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DEVELOPMENT OF PHARMACOGNOSTICAL PROFILE, ANALYTICAL AND PHARMACOLOGICAL EVALUATION OF NEPHROPROTECTIVE MEDICINAL PLANT *PORTULACA OLERACIA*

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Abstract:
The objective of present study deals with the development of a Pharmacognostical profile as well as and analytical and pharmacological evaluation of the Nephroprotective medicinal plant Portulaca oleracea are the goals were to collect and authenticate the investigational plant, evaluate Portulaca oleracea's Pharmacognostical profile (macroscopic and microscopic), conduct a preliminary physico-chemical analysis of the plant, and extract its active ingredients. To standardise plant extracts through phytochemical investigations, develop an active Nephroprotective drug from Portulaca oleracea extracts, and assess Portulaca oleracea extract for Nephroprotective activity in vitro.
Keywords: *Portulaca oleracea, Nephroprotective, Pharmacognostical, GGT, Analytical evaluation.*

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INTRODUCTION:

Once in a while one comes across a plant that is so outstanding that one wonders how on earth it has been overlooked. Purslane (Portulaca oleracea) is one such plant. It is commonly called purslane or pigweed in English language, papas an in Yoruba, babajibji in Hausa, ntioke, ntilimoke, ntiike or idiridi in Igbo. Portulaca oleracea is a member of the Portulacaceae family with more than 120 different species. The use of this plant as a vegetable, spice and medicine has been known since the times of the ancient Egyptians and was popular in England during the Middle Ages, why it has fallen into obscurity is quite strange. It is fascinating that a plant so prevalent around the world has achieved almost identical recognition in each culture for its benefits [1].

It was first identified in the United States in 1672 in Massachusetts. The name Portulaca is thought to be derived from the Latin Porto meaning 'to carry' and lac meaning 'milk', since the plant contains a milky juice10; oleracea from Latin, meaning 'pertaining to kitchen gardens', referring to its use as a vegetable. The use of this plant as a vegetable, spice and medicine has been known since the times of the ancient Egyptians and was popular in England during the Middle Ages.

Classification [1]

Kingdom- plantae
Subkingdom- tracheobionta
Superdivision - spermatophyta
Division- magnoliophyta
Class- magnoliopsida
Subclass- caryophyllidae
Order- caryophyllales
Family - portulacaceae
Genus - portulacae L.
Species- Portulacae oleracea L.

MATERIALS AND METHODS:

It contain GGT (Reckon diagnostic Pvt. Ltd), Diethyl ether (LR) (Central Drug House (CDH) Ltd. Bombay), Ferric chloride (AR) (Central Drug House (CDH) Ltd. Bombay), Formalin (S.D. Fine Chem., Ltd. Bombay), Folin's reagent (Sisco Research Lab. Pvt. Ltd. Mumbai), Mesna (Baxter Oncology Germany), Methanol (LR) (Central Drug House (CDH) Ltd. Bombay), Sodium Potassium Tartarate (S.D. Fine chem. Ltd. Mumbai), Orthophosphoric acid (AR) (S.D. Fine Chem., Ltd. Bombay), Picric acid (AR) (Central Drug House (CDH) Ltd. Bombay), Petroleum ether (60-80^0) (LR) (E. Merck (India) Ltd., Mumbai), Sodium bicarbonate (Central Drug House Pvt. Ltd. Mumbai), Thio barbituric acid (Central Drug House Pvt. Ltd. Mumbai), Tri chloro acetic acid (Central Drug House Pvt. Ltd. Mumbai) all showed be used to analytical and pharmacological evaluation of nephroprotective medicinal plant *Portulaca Oleracia.* And some equipment used for the evaluation of medicinal plant like Analytical balance, UV-Vis Spectrophotometer, Micropipettes, Shaking water bath, Homogenizer, HPTLC, HPLC and Vortex mixture are used for analytical and pharmacological evaluation of nephroprotective medicinal plant *Portulaca oleracea.*

METHODS:

1. PLANT COLLECTION AND AUTHENTIFICATION

Portulaca oleracea plant were collected with the help of manual like clippers, diggers, scrapers, etc. by the lab technicians from Department of Pharmacognosy, College of Pharmacy, Chopda (Jalgaon, Maharashtra) and Herbarium sheets were prepared. The requisite was collected from the department by the student. Further, the plant and its constituents were authenticated by Botanical Survey of India, Pune. Portulaca oleracea were taxonomically identified and authenticated (by D. L. Shirodkar, Botanist) at Botanical Survey of India, Pune, M. H. The plant collection and authentification data shows in **Figure 1 & 2.**

PHARMACOGNOSTIC STUDIES [2, 3]

The Pharmacognostic study was carried out by referring to various official guidelines and academic textbooks. Results of physicochemical parameters were tallied and studied along with the standards in Ayurvedic Pharmacopoeia of India. Macroscopic and microscopic features of plant materials were matched with the identification characters involving microscopic profile of various parts of drugs described in various ICMR (Indian council of medical research) monographs.

