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Evaluation of hair growth promoting activity of petroleum ether extracts of *Hemidesmus indicus* Linn. (Seed) on Wistar Albino rats

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ABSTRACT

In present investigation was carried out to screening of hair growth promoting potentiality of petroleum ether extract of *Hemidesmus indicus* Linn. (Seeds). Preliminary chemical tests and TLC analysis revealed the presence flavonoids and saponins. Hair growth promoting activity of petroleum ether of *Hemidesmus indicus* was screened by considering different parameters which included time taken for covering bald patch, length of hair produced, percentage of hair follicles in anagen and telogen phases, time of hair growth initiation and completion and level of minerals in blood. The petroleum ether extract of *Hemidesmus indicus* showed a very good hair growth promoting activity at a dose of 300 mg/kg which was comparable to that of 2% minoxidil. After 30 days of treatment with test and standard drugs it was observed that, time taken for covering the bald patch, hair growth initiation and completion time and quantitative hair growth were found to be comparable to that of the standard drug. An increase in percentage of hair follicles turning from telogen phase to anagen phase was noted. The control treated group of animals showed poor hair growth for all the parameters.

Keywords: *Hemidesmus indicus* Linn., Anagen, Catagen, Telogen, Minoxidil.

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INTRODUCTION

The utilization of herbal drugs is on the flow and the market is growing step by step. The annual turnover of the Indian herbal medicinal industry is about Rs. 2,300 crore as against the pharmaceutical industry's turnover of Rs. 14,500 crores with a growth rate of 15 percent[1-5]. The export of medicinal plants and herbs from India has been quite substantial in the last few years. India is the second largest producer of castor seeds in the world, producing about 1,25,000 tonnes per annum. The major pharmaceuticals exported from India in the recent years are isabgol, opium alkaloids, senna derivatives, vinca extract, cinchona alkaloids, ipecac root alkaloids, solasodine, Diosgenine/16DPA, Menthol, gudmar herb, mehdi leaves, papian, rauwolfia guar gum, Jasmine oil, agar wood oil, sandal wood oil, etc .The turnover of herbal medicines in India as over-the-counter products, ethical and classical formulations and home remedies of traditional systems of medicine is about $ one billion and export of herbal crude extract is about $ 80 million[6]. The herbal drug market in India is about $1 billion[7].

According to World Health Organization, herbal medicines are lucrative globally and they represent a market value of about US$ 43 billion a year[8]. According to an estimate in 1991, the herbal medicine market in the European countries was about $ 6 billion, with Germany accounting for $ 3 billion, France $ 1.6 billion and Italy $ 0.6 billion while in other countries was 0.8 billion. In 1996, the herbal medicine market in the European countries was about $ 10 billion, in USA about $ 4 million, in India about $ 1 billion and in other countries was $ 5.0 billion[9]. In 1997, the European market alone reached about $ 7.0 billion. The German market corresponds to about 50% of the European market, about $ 3.5 billion. This market is followed by France, $ 1.8 billion; Italy, $ 700 million the United Kingdom, $ 400 million; Spain, $300million; the Netherlands, about $ 100 million[10].

Hair is one of the first things other people notice about us--and is one of the primary ways we declare our identity to others. Both in our personal relationships and in relationships with the larger world, hair sends an immediate

signal that conveys messages about our gender, age, social class, and more[11].

Hair is one of the vital parts of the body derived from ectoderm of the skin, is protective appendages on the body and considered accessory structure of the integument along with sebaceous glands, sweat glands and nails. They are also known as epidermal derivatives as they originate from the epidermis during embryological development[12].

MATERIALS AND METHODS

Collection of Plants

Hemidesmus indicus Linn. (Seeds) were purchased from well-known supplier from local market, Indore (M.P.). The collected crude seeds were identified and authenticated on the basis of macroscopic and microscopic characters at the Department of Botany, Government Agriculture College, Indore; Voucher specimens SCOPE/Phcog/344 have been deposited at the herbarium of the Department of Pharmacognosy of the College for further reference.

The experiments were carried out air dried plant materials which were reduced to moderately coarse powder using mechanical grinder. The powders were then passed through sieve # 40 and stored in an air tight container for further use.

