

## Phcog Rev.: Plant Review

### Therapeutic Uses of *Euphorbia thymifolia*: A Review

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#### ABSTRACT

The extracts of many plants used in traditional medicine contain curative agents That is used in many modern medicines. As part of the quest for potentially valuable Plants of medicinal value. In Sanskrit *Euphorbia thymifolia* means “Laghu dudhika or Raktavindachada”. The *Euphorbia thymifolia* widely used in the Ayurveda to cure many remedies. The leaves and seeds are given in worm case and in certain bowel affection of children. In India they are considered as stimulant and laxative also use in many disease due to presence of different constituents.

**KEY WORDS:** *Euphorbia thymifolia*, Hydrolysable Tannins, Antioxidant, Antiviral.

#### INTRODUCTION

The use of plants for medicines or tonic properties goes back to prehistoric times & has attracted the interest of scientists for centuries. The Vedas give the earliest written record about the science of healing .The reference to medicinal plant is also found in Ebers papyrus (16<sup>th</sup> century B.C.)which lists in detail over 7000 herbal remedies, for example-poppy, castor oil, caraway etc .The Indians were the first to use “chaulmoogra” fruits for its antileprotic activity .The Brazilians employed ‘Ipecacuanha’ for the treatment of dysentery and diarrhea .The root of *Rauwolfia serpentina*, an indigenous plant has since time immemorial been widely used in India and Malaysia as an antidote for insect and snake bites and Mental derangement.

The utility of plants as therapeutic agents in traditional medicine system is still prevalent today. For example, the middle eastern civilization developed the Greco-Arabic system of medicine (Unani system of medicine), which is practiced in the Indian sub continent .Similarly, the Chinese race developed the Chinese system of medicine largely based on it unique system of theories including the concept of **Yen & Yang**; the idea of **Wv, Xing** (the five elements), the theory of influence imparted from nature. The Ayurveda and Sidha system of medicines were contributed by Indians .All these systems procure more than 80% of their medicaments from plants (1).

Though not extensively used in medicine, many of the *Euphorbias* are consumed in the Eclectic practice. Some are excellent old Eclectic drugs, while the *E.hypericifolia* was reintroduced in 1874, by Dr. H. L. True, having staggered under a load of popular appellative some of them being peculiar to each plant named, other being shared by plants bearing no relation to the *Euphorbia*. *Euphorbia Ipecacuanha* is called wild ipecac and *Ipecacuanha spurge*; *E.corollata*-looming spurge large flowering Spurge, snake’s milk, hippo, picac, purge root, milk purslane, emetic root, apple root, Indian physic, ipecac, *Ipecacuanha* and Bowman’s root.

There are many other species of *euphorbia*, though all possess widely diverse characteristics from older medicinal plants .In the dominions of the Mauritanian despot the plants abound as large succulent trees or brushes resembling cacti, but differing from them in having a milky juice, which exudes on the slightest puncture.nearly all the *Euphorbias* are more or less poisonous, and all exude this acrid milky fluid when broken. Like their fellow of the same natural order, the *Ricinus commanis*, or castor oil plant, most of them have cathartic powders, through some astringent.

In hot countries the milk of a certain pieces is employed as a caustic, while another furnishes the natives with an “arrow poison” by simply dipping the weapon in the milky juice. Anthelmentic properties are possessed by the *Euphorbia thymifolia* Linn India.

#### DESCRIPTION

*Euphorbia thymifolia* Linn. Diels syn; *C.thymifolia*

Family:*Euphorbiaceae*

#### Vernacular names

Ayurveda - Chhoti dudhi, Laghu dugdhikaa

Bengal - Dudiya, shweetkeruee, swetkerua

Bombay - Nayata, Nayeti

Ceylon - Cgittirapalavi

English - Chicken weed, dwarf spurge, red caustic creeper, thyme-leafed, Spurge, Asthma plant, Pill-bearing spurge

