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PHARMACOGNOSTICAL AND PHYTOCHEMICAL INVESTIGATION OF *EUPHORBIA PROSTRATA* AIT.

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ABSTRACT

Euphorbia prostrata Ait.(Euphorbiaceae) is a small, prostrate, annual green herb sometime purple tint in colour. It is widely distributed globally and used as antihemorrhoidal, anti-inflammatory, analgesic, hypolipidemic, antidiabetic, antidirroheal, antiasthmatic and for various skin diseases. The microscopy of root shows the presence of obliterated cork cells, phelloderm, cortex, endodermis, phloem, medulary rays and xylem; the stem shows the presence of multicellular trichome, cuticle, epidermis, cortex, endodermis, pericycle, phloem, latex canal, xylem and pith; the leave reveal the presence of multicellular, multiseriate glandular hairs, epidermis, vascular bundles, stomata anomocytic and anisocytic. The vein islet number and vein termination number have also been determined. The powder study reveals the presence of epidermal cells, trichomes, parenchymatous cells, pollen grains, vessels, fibers and stomata. The water soluble, alcohol soluble and petroleum ether extractive values were determined. The total ash, water soluble ash, acid insoluble ash and sulphated ash were also observed. Preliminary phytochemical studies revealed the presence of flavonoids, tannins, glycosides and saponins in the alcoholic extract of the plant. This is the first report on pharmacognostical studies on this plant.

INTRODUCTION: *Euphorbia prostrata* Ait. (Euphorbiaceae) is small annual herb found all over India especially in foot hills of Himalayas. It is native to the West Indies and certain parts of South America and also widely naturalized in many other parts of the world. The two varities found are red and green [1]. These are branched prostrate with many stems spreading from the roots, slender upto 20 cm long, leaves green but occasionally purplish red [2]. It is a reputed drug in the Indian System of Medicine and used in treatment of many diseases of skin [3, 4], digestive system [5], antiasthmatic [6], antidiabetic [7], haemorrhoids [8] etc. It is also used traditionally as snake bite remedy [9].

The various types of phytoconstituents reported in *Euphorbia prostrata* Ait. are like glucoside, galactoside, β-sitosterol, compesterol, stigmasterol, cholesterol, apigenin, luteolin, apigenin-7-glucoside, luteolin-7-glucoside, gallic acid, ellagic acid and tannins [2, 10].

MATERIAL AND METHODS: The plant was collected from the campus of Guru Jambheshwar University of Science and Technology, Hisar, Haryana and identified by Dr. H.B Singh at NISCAIR, New Delhi vide reference no. NISCAIR /RHMD /Consult /-2009- 10/1282/86. The herb was air dried, packed and stored for study.

Pharmacognostic Studies:

Macroscopic: Macroscopic studies were done using simple microscope. The taste, odour, shape of plant parts of fresh and dried plant was observed [11].

Microscopic: Anatomical sections surface preparation of stem, root, leaves for the microscopy were carried out [11-13]. The microscopical features were observed under Zeius Trinocular Microscope (Germany). Quantitative microscopy of leaf was also carried out and upper and lower stomatal number, vein islet number and vein termination number were observed [14, 15].

Powder Study: Plant was oven dried at 40±5^{o}C to make it moisture free and powdered form with the help of electric grinder and powder was passed through sieve no. 60. Standard methods were followed to study the powder characteristics [11]. Flourescent study and various histochemical reaction studies were carried out on plant powder [15, 16].

Physical Evaluation: The physiochemical parameters such as water, alcohol, petroleum ether soluble extractive values, percentage of total ash, water soluble ash, acid insoluble ash and sulfated ash, loss on drying, swelling index, foaming index, bitterness value, crude fiber content and heavy metals concentration were performed and calculated as per WHO guidelines [17].

Preliminary Phytochemical Studies: The dried plant was pulverized and 500 gm of plant sample was extracted successively with 4 litres ethanol using soxhlet apparatus for about 72 hours. Thereafter the marc was subjected to three consecutive aqueous extractions for 24 hours each. Each extract was concentrated and dried over anhydrous calcium chloride and kept aside for phytochemical investigation. The qualitative tests were carried out [12, 18].

RESULTS AND DISCUSSION: Macroscopic characters of *E. prostrata* shows that it is branched, prostrate with many stems spreading from the root, slender upto 20 cm long; leaves green but occasionally purplish red (**fig. 1**). The plant consist hairs occasionally present on the surface, no characteristic odour and taste (**Table 1**).

