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Original Article

Pharmacognostic standardization and antibacterial potential of aerial herbs of *Portulaca grandiflora* Hooker (Portulaceae)

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ABSTRACT

To establish the standardization parameters for complete pharmacognostic evaluation of aerial parts of *Portulaca grandiflora* (*P. grandiflora*) Hook (Portulaceae) an important herb in Indian medicinal system. Present study was carried out by performing some parameters such as morphological, microscopical, physicochemical evaluation, florescence analysis; preliminary phytochemical analysis, thin layer chromatographic study and antimicrobial potential of alcoholic extract of *P. grandiflora* were carried out. Macroscopically the leaves are fleshy leaves, watery, needle shape. Flowers are racemes form; fruits are ovoid with small black colored seed. Chemomicroscopy revealed the presence of Rubiaceous stomata in leaf; Rosette calcium oxalate crystals and protoplast in mesophyll of leaf, cortex and pith of stem and root; pink colored cuticle of stem, collateral vascular bundle with lignified xylem, abundant of starch grains and mucilaginous cells in all aerial parts. Physicochemical evaluation used to determined numerical standards showed a results with total ash (14.5±0.05%), acid insoluble ash (5.24±0.01%), water soluble ash (3.28±0.02%), sulphated ash (10.2±0.05%), ethanol soluble extractive (18.6±0.02%), water soluble extractive (2.7±0.03%), moisture content (24.0±0.02%), swelling index (4.60 ml) and total crude fiber content(48.36±0.05%) in powder of stem. Preliminary phytochemical analysis revealed the presence of alkaloid, steroids, Triterpenoids, flavonoid, tannins, and carbohydrates. The total flavonoid content in alcoholic extract was found to be 0.094 mg/mg. The alcoholic extract of herbs showed significant inhibition of microorganisms. The results of the study can serve as a valuable source of information and provide suitable standards for the identification of this plant material in future investigation and application.

***Keywords*:** *Portulaca grandiflora*, Pharmacognostic study, physicochemical analysis, Preliminary phytochemical evaluation, Antibacterial activity.

INTRODUCTION

Nature is a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Plant-derived substances have recently become a great interest owing to their versatile applications. Medicinal plants are the richest bioresource of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. Medicinal plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals [2] Plant products still remain the principal source of pharmaceutical agents used in traditional medicine [3.]According to the WHO the first step for identification and purification of herbal drugs is the pharmacognostic (macroscopic and microscopic) studies which are essential for any phyto pharmaceutical products used for standard formulation [4].

Majority of crude herbs come from wild sources and it is collected to assess quality parameters by which presence of various phytochemical can be confirmed. Standardization of natural products is complex task due to their heterogeneous composition in form of whole plant. *Portulaca grandiflora* Hooker (Portulacaceae) commonly called as Moss rose in English, Nonia in Hindi, Pung mapan satpi in Manipuri and Gul-e-Shama in Urdu, is a succulent plant, profusely branched approximately 10-30cm high, leaves about 12-35 mm in length and 1- 4 mm in width, linear-

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subulate, thick, and fleshy and spirally arranged. Petiole is mostly short with axillary hairs. Its colorful flowers are 2-3cm across with conspicuous stamens and arranged in a terminal capitulum, surrounded by whorls of leaves. Two sepals are about 6mm long with a very small apical keel. Petals are broadly obovate, deeply notched and posses red, pink, or purple colour. Fruits are sub globose and 4-6mm in diameter. Seeds are very small and shining. Aerial parts are reported to contain various diterpenoids like portulal, portulenone, portulenol, portulene [5] and portulene acetal a minor diterpenoid [6]. The plant is used for the cure of sore throat and skin rashes. It is a putative immunostimulant [7]. It is also used for detoxification. It has also been reported for its efficacy on hepatitis B surface antigen [8].

In spite of the numerous medicinal uses attributed to this plant, there is no pharmacognostical report on the anatomical and other physico-chemical standards required for the quality control of the crude drug. Hence the present investigation includes morphological and anatomical evaluation, determination of physicochemical constants and preliminary phytochemical and antibacterial screening of the alcoholic extracts of *P. grandiflora*.

MATERIALS AND METHODS

Plant material: The plant material was obtained from Nasik district (M.S.) and authenticated by Dr. D. A. Patil, reader and the authorized plant identifier of Department of Botany, SSVPS College, North Maharashtra University, Dhule (M.S) India; a specimen is preserved in the college herbarium (KBHSS/PCG/2012/20).

Organoleptic evaluation: Various sensory parameters of the plant material (such as colour, odour, size, shape, and taste) were studied by organoleptic evaluation**.**

Macroscopic evaluation: Various macroscopic characters of fresh leaves and stem of *P. grandiflora* were recorded such as duration, type of leaf base, presence or absence of petiole and characters of lamina. Lamina consists of characteristic features such as composition, incision, shape, venation, margin, apex, base, surface and texture. The stem is morphologically studied for its size, shape, surface and configuration [9].

Microscopical characterization

Sectioning: Selected samples were stored in a solution containing formalin (5 ml), acetic acid (5 ml) and 70% v/v ethyl alcohol (90 ml). After 24 hours of fixing, the specimens were dehydrated with graded series of tertiary- Butyl alcohol as per the method [10]. Infiltration of the specimens was carried by gradual addition of paraffin wax until tertiary-Butyl alcohol solution attained supersaturation. The specimens were casted into paraffin blocks. The paraffin-embedded specimens were sectioned with the help of Senior Rotary Microtome, RMT-30 (Radical Instruments, India). The thickness of the sections was kept between 10 and 12 µm. The dewaxing of the sections was carried out as per the procedure described by Johanson. [11]. The sections were stained with phloroglucinol –hydrochloric acid (1:1), fast green, and safranin and mounted in glycerin. Powder (# 60) of the dried aerial parts was used for the observation of powder microscopical characters. The powdered drug was separately treated with glycerine, chloral hydrate and water to determine the presence of various tissues [12].

Photomicrograph: Microscopic descriptions of selected tissues were supplemented with micrographs. Photographs of different magnifications were taken with 3V microvision Sr. no. 116710 Microscopic unit. For normal observations, bright field was used. For the study of crystal, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property under polarized light they appear bright against dark background [13].

Fluorescence analysis: Fluorescence analysis of powder aerial part was done by standard procedure [14]. In this analysis the aerial part and its powder were treated with various acidic and basic solvents and were then observed in UV-visible chamber under visible, short wavelength and long wavelength regions simultaneously [15-16]. Fluorescence is an important phenomenon exhibited by various chemical constituents show fluorescence in the visible range in day light. The UV light produces fluorescence in many natural products (e.g. alkaloids like berberine) which do not visibly fluorescence in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation [17]. The changes in appearance and colour were observed and recorded.