Gujarat - Nahanidudheli

Hindi - Chhoti -dudhi

Marathi - Ghakdidudhi, Chothadudhi

Sidha - Ammanpharisi

Sanskrit - Laghududhika, Raktavindachada

Spanish - Golondrina

Unani - Dudhi khurd

#### MORPHOLOGY

A small annual herb, more or less hispidly pubescent; stems prostrate, divariately branched, slender , cylindrical , more or less hairy, forming dense mats up to 20cm. in diameter, grayish green and usually reddish purple tinged on all parts . Leaves opposite, very small, numerous, 3-6 by 2.5-4 nm., obliquely oblong or elliptic-oblong , rounded at the apex,

crenulate, glabrous above, very unequal-sided; petioles very short; stipules fimbriate. Involucres axillary, solitary or 2-3 in an axil, campanulate, 08 mm long; stalk very short; gland minute or 0; limb 0. Capsule 1.5 mm long, obtusely keeled, pubescent; styles short, 2-fid. Seeds 125 mm. long, quadrangular, bluntly pointed, with 5 or 6 transverse furrows (2)

#### ETHNOPHARMACOLOGY

Charaka prescribed Dugdihika as an ingredient of vegetable soup for diarrhoea, painful bleeding piles. The latex of dugdhukia was applied on ring worm and eruptive boils. According to Bhaavaprakasha, dugdhika is expectorant cures aggravated cough, skin disease, parasitic infection, promotes conception possesses aphrodisiac and age-sustaining properties (3).

The leaves and seeds are given in worm cases and in certain bowel affections of children in the Tamil country. In Northern India, they are considered stimulant and laxative. In Konkan, the juice is used to cure ringworm. The Santals use the root as a remedy for amenorrhoea. The expressed juice or the powdered plant is administered internally with wine as a remedy for snake-bite, and it is applied externally to the part bitten (Mhaskar & Caius) (2).

*E. thymifolia* possesses antioxidant and antiviral activities (5). The plant is commonly used as an herbal medicine. It is believed to possess Diuretic, Laxative, and Detumescent, anti-diarrheic, anti-malarial, anti rash, anti dysentery, anti carbuncle detoxification and antihemorrhoidal activity. Extract of *E. thymifolia* prepared with 1.5% HCl could inhibit the growth of Gram positive (*Bacillus subtilis*) and Gram negative (*E. coli*) bacteria (6).

Ether acetate and chloroform extract of this plant were also reported to exhibit activity against the growth of *E. coli* and *Shigella flexneri*. Studies also show that ether acetate extract of *E. thymifolia* possesses antifungal activity.

In 1982 human subjects with contact dermatitis treated with extract of this plant exhibited positive response (7). *E. thymifolia* has also shown beneficial effects when used in the treatment of Diarrhea and Dysentery (2).

#### PREVIOUSLY ISOLATED PHYTOCONSTITUENTS

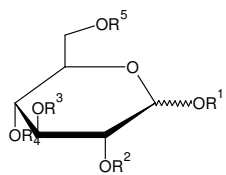
*E. thymifolia* has been thoroughly studied by various workers and a number of inactive as well as active principles belonging to different classes of natural product have been isolated. Myricyl alcohol, Taraxerol and Tirucallol have been isolated from petroleum ether (B.P. 40-60°) extract and Hentriacontane from the ethanolic extract of *E. thymifolia* (8). Several terpenes, anthocyanins, alcohols and steroids have been isolated from *E. thymifolia* Linn identified by their spectral (UV, IR, PMR) data. Whole plant petroleum Ether extract of *E. thymifolia* have 12-deoxyphorbol-13, 20-diacetate, 12-deoxy 8-OH phorbol-13-dodecanoate 20-acetate, and 1-Hexacosanol compounds (9). Epiteraxol, n-hexacosanol, euphorbol, 24-methylene cycloartenol, 12-deoxy 48-OH phorbol-13-dodecanoate 20-acetate, 12-deoxy 48-OH phorbol-13-phenylacetate-20-acetate and Quercetin-3-β-galactoside have been isolated from pet. Ether and ethanolic extract of the plant and identified by co-TLC and spectroscopic studies (10).

Chemical investigation of *Euphorbia thymifolia* has led to the isolation and characterization of new hydrolysable tannin named "Isomallotinic acid", in addition to 15 known tannins (8).