FIG. 1A: *EUPHORBIA PROSTRATA* AIT. HERB

FIG. 1B: *EUPHORBIA PROSTRATA* AIT. ROOT

FIG. 1C: *EUPHORBIA PROSTRATA* AIT. AERIAL PART

T.S. of the root shows the presence of the presence of cork cells, phelloderm, cortex, endodermis, phloem, medulary rays and xylem (**fig. 2 A**); the stem shows the presence of multicellular trichome, cuticle, epidermis, cortex, endodermis, pericycle, phloem, latex canal, xylem and pith (**fig. 2 B**); the leave reveal the presence of multicellular, multiseriate glandular hairs, epidermis, vascular bundles, stomata anomocytic and anisocytic (**fig. 2 C; Table 2**), upper and lower stomatal index is 11.7-18.7 and 17.6-26.3 respectively, vein islet number is found to be 2-5 and vein termination number is found to be 5-13 (**fig. 2 D; Table 3**).

TABLE 1: THE MACROSCOPIC CHARACTERS OF ROOT, STEM AND LEAF OF *E. PROSTRATA* AIT.

Macroscopic characters	Root		Stem		Leaf	
	Fresh	Dried	Fresh	Dried	Fresh	Dried
Condition	Tap root system slender	Tap root system slender	Prostrate, slender, internode	Prostrate, slender, internode	Pubscent, opposite, broad, oblong, glabrous above	Opposite, broad, oblong, glabrous above
Length	8 to 12 cm	5 to 10 cm	17 to 20 cm	15 to 18 cm	2.5 to 5 mm	2 to 3.5 mm
Breadth	0.2 to 0.4 cm	0.1 to 0.25 cm	0.3 to 0.5 cm	0.2 to 0.4 cm	2 to 4 mm broad	1.5 to 3 mm broad
Colour	Pink at base, pale yellowish downwards, creamish from inner side	Light yellowish outside and off from inner side	Green or sometime purple tint from outside, creamish inner side	Purple tint, slightly green patches, creamish from inner side	Green, purplish red	Purplish red
Branching	One primary long vertical root, some secondary root at stem base	One primary long vertical root, some secondary root at stem base	Branched	Branched	Opposite, petiole short, margin serrulate	Opposite, petiole short, margin serrulate
Fracture	Tough	Tough	Fibrous	Fibrous	Fibrous	Fibrous
Taste	Tasteless	Tasteless	Tasteless	Tasteless	Tasteless	Tasteless
Odour	Not characterstic	Not characterstic	Not characterstic	Not characterstic	Not characterstic	Not characterstic

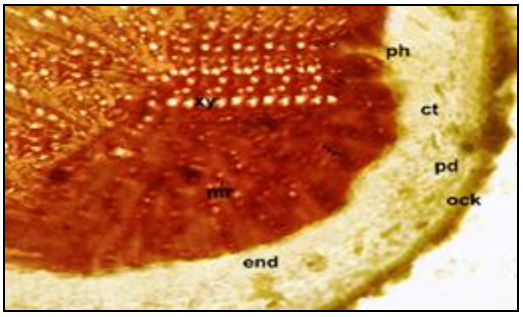

FIG. 2A: T.S. OF *EUPHORBIA PROSTRATA* AIT. ROOT

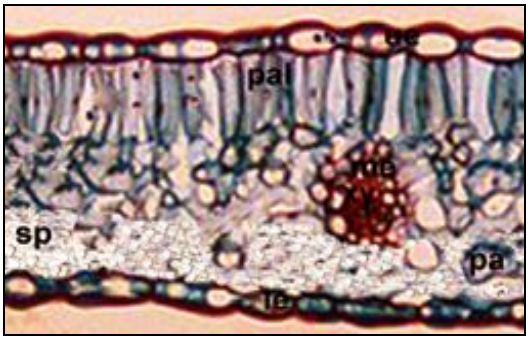

FIG. 2B: T.S. OF LEAF OF *EUPHORBIA PROSTRATA* AIT. THROUGH MIDRIB

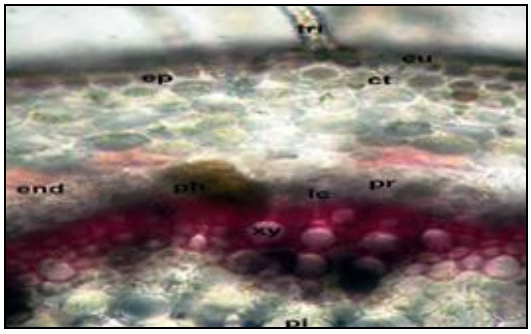

FIG. 2B: T.S. OF *EUPHORBIA PROSTRATA* AIT. STEM

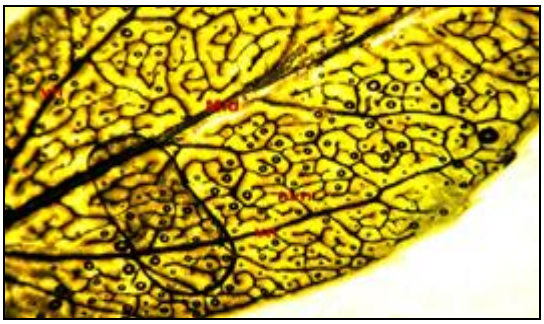

FIG. 2D: DECOLORIZED LEAF ANATOMY

TABLE 2: OBSERVATIONS FROM T.S OF STEM, ROOT AND LEAF OF *E. PROSTRATA* AIT.