Physico-chemical evaluations: Physicochemical parameters of powdered drug were determined and reported as total ash, water-soluble ash and acid-insoluble ash values, moisture content, swelling index and total crude fibres, alcohol and water-soluble extractive values were determined to find

out the amount of water and alcohol soluble components [18-19].

Preliminary phytochemical screening: Coarse powder of the drug (25 g) was subjected to soxhlet for solvent extraction. Extract was concentrated and subjected to various chemical tests to detect the presence of different phytoconstituents [20-21].

Thin layer chromatography of the extracts: The extracts obtained by solvent extraction were subjected to thin layer chromatography in different solvent system. Thin layer chromatographic plates were made with silica gel G and they were activated. The extracts was spotted by means of micropipette and dried, further developed in their respective solvent system for identification of constituents with various solvent system and spraying agent [22]. [Table 1]

Spectroscopy analysis of alcoholic extract of aerial parts for total flavonoid content: In this method, quercetin was used to make the calibration curve. 10 mg of quercetin was dissolved in methanol and then diluted to 6.25, 12.5, 25, 50, 80, and 100 µg/ml. A calibration curve was made by measuring the absorbance of the dilutions at 415 nm (λmax of quercetin) with a Shimadzu UV-1800 spectrophotometer. Aluminium chloride, 1% and potassium acetate, 1M solutions were prepared [23-24].

Stock Solution of Extracts: 100 mg of the each extract was accurately weighed and transferred to 10 ml volumetric flask and made up the volume with methanol.

Preparation of Test Solutions: 0.5ml of each extract stock solution, 1.5 ml methanol, 0.1 ml aluminum chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water were added and mixed well. Sample blank was prepared in similar way by replacing aluminum chloride with distilled water. Sample and sample blank of all four extracts were prepared and their absorbance was measured at 415 nm. All prepared solutions were filtered through whatmann filter paper before measuring

Antimicrobial activity: Microorganisms*: Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger* were selected for the study. The cultures were obtained from National Collection of Industrial Microorganism (NCIM) Pune, India. The cultures of these bacteria were grown in nutrient broth at 37 °C and maintained nutrient agar slants< 12 °C.

Reference standards: Standard drugs used for antimicrobial screening: Gentamicin (5mg/ml) for bacteria and Tioconazole (5 mg/ml) for fungi.

Antimicrobial screening: The samples used for this study was alcoholic extract of the aerial parts of *P. grandiflora* 0.25 g of sample was weighed and dissolved in 5 ml of the solvent used for extraction to give 50 mg/ml. Five (5) other test tubes contained 2.5 ml of the same solvent. From the first tube that contained 50 mg/ml, 2.5 ml of the content was drawn and added into the second test tube to give 25 mg/ml and this was done serially from the first test tube to the sixth until a concentration of 1.5625 mg/ml was achieved in the sixth test tube. The seventh test tube was used to prepare negative control, which contained the solvent of dissolution only. The eighth test tube served as the positive control and contained Gentamicin for bacteria and Tioconazole for fungi.

Agar diffusion: Pour plate for bacteria: The pour plate method was used for antibacterial screening. An overnight culture of each organism was prepared and 0.1 ml of each of the organism was taken into 9.9 ml of sterile distilled water (SDW) to give 10 ml of 1:100 (10^2) dilution. 0.2 ml was then taken into the prepared molten Nutrient Agar (NA) at 45^0C and this was aseptically poured into the sterile plates and allowed to set on the bench for 45 minutes. A sterile cork-borer was used to create wells (or holes) inside the set plates. Different prepared concentrations of the sample as well as the positive and negative controls were introduced using syringes into the wells. The positive control for bacteria was Gentamicin at 5 mg/ml. These were allowed to stay on the bench for two hours before incubation at 37^0C for 24 hours. Clear zones of inhibition were observed. The diameter of the zones of inhibition was measured in millimeter (mm) using a transparent well-calibrated ruler. Average readings were calculated from triplicates analysis.

Agar diffusion: surface plate for fungi: Molten sterile Sabouraud Dextrose Agar (SDA) was poured aseptically into the sterile plates and allowed to cool for 45 minutes. 0.2 ml of 1: 100 dilution of the organism was spread on the surface using a sterile spreader. Then, a sterile cork-borer was used to create wells inside the plates. The same procedure described for anti-bacterial activity above was followed from this stage. All the plates for the fungi were incubated at 28^0C for 48 hours unlike that of bacteria that was incubated at 37^0C for 24 hours. The clear zones of inhibition were observed and recorded using the same method as described in the case of bacteria [25].

RESULTS

Morphological study: Herbaceous plant that grows in the rainy season or up to the top backs, 15-30 cm long, often branched from the base, the color ruasnya smooth-haired, and red or green. Single leaf, where the scattered, not stemmed, at the end of the rod tightly full, to the base of the leaf is rarer. Leaves are thick fleshy, watery, needle shape, a round cylindrical, blunt tip, 1 to 3.5 cm long, green colour. Flowers gathered in groups of 2-8 at the end of the stalk, bloom at eight o'clock in the morning and wilt late in the afternoon, the colour of petals red, and dice, white, orange, or yellow. Fruits are ovoid shape and surface-haired. [figure 1]

Microscopy of P. grandiflora

Transverse section of leaf: Transverse section of the lamina shows the cuticle with deep irregular striations especially on the upper (adaxial) surface. The epidermal cells are tubular to pear shaped. Some epidermal cells contain rosettes of calcium oxalate and also other crystalline structures. Some flakes of whitish mucilage are detected at the epidermal layers. The paracytic stomata are well distributed in both epidermises but they are more on the upper one. The mesophyll encloses almost one layer of palisade tissues underlying the upper epidermal that consists of short and broad parenchyma cells with thin wavy cell walls. They contain rosettes of calcium oxalate and some of them also contain whitish mucilage. The spongy mesophyll consists of few sub-spherical cells and they are rich in calcium oxalate rosettes; some spongy cells that are adjacent to the vascular bundles are pear-shaped. The vascular strands are intricate and they contain narrow spirally and scalariformly thickened vessels. The accompanying fibres are short and unlignified [figure 2-5].