Macroscopic studies

Collected specimens were investigated for organoleptic features. Macroscopic identity of a medicinal plant material is based on the visual appearance with naked eyes and external characters were observed like shape, size, colour, taste, apex, surface, base, margin, venation, texture, fracture and odour.

a) **Size:** Vernier Callipers were used to measure the dimensions of the seed
b) **Colour:** Colour of fresh seed sample was examined under lab conditions.
c) **Surface characteristics, fracture and texture**

characteristics: Seed Sample was studied under Compound Microscope at 10 x. Surface characteristics were identified by touch. Seeds were bent to obtain information on brittleness and the appearance of the fracture plane- whether it is rough, fibrous, granular, smooth etc.

d) **Odour:** The seed was powdered and was placed on bare palms. Strength of odour (none, weak, distinct, strong) and the odour sensation (aromatic, fruity, musty, mouldy, rancid, etc) was determined by the researcher.

e) Taste: The taste of powdered seed was evaluated by placing a pinch of the powder on tongue which was carried to all regions of tongue which have specialized taste buds corresponding to different types of taste such as sweet, bitter, astringent and acrid etc. Mouth was rinsed with mouthwash before and after this test. The macroscopic data shows in **Figure 3.**

Microscopic studies

This study involves the examination of the cell (form and arrangement) in a crude drug. The plant drugs are generally used in powdered or comminute form where the macro morphology is destroyed, so the evaluation of the microscopically cell characters is essential. Cytomorphological characters play a major role in drug identification.

a) **Transverse section:** The seed was soaked overnight and was cut manually using a sharp blade and potato as pith when required. Thin sections were decolorized using chloral hydrate and stained with safranin or phloroglucinol and hydrochloric acid (1:1). Temporary slides were prepared by mounting section in glycerine and covered with cover slip free from any air bubble. Prepared slides were visualized under microscope at magnification 10 x.

b) Powder microscopy: Fine powder of the seed was boiled with chloral hydrate for 5-10 minutes in order to remove plant pigments, and then further stained with various stains like phloroglucinol. The slide was analysed by using Stage Microscopy. The microscopic data shown in **Figure 4.**

Physicochemical Studies [4, 5]

a) Foreign matter analysis

Was performed visually and was scrutinized if any other parts except for seeds were available in the material.

b) Determination of ash values

i) **Total ash value:** The total ash method is designed to measure the total amount of material remaining after ignition this includes both ‘‘physiological ash’’ which is derived from the plant tissue itself, a ‘‘non- physiological’’ ash which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface. 2-4 g of coarse powder of seed was weighed in a tarred crucible. The material was ignited until ash became white indicating the absence of carbon by heating at 500-600˚C. It was then cooled in a desiccator and weighed. The content of total ash was calculated as percent yield.

ii) **Acid insoluble ash:** Acid insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present. 25mL of hydrochloric acid was added to the crucible containing total ash covered with watch glass and boiled gently for 5 min. The watch glass was then rinsed with 5mL of hot water and this liquid was then added to the crucible. Further insoluble matter was collected on an ash less filter paper and washed with hot water until the filtrate became neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in desiccator for 30 min, and then weighed. The content of acid-insoluble ash was calculated in mg per g of air-dried material.

iii) **Water soluble ash:** Water soluble ash is the difference in weight between the total ash and residue after the treatment of total ash with water. To the crucible containing total ash, 25mL of water was added and boiled for 5 min. The insoluble matter was then collected on an ash less filter paper, washed with hot water and ignited in a crucible for 15 min at a temperature not exceeding 450˚C. The weight of this residue was subtracted from the weight of total ash. The content of water-soluble ash was calculated in mg per gram.

c) Determination of moisture content

Loss on drying: 10 gm. of seed powder was weighed and was distributed evenly on a slab in Hot Air Oven. It was heated to 100-105˚C. Then the powder was removed from the oven and was weighed. % LOD was calculated.

d) Determination of Extractable matter

Extractable matter is helpful in evaluating the constituents of crude drug, which cannot be determined by any other means. It also indicates the nature of the constituent present in the drug. This method determines the amount of constituents extracted with solvents (petroleum ether, methanol and water etc.) from a given amount of medicinal plant material.

I. **Hot Extraction:** 10 g of accurately weighed air dried coarse powder of seed was placed in a glass stopper conical flask with 100 mL of solvent and was allowed to stand for 1 hour. Reflux condenser was attached to the flask and boiled gently for next 1 hour; cooled and was weighed. Readjusted to the

original total weight with the solvent. Shaken well and filtered. 25mL of the filtrate was then transferred to the tarred Petri plate and evaporated to dryness on a water bath. After drying at 105^0C for 6 hours it was cooled in a desiccator for 30 minutes. Content of extractable matter was weighed and calculated in mg/g of air-dried material.

II. **Cold Maceration:** 10 g of accurately weighed air dried coarse powder of seed was placed in a glass stopper conical flask. Further it was allowed to macerate with 100 mL of the solvent for 6 hours, shaken frequently and then allowed to stand for 18 hours. Filtered rapidly and 25mL of filtrate was transferred to a tarred flat-bottomed dish and evaporated to dryness on a water bath. After drying at 105^0C for 6 hours it was cooled in a desiccator for 30 minutes and then weighed without delay. Content of extractable matter was calculated and recorded in mg/g of air-dried material

e) Swelling index

Swelling index is the volume in mL taken up by the swelling of 1 g of plant material under specified condition. 1 g of coarsely powdered seed was accurately weighed and taken into 25 mL measuring cylinder having inner diameter of 16 mm. 25 mL of water was added and the mixture was shaken thoroughly every 10 minutes for 1 hour. It was allowed to stand for 3 hours at room temperature. The determination of swelling index was done in a triplicate and the mean value of the individual determination was calculated related to 1g of plant material.

f) Foaming index

Many medicinal plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The Foaming ability of an aqueous decoction of plant material and their extracts is measured in terms of foaming index. About 1g of coarsely powdered plant material was allowed for moderate boiling for 30 minutes. Cooled and filtered into 100 mL volumetric flask. The decoction was then poured in 10 test tube f) Foaming index:

Many medicinal plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The Foaming ability of an aqueous decoction of plant material and their extracts is measured in terms of foaming index. About 1g of coarsely powdered plant material was allowed for moderate boiling for 30 minutes. Cooled and filtered into 100 mL volumetric flask. The decoction was then poured in 10 test tube starting from 1mL to10 mL and the volume of liquid in each tube was make up or adjusted with water to 10 mL. Tubes were then stoppered and shaken in a lengthwise longitudinal motion for 15 sec at a rate of two shakes per second. Further, all the test tubes were allowed to stand for 15 minutes and the height of foam was measured in each test tube.

