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PHARMACOGNOSTICAL EVALUATION OF *URARIA PICTA, URARIA LAGOPOIDES* AND *PSEUDARTHRIA VISCIDA* AS PER WHO GUIDELINES

Disha A. Mool, Apurva C. Somkuwar, Ashwati A. Meshram, Trushna S. Bhoyar

[1,2]Assistant Professor, [3,4]Student
Department of Pharmacognosy,
Maharashtra Institute of Pharmacy, Betala, Bramhapuri

Abstract : The present study was undertaken to evaluate the pharmacognostical characteristics of Uraria picta, Uraria lagopoides, and Pseudarthria viscida in accordance with World Health Organization (WHO) guidelines for the standardization and quality control of medicinal plant materials. These medicinal plants are widely used in traditional systems of medicine for the treatment of inflammatory disorders, fever, wounds, urinary ailments, and general debility. The study aimed to establish diagnostic standards for proper identification, authentication, and differentiation of these species. Pharmacognostical evaluation included macroscopic, microscopic, physicochemical, and preliminary phytochemical investigations. Organoleptic characters such as colour, odour, taste, texture, and appearance were recorded. Microscopic studies of transverse sections and powdered drug samples revealed characteristic cellular structures including epidermal cells, trichomes, vascular bundles, fibres, starch grains, calcium oxalate crystals, and lignified tissues useful for identification. Physicochemical parameters such as ash values, extractive values, moisture content, and foreign matter analysis were determined according to WHO prescribed methods. Preliminary phytochemical screening indicated the presence of important bioactive constituents including alkaloids, flavonoids, tannins, glycosides, saponins, phenolic compounds, and terpenoids. The findings of the study provide valuable pharmacognostical standards for the authentication and quality assessment of these medicinal plants and may serve as a reference for future herbal drug standardization, formulation development, and pharmacological investigations. The study also supports the importance of WHO guidelines in ensuring the safety, purity, and efficacy of herbal medicines.

Keywords: *Uraria picta, Uraria lagopoides, Pseudarthria viscida*, Pharmacognostical evaluation, WHO guidelines, Microscopy, Physicochemical parameters, Phytochemical screening, Standardization, Medicinal Plant

INTRODUCTION

India is the world's most diversified country, with a wide variety of plant species as its heritage. In India, there are several medicinal plants that provide a rich source of active phytochemicals. Many people have been using herbal plants for health and medical purposes as basic healthcare for thousands of years. Herbal formulations are considered very safe because they have no or very few toxicity and negative effects. As a result, there is a worldwide trend toward using natural medicines to treat acute and chronic illnesses (Ayurvedic pharmacopoeia of India). In this period of phytotherapy, herbal medications have become one of the totems. Medicinal plants have huge commercial potential all around the world. With the rise in interest in herbal remedies, the amount of literature on the subject has exploded, and quality control has become critical in this regard all over the world

Ayurveda is an Indian system of traditional medicine that is also used as an alternative treatment in other areas of the world. Ayurveda is derived from the Sanskrit words ayus, which means "life," and Veda, which means "knowledge." Asava, arishta, rasa, curna, parpati, lepa powders, medicated oils, and other Ayurvedic formulations are only a few examples. "Dasamula" is a term used in several of these formulations, where "dasa" means ten and "mula" denotes roots. "Dasamula" is a unique ten-root combination, as the name suggests.

According to trade statistics gathered over the years, demand for various constituents in Dasamula has outpaced supply, necessitating the purchase of higher quantities of ingredients used in herbal formulations. Given this, it has been noted that many of the ingredients' original species are not geographically available in the numbers required. As a result, they are often exchanged or even contaminated with other species with similar or dissimilar structural and chemical characteristics. *Uraria picta* is a key ingredient in the Ayurvedic Dasamula recipe. *Uraria lagopoides* and *Pseudarthria viscida* are two substitutes or adulterants for *Uraria picta*. Both plants are essential in indigenous medicine for their biological activity, and they are used in many herbal formulations in India. *Uraria picta* flowers are purple in colour. Between August and October, the plant blooms and bears fruit. The *Uraria lagopoides* flowering stage is visible around November. *Pseudarthria viscida* flowers are brilliant purplish or pink in colour and occur in the cold season (October to December) or on rare occasions almost all year

In Ayurveda, *Uraria picta* is traditionally used as an anti-inflammatory herb. The plant also has a high antioxidant capacity. *Uraria picta* is effective in many ways and has a wide range of therapeutic characteristics Asthma, dysentery, delirium, ulcers, malarial fevers, bone fractures, chest discomfort, and diarrhoea have all been treated using *Uraria lagopoides*. In the indigenous medical system, this herb is used to treat a variety of diseases *Pseudarthria viscida* is a rejuvenating tonic that is used as an astringent, emollient, thermogenic, digestive, constipative, anthelmintic, anti-inflammatory, aphrodiasiac, cardiotonic, febrifuge, and anthelmintic. Antifungal, antioxidant, analgesic, and antipyretic properties have been discovered in the plant. In the treatment of headaches and hemicranias, the root juice is administered as a nasal-drop

NEED OF STANDARDIZATION

In the global perspective, there is a shift towards the use of medicine of herbal origin, as the dangers and the shortcoming of modern medicine are getting more apparent. It is the cardinal responsibility of the regulatory authorities to ensure that consumers get the medication, which guarantees purity, safety, potency and efficacy. The regulatory authorities rigidly follow various standards of quality prescribed for raw materials and finished products in pharmacopoeias, formularies and manufacturing operation through statutory imposed good manufacturing practices. These procedures logically would apply to all types of medication

Whether included in modern system of medicine or one of the traditional systems. Though herbal products have become increasingly popular throughout the world, one of the impediments in its acceptance is the lack of standard quality control profile. The quality of herbal medicine that is, the profile of the constituents in the final product has implications in efficacy and safety. However, due to the complex nature and due to the complex nature and inherent variability of the constituents of plant-based drugs, it is difficult to establish quality control parameter though modern analytical technique are expected to help in circumventing this problem. Furthermore, the constituents responsible for the claimed therapeutic effects are frequently unknown or only partly explained. This is further complicated by the use of combination of herbal ingredients as being used in traditional practice. It is common to have as many as five different herbal ingredients in one product. Thus batch to batch variation starts from the collection of raw material itself in the absence of any reference standard for identification. These variations multiply during storage and further processing. Hence for herbal drugs and products, standardization should encompass the entire field of study from cultivation of medicinal plant to its clinical application. Plant materials and herbal remedies derived from them represent substantial portion of global market and in this respect internationally recognized guidelines for their quality assessment and quality control are necessary.

MATERIAL AND METHOD

Collection and Authentication of plant material

The biomass of plants of *Uraria lagopoides* and *Pseudarthria viscida* were gathered from the Kesav Shrushti Kandiwali, Mumbai. It was confirmed by Dr. Nitin Dongarwar Head of Department Botany University campus RTMNU, Nagpur. The dried plant material of *Uraria picta* purchased from Trust Herb Amazon.in and it was used for extraction process.

Morphological Evaluation

The morphological evaluation of the plants was carried out. The various parameters like colour, odour, taste, dimensions etc. were studied.

Microscopical Evaluation

In microscopic evaluation the Transverse Section (TS) of the Leaves and Stem mounted on the slide utilizing HCl, Phloroglucinol and Glycerine then each slide was secured with cover slip and analyzed under advanced Microscope equipped with 10X, 15X, 45X, and 100X objectives.

Proximate analysis

Foreign organic matter: 100 g of drug was weighed and spreaded the sample on a white tile without overlapping. Inspected the sample with naked eye or by lens (10 x or above). Separated the foreign organic matter. After completed separation, weighed the foreign organic matter and calculated the percentage w/w present in the sample.

Loss on drying (Moisture content): An accurately wieghed quantity of about 1-2 g of powdered drug was taken in a tared glass petridish. The powder was distributed evenly. The Petri dish kept open in oven and the sample was dried at 105° for 2hrs.until a constant weight was recorded. Then it was cooled in a desicator to room temperature, weighed and recorded

Ash values

The total ash, water-soluble ash values and acid insoluble ash were determined from air-dried samples.

Extraction

Extraction, as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use.

Maceration Extraction: Coarsely powdered plant material of were kept in contact with petroleum ether, chloroform, ethanol, Hydroalcoholic (Ethanol: water 7:3) and Aqueous water in a stoppered container for 42 hrs period with frequent shaking on Rotary shaker. This cycle was repeated several times in order to exhaust the plant material. Then the extractive solutions were pooled, filtered and concentrated to dryness under water bath. Then extract was further transferred into sterile bottles and refrigerated until use.

Qualitative evaluation of phytoconstituents

The qualitative preliminary phytochemical tests were performed for testing different chemical groups present in extracts. The primary metabolites like proteins, carbohydrates and fixed oils and fats, were analyzed for their presences as per the standard procedures similarly, the secondary metabolites like, alkaloids, flavonoids, saponins, phenolic, tannins, terpenoids and glycosides were also analyzed.

1) Detection of alkaloids

a) Mayer's Test

The extracts were treated with a few drops of Mayer's reagent. Formation of a creamy white precipitate indicated the presence of alkaloids.

b) Dragendroff Test

Extracts were treated with a few drops of Dragendroff reagent. Formation of orange red precipitate indicated the presence of alkaloids.

2) Detection of Carbohydrates

a) Molisch's Test Filtrates were treated with 2 drops Molisch's reagent in a test tube and 2ml of Conc. sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicated the presence of Carbohydrates

b) Benedict's test

Filtrates were treated with Benedict's reagent and heated on water bath. Formation of orange red precipitate indicated the presence of reducing sugars.

