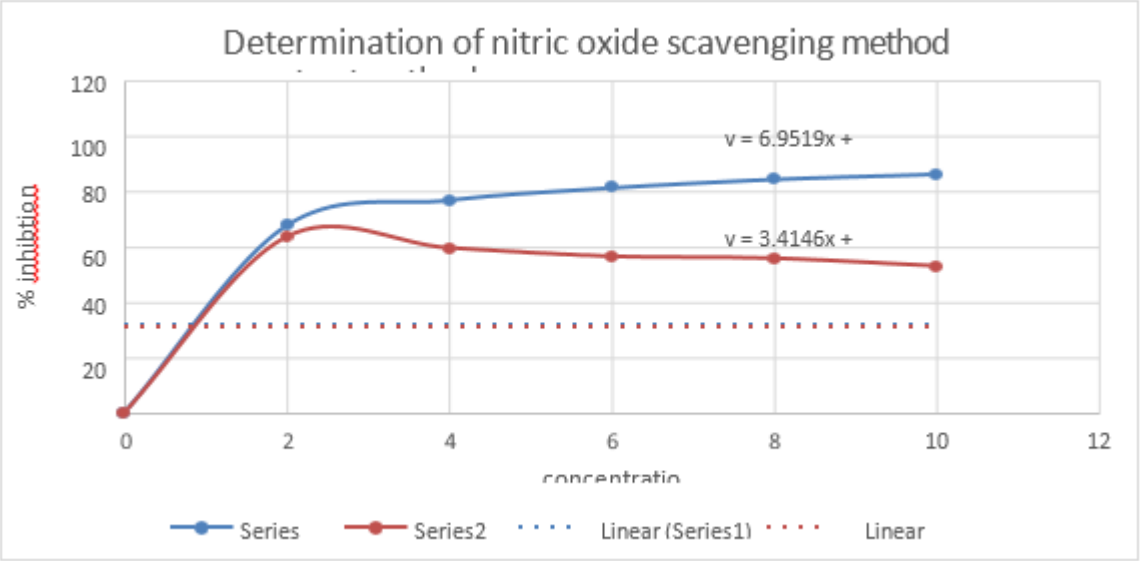


Fig 12: Determination of nitric oxide scavenging method

Table 10: Determination of total antioxidant capacity of HAEAP

S.NO	Concentration of ascorbic acid and HAEAP	% inhibitionof Ascorbic acid	% inhibition of HAEAP
1	10	94.36±0.00088	36.13± 0.001579
2	20	97.06±0.00761	39.25± 0.00068
3	30	97.43±0.00348	40.21± 0.001246
4	40	99.71±0.00066	32.78± 0.00085
5	50	92.71±0.00088	38.37± 0.00176
	IC50	**0.64µg/mL**	**24.67µg/mL**

It is evaluated that total antioxidant Capacity of was found to be *Adenanthera pavonina*, HAEAP 24.67µg/mL against % inhibition of ascorbic acid 0.64µg/mL

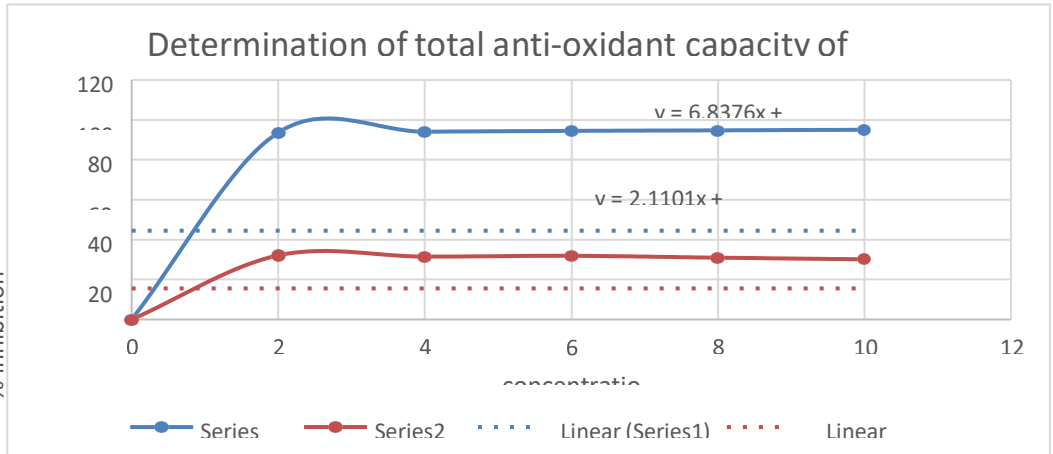


Fig 13: Determination of total anti-oxidant capacity of HAEAP

CONCLUSION

The present research article draws some pharmacoepial monographs for this medicinal plant which had been widely used by tribal of Irula, India to treat to gout. The observed parameters coincides with the pharmacognostical reviews. Therefore this medicinal plant can betuned in to herbal preparation to treat such diseases. In the present study some of the additional microscopical characters had been investigated which would further add additional scientific/ Pharmacognostical information such as hypostomata, thick walled uniserate simple trichomes, vascular bundles, crystal fibres, Simple pitted reticulate vessels, prismatic crystals of calcium oxalate, sclereids Were found. Quantitative microscopical parameters such as epidermal number, vein islets number, vein termination number, stomatal index, stomatal number & palisade ratio were derived for this plant which disparates from previous research. The present phytochemical screening revealed the presence of carbohydrates, phenol, sterols, tannin, protein, flavonoids accords with the previous publication. The quantitative estimation of gallic acid, tannic acid and rutin derived for this plant deviates from fore mentioned literature which may be due to the different extract. In-vitro antioxidant activity was estimated by hydrogen peroxide, nitric oxide method and total antioxidant capacity was observed that HAEAP which would showed mild antioxidant effect in comparison with ascorbic acid used as standard.

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