Foaming index= 1000/a

Where, a= the volume in mL of decoction used for preparing the dilution in the tube where foaming to a height of 1cm will be observed.

Standards

•The foaming index is taken as less than 100 if the height of foam in every tube is less than 1cm.

•The foaming index is taken as 1000 if the height of the foam in every test tube is found to be more than 1cm.

- In a case where the height of foam in any tube is 1cm, the volume of plant material decoction in that tube is used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result. The physicochemical studies data show in **Table 2.**

2.* EXTRACTION OF ACTIVE CONSTITUENTS FROM *PORTULACA OLERACEA STEM, ROOTS

- Collected plant parts were air dried under shade and then ground to a coarse powder using a grinder. Finely powdered samples were extracted using Soxhlet method.
- Powdered sample (100 g) was extracted through Soxhlet apparatus for 16-18 hrs. with acetone, ethanol and methanol solvent systems separately. The total extract was condensed in dry bath and kept as sample stock solution

3. PRELIMINARY PHYTOCHEMICAL STUDY OF *PORTULACA OLERACEA* [5]

acetone, ethanol and methanol fractions were subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloids, glycosides, flavonoids, steroids, saponins, amino acids, carbohydrates, triterpenoids and tannins by using following procedures (Kokate et al., 2008).

Preliminary phytochemical screening involves the identification of the bioactive components present in the samples by using the standard method. The components then were separated from the co extractives. Qualitative Phytochemical tests were conducted to test the powdered form of the plant samples (methanol, ethanol, acetone and aqueous) using standard methods. The preliminary phytochemical study data show in **Table 3.**

4. ANALYTICAL EVALUATION OF MEDICINAL PLANT *PORTULACA OLERACIA* [6]

Validation of HPTLC method for estimation of *P. oleracea*

Sample preparation

The seeds of *P. oleracea* were dried, powdered and stored in air tight jars until use. 2 gm of dried powdered drug was taken in to 100 mL round bottom flask and charged with 50 mL of water by using refluxed condenser. The process was done in triplicate by adding fresh solvent. The extract was concentrated and dried residue was reconstituted using water in a 10 mL volumetric flask and used for HPTLC analysis

HPTLC instrumentation

Precoated HPTLC plates were used for application of samples. Calculated quantities of every extracts were mixed together in their relevant solvent. Linomat V applicator was used in sample application. The selected and optimized solvent system for TLC study was chosen for HPTLC analysis.

Chromatographic conditions

The chromatographic conditions necessary to acquire an efficient resolution by selected mobile phase discussed underneath.

Stationary phase: HPTLC precoated, silica gel G 60 F254 (Merck, Germany) Size: 10 x 20 cm (0.2 µm thickness)

Developing chamber: Twin trough glass chamber Mode of application: Band

Band size: 4 mm

Separation technique: Linear ascending

Temperature: 20 ± 50ºC

Saturation time: 30 min

Scanning wavelength: 254 nm / 366 nm Scanning mode: Absorbance / Reflectance

The samples were applied using syringe on precoated silica aluminium plate G60F254. The band width of 3.0 mm, space between two bands 5.0 mm, application rate of 120 nL sec-1 and slit 3.0 × 0.30 mm and 20 mm sec^{-1} scanning speed was employed for the analysis. The mobile phase toluene: ethyl acetate: formic acid (5: 4: 1, *v/v/v*) and 20 mL of mobile phase was used for chromatography. A current of hot air was used with the help of an air dryer to dry the TLC plate.

5. In-vitro nephroprotective activity of plant extracts by GGT assay method [7]

In living system Gama glutamyl transpeptidase (GGT) is one of the most sensitive enzymes. Glycylglycine and Gama glutamyl-p-nitroanilidlide (GPNA) are converted by the action of GGT to p-nitro aniline and L-gamma glutamyl glycylglycine. Increase in the rate of absorbance at 405 nm due to the release of p-nitro aniline is directly proportional to the GGT activity (Hanigan *et al.*, 1996). The analytical evaluation of medicinal plants data show in **Figure 5, 6, 7 & 8 and Table 4, 5.**

Table 1. Reagent composition for GGT assay methods

Active ingredients	Concentration
Reagent -1	
Soluble GPNA	3 m mol L^{-1}
Reagent -2	
Buffer	100 m mol L^{-1}
Glycyl glycine (Ph. 8.5±0.1 at 25 ^{0}C)	30 m mol L^{-1}
1 GGT (Substrate)	2 (10 tablets)
2 GGT (Buffer)	2
Reconstitution vial	1

Sample preparation

Working reagent preparation

Reconstitute one tablet of 1-GGT with 1.1 mL of 2-GGT to prepared working reagent. Mix gently to dissolve the contents and use after 5 min.

Preparation of plant extract

Each plant extract (2 mg mL^{-1}) was accurately weighed, transfer in to test tubes and separately mixed with DMSO to make up the volume 5 mL^{-1}. This was then accurately transfer in to another test tube with 2 mL of DMSO in increasing order and the volume should be 3 mL whilst in the last test tube volume should be 4 mL (0.78 ug mL^{-1} to 500 ug mL^{-1}). The *in-situ* extracts were used for the analysis.