3) Detection of Glycosides

a) Keller kelliani test

Extract were treated with chloroform and evaporate it to dryness. Separately 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride was added and transferred to a small test tube added with carefully 0.5 ml of concentrated Sulphuric acid by the side of the test tube, blue colour appears in the acetic acid layer indicating the presence of glycosides.

b) Borntrager's test

Extract were boiled with 1 ml of dilute Sulphuric acid in a test tube separately for 5 min, filtered while hot, pipette out the supernatant or filtrate, cooled and shaken with an equal volume of dichloromethane. The lower levels of dichloromethane separated and shaken with half its volume with dilute ammonia. A rose pink to red color appeared in the ammonical layer, indicating the presence of glycosides.

4) Detection of saponins

Foam test

Small amount of extract was shaken with little quantity of water. If foam produced persists for ten minutes, it indicated the presence of saponins**.**

5) Detection of phytosterols

Salkowski's Test

Extract were treated with chloroform and treated with few drops of conc. Sulphuric acid. Shaken and allowed to stand. Appearance of golden yellow indicated the presence of phytosterols (triterpenes).

6) Detection of phenolic acids and tannins

a) Ferric chloride test

Extracts were treated with few drops of ferric chloride solution. Formation of blackish violet or pinkish red colour indicated the presence of tannins or phenolic acids. {Note: Formation of emerald green to brownish black colour could also indicate the presence of phenolic acids or tannins.

b) Gelatin Test

Extracts were treated with few drops of 1%gelatin solution containing 10% sodium chloride. Formation of white precipitate indicated the presence of tannins.

7) Detection of proteins and amino acids

a) Xanthoproteic Test

The extracts were treated with few drops of Conc. Nitric acid solution. Formation of yellow colour indicated the presence of proteins.

b) Ninhydrin test

To the extract, 0.25% ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicated the presence of amino acid.

8) Detection of Flavonoids

a) Alkaline Reagent Test

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colorless on addition of dilute acid, indicated the presence of flavonoids.

b) Shinoda Test

To the alcoholic solution of extracts, a few fragments of magnesium ribbon and Conc.HCl were added. Appearance of magenta colour after few minutes indicated the presence of flavonoids.

9) Detection of Gums and Mucilage

Alcohol precipitation

Extract were treated with alcohol; formation of precipitate indicate the presence of Gums and mucilage.

Quantitative evaluation of phytochemical constituents

1) Estimation of Alkaloids

Procedure:

1. Take 0.4, 0.6, 0.8, 1.0 and 1.2 ml atropine solution in separate test tube.
2. Add 5 ml of Phosphate Buffer Solution (pH 4.7) and 5 ml of BCG solution.
3. Shake well and extract the yellow colored complex with chloroform.
4. Separate chloroform and make up the volume to 10 ml.
5. Measure the absorbance at 470 nm against blank.
6. Now prepare the methanolic extract of plant material. Dry and dissolve in 2N HCL. Filter and wash with chloroform. Adjust the pH neutral with 0.1 N NaOH .
7. Now add 5 ml of Phosphate Buffer Solution (pH 4.7) and 5 ml of BCG solution.
8. Shake well and extract the yellow colored complex with chloroform.
9. Separate chloroform and make up the volume to 10 ml and measure the absorbance at 470nm.
10. Calculate concentration of total alkaloids from calibration curve of atropine standard

2) Estimation of Flavonoids

Procedure

1. Prepare the calibration curve of standard Quercetin (10-100 µg/ml in methanol).

2. Mix 0.5 mL standard solution with 1.5 mL of 95% ethanol, 0.1 mL of 10% aqueous aluminum chloride, 0.1 mL potassium acetate and 2.8 mL of distilled water.
3. Incubate for 30 min. at room temperature. Measure the absorbance of the reaction mixture at 415 nm with a UV spectrophotometer.
4. To prepare blank solution substitute 10% aluminum chloride with the same amount of distilled water.
5. Similarly, treat 0.5 mL of plant extract samples with aluminum chloride for determination of flavonoids content from calibration curve.

3) Estimation of phenol

Procedure

1. Prepare calibration curve of standard Gallic acid (10-100 µg/mL in water).
2. Prepare 1 mg/mL of extract solutions.
3. Mix 1 mL of each sample with 0.25 mL of Folin- Ciocalteu's reagent and 1.25 mL of 20% sodium carbonate solution.
4. Allow the mixture to react for 40 min. at room temperature.
5. After the reaction period, the contents are mixed and measure the blue color at 725 nm in comparison with standards. Calculate the amount of total phenols from calibration curve as a Gallic acid equivalent by the following formula: T= C.V. / M
6. Where, T= total content of phenolic compounds, milligram per gram plant extract, C= the concentration of gallic acid established from the calibration curve, milligram per milliliter; V= the volume of extract, milliliter; M= the gram weight of plant extract.

4) Estimation of protein

Procedure

1. Prepare the concentration of standard protein (albumin or gamma globulin) between 5 to 100µg.
2. Add 5 mL Bradford reagent and incubate for 5min. (Note: If sample are not readily soluble in the colour reagent then use 1 M NaOH).
3. Measure the absorbance at 595 nm. Prepare a standard calibration curve of the standard.
4. Repeat same procedure for sample and determine the amounts of protein in the sample from the standard calibration curve.

5) Estimation of Tannins

Procedure

1. Take 1, 2, 3, 4 and 5 ml working standard solution of tannic acid in a separate test tube.
2. Add 0.5 ml of Folin-Denis reagent and 1ml sodium carbonate to each test tube.
3. Make volume of each test tube up to 10ml. Blue colour solution is formed. Measure the absorbance at 700 nm within 30 min. against the reagent blank prepared in a similar manner without the tannic acid.
4. Calculate the percentage of total tannin from calibration curve of tannic acid.

6) Estimation of Carbohydrate

Procedure

1. Take 100 mg of the glucose into test tube. Add 5ml of 2.5 N HCL and boil in water bath for 3 hours to hydrolyze the sugars. Cool to room temperature.
2. Add sufficient quantity of solid sodium carbonate until the effervescence ceases. This indicates complete neautralisation. Filter and make the volume up to 100 ml.
3. Pipette out 0.2, 0.4, 0.6, 0.8 and 1ml of working standard into a series of test tubes.
4. Similarly prepare sample to be studied. Pipette out 0.1 and 0.2 ml of this solution in two separate test tubes. Make the volume up to 1 ml with water.
5. Set the blank with all reagents without sample.
6. Add 1 ml of phenol solution to each test tube and shake well. Then add 5 ml of sulphuric acid 96% and again shake well.
7. Keep aside for 10 min. Shake each test tube and keep in water bath at 25-30°C for 20 min. Now measure the colour at 490 nm.
8. Calculate the total amount of total carbohydrate in the sample from glucose standard graph.
9. Absorbance corresponds to 0.1 ml of the test= x mg of glucose.
10. 100 ml of the sample solution contains= x/0.1 × 100 mg of glucose= % of total carbohydrate present.

Result and discussion

1. Morphological evaluation

Table No.1: Morphological evaluation of *Uraria picta, Uraria lagopoides and Pseudarthria viscida*

Sr. No.	Parameters	Characteristics		
		Uraria picta	***Uraria lagopoides***	***Pseudarthria viscida***
1.	Leaf color	Yellowish green	Green	Green
2.	Taste of leaf	Bitter	Bitter	Sweet and bitter
3.	Flower color	Purple	Pink	Pink or violate
4.	Stem color	Lemon yellow to brown	Greenish	Greenish

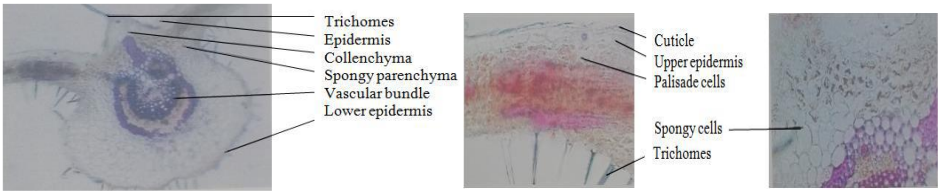
2. Microscopical evaluation

The microscopic examination of plant tissues represent a uniquely new technique that offered a more in-depth and comprehensive view of the plant than was ever previously possible. With a microscope, even the contents of the cells could, for the first time, be visualized in a manner previously not attainable with the naked eye. Microscopy allowed detailed descriptions of plant anatomy

and complemented the botanical illustrations and macroscopic characterizations of early herbals, adding a new dimension to the physical characterization of plants.

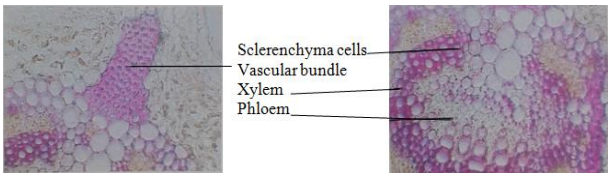
3. Microscopy of *Uraria picta*

3.1. Leaf: Leaf is dorsiventral. Stomata are of paracytic type and present on both the epidermis. Epidermis is single layered with thin cuticle. Two layers of elongated palisade cells are present below the upper epidermis followed by 3-5 layers of spongy parenchyma cells. Leaf bears unicellular covering trichomes abundant on the lower epidermis with covered apex. Midrib region shows collenchymatous cells on both sides below the epidermis. Midrib region shows patches of sclerenchyma cells. Vascular bundles are seen scattered.