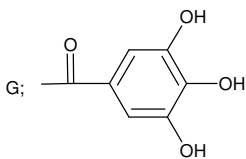
Table 1: Hydrolysable tannins present in *Euphorbia thymifolia*

Sl.No	CHEMICAL CONSTITUENTS	OPTICAL ROTATION ° [α] <sub>D</sub>
1.	2,3-di O-galloyl D-glucose[13]	+133
2.	1,2,3-tri O-galloyl B D-glucose	+286
3.	1,3,4,6-tetra O-galloyl B D-glucose[14]	+38.2
4.	1,2,3,4,6-penta O-galloyl B D-glucose[15]	+18.0
5.	2,3(s)-4,4',5',6,6' hexahydroxydiphenoyl (HHDP) D- glucose[16]	+45.3
6.	3,O galloyl 4,6(s)HHDP D glucose[17]	+40.6
7.	Pedunculagin[18]	+86.0
8.	1-desgalloyleugeniin	+100.8
9.	Eugeniin[15]	+62.2
10.	Rugosin [19]	+124.0
11.	Corilagin[16]	-203.1
12.	Geranin[20]	-147.8
13.	Bixanin	-63.9
14.	Desgalloylstachyurin[21]	-26.5
15.	Casuariin[21]	+129.3
16.	1-O-galloyl-3,6-(r)-valoneayl-B-D glucose	-37.5

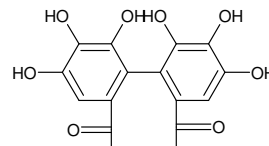
HHDP: (s)-4, 4', 5', 6, 6' hexahydroxydiphenoyl



Basic structure

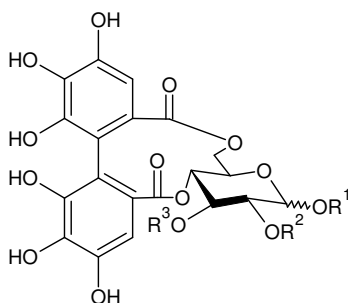


G=galloyl

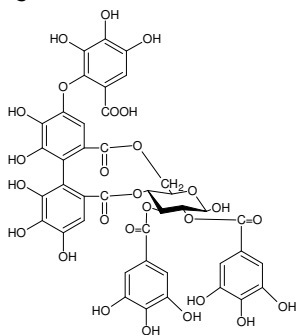


(s)HHDP

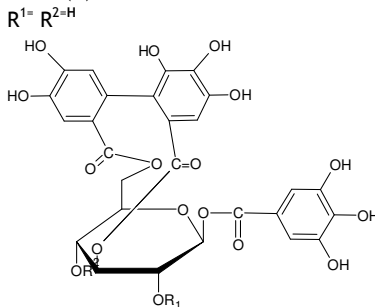
	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	R <sup>4</sup>	R <sup>5</sup>
1	2, 3-di O-galloyl D-glucose	OH	G	G	H
2	1, 2, 3-tri O-galloyl B D-glucose	(B) G	G	G	H
3	1, 3, 4, 6-tetra O-galloyl B D-glucose	(B) G	H	G	G
4	1, 2, 3, 4, 6-penta O-galloyl B D-glucose	(B) G	G	G	G
5	2, 3(s) HHDP D- glucose	OH	(S) HHDP	H	H



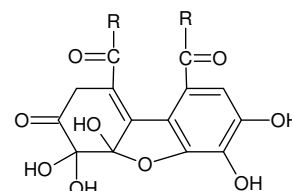
	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	
6	3, O galloyl 4, 6(s) HHDP D glucose	OH	H	G
7	Pedunculagin	OH	(S) HHDP	G
8	1-desgalloyleugenin	H	G	G
9	Eugenin	(B) G	G	G



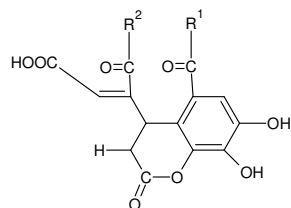
10 Rugosin B



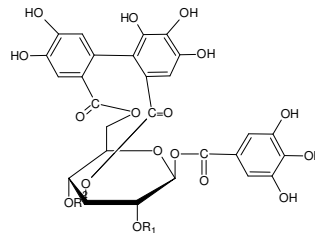
11 Corilagin



12 Geranin



13 Bixanin



14	Desgalloylstachyurin	OH	H
15	Casuariin	H	OH

Fig. 2: Hydrolysable tannins of *Euphorbia thymifolia*

## PHARMACOLOGICAL ACTIVITIES

### Antimicrobial activity

Fresh plants of *Euphorbia thymifolia* were collected. The dried powdered plants were extracted with 1.5% HCl, by soaking for about 24 hrs, Liquor ammonia was added to the extent till alkaline and precipitate separated out [A].