Methodology

GGT inhibition study was done to determine the effect of mesna–disulfde heteroconjugates on the enzyme's activity *in vitro*. A spectrophotometric assay based method was utilized to calculate the percentage inhibition of gamma-glutamyl transpeptidase (GGT) activity, mediated by mesna and aqueous extract of plants material at 405 nm. Different concentrations of plants extract (3.9–500 ug mL^{-1}) and mesna (3.9–500 ug mL^{-1}) were generated in situ and incubated with GGT. A graph was plotted against log concentration and mean percentage inhibition of each concentration. The 50% inhibitory dose value (IC50) was found by interpolation by using graph pad (Prism 6 software) and compared with standard. The mesna and plants extract data show in **Table 6 & Figure 9.**

RESULT AND DISCUSSION:

1. COLLECTION, IDENTIFICATION AND AUTHENTICATION

There are several species available from same genus as well as there could be resemblance in physical appearance with other plants so the authentication of the plants under study is essential part of the protocol. Authentication ensures the correct plant species and plant parts used as raw materials for scientific study of medicinal plants. The plant Portulaca oleracea was collected from Department of Pharmacognosy, College of Pharmacy, Chopda (Jalgaon, Maharashtra) and was identified and authenticated by Botanical Survey of India, Pune.

2. PHARMACOGNOSTIC EVALUATION

WHO has emphasized the need to ensure the quality of medicinal plants and therefore, designed the series of tests for plant standardization. Morphologic and microscopic features of Portulaca oleracia was matched with the identification characters described in volumes of Ayurvedic Pharmacopoeia of India. Results of physicochemical parameters, morphologic and microscopic features of Portulaca oleracia were matched with the standards given in Ayurvedic Pharmacopoeia of India. These studies will help in the identification of the plant materials or for ensuring whether the plant is correct and meets prescribed standards.

Macroscopic and microscopic evaluation

Macroscopic examination of the selected plant species was carried out under sunlight and artificial light by naked eye. Samples were examined thoroughly using magnifying lens (6x to 10x). Microscopic characters of transverse section and of dried powder of selected plant part were visualized under 10x or 40x magnification. The plant drugs are generally transported and used in powdered or comminuted form where the macro morphology is destroyed. So it is necessary to get familiar with powder microscopical features of plants in order to detect any kind of adulteration and substitution. Microscopic examination includes the detection of the type and arrangement of different features present in crude drug for e.g. trichomes, epidermis, stomata, fibers, vessels, vascular bundle, cork, cortex, type of cells (parenchyma, collenchymas, sclerenchyma etc), crystals etc.

a) Macroscopic features

The macroscopic and anatomical features are often so diagnostic and are very useful for authentication at a microscopic level to distinguish closely related species. The results showed seeds were smooth, tiny about 0.2 cm to 0.3 cm long, outer surface is black to shiny in color with characteristic odour, sour, acrid and somewhat astringent in taste **(Fig. 1).**

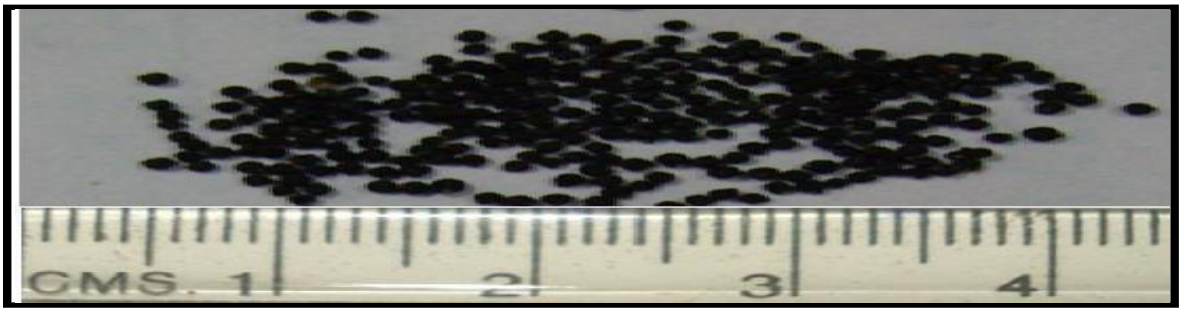

Fig. 1. Macroscopical characters of *P. oleracea* seeds

b) Microscopic Features

The microscopic results confirmed the existence stellulate-tessellate epidermal cells (Fig. 2 A). Paradermal section showed crystals (Fig. 2 B). The cells of epidermal province were rectangular to multilateral, to some extent lobed; their antidinal walls (Fig. 2 C) were lean and somewhat curvy. The testa cells were elliptic with curved tipped and were freely packed (Fig. 2 D). Few silky wall granules (Fig. 2 E) were also present. The parenchyma cells were pitch shaped to elliptic silky walled to granulous in their surfaces and packed with resinous material (Fig. 2. F).