Magnified view of lamina region

Magnified view of lamina and midrib region

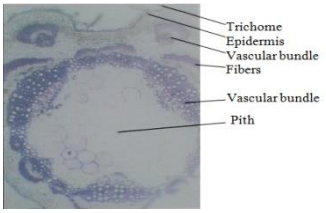


Magnified view of midrib region showing patch of sclerenchyma cells

Magnified view of midrib region showing vascular bundle

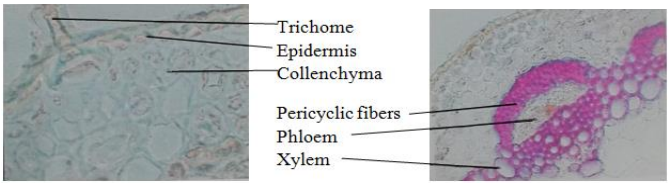
Fig No.1.Transverse section of Leaf of *Uraria picta*

3.2. Stem: T.S. of stem is circular in outline showing single layer of epidermis with covering trichomes. Collenchyma underlies epidermis, which is 3-5 layered. Epidermis and Collenchyma is followed by parenchymatous cortex. Within the cortex, scelrenchymatous patches are seen arranged in a ring followed by vascular bundles. Xylem vessels with spiral and scalar from thickening are seen. Pith is composed of parenchymatous cells.



Magnified view of epidermis showing trichome

Magnified view of cortex showing scelrenchymatous patches and vascular bundles



Magnified view of vascular bundle region

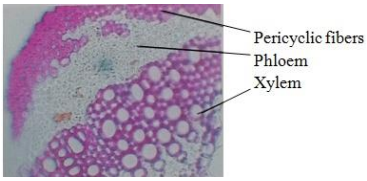
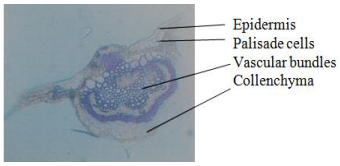


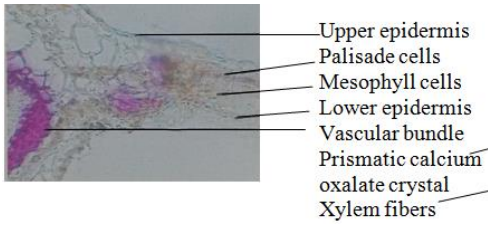
Fig No.2. Transverse section of stem of *Uraria picta*

4. Microscopy of *Uraria lagopoides*

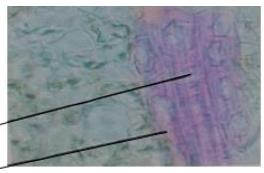
4.1. Leaf: Leaf is dorsiventral. Epidermal cells are covered with thin cuticle. It consists of upper and lower epidermis. Upper epidermis shows palisade cells, which lie underneath. Mesophyll consists of 2-3 layers of spongy parenchyma with intercellular spaces and some cells of which contains prismatic calcium oxalate crystals. The midrib region shows collenchyma below epidermis on both surfaces. Midrib region shows vascular bundles. The phloem is present on dorsal side and xylem and ventral side.



Magnified view of lamina region



Magnified view of surface region showing prismatic calcium oxalate crystals



Magnified view of midrib region showing vascular bundles

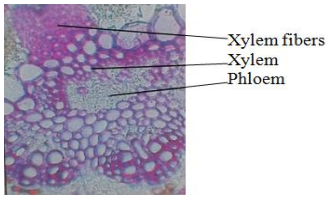
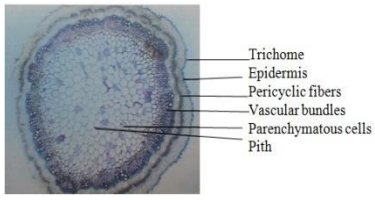


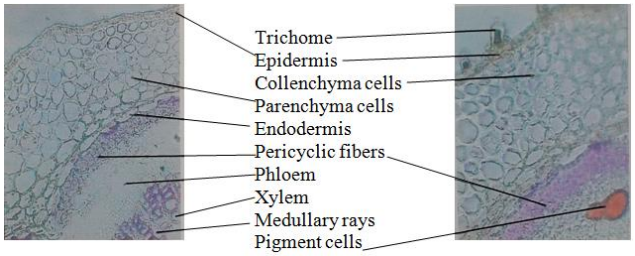
Fig No.3.Transverse section of Leaf of *Uraria lagopoides*

4.2. Stem: Transverse section shows thin striated cuticle. Epidermis bears trichomes, which are glandular in shape. Epidermis is composed of thin-walled, polygonal cells. Collenchyma underlies epidermis below the ridges. The cortex consists of loosely arranged thin walled parenchymatous cells and pigment cells. Next is a single layer of thin walled elongated cells of endodermis. Phloem consists of pericyclic fibers, which occur in groups and phloem parenchyma. Xylem consists of vessels with reticulate and pitted thickening. Medullary rays are 2-3 celled wide. Pith is composed of loosed arranged parenchyma cells.

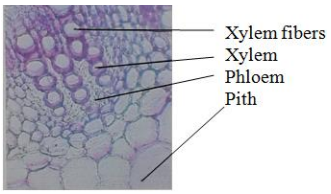


Magnified view of epidermis and cortex region

Magnified view of epidermis showing trichome and cortex showing pigment cells



Magnified view of vascular bundle region



Magnified view of pith

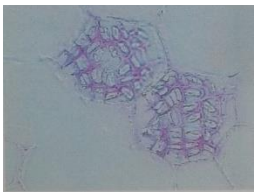
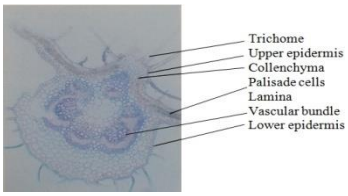


Fig No.4.Transverse section of stem of *Uraria lagopoides*

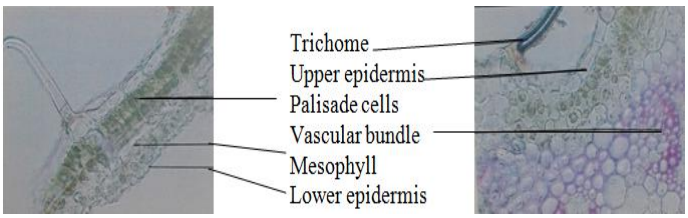
5. Microscopy of *Pseudarthria viscida*

5.1. Leaf: T.S. of leaf of *Pseudarthria viscida* shows single layered thin walled epidermal cells showing numerous covering trichomes. Epidermal cell with thin cuticle is present. Below the upper epidermis, 2 layers of elongated palisade cells are present. Mesophyll consists of spongy parenchymatous cells. The midrib region shows collenchymatous cells below the upper and lower epidermis 6 vascular bundles are present in the midrib region of which 3 are large and remaining 3 are small. The pericyclic fibers surround the vascular bundles forming a discontinuous ring. The phloem parenchyma surround the xylem cortical parenchymatous cells occupy the space between collenchyma of vascular bundles.



Magnified view of lamina showing trichome

Magnified view of midrib region showing trichome



Magnified view of midrib region showing vascular bundle

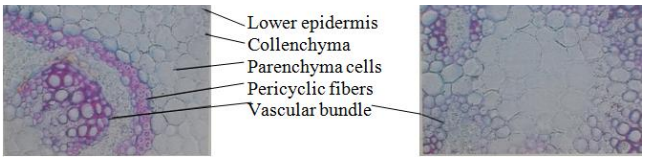
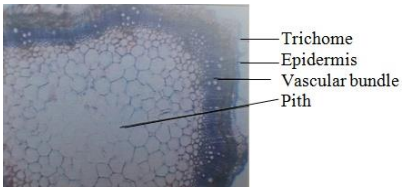
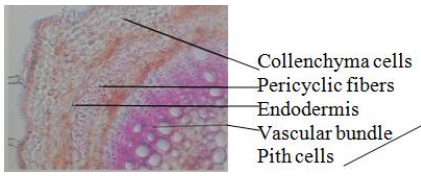


Fig No.5. Transverse section of Leaf of *Pseudarthria viscida*

5.2. Stem: T.S. of stem of *Pseudarthria viscida* shows single layered epidermis having covering trichomes. Few layers of collenchyma cells are present below the epidermis. Single layer of endodermis with elongated cells is seen. The phloem consists of pericyclic fibers and phloem parenchyma. Xylem consists of vessels and fibers. Pith is composed of large rounded to polygonal parenchyma cells.



Magnified view of epidermis and cortex



Magnified view of pith

Magnified view of epidermis showing trichome

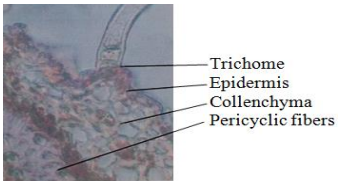


Fig No.6.Transverse section of stem of *Pseudarthria viscida*

6. Proximate analysis

All the three plants were subjected to proximate analysis to evaluate their foreign organic content, moisture content, total ash, water soluble ash, acid insoluble ash, alcohol soluble extractive value and water-soluble extractive value. The physicochemical values were found to be within the prescribed limits of API.

Table No.2: Proximate analysis of *Uraria picta, Uraria lagopoides and Pseudarthria viscida* crude drug

Standards(%w/w)	*Uraria picta*	*Uraria lagopoides*	*Pseudarthria viscida*	Limits
Foreign Organic matter	Nil	Nil	Nil	Not more than 2%
Moisture content	7.8 ± 0.1	6.0 ± 0.2	8.83 ± 0.26	Not more than 9%
Total ash	6.9 ± 0.5	8.2 ± 0.7	6.03 ± 0.3	Not more than 20%
Water soluble ash	3.87 ± 0.1	3.15 ± 0.2	3.20 ± 0.2	Not more than 12%
Acid insoluble ash	2.3 ± 0.3	1.10 ± 0.1	1.06 ± 0.2	Not more than 5%
Alcohol soluble extractive value	1.87 ± 0.04	2.30 ± 0.05	2.56 ± 0.05	Not more than 3%
Water-soluble extractive value	10.24 ± 0.15	8.89 ± 0.45	9.81 ± 0.57	Not more than 12%

7. Extraction

Extractions are a way to separate a desired substance when it is mixed with others. The mixture is brought into contact with a solvent in which the substance of interest is soluble, but the other substances present are insoluble. Extraction is done by Soxhlet's apparatus and cold maceration method. Soxhlet's extraction is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material. Soxhlet's extraction is done by using petroleum ether, chloroform, ethanol, Hydroalcoholic (Ethanol: water 7:3) and Aqueous. Maceration is an extractive technique that is conducted at room temperature. It consists of immersing a plant in a petroleum ether, chloroform, ethanol, Hydroalcoholic (Ethanol: water 7:3) and Aqueous in a stoppered container for 72 hrs period with frequent shaking on Rotary shaker. Physical characteristics and % yield of *Uraria picta, Uraria lagopoides and Pseudarthria viscida* extract.