The precipitate was washed with water and then extracted with chloroform, till residue was free of alkaloids, then concentrated the chloroform extract at low temp. (50°C).designated as fract.'a'.After that precipitate was further extracted with amyl alcohol. The alcoholic extract was concentrated and tested for presence of alkaloids this was designated as fract.'b'.

To the decanted clear liquid [A], 2N sodium hydroxide solution was added when brown precipitate was obtained; the precipitates were allowed to stand. The upper clear liquid was decanted off and the precipitates extracted with chloroform. The chloroform extract was concentrated and designated as fraction 'b'.

Organism used for the investigation were

(1)*E.coli* (2) *B.subtilis* (3) *B.cereus* (4) *Staph .aureus*.

Solid residues of fract.'a'&'c'were dissolved in chloroform and that of fract. 'b' was dissolved in amyl alcohol .Filter papper were cut in small discs of about 0.5cm. Diameter and sterilized. Four test tubes, each containing 15 ml of sterilized nutrient agar, were taken and incubated with young culture of the test organisms and then plated. When the agar solidified , the paper discs containing 'a', 'b' and 'c' were placed carefully on the agar at 3 corners of the plate .The Petri dishes were kept in refrigerator overnight for diffusion of the incubator at 30 0 C for 12 hrs. The organism multiplied there and the action of fraction on them was noted .Formation of clear zone showed inhibition of growth and the diameter of the inhibition zone was recorded in millimeter.

So the conclusion from that zone of inhibition of the three fraction 'a' was comparatively more active (6)

### Antibacterial activity

*Euphorbia thymifolia* Linn. Was extracted with rectified spirit, and the extract partitioned between petroleum ether, chloroform, ethyl acetate and water. In vitro test of these fractions showed that 450µg/ml of ethyl acetate extract and 750µg of chloroform extract could inhibit the growth of *Escherichia coli* and *Shiglla Flexner* .Ethyl acetate extract appeared to be more potent than the chloroform extract. The ethyl acetate extract was found to be active against *Sh.flexneri* in vivo. It was observed that 1500µg of the ethyl acetate extract per day could cure 80% of infected mice. In paper disc method , of four solvent extracts of *E. thymifolia* Linn, ethyl acetate (ETE) and chloroform (ETC) extracts were found to be more active against the test organism.

*E.coli* & *sh. flexneri*. These results demonstrate that the plant contains antibacterial principles that are active against *E.coli*& *sh. Flexneri*. The active ingredient could be extracted from the plant by chloroform and ethyl acetate .The ethyl acetate extract was more potent than the chloroform extract. The minimum inhibitory concentration extract (750µg/ml) also indicates the higher potency of the former. [22]

### Antioxidant and antiviral activities

Antioxidant and antiviral activities of *E. thymifolia* (Euphorbiaceae) were investigated in this study. The result showed that all of the fractions (MeOH,CHCl<sub>3</sub>,EtOAc,n-Butanol and water )and pure compounds (3-o-galloyl 4,6-(s)-HHDP-D-glucose, Rugosin B and 1,3,4,6-tetra -O-galloyl-D-glucose) tested possessed antioxidant activities, with the exception of the organic aq. fraction in the lipid and anti-super oxide formation assays. The range of IC<sub>50</sub> of ant-lipid formation, anti-super oxide formation and free radical scavenging assays for all fractions and pure compounds were 2.81-7.63, 0.03-2.18 and 0.013-2.878 mg/ml, respectively. Electron spin resonance studies showed that water extracts and pure compound of *E.thymifolia* exhibited super oxide radical and hydroxyl radical scavenging activities. Besides antioxidant activities, 3-o-galloyl 4, and 6-(s)-HHDP-D-glucose and EtOAc fraction also showed anti-HSV-2 activity. Thus *E. thymifolia* was concluded to possess antioxidant and anti-HSV-2 activities (8).