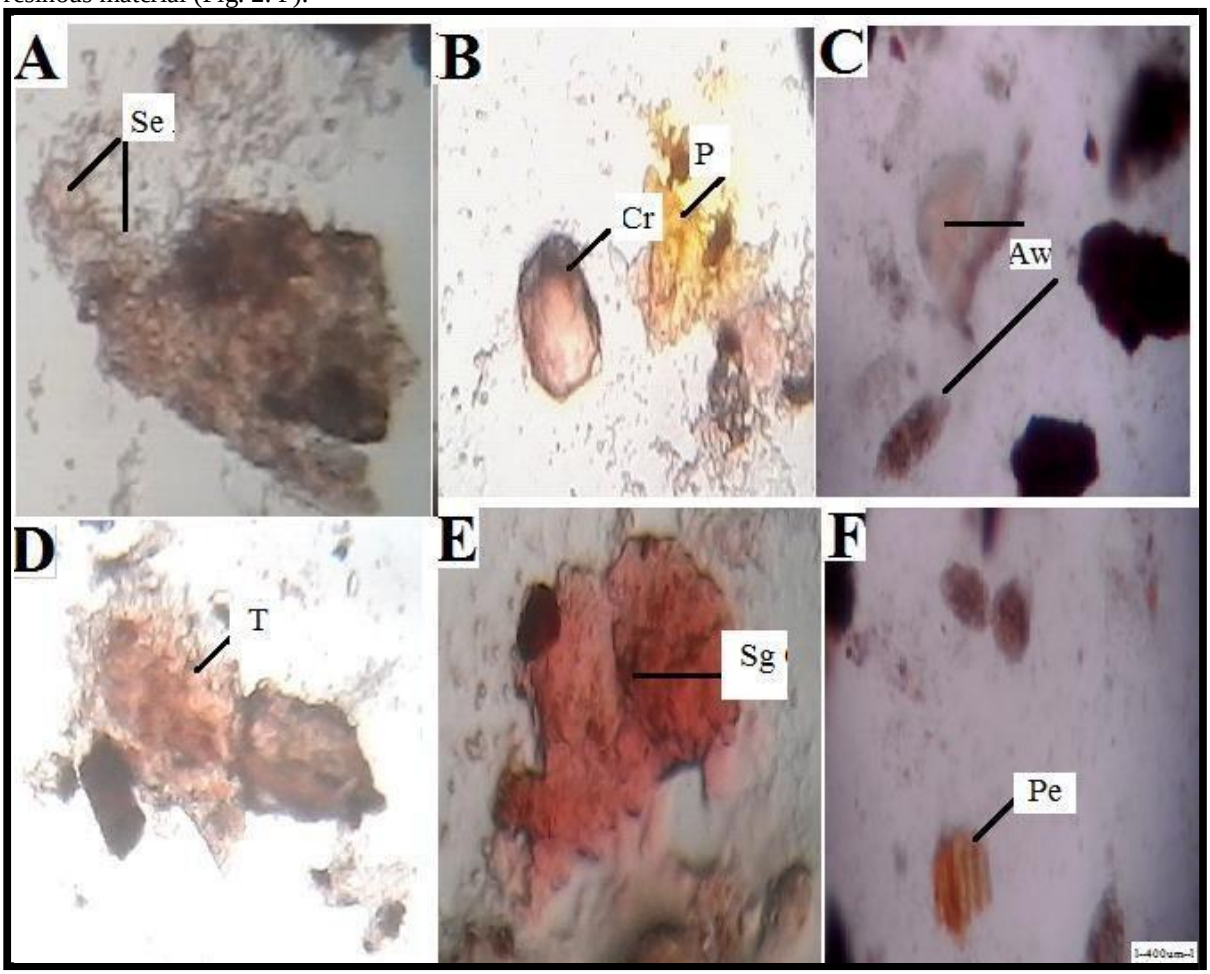

Fig. 2.T.S. of *P. oleracea* seed (A) Se- stellulate-tessellate epidermal cells, (B) [Cr- crystals at paradermal section; P- parenchyma with resinus material], (C) Aw- antidinal walls, (D) T- testa, (E) Sg- silky granules, (F) Pe- parenchyma with elliptic silky cells

3. PHYSICOCHEMICAL EVALUATION

All physicochemical parameters were repeated for three times and are reported in (Table 29) by means of SD (standard deviation). The Karl Fischer method was used for fortitude of water to confirm the product superiority. The results confirmed that the loss on drying was 5.45 % and moisture content was 4.07 %. Inorganic materials, like carbonates, silicates, oxalates and phosphates can be validate by the grit of total ash. Ash value designate about adulteration, value and purity of the plant material and their products. The acid insoluble ash is responsible for silica while high acid insoluble ash mainly for the contamination with sandy materials. Inorganic elements can be calculated by means of water-soluble ash.

The total ash in P. oleracea sample was found 6.97% w/w, whereas acid insoluble and water-soluble ash were 3.62% w/w and 3.16±0.05% w/w, respectively. It was considered that the drug was found acidic in nature at the pH of 1.0 and 10% i.e. 6.7 and 5.5 respectively. The yields measure the quantity of phytoconstituents in an extract with their compatible. Fixed oil, resin and volatile substances are come out in the petroleum ether extract, while at 1050C all the volatile substances are evaporated though fixed oil, resins remain. The high yield was set up in methanol extract (19.15% w/w) when hot extraction was conceded where lowest amount of yield was set up in petroleum ether (0.98% w/w) when cold extraction was conceded **(Table 2)**

Table 2. Results of physicochemical evaluation of P. oleracea (n=3)

Sr. No	Parameters	% w/w(Mean ± SD*)
1	LOD	5.45±0.02
2	Moisture content	4.07±0.64
3	**Ash values**	
	Total ash	6.97±0.04
	Water soluble ash	3.16±0.05
	Acid insoluble ash	3.62±0.01
4	**pH**	
	10 % solution	5.5±0.10
	1 % solution	6.7±0.15
5	**Cold extraction**	
	Petroleum ether	0.98±0.13
	Chloroform	5.79±0.41
	Methanol	10.89±0.37
	Aqueous	15.45±0.28
6	**Hot extraction**	
	Petroleum ether	2.79±0.15
	Chloroform	7.39±0.47
	Methanol	19.15±0.46
	Aqueous	27.63±0.34
7	**Successive extraction**	
	Petroleum ether	2.18±0.23
	Chloroform	6.26±0.51
	Methanol	15.0±0.91
	*SD	

4. PRELIMINARY PHYTOCHEMICAL SCREENING

Acetonic, methanolic, ethanolic and aqueous extract were subjected for preliminary phytochemical screening to access the presence of different plant secondary metabolites using qualitative tests summarized in table 3.