Table No. 3.Physical characteristics and % yield of plant extract

Solvent	Colour of extract	Odour	Consistency	Sense of touch	% yield of *U.P.*	% yield of *U.L.*	% yield of *P.V.*
Petroleum ether	Dark green	Characteristic	Semisolid	Sticky	12.1	11.1	11.5
Chloroform	Yellowish brown	Characteristic	Semisolid	Sticky	4.7	4.2	5.7
Ethanol	Dark green	Characteristic	Semisolid	Sticky	19.2	18.6	22.9
Hydroalcoholic	Dark brown	Characteristic	Semisolid	Sticky	11.2	9.2	11.4
Aqueous	Dark green	Characteristic	Solid	Dry	31.7	25.7	27.1

8. Qualitative evaluation of phytoconstituents

8.1. Qualitative phytochemical evaluation of the extract of *Uraria picta*

Table No.4.Qualitative phytochemical evaluation of the extract of *Uraria picta*

Chemical Constituents	Tests	Pet. ether	Chloroform	Ethanol	Hydroalcoholic	Aqueous
Alkaloids	Mayers	-	+	+	-	-
	Dragendroff	-	+	+	-	-
	Wagners	-	+	+	-	-
	Hagers	-	+	+	-	-
Carbohydrates	Molisch	+	-	-	+	+
	Benedict	+	-	-	+	+
	Fehling	-	-	-	+	+
Saponins	Foam test	-	-	+	-	+
Phenolic acids and Tannins	Ferric chloride	-	-	+	+	+
	Gelatin	-	-	+	+	+
	Lead acetate	-	-	+	+	+
Proteins and amino acids	Xanthoproteic	-	-	-	+	+
	Ninhydrin	-	-	-	-	-
	Biuret	-	-	-	+	+
Flavonoids	Alkaline reagent	-	-	+	+	+
	Lead acetate	-	-	+	+	+
	Shinoda	-	-	+	+	+

8.2. Qualitative phytochemical evaluation of the extract of *Uraria lagopoides*

Table No.5. Qualitative phytochemical evaluation of the extract of *Uraria lagopoides*

Chemical Constituents	Tests	Pet.ether	Chloroform	Ethanol	Hydroalcoholic	Aqueous
Alkaloids	Mayers	-	+	+	-	+
	Dragendroff	-	+	+	-	+
	Wagners	-	+	+	-	+
	Hagers	-	+	+	-	+
Carbohydrates	Molisch	+	-	-	+	+
	Benedict	+	-	-	+	+
	Fehling	-	-	-	+	+
Saponins	Foam test	-	-	+	-	+
Phenolic acids and Tannins	Ferric chloride	-	-	+	-	+
	Gelatin	-	-	+	-	+
	Lead acetate	-	-	+	-	+
Proteins and amino acids	Xanthoproteic	-	-	-	+	+
	Ninhydrin	-	-	-	+	+
	Biuret	-	-	-	+	+
Flavonoids	Alkaline reagent	-	-	+	-	+
	Lead acetate	-	-	+	-	+
	Shinoda	-	-	+	-	+

8.3. Qualitative phytochemical evaluation of the extract of *Pseudarthria viscida*

Table No.6.Qualitative phytochemical evaluation of the extract of *Pseudarthria viscida*

Chemical Constituents	Tests	Pet. ether	Chloroform	Ethanol	Hydroalcoholic	Aqueous
Alkaloids	Mayers	+	-	-	-	+
	Dragendroff	+	-	-	-	+
	Wagners	+	-	-	-	+
	Hagers	+	-	-	-	+
Carbohydrates	Molisch	+	-	-	-	+
	Benedict	+	+	-	-	+
	Fehling	+	-	-	-	+
Saponins	Foam test	-	-	+	-	+
Phenolic acids and	Ferric chloride	-	-	+	-	+

Tannins	Gelatin	-	-	+	-	+
	Lead acetate	-	-	+	-	+
Proteins and amino acids	Xanthoproteic	+	-	+	-	+
	Ninhydrin	+	-	+	-	+
	Biuret	+	-	+	-	+
Flavonoids	Alkaline reagent	-	-	+	+	+
	Lead acetate	-	-	+	+	+
	Shinoda	-	-	+	+	+

9. Quantitative Estimation of Phytoconstituents

The extracts of the plant material were subjected to quantitative phytochemical evaluation in order to find out the total alkaloids, flavonoids, phenol, tannin, carbohydrate and total protein content present.

9.1. Total Alkaloid Content

Table No.7.Standard curve of Atropine

Sr. No.	Conc. of Std mg/ml	Abs at 470 nm
1	0.2	0.101
2	0.4	0.198
3	0.6	0.269
4	0.8	0.339
5	1	0.390

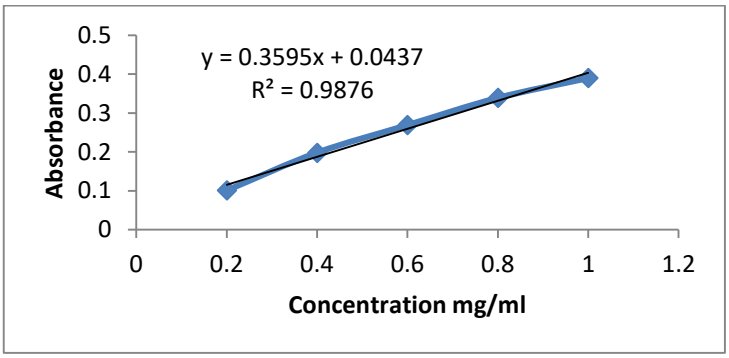


Fig No.7.Standard curve of Alkaloid

Table No. 8.Total Alkaloid content in extract

Sr. No.	Different Extract	Absorbance	µg/ml
1	Chloroform Extract of *Uraria picta*	0.268	0.626
2	Ethanolic Extract of *Uraria picta*	0.295	0.701
3	Chloroform Extract of *Uraria lagopoides*	0.275	0.646
4	Ethanolic Extract of *Uraria lagopoides*	0.309	0.740
5	Aqueous Extract of *Uraria lagopoides*	0.291	0.690
6	Pet ether Extract of *Pseudarthria viscida*	0.298	0.710
7	Aqueous Extract of *Pseudarthria viscida*	0.313	0.752

9.2. Total Flavonoid Content

Table No. 9.Standard curve of Quercetin

Sr. No.	Conc. Of Std mg/ml	Abs at 415 nm
1	0.1	0.110
2	0.2	0.182
3	0.4	0.363
4	0.6	0.511
5	0.8	0.661
6	1	0.780

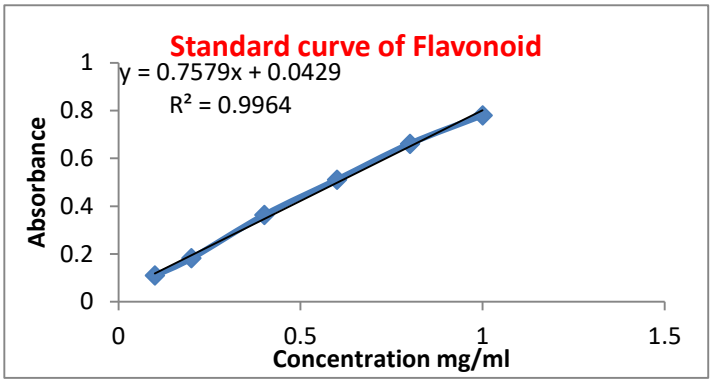


Fig No.8.Standard curve Quercetin

Table No.10.Total Flavonoid content in extract

Sr. No.	Different Extract	Absorbance	µg/ml
1	Ethanolic extract of *Uraria picta*	0.568	0.694
2	Hydroalcoholic Extract of *Uraria picta*	0.695	0.862
3	Aqueous Extract of *Uraria picta*	0.559	0.682
4	Ethanolic Extract of *Uraria lagopoides*	0.612	0.752
5	Aqueous Extract of *Uraria lagopoides*	0.590	0.723
6	Ethanolic Extract of *Pseudarthria viscida*	0.578	0.708
7	Hydroalcoholic Extract of *Pseudarthria viscida*	0.618	0.842
8	Aqueous Extract of *Pseudarthria viscida*	0.549	0.669

9.3. Total Phenol Content

Table No.11.Standard curve of Gallic acid

Sr. No.	Conc. Of Std mg/ml	Abs at 725 nm
1	0.1	0.118
2	0.2	0.209
3	0.4	0.359
4	0.6	0.566
5	0.8	0.755
6	1	0.918