### Anti-lipid per oxidation activity

The anti-lipid per oxidation activity of *E. thymifolia* was evaluated according to the method of Ohkawa et al (22) and Schinella et al (23).The reaction mixture,which comprised 0.25ml supernatant of liver homogenate (1mg protein),0.10ml tris-Hcl buffer (pH 7.2),0.05ml of test extract and 0.10ml of FeCl<sub>2</sub>-ascorbic acid to induce nonenzymatic lipid per oxidation for the production of malonaldehyde(MDA), was incubated at 37<sup>o</sup> for 1 hr in a capped tube.0.5ml of 0.1M HCl, 0.2ml of 9.8%SDS,0.9ml of distilled water and 0.2 ml of 0.6%TBA were then added to each tube. The tube was vigorously shaken before it was placed in a boiling water bath (100°C) for another 30 min. After cooling, 5 ml n-Butanol was added into tube and then centrifuged at 3,000 rpm for 25 min to remove flocculent precipitate. Lipid peroxide concentration was determined by HAD-TBA adduct at 532 nm using a Hitachi U-2000 spectrophotometer. To evaluate the anti-lipid per oxidation activity of *E. thymifolia*, its fractions and pure compounds were incubated together with rat liver homogenate and FeCl<sub>2</sub> ascorbic acid (8).

### Anti -super oxide radical formation

Anti-super oxide radical formation activity of *E. thymifolia* was evaluated by Spectro photometric measurement of the formation of uric acid from Xanthine / xanthine oxidase system (24, 25). The samples were first dissolved with DMSO, and then diluted to desire concentrations with PBS 50µl of sample solution, 400 µl of xanthine /PBS and 530µl of water were added into tube. The tube was then vigorously mixed and 20µl of 1-unit xanthine oxidase solution was then added .After vigorously mixing the solution was screened for 1 min at 295nm. The IC<sub>50</sub> of each sample was calculated from the regression line (8).

### Free -Radical scavenging activity

Free -Radical scavenging activity was assayed spectrophotometrically by the cytochrome C reduction method as described by McCorgand Fridovich (26) And Yu et al; (27). When xanthine oxidase converts xanthine to uric acid, the super oxide anion produced would reduce ferricytochrome C to ferrocycytochrome C. Since

ferrocytochrome C shows maximum absorption at 550nm, the super oxide anion scavenging activity of *E. thymifolia* can be evaluated using the cytochrome C reduction method. Briefly, samples were dissolved in DMSO and diluted to various conc. With PBS. 50 µl of sample solution, 400µl of working solution, 530µl of distilled water and 20µl of 1-unit xanthine oxidase solution were mixed vigorously and then screened for 1 min at 550nm. The IC<sub>50</sub> of each sample was calculated from the regression line (28, 8).

#### **Super-oxide anion and Hydroxyl radical scavenging activity**

Super oxide radical was generated from the hypoxanthine.Xanthine oxidase reaction system and trapped by DMPO. The product spin adduct (DMPO-OOH); was analyzed by using ESR spectrometer (29, 30, 31). Solutions of 2.0mHPX/PBS (A), 5.5 mM DETAPAC (B) various concentrations of test extracts of SOD (C) and 0.4 unit/ml XOD/PBS(D) were prepared before use .Solution D was stored in an ice bath to prevent any inactivation of enzyme. 50µl of A, 35µl of B, 50µl C and 15µl of DMPO were transferred into a test tube. 50µl of D was added to the mixed solution to produce DMPO-OOH.

During Hydroxyl radical scavenging studies ,1 mM ferrous sulfate(A),5.5mM DETAPAC(B),various conc. Of test extracts or ascorbic acid(C) and 1mM hydrogen peroxide(D) were prepared just before use.37.5µl of A ,37.5µl of B, 50µl of C and 20µl of DMPO were pipetted into a test tube.75µl of D was then added to the mixed solution to generate spin adduct(DMPO-OH). DMPO-OH was analyzed using an ESR spectrometer (8).