Preparation of Extracts

The powdered material was extracted by petroleum ether solvent using soxhlet apparatus. The extracts were filtered, concentrated on water bath, dried in vacuum and stored in refrigerator for subsequent experiments. Yield value was calculated for each herb.

Characterization of Extracts

The extracts were characterized by TLC (thin layer chromatographic) studies on silica gel-G plates benzene ethyl acetate (60:30v/v) as mobile phase gave best resolution for petroleum ether extract of both extracts after derivatization with anisaldehyde.

Pharmacognostic Studies

Macroscopy

Color, odor, taste and shape were determined by oraganoleptic evaluation.

Microscopy

The coarse powder was boiled with chloral hydrate to remove the coloring matter and then mounted on a glass slide using phloroglucinol, dilute hydrochloric acid and glycerin, covered with a cover slip. Then the powder was viewed under compound microscope and the microscopic characters of the powder were observed. The mount was made free from air bubbles to determine the types of cells, the nature of cell wall present and cell contents etc [13].

Physicochemical Studies

Ash Values

Ash values are useful in determining the quality and purity of the crude drugs, especially in the powdered form. The ash content of a crude drug is generally taken as the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may be also include inorganic matter added for the purpose of adulteration. Hence, ash value furnishes a basis for judging the identity and purity of the crude drug and gives information relative to its adulteration with inorganic matter[14].

Total Ash Value

Three gm of the air dried drug powder was placed in a tarred silica crucible and incinerated at a temperature not exceeding 450^{o}C until free from carbon, cooled and it was again weighed. The percentage of ash was calculated with reference to air dried drug.

Acid Insoluble Ash

The total ash was boiled with 25 ml of 2M HCl for 5 min. The insoluble matter was collected in an ash less filter paper, then it was washed with hot water, ignited and cooled in a desiccator and weighed and the percentage of acid insoluble ash was calculated with reference to air dried drug.

Water Soluble Ash

The total ash was boiled for five minutes with 25 ml of water and the insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temp not exceeding 450^{0}C. The weight of the insoluble matter was subtracted from the weight of the total ash and the difference in weight represents the water soluble ash. The percentage of water-soluble ash was calculated with reference to the air dried drug[15].

Extractive Values

Extractive values of crude drugs are useful for their evaluation. These values indicate the amount and nature of the constituents present in the crude drugs.

Water Soluble Extractive Value

5 gm of the air-dried, coarsely powdered drug was macerated with 100 ml of water in a closed flask for 24 hours, shaken frequently during the first 6 hours and allowed to stand for 18 hours. Thereafter, it was filtered taking precautions against loss of water; 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105^{o}C and weighed. The percentage of water-soluble extractive with reference to the air dried drug was calculated[16].

Alcohol Soluble Extractive Value

5gm of the air dried, coarsely powdered drug was macerated with 100 ml of alcohol (95%v/v) and refluxed for 2 hrs. Then it was filtered and 25 ml of filtrate was transferred in a porcelain dish and evaporated to dryness on a water bath and dried completely in an oven at 105^{o}C and finally weighed. The percentage of alcohol-soluble extractive with reference to the air dried drug was calculated[17].

Loss on Drying

The zero % coincidence and 100 % were set by rotating the knob in such a way that the marked line, the pointer and the 100% mark, all coincided each other. Then scale was brought to zero and coincided with line marked on it. The lid cover was opened and the test material put in the pan, till the pointer slide down and came to zero mark. The lid was closed and the temperature started by the thermostatically controlled knob. This knob was adjusted to get constant temperature and the material allowed drying. The pointer remained stable when the drying was over to constant weight and reading on scale was noted.

Fluorescence Analysis

In the near- violet region of the spectrum (3000–4000 Aº) some of the phytoconstituents show more or less brilliant colouration when exposed to radiation. This phenomenon of emitted visible wavelengths as a result of being excited by radiation of a different wavelength is known as fluorescence.

Sometimes the amount of Ultra-violet light normally present with visible light is sufficient to produce the fluorescence, but more often a more powerful source of ultra-voilet light is necessary, e.g. a mercury vapour lamp. It is often to make use of this phenomenon for the qualitative examination of herbal drugs[18].

Preliminary Phytochemical Screening

The plant materials were subjected to various tests for the presence of phytosterols.