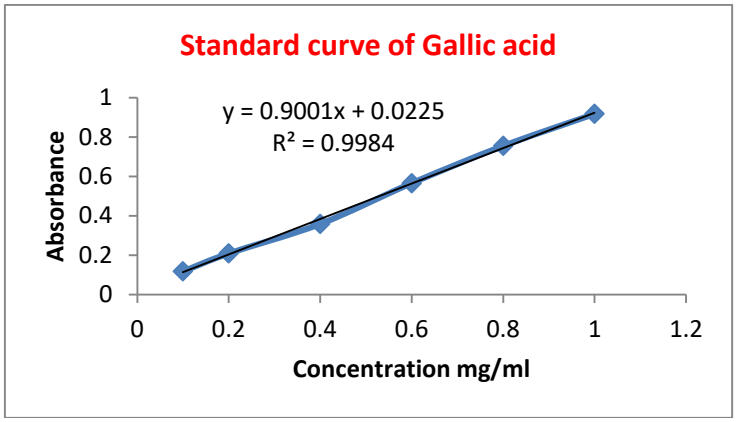


Fig No.9.Standard curve of Gallic acid

Table No12.Total Phenol content in extract

Sr. No.	Different Extract	Absorbance	µg/ml
1	Ethanolic extract of *Uraria picta*	0.564	0.602
2	Hydroalcoholic Extract of *Uraria picta*	0.559	0.596
3	Aqueous Extract of *Uraria picta*	0.617	0.661
4	Ethanolic Extract of *Uraria lagopoides*	0.578	0.617
5	Aqueous Extract of *Uraria lagopoides*	0.602	0.644
6	Ethanolic Extract of *Pseudarthria viscida*	0.615	0.658
7	Aqueous Extract of *Pseudarthria viscida*	0.549	0.585

9.4. Total Carbohydrate content

Table No13.Standard curve of Dextrose

Sr. No.	Conc. Of Std mg/ml	Abs at 490 nm
1	0.1	0.113
2	0.2	0.206
3	0.4	0.354
4	0.6	0.565
5	0.8	0.753
6	1	0.917

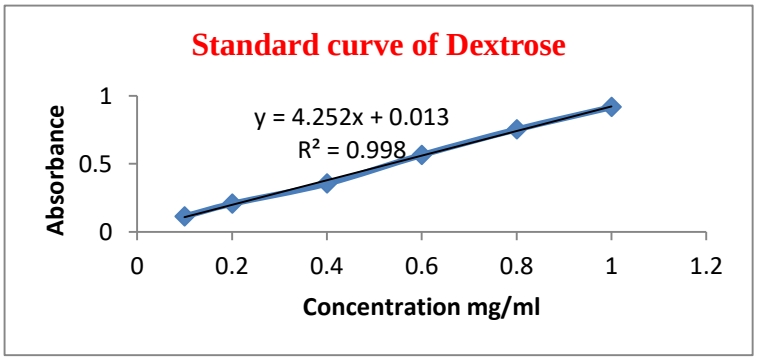


Fig No.10.Standard curve of Dextrose

Table No14.Total Carbohydrate content in extract

Sr. No.	Different Extract	Absorbance	µg/ml
1	Pet ether Extract of *Uraria picta*	0.201	0.444
2	Hydroalcoholic Extract of *Uraria picta*	0.267	0.059
3	Aqueous Extract of *Uraria picta*	0.265	0.059
4	Pet ether Extract of *Uraria lagopoides*	0.298	0.067
5	Hydroalcoholic Extract of *Uraria lagopoides*	0.211	0.046
6	Aqueous Extract of *Uraria lagopoides*	0.209	0.060
7	Pet ether Extract of *Pseudarthria viscida*	0.244	0.054
8	Chloroform Extract of *Pseudarthria viscida*	0.214	0.047
9	Aqueous Extract of *Pseudarthria viscida*	0.237	0.052

10. In-vitro Free Radical Scavenging Activity

The various extracts of *Uraria picta, Uraria lagopoides and Pseudarthria viscida* was investigated for the effects on the *In-vitro* generation of free radicals and antioxidant profile. The results of the study showed that the maximum extent inhibition of free radical generation and maximum amount of antioxidant capacity, identification of nature of the active principle and extracts of all three plants was analyzed using antioxidant profile against a battery of oxidant moieties that included radicals like DPPH, NO, and reducing power assay.

10.1DPPH free radical scavenging activity

Table No15.% Inhibition Concentration (50%) of Standard Ascorbic acid when treated with DPPH reagent for radical scavenging activity

Sr. No.	Conc.of Ascorbic acid (µg/ml)	% Inhibition	IC 50 Value (%)
1.	0	0	65 µg/ml
2.	10	58.13	
3.	20	60.55	
4.	40	64.01	
5.	60	67.82	
6.	80	76.81	
7.	100	77.16	

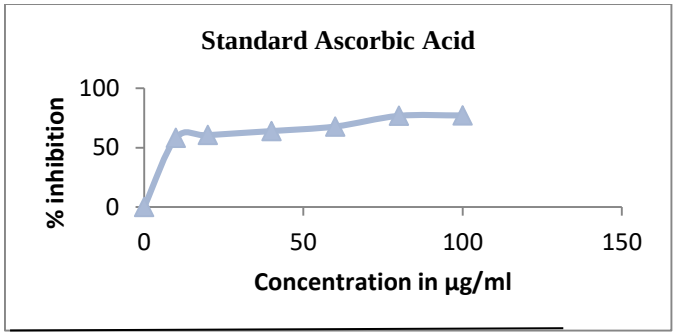


Fig No.11.% Inhibition of standard ascorbic acid

Table No16.% Inhibition Concentration (50%) of *Uraria picta* plant extract (Coded with UP-PE,UP-$CHCl_3$, UP-EtOH, UP-HA,UP-AQ)when treated with DPPH reagent for radical scavenging activity

Concentration µg/ml	UP-PE Extract	UP-$CHCl_3$ Extract	UP-EtOH Extract	UP-HA Extract	UP-AQ Extract
0	0	0	0	0	0
10	48.39	47.83	49.62	48.38	49.66
20	53.94	52.49	57.28	53.12	55.08
40	62.26	54.15	67.79	61.34	61.45
60	66.04	61.13	69.81	66.22	68.26
80	72.01	73.01	75.47	71.01	74.76
100	73.33	74.9	79.24	73.98	76.23
IC50 value	**62 µg/ml**	**63 µg/ml**	**68 µg/ml**	**61 µg/ml**	**69 µg/ml**

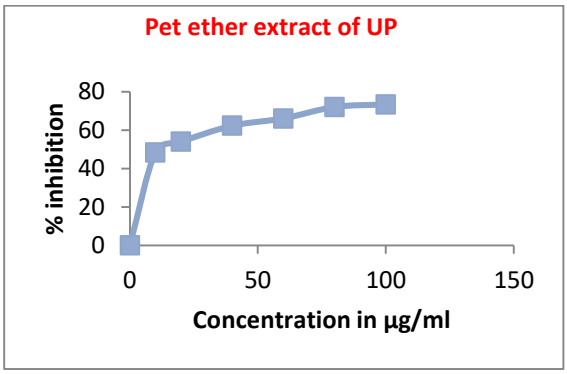


Fig No.12.Inhibition of Pet. Ether extract of UP

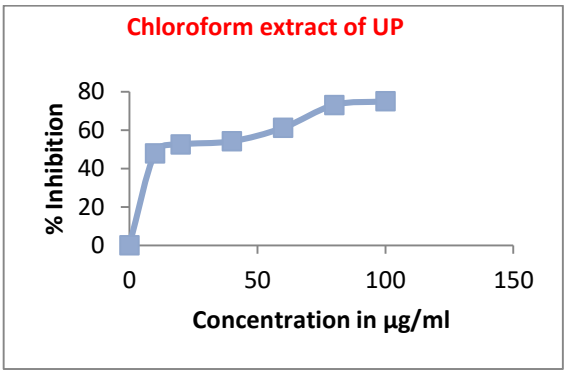


Fig No.13.Inhibition of Chloroform extract of UP

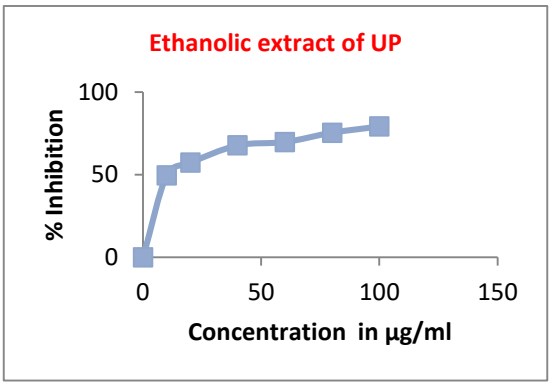


Fig No.14.Inhibition of Ethanolic extract of UP

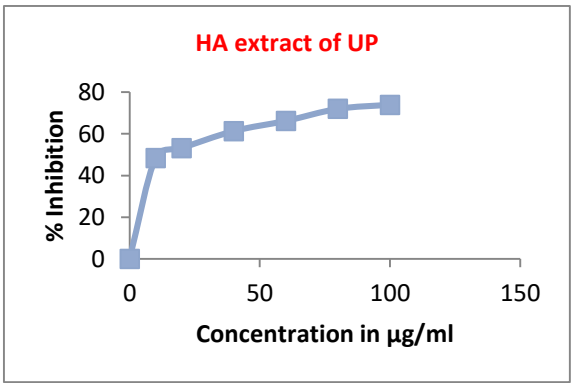


Fig No.15.Inhibition of Hydroalcoholic extract of UP

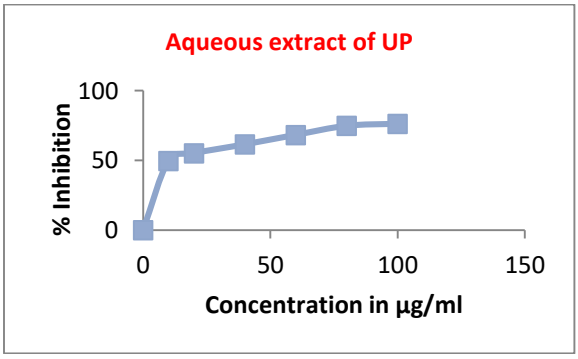


Fig No.16.Inhibition of Aqueous extract of UP

Table No17.% Inhibition Concentration (50%) of *Uraria lagopoides* plant extract (Coded with UL-PE,UL-$CHCl_3$, UL-EtOH, UL-HA,UL-AQ)when treated with DPPH reagent for radical scavenging activity