Transverse section of stem: The epidermal cell is rectangular to square with thick smooth and pink colored cuticle. Cell wall is moderately thickened. Periderm consists of many layers; its cells are rectangular to square. The cortex consists of *lamellar collenchyma* and polygonal parenchyma. Pericycle comprises of polygonal cells and small cells of parenchyma. Vascular system is bicollateral. The phloem cells are arranged in groups and present in form of strands. Xylem appears in form of continuous cylinder. Intra-xylary phloem is present in form of strand and at the periphery of the pith. The pith is non-lignified and composed of polygonal parenchyma. Rosettes of calcium oxalate are abundant in pith and parenchyma of the cortex region. [figure 6]

Transverse section of root: In transverse section of stem shows cortex composed of 9-10 layer of thin walled cellulosic parenchyma which was polygonal thin walled and non- lignified. Number of fibers, starch grains, protoplast and cluster of rosettes type calcium oxalate crystals were embedded in this region. In the stellar region vascular bundles were arranged radially associated with lignified pericyclic fiber, lignified xylem and phloem. The vascular bundles are around 13-15, collateral, conjoint, open and arranged in ring. Xylem is well developed consists of cylindrical lignified vessels with simple pits, tracheids and parenchyma. Xylem was differentiated centrifugally and the Protoxylem was endarch. Phloem was differentiated centripetally and protophloem was exarches containing sieve tubes and companion cells. The center or medulla occupied by pith consisting oval parenchymatous cells. Crystals, protoplast and starch grains were abundant in this region. Transverse section of stem after staining with fast green shows dark blue color of lignified pericyclic fiber, violet color of epidermis, green color of xylem and faint blue collenchymatous tissue. T.S. staining with safranin shows dark red color of cellulosic collenchyma, lignified xylem and pericyclic fiber. Starch grains shows dark blue color with dilute iodine solution [table 2, 3] [figure 7].

Fluorescence analysis: The fluorescence studies for the powder drug by treating it with different chemical reagents and the results were reported (Table 4). Fluorescence characteristic of powdered drug was observed in visible, short and long ultra-violet light for resolution of doubtful specimen [table 4].

Physico-chemical evaluation: Physiochemical parameters were evaluated & the result for each parameter is depicted in [table 5].

Phytochemical screening: Preliminary evaluation the ethanolic extract exhibit the presence of steroid, terpenoids, tannins, flavonoids, alkaloids and aqueous extract showed the presence of mucilage, flavonoid, and alkaloids. [Table 6]

Thin layer chromatography: The plate was developed in respective mobile phase upto 80% of height and sprayed with respective spraying reagent. Alcoholic extract showed light blue, violet and greenish yellow color spot for phytosterols, terpenoids and flavonoids respectively compared with available literature (figure 8-11).

Spectroscopical analysis of alcoholic extract of flavonoids content: To perform the calculations of total flavonoid content in the studied plant using Kiranmai et al., method, a standard curve is needed which is obtained from a series of different quercetin concentrations in [table 7]. Concentration

values of extracts were obtained from Quercetin standard curve, by interpolating to the X- axis in [graph 1]. TFC was calculated by using the following formula,

$$\textbf{TFC} = \frac{r \text{ X df X v X } 100}{W}$$

Where,
r - Result obtained from the standard curve
df - Dilution factor
v - Volume of stock Solution
100 - For 100 g dried plant
w - Weight of plant used in the experiment
The total flavonoid content in alcoholic extract was found to be 9.49 (µg of quercetin/mg of extract)

Antimicrobial screening: The zones of inhibition measured during the antimicrobial test by Agar well diffusion method are presented in [table 8]. It was observed that all the tested samples possessed broad spectrum antimicrobial activities on both gram positive and gram negative bacteria and the fungi used. Integer 1-6 represents alcoholic extract of *P. grandiflora* Linn. at various concentrations (mg/ml)viz: (1) 50 (2) 25, (3) 12.5, (4) 6.25, (5) 3.125, (6) 1.5625, positive control : [Gentamicin at 10 µg/ml for bacteria or Tioconazole (70%) for fungi], - = no inhibition
E.coli- Escherichia coli, B. sub- Bacillus subtilis, Ps.a- Pseudomonas aeruginosa, C.a – Candida albicanas, A.n– Aspergillus niger [graph 2].

DISCUSSION

Enthnomedicinally, the aerial parts of this herb were used by local peoples in the treatment of various disease conditions without standardization. The standardization of crude drug is an integral part for establishing correct identity. Before any crude drug can be included in an herbal pharmacopoeia, Pharmacognostic parameters and standards must be established. Microscopic methods are one of the simplest and cheapest methods to start with for establishing the correct identity of source materials [26].

The Pharmacognostic standards for aerial parts of *P. grandiflora* are carried out first time in this study. The macroscopical character of leaves and flowers can serve as diagnostic character. Microscopical studies indicated the presence of rosette calcium oxalate crystal and starch grains in cortex, pith and mesophyll of leaf, rubiaceous stomata in leaf. Bi co-lateral fibrovascular bundles are present in stem. The water storage cell (protoplast) in cortex and collenchymas is the characteristics of the plant. Ash value and extractive value can be used as reliable aid for detecting adulteration. These studies help in the identification of the plant materials [27].

Ash values of drug give an idea of earthy matter or the inorganic composition and other impurities present along with the drug. Extractive values primarily helpful for the determination of exhausted and adulterated drugs. Extractive value also useful to evaluate chemical constituents present in the crude drug and also estimation of specific chemical constituents soluble in particular solvent [28]. The percentage yield of alcohol soluble extractive of aerial parts of *P. grandiflora* is higher than water soluble extractive. The percentage of active chemical constituents in crude drug is mentioned on air dried basis. Hence, the moisture content of the crude drug should be determined and should also be controlled. The moisture content of drug should be minimized in order to prevent decomposition of crude drug either due to chemical change or microbial contamination. The fluorescent analysis of powder drug with various chemicals is very rapid methods to identify the doubtful specimens. All crude drugs are standardized for its active constituents. An extract is referring to concentrated, well dried preparation of active constituents of medicinal crude drug. The concept of standardized extract definably provides scientific validation of crude drug. Here, preliminary phytochemical analysis of extract confirmed the presence of sterols, carotenoids, polyphenolic acids, flavonoids, polysaccharides, reducing agents and triterpenoids. Additionally, the alcoholic extract of herb was examined to identify the total flavonoid content by aluminum chloride colorimetric method. The alcoholic extract showed varying degrees of inhibition against all the bacterial stains. It showed that MIC for *Pseudomonas aeruginosa* is found to be more followed by *Candida albicans* as compared with other tested microorganisms. In general, extract of the aerial herb was exhibited considerable antibacterial activity. This analysis suggests that aerial herb extract of *Portulaca grandiflora* probably contain active agents and provides the basis for their use as a cure for some human ailments. In conclusion, these parameters which are being reported for the first time could be useful in setting some diagnostic indices for the identification and preparation of a monograph of the *P. grandiflora* plant.

Conflict of interest statement: We declare that we have no conflict of interest.