***Liebermann's Test*:** A small fraction from the respective extract was taken with about 3ml of acetic anhydride. Heat & cool. Add a few drops of conc.H_2SO_4 blue color appears.

Lieberman's Burchards *Test*: A small fraction from the respective extract was taken with about 1 ml of chloroform & adds 1-2 ml acetic anhydride and 2ml of conc.sulphuric acid from side of test tube.

***Salkowski Test*:** A small fraction of extract was taken with about 2 ml of chloroform &few drops of conc. sulphuric acid. Shake well .chloroform layer appear red and acid layer shows greenish yellow fluorescence[19].

Pharmacological Studies

Animals

Male Wistar rats (100-120g) were used in experiments. The animals were housed in polypropylene cages under standard conditions (12 h light; 12 h dark cycle; 25± 5^0 C; 35-60% humidity). They were fed with standard rat pellet diet (Pranav Agro Industries Ltd., Maharashtra, India) and water *ad libitum*. Experimental protocol was approved by the Institutional Animal Ethical Committee. Animal ethical norms were strictly followed during all experimental procedures.

Dermal Acute toxicity study (According to OECD guidelines 434)

Preparation of animals: Approximately 24 hours before the study, fur was removed from the dorsal area of the trunk of the test animals by clipping or shaving. At least 10 % of the body surface clears for the application of the test substance.

Administration of Doses

The test substance was applied uniformly over an area which is approximately 10 % of the total body surface area. Test substance was held in contact with the skin with a porous gauze dressing and non-irritating tape throughout a 24-hour exposure period. The test site was further covered in a suitable manner to retain the gauze dressing and test substance and ensure that the animals cannot ingest the test substance.

Animals were observed immediately after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention gave during the first 4 hours and daily thereafter, for a total of 14 days.

After studies it was observed that the petroleum ether extracts of both extracts when applied in a concentration of up to 5% did not show any toxic side effects or erythema on skin surface. Thus, the prepared extracts were considered safe for topical administration.

Preparation of samples

Petroleum ether extracts of *Hemidesmus indicus* Linn. incorporated in liquid paraffin by sonication process[20].

Treatment

Animals were divided into 6 groups: Group I was applied no treatment and served as control, Group II was applied 2% petroleum ether extract of Abrus precatorius in liquid paraffin, Group III 5%. petroleum ether extract of *Abrus precatorius* in liquid paraffin,Group *IV* was applied 2% petroleum ether extract of *Hemidesmus indicus* in liquid paraffin, Group V5% petroleum ether extract of *Hemidesmus indicus* in liquid paraffin. Group VI served as positive control and applied alcoholic solution of minoxidil(Tugaine).

Application of Test samples

Hairs on dorsal side of the animals were removed using marketed hair remover (Anne French) to assure complete denual of 4 sq cm area .Finally denuded skin was wiped off with surgical spirit. Equal quantity (1 ml) of prepared and 1 ml of standard Minoxidil solution was applied to the denuded area of albino rats once a day for 21days[21].

Statistical Treatment

Data are reported as mean + SEM. Statistical analysis of data was carried out by one way ANOVA comparing all test groups versus control followed by Dunnet's test

Qualitative Hair Growth Study

Qualitative hair growth was evaluated by visual observation of two parameters: hair growth initiation time i.e. minimum time to initiate perceptible hair growth and hair growth completion time i.e. minimum time taken to cover the denuded skin region with new hair completely. The investigator was blinded about the treatments to various groups under investigation. Hair growth initiation and completion time was recorded for each group of animals and compared with positive control Tugaine(standard 5% ethanol solution of minoxidil) and control (with no treatment)[22].

Quantitative Hair Growth Study

The method described by Uno was followed for the quantitative evaluation of drug extract. One rat from each group was euthanicated after the 21 days of treatment, skin biopsies were taken from the shaved area, and specimen preserved in 10% formalin. The specimen was fixed on paraffin wax and blocks prepared for microtomy. After fixation, vertical sections of the skin were cut with the help of semiautomatic rotary microtome (Remi- 1871, India). The sections were stained with hematoxylin and eosin. The number of hair follicles per milllimeter area of skin and ratio of hair follicles in different cyclic phases i.e. anagen (active growth phase) and telogen (resting phase) were determined using the microscope. Hair folliculogram was prepared by observing growth cycle of 100 hairs and length of hair follicle[23].