#### **Anti-viral activity (XTT assay)**

The antiviral activity of fractions and pure compounds from *E. thymifolia* was assayed using XTT (Sodium 3'-[phenylamino-carbonyl 3, 4-tetrazolium]-bis (4methoxy-6-nitro) benzene sulfonic acid) method [33]. Briefly, 10<sup>4</sup> cells/well were seeded into 96-well culture plates. After 4 hr. of incubation, the cell monolayer was infected with HSV-2 at multiplicity of infection (MOI) =0.5. Various concentrations of fractions and pure compounds were then added. The plate was incubated at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> for 2 hr. The media then aspirated and XTT reagent was added. The trays were reincubated for an additional 2hr. to allow the production of formazan. Optical densities were measured with EIA reader at a test wavelength of 492 nm and a reference wavelength of 690nm. The antiviral activity of each fraction or pure compounds from *E. thymifolia* was determined by following formula:-

$$\text{Antiviral Activity \%} = \frac{(OD_t)_{HSV} - (OD_c)_{HSV}}{(OD_c)_{Mock} - (OD_c)_{HSV}} \times 100$$

The minimum concentration of each fraction or pure compound from *E. thymifolia*, which was required to inhibit 50% of virus growth, was also calculated (8).

#### **Plaque reduction assay**

A plaque reduction assay was performed according to previously described procedures (28) with minor modification. Vero cells were seeded into 24-well culture plates at a density of 10<sup>5</sup> cells/well and incubated at 37°C with 5% CO<sub>2</sub> unit reading at least 95% confluency. The cell monolayer was

then infected with 100 PFU HSV -2 in the absence or presence of fractions and pure compounds from *E. thymifolia* and further incubated for 1 hr at 37°C with 5% CO<sub>2</sub>. After 1 hr adsorption, the cell monolayer was overlaid with overlay medium. The overlay medium was removed 2 days latter, and stained with 10% formalin and 1 % crystal violet, respectively. The anti viral activity of fractions and pure compounds from *E. thymifolia* was determined by the following formula.

$$\text{Percentage of inhibition} = \frac{(\text{No. of Plague})_{\text{tested}}}{(\text{No. of Plague})_{\text{control}}} \times 100\%$$

The minimal concentration of fractions and pure compounds from *E. thymifolia* required to reduce the plaque number by 50% (IC<sub>50</sub>) was calculated by regression analysis of the dose response curves generated from the data (33, 8).

#### **Cytotoxicity assay**

The effect of fractions or pure compounds from *E. thymifolia* against Vero cell viability was measured using XTT method (34). The cytotoxic concentration of each extract toward vero cell lines was calculated by following formula:

$$\text{Percentage of survival cell} = \frac{OD_t}{OD_c} \times 100\%$$

Where OD<sub>t</sub> and OD<sub>c</sub> indicate the absorbance of the test compounds and solvent control respectively. The 50% cytotoxic concentration, which was expressed as the concentration that achieved 50% Cytotoxicity against vero cell lines, was calculated from the regression line (8).

The ethyl acetate extract (EtOAc) and 3-O-galloyl (s)-HHDP-D-glucose of *E. thymifolia* Linn have been shown to exhibit anti-herpes simplex virus activity in vitro. In the present study, the mode of these two compounds in suppressing HSV-2 multiplication The EtOAc extract significantly reduced virus infectivity at a concentration of 4.0µg/ml, where as 3OG46HG obviously diminished virus infectivity at concentration of 5.0µg/ml. The EtOAc extract and 3OG46HG of *E. thymifolia* are concluding to inhibit HSV-2 multiplication by reducing virus infectivity (35).

The antioxidant activity of the ethanol extract of the whole plant of *E. thymifolia* Linn has been evaluated in both in vivo & in vitro experimental models, by estimating the malondialdehyde content of the lipid per oxidation. The ethanolic extract of the plant showed significant inhibition of lipid peroxidant level comparable to that vit. E used as standard (36).

#### **SUMMARY AND CONCLUSION**

In the present review we have made an attempt to congregate the botanical, phytochemical, ethnopharmacological and pharmacological information on *E. thymifolia*, a medicinal herb used in the Indian system of medicine, survey of literature revealed the presence of phenolics groups, hydrolysable tannins, flavonoids & steroids. Research on hydrolysable tannins have shown promising activities like Antioxidant, Antiviral, Anti HSV-2 activity, Cytotoxic activity, Ant lipid peroxidase activity etc. All these activities are due to presence of Galloyl moiety present in all hydrolysable tannins. Further studies are going on the plant to demonstrate more activity in plant constituents. There fore

there are many uses of the plant prescribed in Ayurveda on that base go for further studies

This review will definitely help for the researcher as well as practitioners, dealing with this plant, to know its nature proper usage.

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