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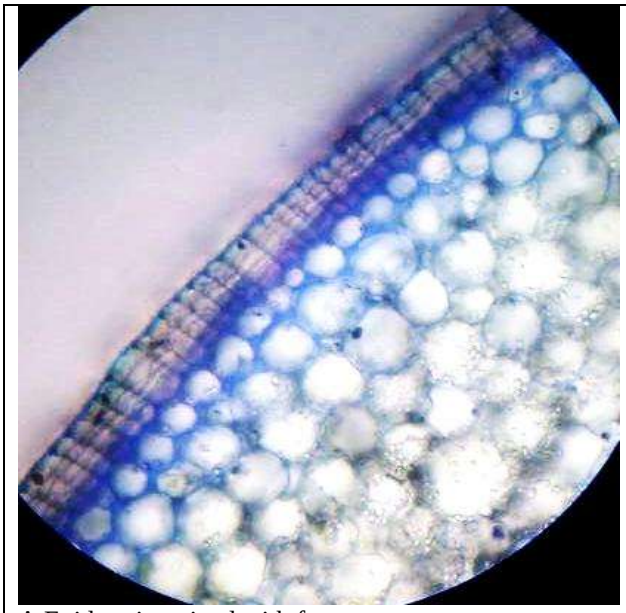
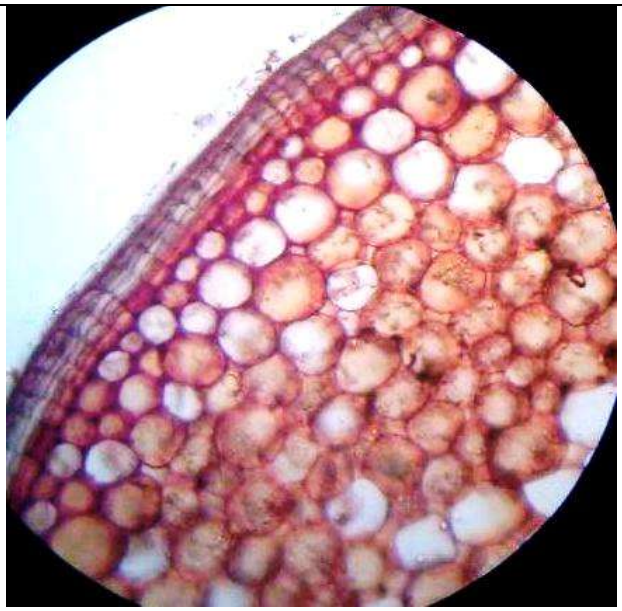
Table 1. Preliminary phytochemical investigation

Stationary phase	Phytoconstituents	Mobile phase	Visualization
Silica Gel G	Steroids	1.Toluene: Ethyl acetate (8:2)	5 % Ethanolic H_2SO_4
		2.Chloroform : Ethyl acetate (60:40)	Anisaldehyde- H_2SO_4 reagent
	Triterpenoids	1.Chloroform: Methanol (8:2)	Vanillin-H_2SO_4 reagent
		2. Toluene : Ethyl acetate (8:2)	
	Flavonoids	1.Chloroform : Ethyl acetate (60:40)	Under UV lamp 254nm
		2.Ethyl acetate : Methanol : Water (99:18:2)	

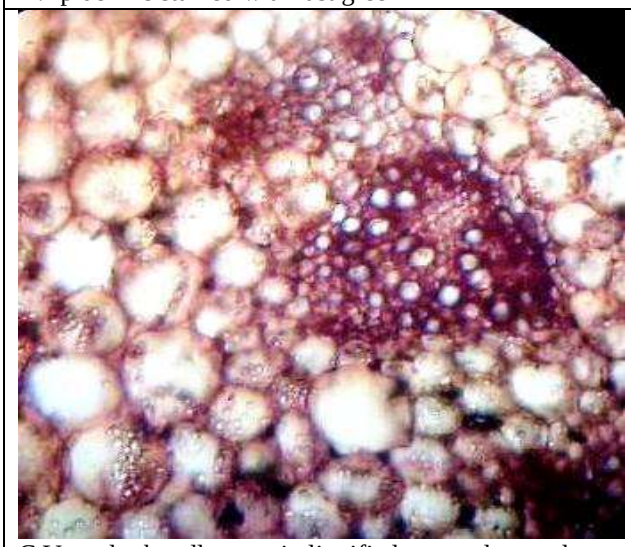
Table 2 Shows close view of *P. grandiflora* stem



A.Epidermis stained with fast green

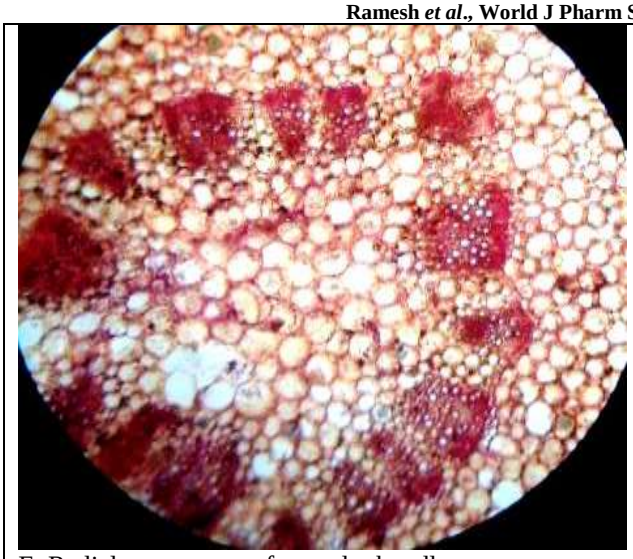


B. Epidermis stained with safranin

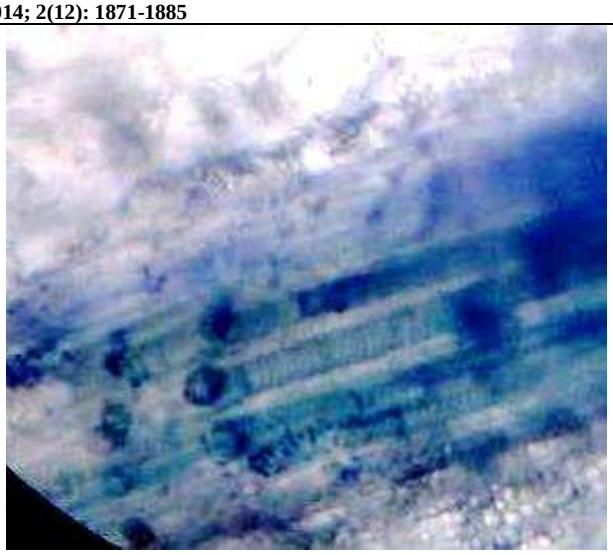


C.Vascular bundle contain lignified metaxylem and protoxylem.