RESULTS

Pharmacognostical Studies

Macroscopic Characteristics

The crude drugs were examined and following results were found.

Table 1: Macroscopic Characteristics of Crude Drugs

S. No.	Characteristics	*Hemidesmus indicus*
1.	Colour	Reddish Brown
2.	Odour	Agreeable
3.	Taste	Bitter
4.	Shape	Cylindrically Tortuous
5.	Size	Varies
6.	Surface	Longitudinally Fissured & Transversely Cracked

Microscopic Characteristics

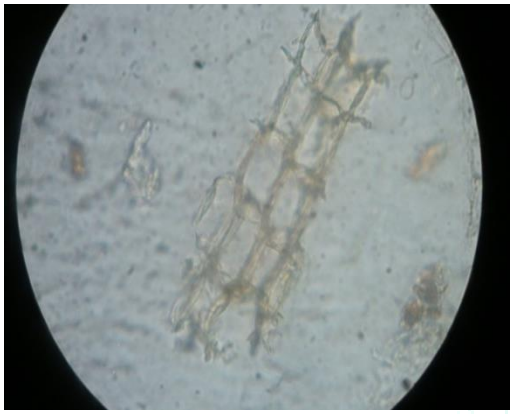


Figure 1: Brick shaped thin rectangular cork

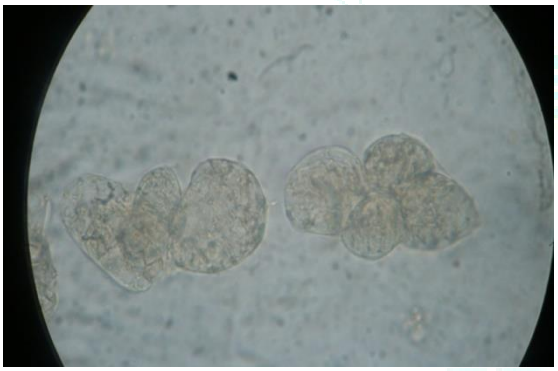


Figure 2: Phloem Parenchyma cells

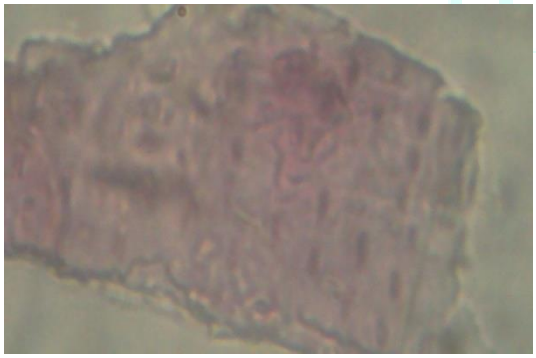


Figure 3: Fragment of Reticulate Xylem Vessels

Figure 4: Unicellular Trichome

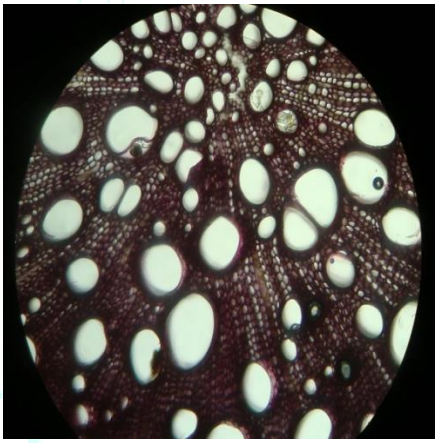


Figure 5: T. S of Seed: Tracheas & Phloem

Physicochemical Studies:

Table 2: Physicochemical Parameters of *Hemidesmus indicus* Seed

S. N.	Physicochemical Parameters	Result
1.	Total ash	2.60% w/w
2.	Acid insoluble ash	15.50% w/w
3.	Water soluble ash	10.66% w/w
4.	Loss on drying	
5.	Water soluble extractive value	11% w/w
6.	Alcohol soluble extractive value	17%