Concentration µg/ml	UL-PE Extract	UL-$CHCl_3$ Extract	UL-EtOH Extract	UL-HA Extract	UL-AQ Extract
0	0	0	0	0	0
10	48.8	49.5	49.81	50.78	48.10
20	52.2	61.3	60.98	56.45	59.89
40	58.89	64.44	63.45	60.94	62.25
60	64.21	73.67	71.63	62.10	64.04
80	75.58	77.24	76.30	73.64	75.43
100	78.66	78.87	78.52	74.98	76.09
IC50 value	**64 µg/ml**	**56µg/ml**	**65 µg/ml**	**60 µg/ml**	**63µg/ml**

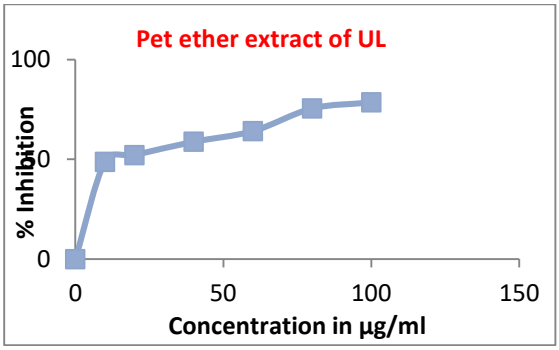


Fig No.17.Inhibition of Pet. ether extract of UL

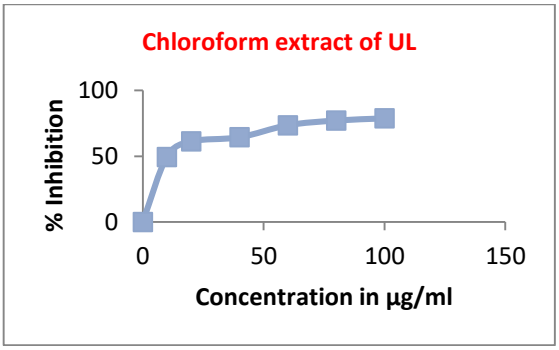


Fig No.18.Inhibition of Chloroform extract of UL

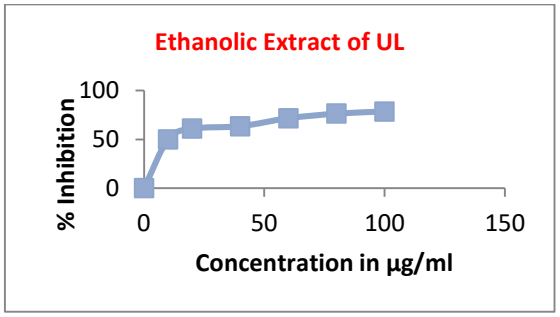


Fig No.19.Inhibition of Ethanolic extract of UL

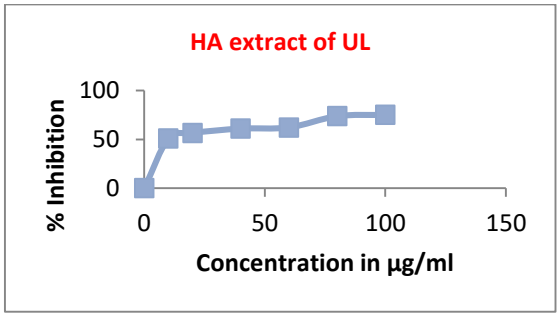


Fig No.20. Inhibition of Hydroalcoholic extract of UL

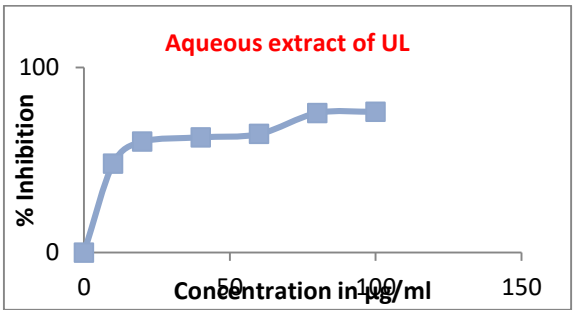


Fig No.21.Inhibition of Aqueous extract of UL

Table No18.% Inhibition Concentration (50%) of *Pseudarthria viscida* plant extract (Coded with PV-PE, PV-$CHCl_3$, PV-EtOH, PV-HA,PV-AQ) when treated with DPPH reagent for radical scavenging activity

Concentration µg/ml	PV-PE Extract	PV-$CHCl_3$ Extract	PV-EtOH Extract	PV-HA Extract	PV-AQ Extract
0	0	0	0	0	0
10	50.85	51.34	47.67	49.88	48.53
20	59.15	56.04	54.78	55.24	52.76
40	63.53	62.51	58.34	59.66	53.46
60	72.26	70.53	62.56	63.70	55.97
80	77.60	72.67	67.08	66.33	57.38
100	78.23	78.23	68.04	71.35	58.06
IC50 value	**59 µg/ml**	**58 µg/ml**	**64 µg/ml**	**70 µg/ml**	**74 µg/ml**

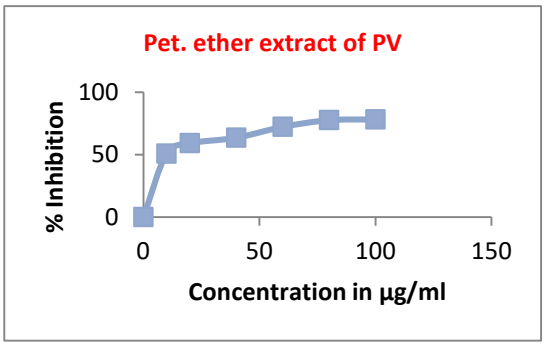


Fig No.22.Inhibition of Pet. ether extract of PV

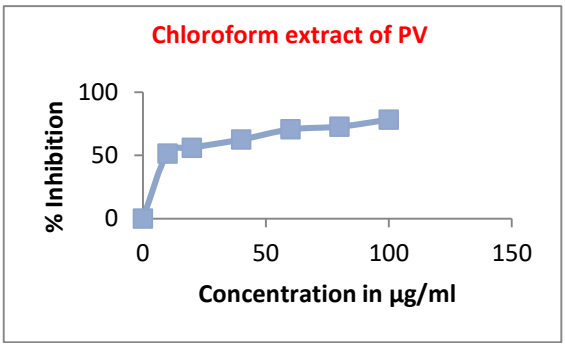


Fig No.23. Inhibition of Chloroform extract of PV

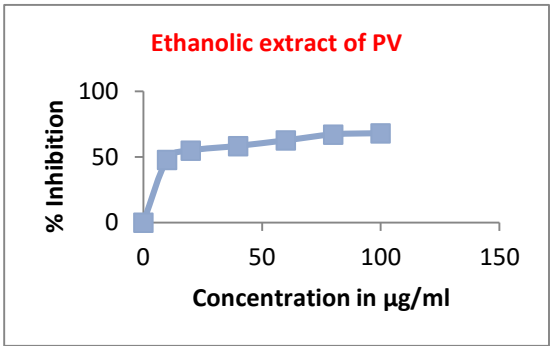


Fig No.24. Inhibition of Ethanolic extract of PV

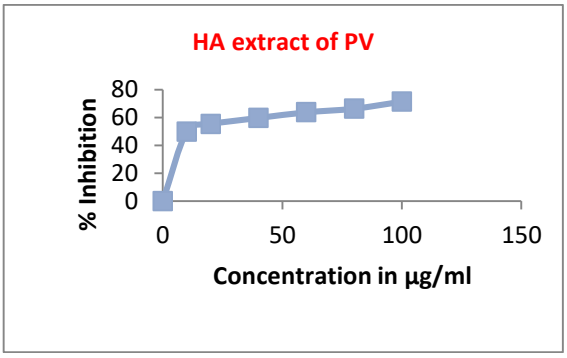


Fig No.25.Inhibition of Hydroalcoholic extract of PV

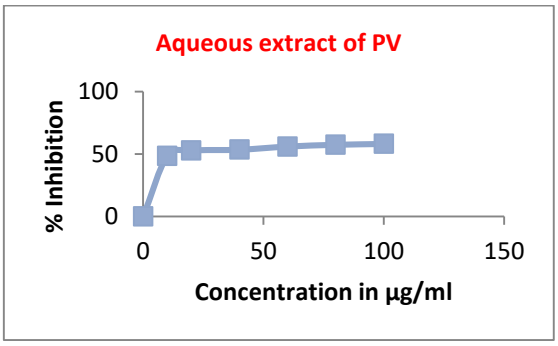


Fig No.26.Inhibition of Aqueous extract of PV

10.2. Nitric Oxide scavenging activity

The extracts of *Uraria picta, Uraria lagopoides and Pseudarthria viscida* effectively reduced the generation of nitric oxide from sodium nitroprusside. IC_{50} value of *Uraria picta, Uraria lagopoides and Pseudarthria viscida* extracts showed nitric oxide scavenging activity as the standard Ascorbic acid was showed. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside, which reacts with oxygen to produce nitrite ions that can be measured by using Griess reagent. The absorbance of the chromophore was measured at 546 nm in the presence of the extract

Table No19.% Inhibition Concentration (50%) of *Uraria picta* plant extract (Coded with UP-PE,UP-$CHCl_3$, UP-EtOH, UP-HA,UP-AQ) when treated with Nitric Oxide reagent for radical scavenging activity

Concentration µg/ml	UP-PE Extract	UP-$CHCl_3$ Extract	UP-EtOH Extract	UP-HA Extract	UP-AQ Extract
0	0	0	0	0	0
10	32.39	41.83	37.52	36.97	42.12
20	38.06	52.48	45.18	48.40	58.67
40	48.16	65.55	55.59	58.32	66.43
60	52.84	71.43	61.01	68.21	78.57
80	58.51	73.31	76.47	70.12	79.80
100	60.12	80.17	80.34	74.22	88.34
IC50 value	**64 µg/ml**	**52 µg/ml**	**54 µg/ml**	**61 µg/ml**	**62 µg/ml**