FEATURES			
Stem	Root	Leaf	Powder (Whole plant)
Unicerate multicellular trichomes	Endodermis	Epidermis	Unicerate multicellular trichomes
Cortex	Cork cells	Vascular bundles	Lignified sclerenchymatous Cells
Pericyclic fibers	Cambium	Stomata (anomocytic)	Spiral lignified vessels
Parenchymatous pith	Short medullary rays	Glandular hairs	Starch granules
			Cork Cells
			Lignified fibers
			Stomata (anomocytic)
			Parenchymatous cells

TABLE 3: QUANTITATIVE MICROSCOPY OF LEAVES OF *E. PROSTRATA* AIT.

Determination	Range	Mean*
Upper epidermal stomatal index	11.7-18.7	15.2
Lower epidermal stomatal index	17.6-26.3	21.95
Vein islet number	2-5	3.5
Vein termination number	5-13	9

*Mean value of 10 counts

The various diagnostic characters of plant powder was yellowish-green, tasteless with oily odour which shows the presence of epidermal cells, trichomes, parenchymatous patches, pollen grains, vessels, fibers, stomata (**fig. 3**).

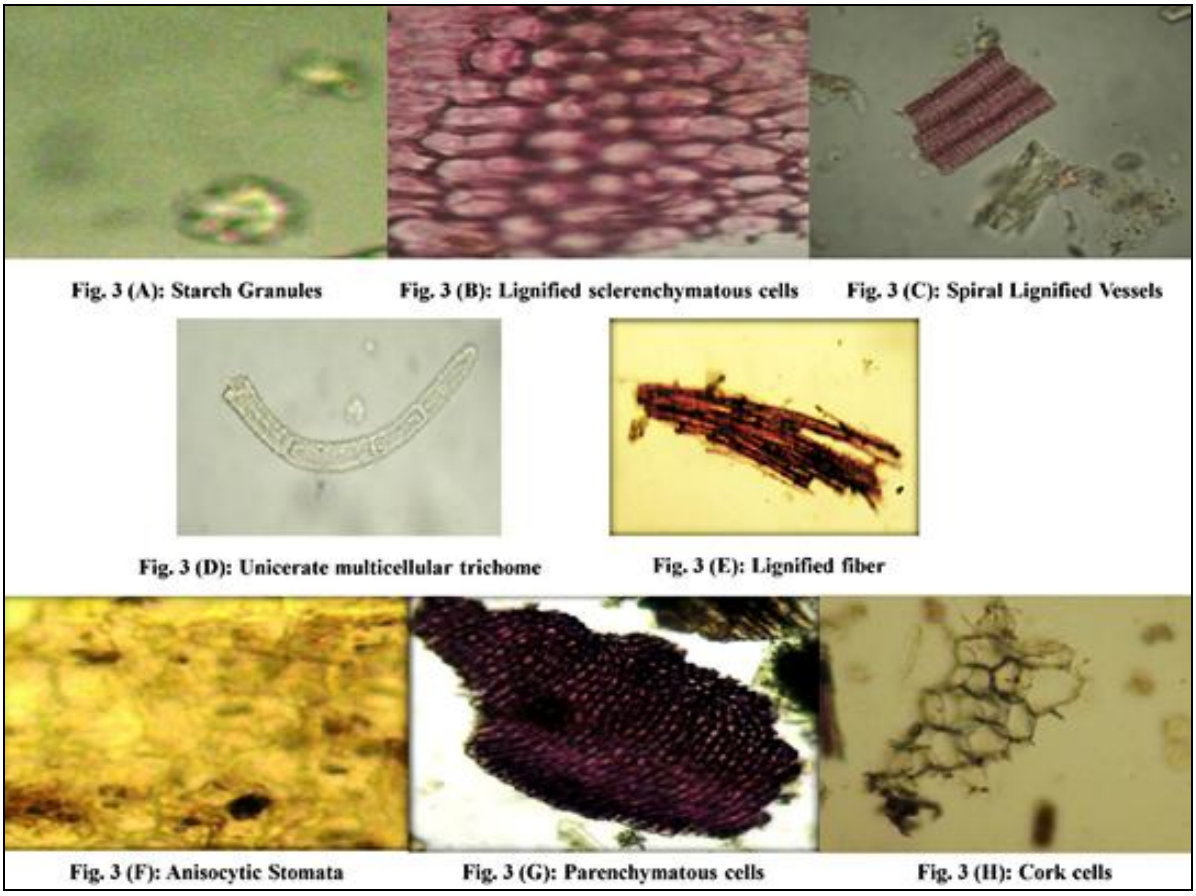

FIG. 3: POWDER CHARACTERISTICS OF *EUPHORBIA PROSTRATA* AIT.