Table 3: Preliminary phytochemical screening

Phyto. constituents	Acetone extract	Ethanol extract	Methanol extract	Aqueous extract
Alkaloids	-	+	+	+
Glycosides	-	-	-	-
Proteins and Amino	+	+	-	+
acids Carbohydrates	-	-	-	-
Tannins	-	+	+	+
Fats and Fixed oils	+	+	-	+
Acids	+	-	-	+
Saponins	+	+	+	+
Steroids	-	+	-	+
Flavonoids	+	+	+	+
Phenols	+	-	+	-

Qualitative Phytochemical tests were conducted to test the powdered form of the plant samples (methanol, ethanol, acetone and aqueous) using extracts.

5. High performance thin layer chromatography (HPTLC) profiling of P. oleracea

Satisfactory and well resolve results were obtained with solvent system hexane: ethyl acetate (80:20, v/v), chloroform: methanol (90:10, v/v) and toluene: ethyl acetate: formic acid (60:35:05, v/v/v) for methanolic, chloroform and petroleum ether extract respectively. The developed plate then scanned at 254 nm and 366 nm and in visible range (450nm). The adequate results were found for methanolic extract at wavelength 254 nm (Fig. 3 A). The satisfactory and compact spots at wavelength 366 nm were found for chloroform extract (Fig.3 B). Good results for petroleum ether extracts were found after sprayed with anisaldehyde sulphuric acid as spraying agent (Fig.3 C). After drying in to air the developed plate were subjected in an oven at 110 0C for 10 min to get compact spots. Total 10, 09 and 08 plants constituents were found in HPTLC fingerprints of methanolic, chloroform and petroleum ether extracts of P. oleracea (Table. 4).

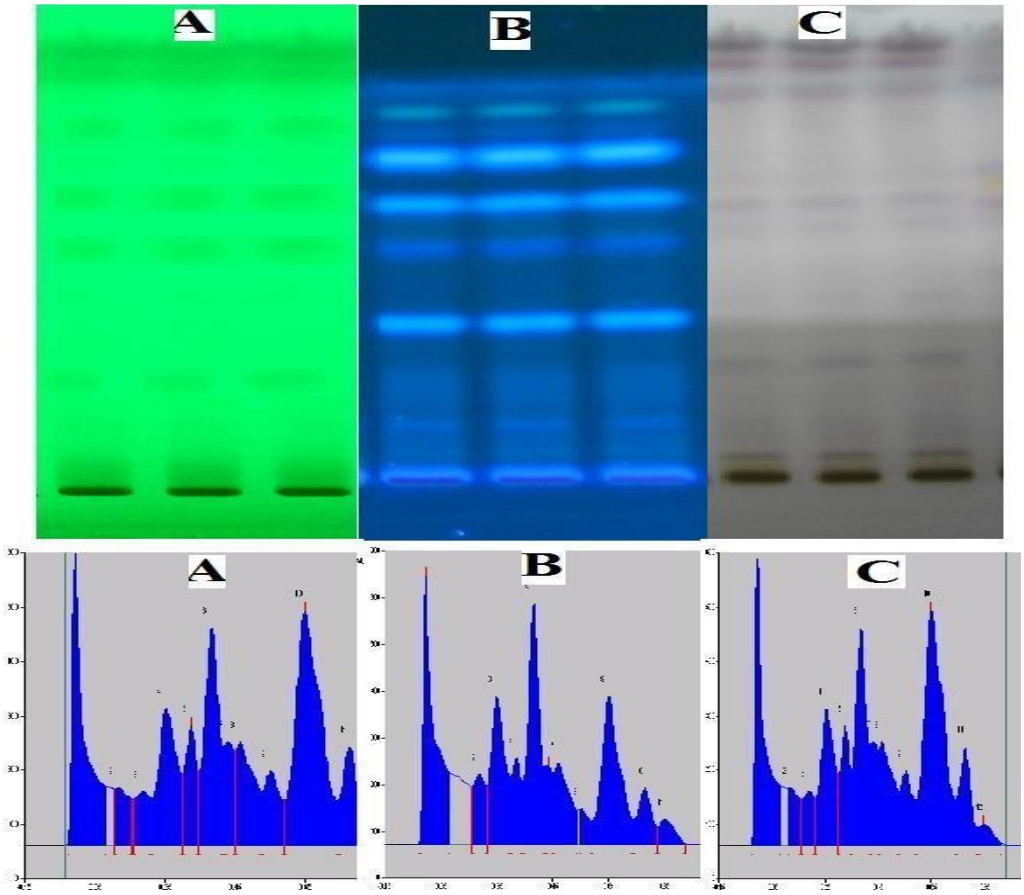

Fig. 3: HPTLC Photographs and chromatograms of (A) methanolic, (B) chloroform (C) petroleum ether extracts of P. oleracea

Table 4. Results of HPTLC fingerprint study of P. oleracea extracts

***P. oleracea* extract**	**Solvent system**	**Detection wavelength (nm) and visualizing agent**	**No of spot and Rf values**
Methanol extract	Hexane: Ethyl acetate (80:20, v/v)	254	(**10**) 0.19, 0.33, 0.41, 0.61, 0.73, 0.74, 0.67,0.73, 0.83, 0.33
Chloroform extract	Chloroform: Methanol (90:10, v/v)	366	(**9**) 0.31, 0.55, 0.35, 0.54, 0.42, 0.51, 0.41,0.33, 0.53
Petroleum ether extract	Toluene : Ethyl acetate: Formic acid (60:35:05, v/v/v)	Anisaldehyde sulphuric acid	(**8**)0.15,0.21,0.43,0.25 0.36, 0.45, 0.33, 0.53,

In-vitro antioxidant and nephroprotective activity

In-vitro antioxidant activity by reversed phase HPLC

The electron donation ability of natural products can be measured by 2, 2-diphenyl-1- picrylhydrazyl radical (DPPH) purple-coloured solution bleaching. The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test. Results of this study suggest that the plant extract rich in phenolic or flavonoids constituents are capable of donating hydrogen to a free radical to scavenge the potential damage. The developed HPLC method used for screening antioxidant activity was found to be simple, sensitive and specific for DPPH free radical scavenging activity. Method allowing short run time and rapid determination of radical scavenging activity of different plants extract. Radical Scavenging (%) was calculated using the formula quoted in experimental method.