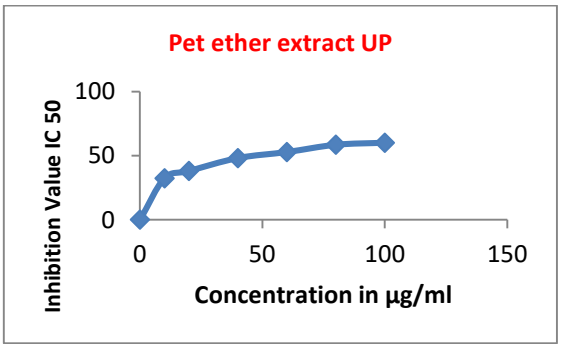


Fig No.27. IC50 values of Pet. ether extract of UP

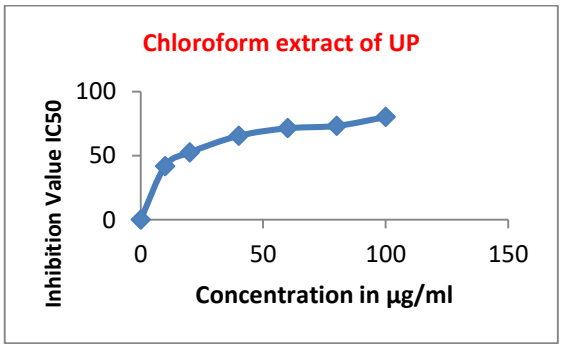


Fig No.28.IC50 values of chloroform extract of UP

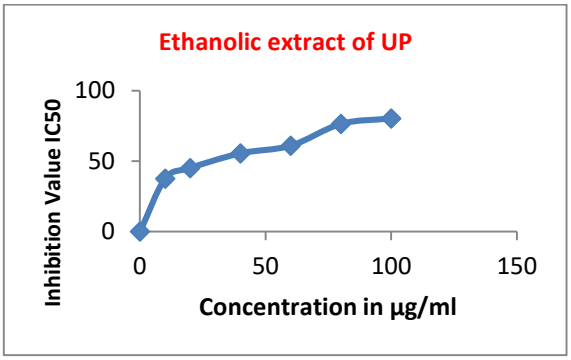


Fig No.29.IC50 values of Ethanolic extract of UP

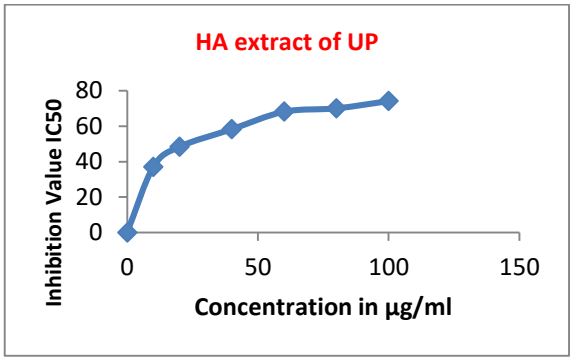


Fig No.30. IC50 values of Hydroalcoholic extract of UP

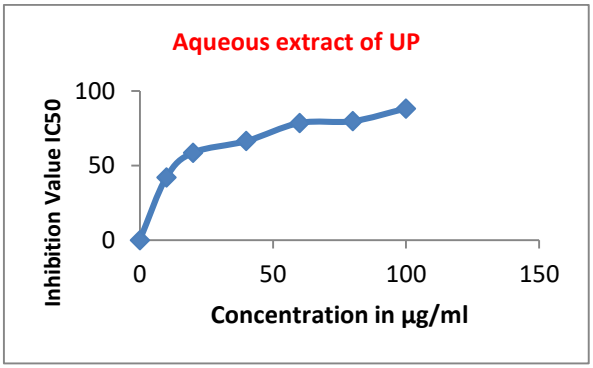


Fig No.31.IC50 values of Aqueous extract of UP

Table No 20.% Inhibition Concentration (50%) of *Uraria lagopoides* plant extract (Coded with UL-PE,UL-$CHCl_3$, UL-EtOH, UL-HA, UL-AQ)when treated with Nitric Oxide reagent for radical scavenging activity

Concentration µg/ml	UL-PE Extract	UL-$CHCl_3$ Extract	UL-EtOH Extract	UL-HA Extract	UL-AQ Extract
0	0	0	0	0	0
10	36.80	47.80	38.20	46.50	58.61
20	47.22	58.21	58.29	58.98	70.67
40	51.76	60.92	65.81	70.88	82.77
60	65.76	72.69	68.55	79.76	84.04
80	72.70	85.31	73.76	82.11	90.06
100	76.01	90.13	75.83	85.34	47.73
IC50 value	**60 µg/ml**	**57 µg/ml**	**56 µg/ml**	**74 µg/ml**	**79 µg/ml**

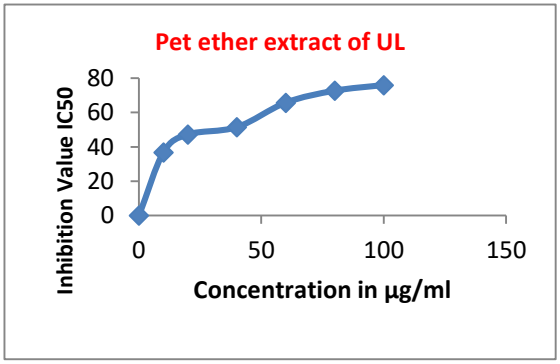


Fig No.32.IC50 values of Pet. ether extract of UL

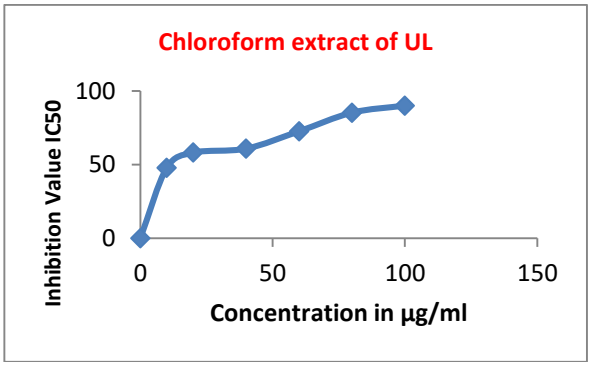


Fig No.33.IC50 values of Chloroform extract of UL

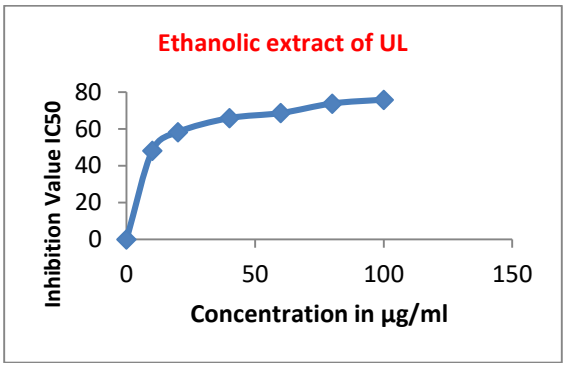


Fig No.34. IC50 values of Ethanolic extract of UL

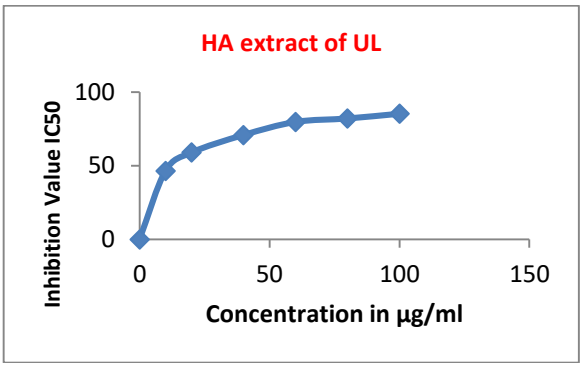


Fig No.35.IC50 values of Hydroalcoholic extract of UL

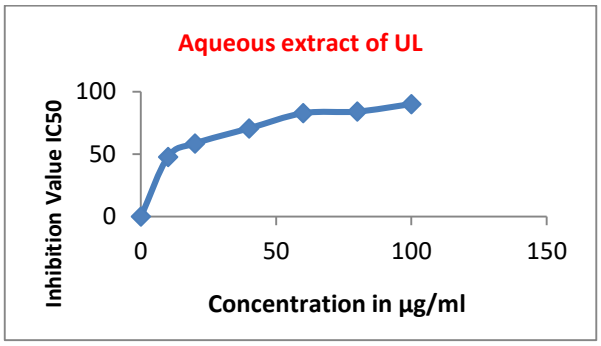


Fig No.36.IC50 values of Aqueous extract of UL

Table No21.% Inhibition Concentration (50%) of *Pseudarthria viscida* plant extract (Coded with PV-PE, PV-$CHCl_3$, PV-EtOH, PV-HA, PV-AQ) when treated with Nitric Oxide reagent for radical scavenging activity

Concentration µg/ml	PV-PE Extract	PV-$CHCl_3$ Extract	PV-EtOH Extract	PV-HA Extract	PV-AQ Extract
0	0	0	0	0	0
10	37.90	45.71	34.58	41.35	39.32
20	56.86	59.34	48.07	53.56	47.65
40	65.88	67.45	59.42	66.54	50.14
60	68.05	78.91	62.83	71.87	56.46
80	73.99	80.29	68.14	73.83	61.08
100	75.87	87.27	70.66	80.19	68.07
IC50 value	**51 µg/ml**	**78 µg/ml**	**60 µg/ml**	**59 µg/ml**	**64 µg/ml**