The physical parameters are necessary in detecting adulteration or improper handling of the drugs. The moisture content is not too high, thus it could discourage bacteria, fungi or yeast growth. Heavy metals concentration is within the limit which proves its non-toxic effects (**Table 4**). The total ash determines the purity of the drugs.

TABLE 4: HEAVY METAL CONCENTRATION IN *EUPHORBIA PROSTRATA* AIT.

Heavy metal	Concentration(ppm)
Lead	0.0041
Cadmium	0.0026
Nickel	0.0093
Mercury	0.0005
Iron	0.4264
Copper	0.511
Sodium	1.52
Magnesium	0.690
Arsenic	0.0019

Water soluble, alcohol soluble and petroleum ether extractive values were found to be 34±1.0%, 12±0.9% and 7±0.4% respectively. The total ash, water soluble ash, acid insoluble ash and sulphated ash were observed to 11±0.3%, 12±0.26%, 1±0.2% and 2±0.2% respectively. The total moisture content was found to be 14±0.7.

Swelling index, foaming index and crude fibre content were found to be 2.7 ml/mg, Less than 100 and 50.8%, respectively (**Table 5**). Qualitative tests reveal the presence of flavonoids, tannins, glycosides and saponins (**Table 6**). The fluorescent behavior and histochemical colour reaction studies were noted (**Table 7, 8**). This Pharmacognostical evaluation will provide valuable information for future studies.

TABLE 5: PHYSIOCHEMICAL PARAMETERS OF *E. PROSTRATA* AIT.

Parameter	Determined values*
Ethanol soluble extractives	12±0.9 (% w/w)
Water soluble extractive	34±1.0 (% w/w)
Petroleum Ether soluble	7±0.4 (% w/w)
Total ash	10±0.3 (% w/w)
Water soluble ash	9.1±0.26 (% w/w)
Acid insoluble ash	0.9±0.1 (% w/w)
Sulphated ash	2±0.2 (% w/w)
Moisture Content	14±0.7 (% w/w)
Swelling Index	2.7 ml/mg
Foaming Index	Less than 100
Crude Fiber content	50.8%

*Mean value of three counts

TABLE 6: QUALITATIVE CHEMICAL TESTS OF *E. PROSTRATA* AIT.

Phytoconstituents	P	C	A	Aq
Alkaloids	–	–	–	–
Flavonoids	–	–	+	+
Carbohydrates	–	–	+	+
Sterols	–	+	+	–
Glycosides	–	+	+	–
Saponins	–	–	+	+
Phenolic compounds and Tannins	–	–	+	+
Proteins and Amino acids	–	–	–	+
Resin	–	–	–	–

P: Petroleum ether extract, C: Chloroform extract, A: alcoholic extract, Aq: Aqueous extract. "+" Present, "-" Absent.

TABLE 7: FLUORESCENT BEHAVIOR OF *E. PROSTRATA* POWDER UNDER VISIBLE AND UV LIGHT

Treatment	Colour		
	Visible	Short UV (254 nm)	Long UV (365 nm)
Powder as such	Yellow Green	Light Green	Brownish Black
Powder + 1N Hydrochloric acid	Dark Green	Brownish Black	Black
Powder + Picric acid	Yellowish Green	Yellowish Green	Black
Powder + Acetic Acid	Dark Green	Dark Green	Reddish Black
Powder + 50% Sulphuric Acid	Greenish Black	Dark Brown	Brownish Black
Powder + 1N Sodium Hydroxide	Green	Purple Green	Purple Black
Powder + Methanol	Yellowish Green	Dark Green	Purple Black
Powder + 1N Nitric Acid	Green	Dark Green	Black

TABLE 8: HISTOCHEMICAL COLOUR REACTIONS ON *E.PROSTRATA* POWDER

Reagents	Test for	Color change in histochemical reaction	Degree of change	Inference
Potassium iodide & Iodine solution	Proteins	Light yellow	Black	Absent
Iodine solution	Starch	Blue black	Brownish Black	Present
Aqueous potassium hydroxide solution (10%)+ Sulphuric acid	Suberin	Brown	Green	Absent
Ferric chloride solution	Tannins	Black	Greenish Black	Present
Wagner's reagent	Alkaloids	Yellow	No change	Absent
Dragendroff's reagent	Alkaloids	Orange	No change	Absent
Dilute Sulphuric acid	Sterols	Dark red	Red changes to Black	Present
Chloroform+ Dilute sulphuric acid	Sterols	Dark red	Reddish black	Present
Libermann Burchard's reagent	Terpenes	Pink	Dark pink	Present

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