Table 3: Florescence Analysis of Powder of Seeds of *Hemidesmus indicus*

Powdered drugs	Visible /day light	Short UV 254nm	Long UV 365nm
Powder as such	Light brown	Light brown	Dark brown
Ammonia Solution+ powder	Brown	Light green	Dark brown
Iodine solution +powder	Light brown	Light green	Black
1N NaOH+ powder	Light brown	Light green	Black
conc.HCL+ powder	Dark brown	Light green	Black
Distilled water+ powder	Light brown	Pale brown	Light brown

Preliminary Phytochemical Screening

The petroleum ether extracts of *Hemidesmus indicus* seed were subjected to phytochemical tests to detect the presence of phytosterol as described earlier in methodology and the results obtained, are shown in Table 4.

Table 4: Results of Preliminary Phytochemical Screening of Extracts of *Hemidesmus indicus*

S. No.	Tests for Phytosterols	Petroleum Ether Extract of *Hemidesmus indicus*
1.	Salkowski reaction	+
2.	Liebermann Burchard reaction	+
3.	Liebermann reaction	+

(+): Present, (-): Absent.

Pharmacological Activity

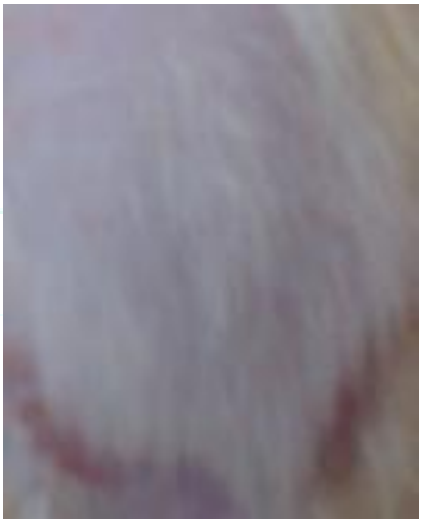
All experimental data are expressed as mean ± SEM. Statistical analysis were carried out by using one way ANOVA using Dunnett's t-test.

Result of Acute Dermal Toxicity Studies

Petroleum ether extracts were applied on the surface of denuded skin for 14 days at 5% concentration and observation made for skin rashes, inflammation, or allergic reactions thus, the prepared extracts were safe for topical administration. Permission from the institutional ethical committee was obtained for animal experimentation.

After 14 Day

Denuded area on 0 day



After 21 day

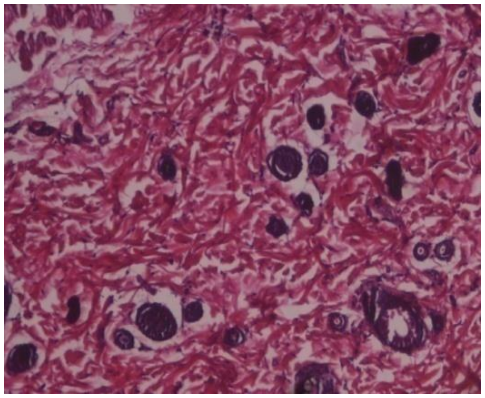
After 7 day

In vivo hair growth study showing hair growth initiation day photographs of rats treated petroleum ether extracts of *Hemidesmus indicus* on 0, 7 ,14, 21day.

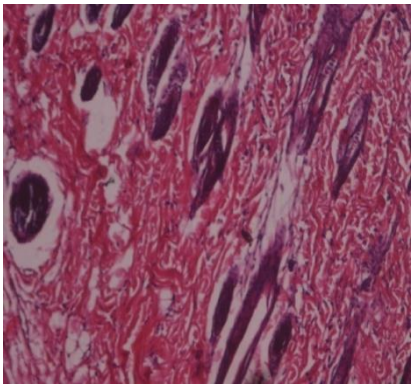
Table 5: Hair Growth Promoting Activity

S. No	Treatment	Hair growth initiation time (days)	Hair growth completion time (days)
1.	Control	10±0.70	17±0.44
2.	Standard	6±0.70	10±0.44
3.	Petroleum Ether Extract of *Hemidesmus indicus* 2%	8±0.44	14±0.54
4.	Petroleum Ether Extract of *Hemidesmus indicus* 5%	6±0.31	12±0.44