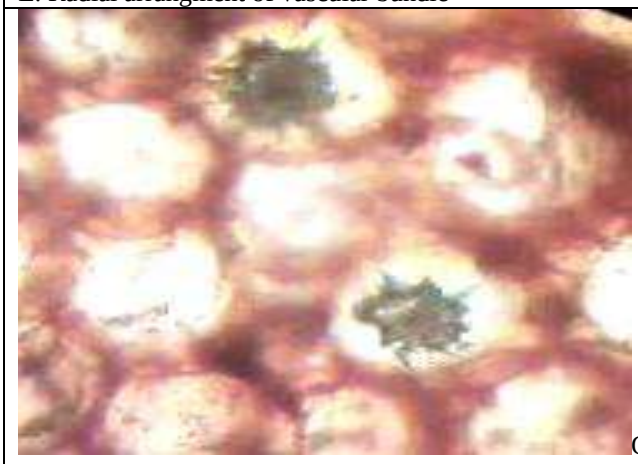
D. Starch grains showed blue color with dilute iodine solution.



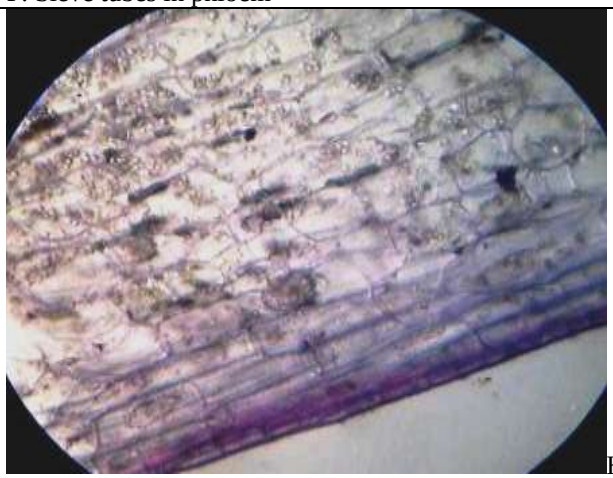
E. Radial arrangment of vascular bundle



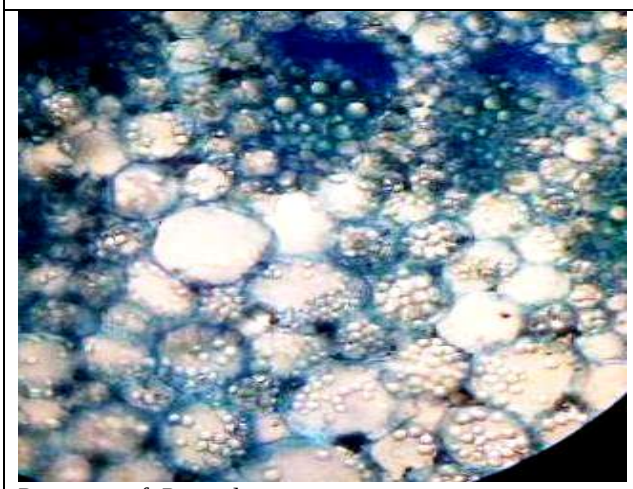
F. Sieve tubes in phloem



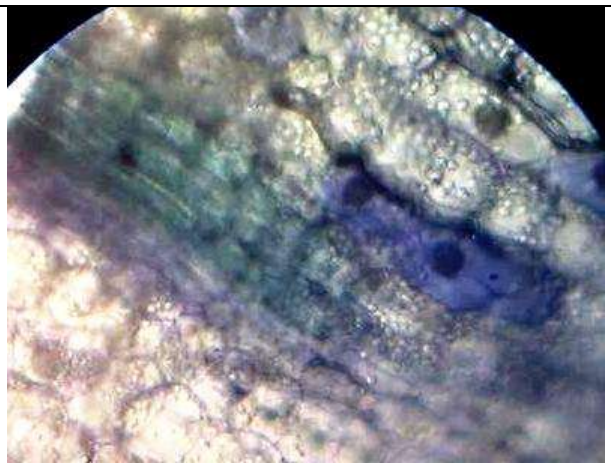
G. Rossettes calcium oxalate crystals



H. Longitudnal scetion



Presence of Protoplast



L.S. shows crystals, xylem vessels

Table 3: Histochemical color reaction on T.S. of *P. grandiflora*

Reagents	Color	Histological zones
Phloroglucinol: HCl (1:1)	Pink	Lignified xylem
Safranin	Dark red Yellow	Lignified xylem and pericyclic fiber, cellulosic collenchyma. Spongy parenchyma
Fast green	Green Violet Blue	Lignified xylem, Cuticle Cellulosic collenchyma and pericyclic fiber
Weak iodine solution	Dark blue	Starch grains in cortex and stellar region
Anilline – H_2SO_4	Yellow	Xylem
Conc. H_2SO_4	Green	Cellulose
Million's reagent	White	Protein

Table 4 Florescence analysis of powdered of *P. grandiflora* Hooker

Sr. no.	Treatment	Colour in Daylight	Colour in UV(254nm)
1.	Powder as such	Pale green	Light green
2.	Powder + NaOH in water	Yellow	Dark green
3.	Powder + NaOH in alcohol	Brown	Yellowish green
4.	Powder + 1N HCl	Yellowish brown	Light green
5.	Powder + 50% H_2SO_4	Yellowish orange	Light green
6.	Powder + 50% HNO_3	Yellowish orange	Light brown
8.	Powder + 5% I_2 solution	Bluish black	Dark brown

Table 5: Physico-chemical evaluation of powder drug

Sr. no.	Parameters	Results (%w/w)
1.	Total ash	14.5 ± 0.05%
2.	Acid insoluble ash	5.24± 0.01 %
3.	Water soluble ash	3.28 ± 0.02 %
4.	Sulphated ash	10.22± 0.05 %
5.	Alcohol soluble extractive value	18.6 ± 0.02%
6.	Water soluble extractive value	2.7± 0.03 %
7.	Moisture content	24 ± 0.02 %
8.	Swelling index	4.60 ml
9.	Total crude fibres	48.36±0.05%

Table 6 Qualitative preliminary phytochemical screening

Sr. no.	Chemical Test	Alcoholic extract
1.	Steroids	+
2.	Terpenoids	+
3.	Alkaloids	+
4.	Saponin	-
5.	Tannins	+
6.	Proteins	-
7.	Flavonoids	+
8.	Mucilage	-

+: present, -: absent

Table7 Results of calibration curve

Sr. No.	Concentration of plant Extract(µg/ml)	Absorbance at 415nm
1.	400	0.094
2.	1000	0.243