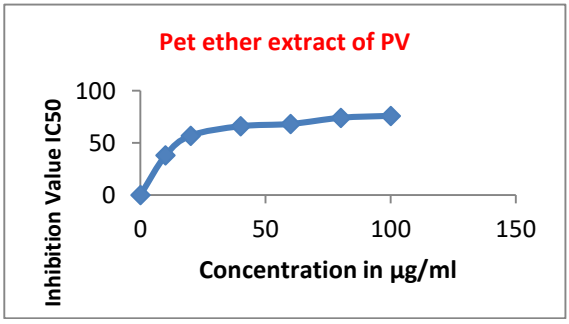


Fig No.37.IC50 values of Pet. ether extract of PV

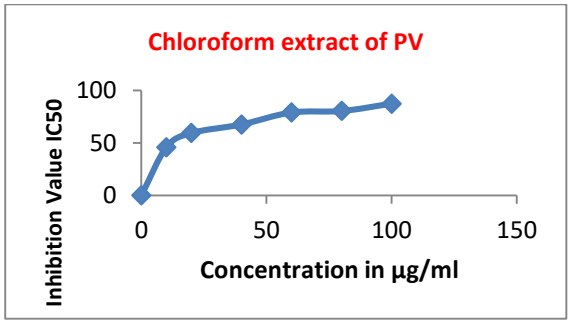


Fig No.38. IC50 values of Chloroform extract of PV

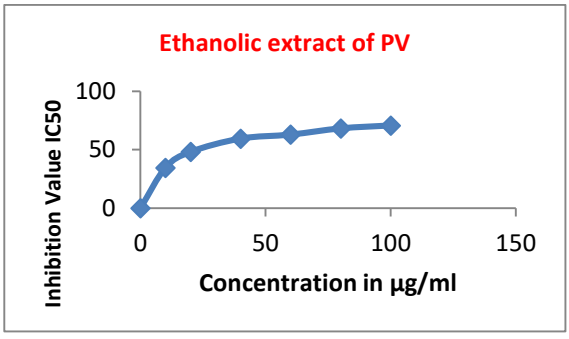


Fig No.39.IC50 values of Ethanolic extract of PV

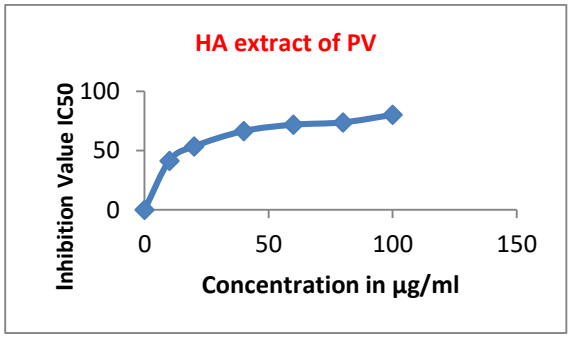


Fig No.40.IC50 values of Hydroalcoholic extract of PV

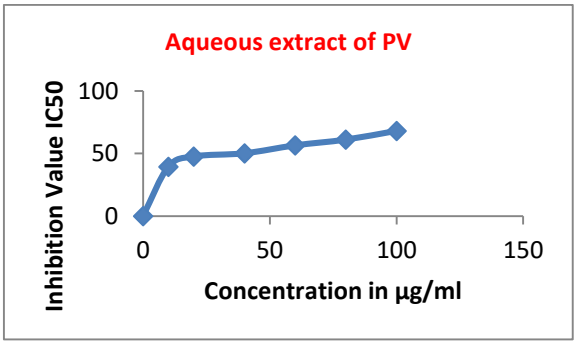


Fig No.41.IC50 values of Aqueous extract of PV

10.3.Reducing power

To find the active species which is capable of donating hydrogen and subsequently its leads to the reducing power activity was determined. The high reducing power is indicative of the hydrogen donating ability of the active species present in the extracts. For the measurement of the reducing ability, the different concentration of *Uraria picta*, *Uraria lagopoides* and *Pseudarthria viscida* as compared to the standard ascorbic acid. The reducing capacity serves as significant indicator of its potential antioxidant activity. TableNo.22. shows the reducing power by different extract.

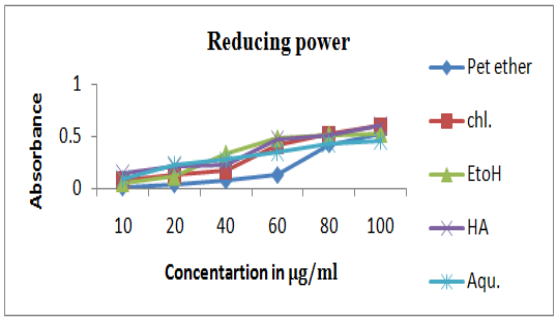


Fig No.42.Reducing power at different conc. of *Uraria picta*

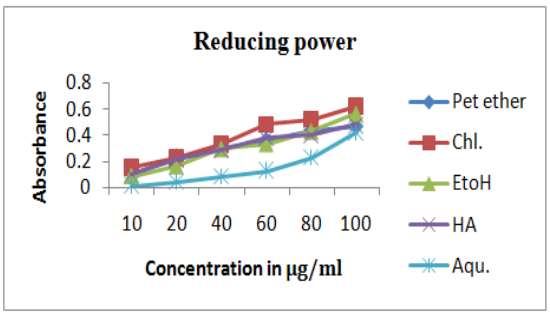


Fig No.43.Reducing power at different conc. of *Uraria lagopoides*

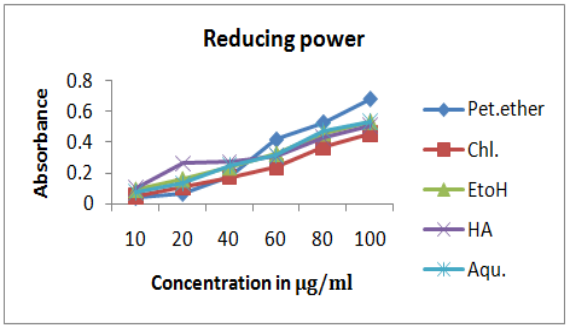


Fig No.44.Reducing power at different conc. of *Pseudarthria viscida*

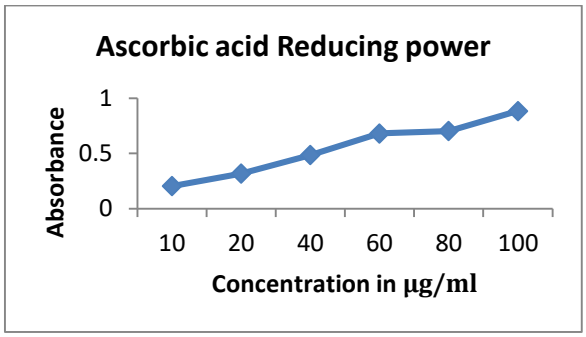


Fig No.45. Reducing power at different conc. of Ascorbic acid

Table No 22.Reducing power of different extracts of *Uraria picta, Uraria lagopoides* and *Pseudarthria viscida*

Sr. No.	Extracts	Concentration (µg/ml)					
		10	20	40	60	80	100
1	*Uraria picta* Petroleum ether extract	0.018	0.048	0.085	0.137	0.427	0.528
2	*Uraria picta* Chloroform extract	0.076	0.124	0.170	0.413	0.522	0.603
3	*Uraria picta* Ethanol extract	0.058	0.118	0.337	0.481	0.517	0.524
4	*Uraria picta* Hydroalcoholic extract	0.144	0.217	0.232	0.475	0.520	0.612

5	*Uraria picta* Aqueous extract	0.094	0.234	0.282	0.357	0.437	0.465
6	*Uraria lagopoides* Petroleum ether extract	0.108	0.234	0.297	0.359	0.434	0.465
7	*Uraria lagopoides* Chloroform extract	0.153	0.228	0.332	0.483	0.523	0.624
8	*Uraria lagopoides* Ethanol extract	0.087	0.164	0.297	0.335	0.431	0.567
9	*Uraria lagopoides* Hydroalcoholic extract	0.103	0.216	0.287	0.381	0.404	0.484
10	*Uraria lagopoides* Aqueous extract	0.015	0.042	0.083	0.128	0.230	0.424
11	*Pseudarthria viscida* Petroleum ether extract	0.045	0.072	0.184	0.423	0.534	0.687
12	*Pseudarthria viscida* Chloroform extract	0.054	0.116	0.178	0.245	0.367	0.456
13	*Pseudarthria viscida* Ethanol extract	0.098	0.165	0.243	0.330	0.463	0.538
14	*Pseudarthria viscida* Hydroalcoholic extract	0.104	0.265	0.274	0.314	0.434	0.512
15	*Pseudarthria viscida* Aqueous extract	0.076	0.134	0.252	0.316	0.479	0.538
16	Ascorbic acid	0.204	0.318	0.485	0.681	0.703	0.884

11.Conclusion

This study authenticated *Uraria picta*, *Uraria lagopoides*, and *Pseudarthria viscida* through detailed morphological and microscopical (T.S. of leaf and stem) evaluations, while identifying minor deviations from the Ayurvedic Pharmacopoeia standards in their physicochemical profiles. *U. picta* exhibited the highest water-soluble extractive value, indicating rich polar active constituents, whereas *P. viscida* yielded the highest alcohol-soluble extractive value. Preliminary testing confirmed the presence of key phytochemicals—including alkaloids, flavonoids, phenolic, and tannins—across all species. Furthermore, in-vitro assays demonstrated strong DPPH free-radical scavenging activity, with the aqueous extracts of *U. picta* (IC_{50}: 69 µg/ml) and *P. viscida* (IC_{50}: 74 µg/ml), alongside the ethanolic extract of *U. lagopoides* (IC_{50}: 65 µg/ml), proving to be the most potent antioxidants.

12.References

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