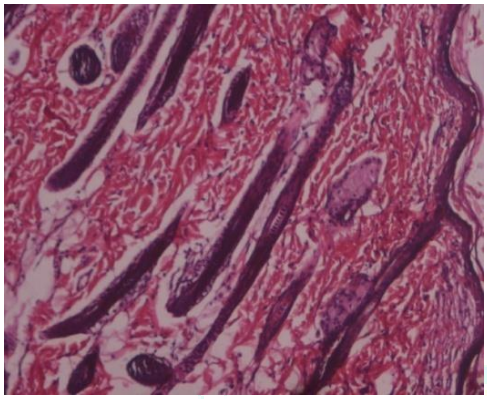
Graph:



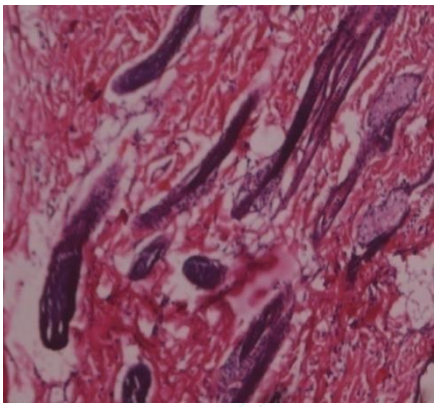
I. Control



III *Hemidesmus indicus* 2%



II. Standard



IV *Hemidesmus indicus 5%*

Table 6: Study of % Population of Hair Follicles in *Hemidesmus indicus*

S. No.	Group	% population of hair follicles		
		Anagen	Catagen	Telogen
	Control	29	6	65
	Standard	65	3	32
	Petroleum Ether Extract of *Hemidesmus indicus* 2%	44	4	52
	Petroleum Ether Extract of *Hemidesmus indicus* 5%	56	5	39

Table 7: Length v/s Percentage Population of hair follicles

S. No	Length of Hair Follicles (mm)	Control	Standard	2% HI 1	5% HI 2
1.	0.1	6	4	6	2
2.	0.15	9	7	6	4
3.	0.2	14	10	11	4
4.	0.25	12	6	12	8
5.	0.3	7	4	4	9
6.	0.35	7	5	12	7
	0.4	4	4	5	11
8.	0.45	8	7	2	5
9.	0.5	4	11	4	5
10.	0.55	5	7	6	7
11.	0.6	8	5	7	7
12.	0.65	7	4	6	7
13.	0.7	4	7	2	6
14.	0.75	2	6	3	4
15.	0.8	2	3	1	5
16.	0.85	1	4	2	4
17.	0.9	-	3	3	2
18.	0.95	-	2	1	1
19.	1	-	1	-	1

DISCUSSION

The plant *Hemidesmus indicus* belonging to the family Apocyanacae were studied for its pharmacognostical, physicochemical, phytochemical studies and hair growth promoting activity. Pharmacognostical studies of plant namely: macroscopy, microscopy is valuable source of information and provides suitable standards for the authentication of this plant material for future investigation.

Determination of ash value is for detecting adulterated or low grade products, exhausted drugs and excess of earthy and sandy matter. The physicochemical parameter like ash value such as total ash was found to be w/w, acid insoluble ash was found to be w/w and water soluble ash was found to be % w/w. The total ash usually consists of carbonates, phosphates, silicates and silica which include both physiological ash (which is derived from the plant tissue itself) and non-physiological ash (which is residue of some adhering material to the plant surface, e.g., sand and soil). Acid insoluble ash gives the evidence of the presence of silica, especially sand and siliceous earth. Water soluble ash detects the presence of material that has been exhausted with water.

The extractive value such as water soluble extractive value was found to be % w/w and alcohol soluble extractive value was found to be % w/w. The extractive value determines the amount of active constituents in a given amount of medicinal plant material extracted with solvent. The moisture content was found to be % w/w. An excess of water in medicinal plant materials will lead to microbial growth, the presence of fungi or insects deterioration and following hydrolysis. Therefore, limits for the amount of water should be set for every given plant material.

The extracts obtained by solvent extraction were subjected to preliminary phytochemical analysis, which revealed the presence of beta sitosterol in seeds &root part of plant species.

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