Table 8 Antimicrobial activity of alcoholic extract of *P.grandiflora*

Zone of inhibition(mm)					
Concentration (mg/ml)	***Microorganism***				
	B. subtilis	***E. coli***	***Ps. aeruginosa***	***C. albicans***	***A. niger***
1	19	14	30	20	18
2	14	12	24	18	16
3	12	10	20	16	13
4	10	-	18	12	10
5	-	-	14	10	
6	-	-	10	-	
Standard (Gentamicin) Or Ticonazole	-	-	-	-	
Control	38	36	38	28	28

Figure 1. *Portulaca grandiflora*

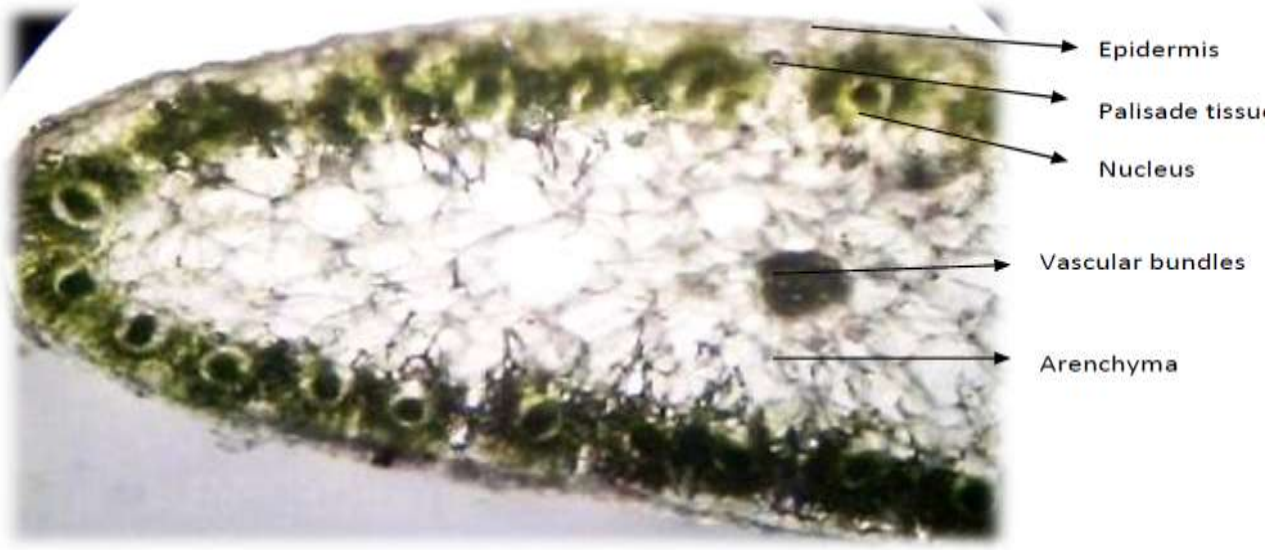


Figure 2: Transverse section of leaf of *P. grandiflora*