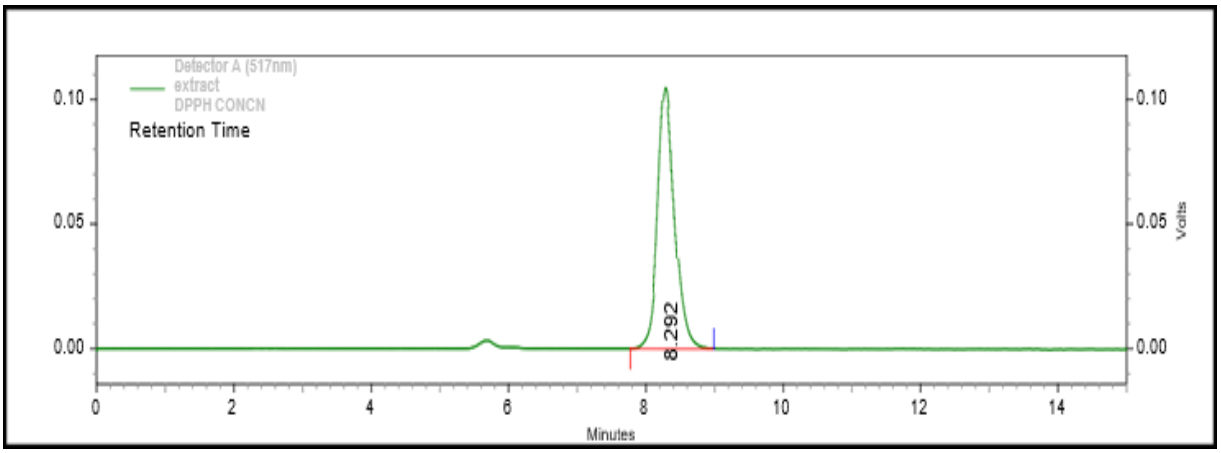

Fig. 4: HPLC chromatogram of DPPH at RT 8.292 and at wavelength of 517 nm; mobile phase; methanol: water (80: 20 v/v); flow rate 1 mL min-1; injection volume 20 µl

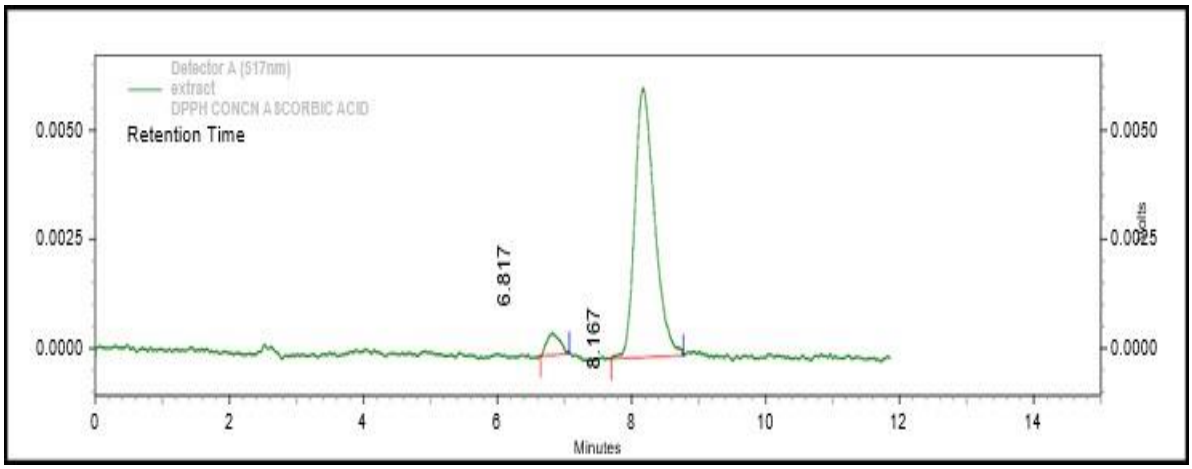

Fig. 5: HPLC chromatogram of DPPH with ascorbic acid at RT 8.292 and at wavelength of 517 nm; mobile phase; methanol: water (80 : 20 v/v); flow rate 1 mL min-1; injection volume 20 µl

DPPH radical scavenging activity of aqueous extract of P. oleracea (AEPO)

The radical scavenging activity of standard ascorbic acid and AEPO were determined by HPLC method at 517nm. A reduction in peak area revealed comparable radical scavenging activity of ascorbic acid at 20µg mL-1(IC50 16.76) and AEPO at 40 µgmL-1 (IC50 27.67)

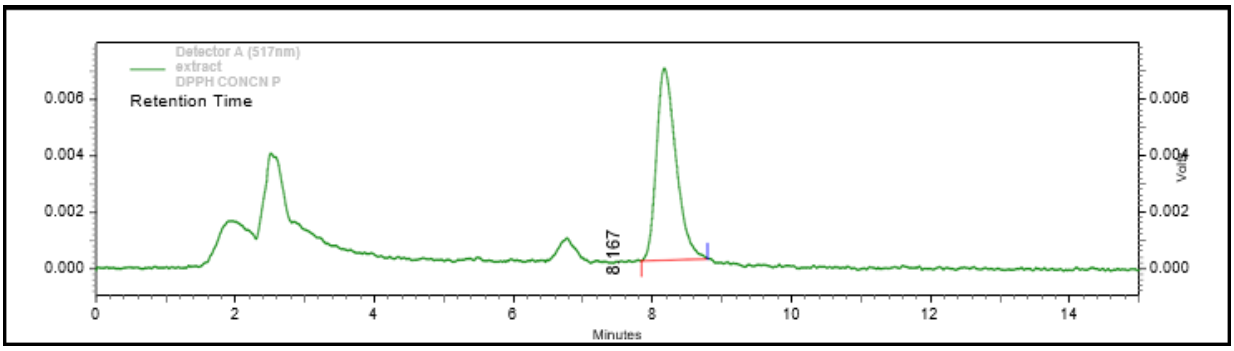

Fig. 6: HPLC chromatogram of DPPH with AEPO at RT 8.167 and at wavelength 517nm; mobile phase; methanol: water (80:20 v/v); flow rate 1mLmin-1; injection volume 20µl