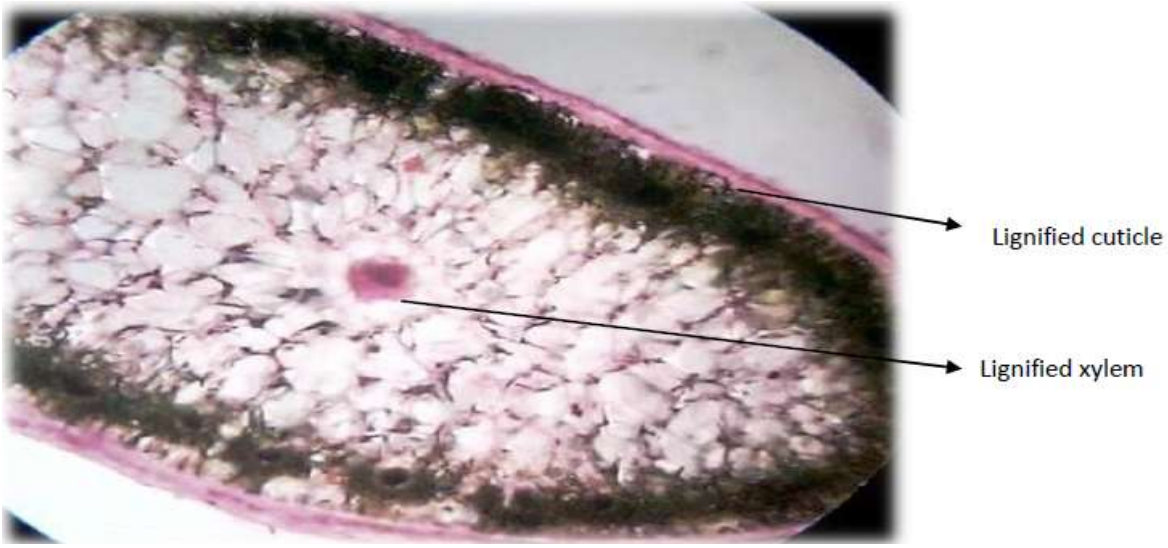


Figure 3: T.S. stained with Saffranin shows lignified vascular bundle at center

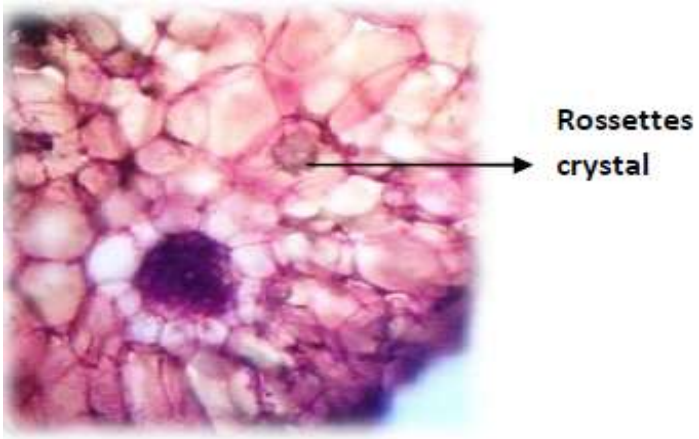


Figure 4 :Calcium oxalate crystals

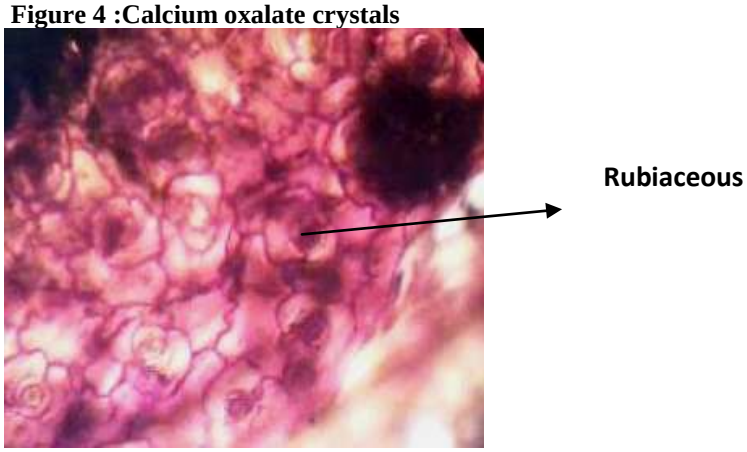


Figure 5: Rubiaceous stomata

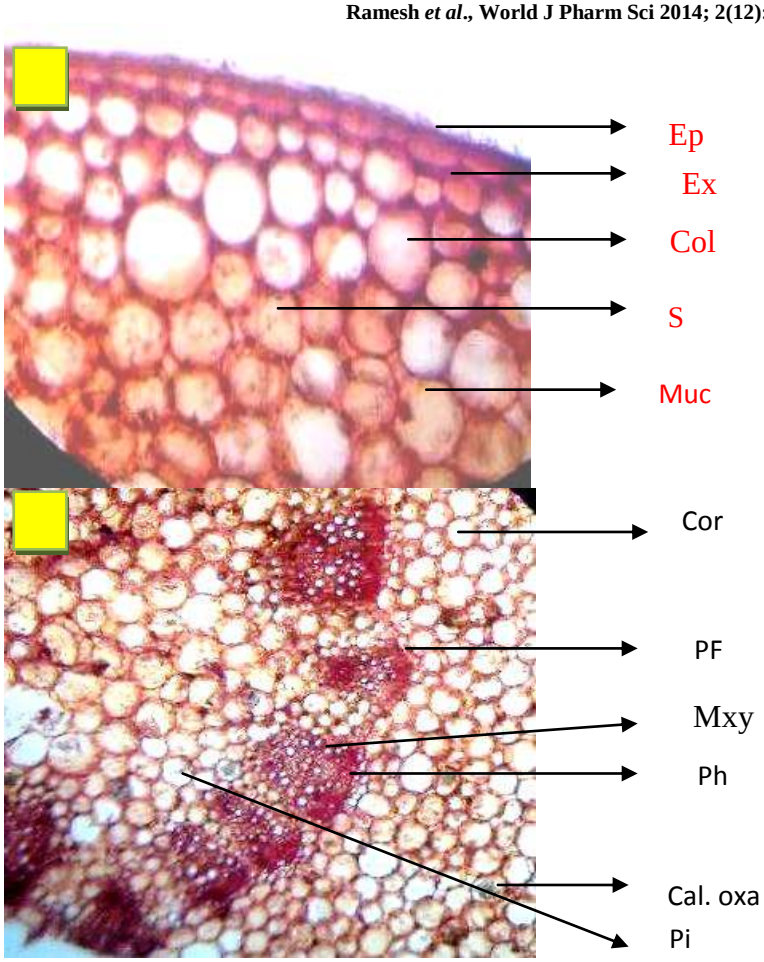


Figure 6: Transverse section of stem of *Portulaca grandiflo*

Ep: Epidermis, Ex: exodermis, Col: Collenchyma, S: starch, Muc: Mucilage, Cor: Cortex, PF: Pericyclic fiber, Mxy: metaxylem, Ph: phloem, Cal. Oxa: calcium oxalate crystals, Pi:Pith.

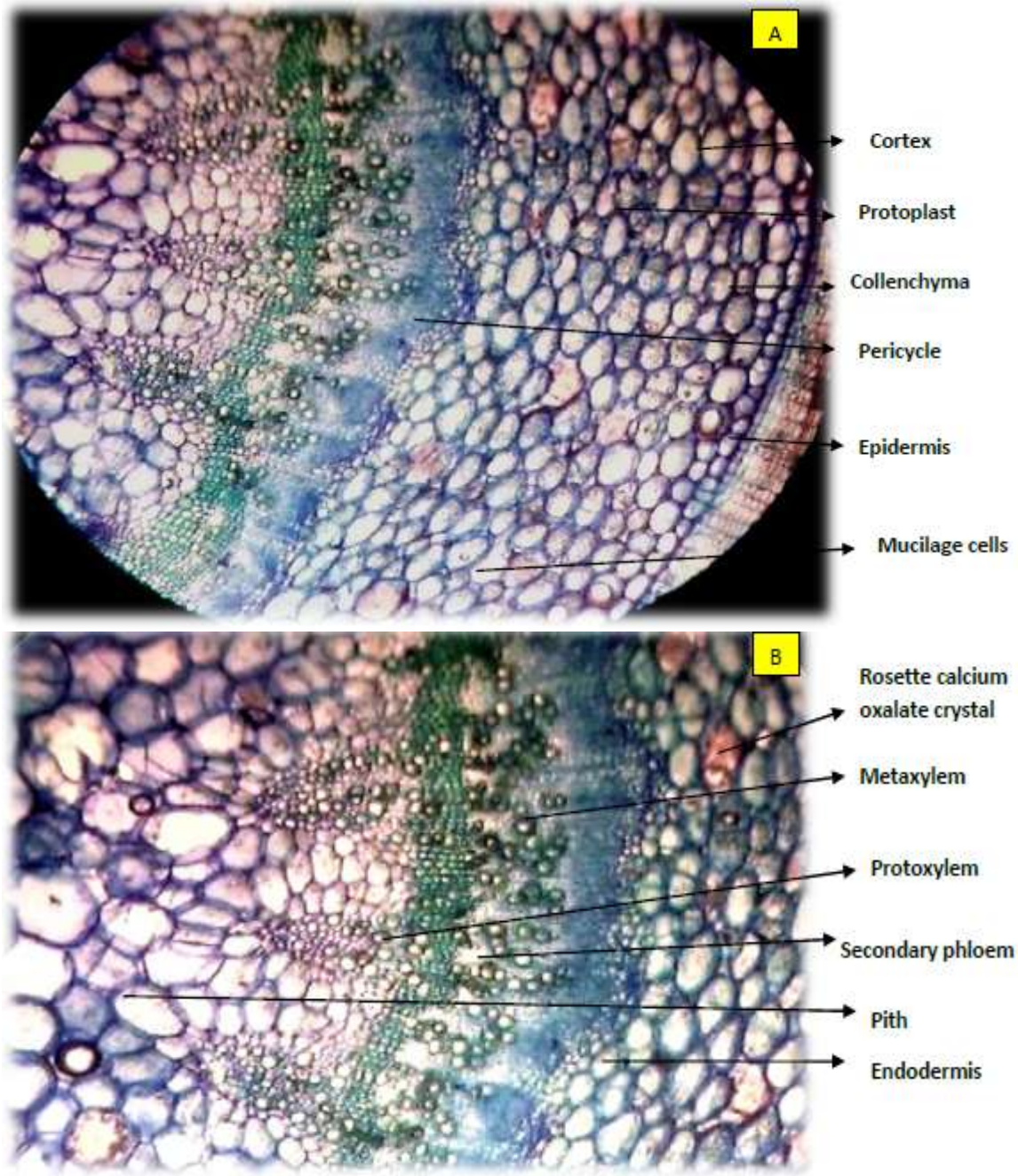


Figure 7 Transverse section of root of *P. grandiflora*

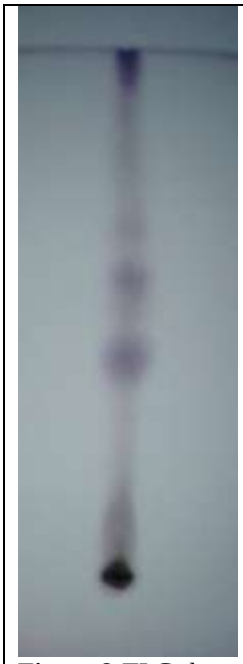
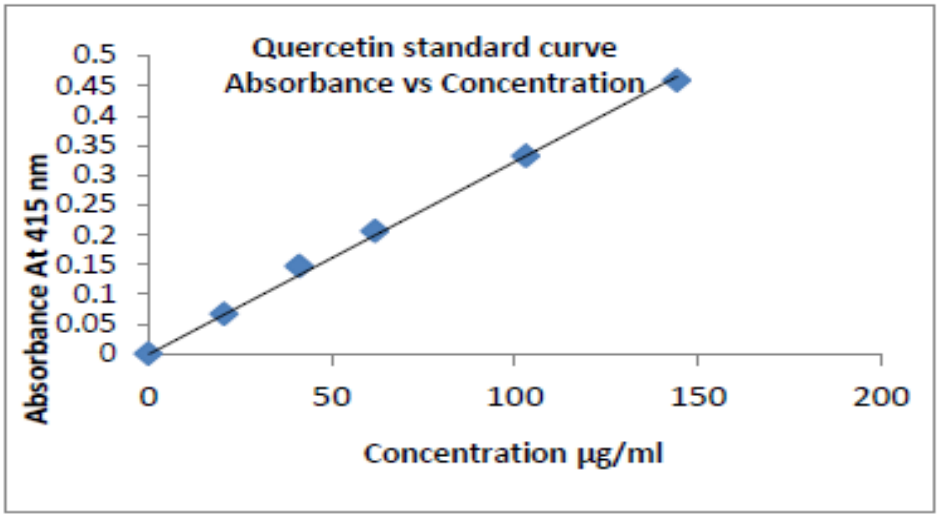


Figure 8:TLC shown pattern of steroid and triterpenoid after spaying with anisaldhyde –H_2SO_4 of ethanolic extract

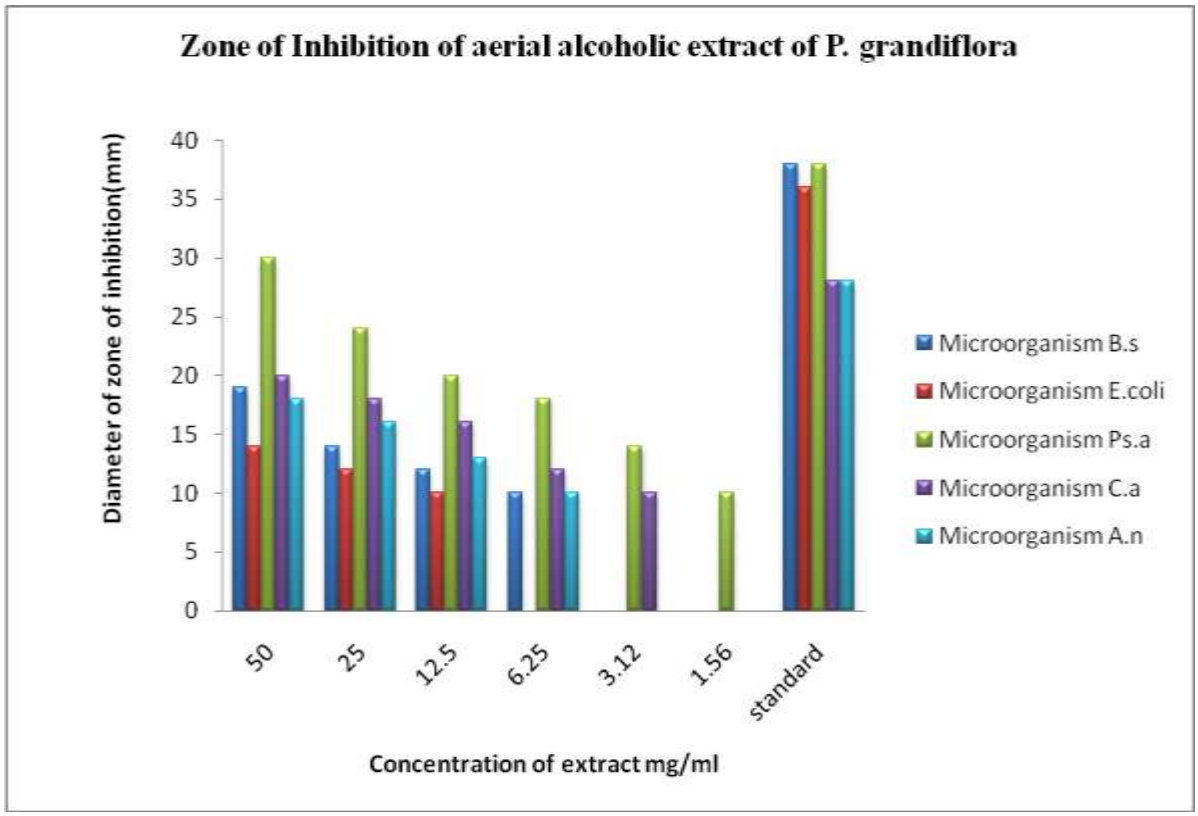
Figure 9: TLC shown pattern of flavonoid under UV 366nm in ethanolic extract**.**

Figure 10 : TLC shown pattern of triterpenoid after spaying with vanillin –H_2SO_4

Figure 11 : TLC shown pattern of steroid after spaying with 50% ethanolic –H_2SO_4



Graph 1. Calibration curve of quercetin



Graph 2. Antimicrobial activity (zone of inhibition) of alcoholic extracts of aerial parts of *P.grandiflora* Hooker at a concentration 50mg/ml.

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