Table 5. DPPH free radical scavenging activity of all plant extracts at 517 nm by HPLC method
Effects of mesna and AEPO on GGT activity

Concentration µg mL^{-1}	Percentage inhibition ± SD	
	Ascorbic acid	*P. oleracea*
5	18.27±0.60	7.23±0.20
10	34.86±0.57	20.59±0.69
20	52.41±1.02	45.40±1.09
30	65.10±1.09	49.22±0.18
40	74.40±1.43	57.50±1.12
60	81.28±1.15	66.30±1.03
80	86.50±0.85	76.89±1.12
100	92.65±1.15	83.33±0.34

A 50% inhibition in GGT activity was observed when 62.5 $ugmL^{-1}$ mesna (IC_{50}61.29) and AEPO 250 $ugmL^{-1}$ (IC_{50}140.8) was incubated with the crude human GGT for 15 min at 25°C. The finding suggesting that mesna and plant extract had inhibitory effects on GGT. The results of the effects of mesna and AEPO on GGT

Table 6. Effects of mesna and plants extract on GGT activity at different concentrations

Concentration µgmL^{-1}	Percentage inhibition ± SD	
	Mesna	*P. oleracea*
3.9	5.68 ± 0.57	2.18 ± 2.68
7.8	9.32 ± 0.41	6.70 ± 1.57
15.6	12.12 ± 0.47	10.23 ± 1.52
31.2	24.71 ± 0.06	17.32 ± 0.41
62.5	51.82 ± 0.90	26.31 ± 5.08
125	61.68 ± 0.47	46.84 ± 0.93
250	87.44 ± 0.15	73.18 ± 0.81
500	90.18 ± 0.10	82.24 ± 0.38
IC50	62.29	140.8

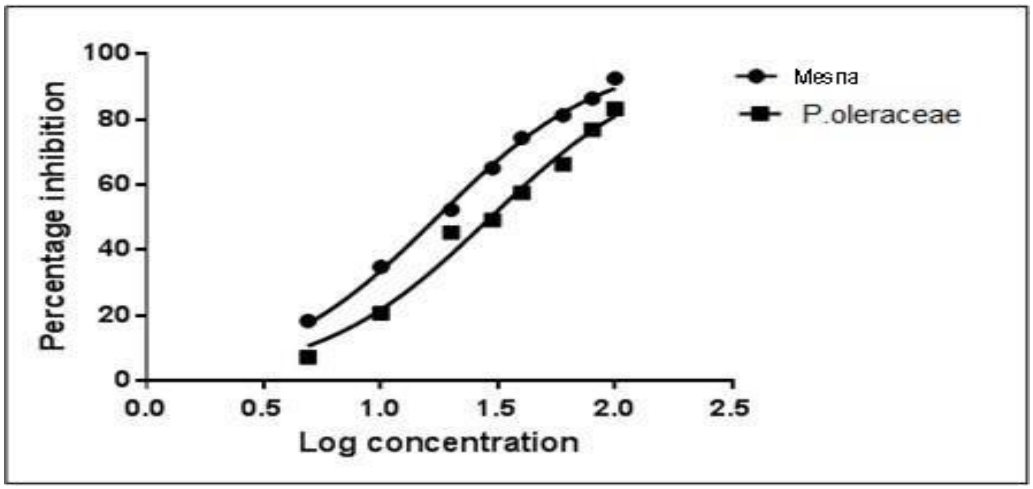

Fig. 7. Comparative GGT activity of mesna and P. oleracea extract at 405 nm

CONCLUSION:

The aim to this present study is Development of Pharmacognostical profile, Analytical and Pharmacological evaluation of Nephroprotective medicinal plant *Portulaca oleracea*. The objectives of the study were Collection and Authentication of the investigational plant, Pharmacognostical profile (Macroscopic & Microscopic) evaluation of Portulaca oleracea, Preliminary physico-chemical study of Portulaca oleracea, Extraction of active constituents from Portulaca oleracea, To develop an active Nephroprotective agent from Portulaca oleracea extracts, Standardization of plant extracts by phytochemical studies, and In vitro pharmacological evaluation of Portulaca oleracea extract for its Nephroprotective activity.

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ABBRIVATION

1. **GGT :** Gama glutamyl transpeptidase
2. **CDH:** Central Drug House
3. **UV-Vis :** Ultra Violet-Visible Spectroscopy
4. **HPLC:** High Performance Liquid Chromatography
5. **HPTLC:** High Performance Thin Layer Chromatography
6. **ICMR:** Indian Council of Medical Research
7. **LOD:** Loss on Drying
8. **H2SO4:** Sulphuric acid
9. **HCl:** Hydrochloric acid
10. **NaOH:** Sodium Hydroxide
11. **KOH:** Potassium Hydroxide
12. **NH4OH:** Ammonium Hydroxide
13. **TLC:** Thin Layer Chromatography
14. **DMSO:** Dimethyl Sulfoxide

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