

REVIEW ON CISSUS QUADRANGULARIS

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ABS TR ACT

Cissus Quadrangularis (Linn) has been used by the common man in India, and neighboring countries, Pakistan, Sri Lanka, Malaysia for promotion of fracture healing and well known as "Hadjod". It is also known as Vitis quadrangularis Wall. Belongs to family Vitaceae. It is a common perennial climber, which is distributed throughout India, particularly in tropical regions. It requires warm tropical climate and propagated by stem cuttings. The plant is prescribed in the ancient Ayurvedic literature as a general tonicand analgesic, with specific bone fracture healing properties. The plant is believed to be useful in helminthiasis, anorexia, dyspepsia, colic, flatulence, skin diseases, leprosy, hemorrhage, epilepsy, convulsion, hemoptysis, tumors, chronic ulcers, swellings. The scrutiny of the present overview revealedpharmacognostical, phytochemical and some notable pharmacological activities of the plant such as Anti-osteoporotic activity, antioxidant, free radical scavenging, antimicrobial, antibacterial, bone healing, antiulcer, analgesic and anti-inflammatory, diuretic, Anabolic and And-rogenic activity and toxicity studies.

INTRODUCTION

Cissus quadrangularis (Linn) has been used by the common man in India, and neighboring countries, Pakistan, Sri Lanka, Malaysia for promotion of fracture healing and well known as "Hadjod". It is also known as Vitis quadrangularis Wall. Belongs to family Vitaceae. It is a common perennial climber, which is distributed throughout India, particularly in tropical regions. It requires warm tropical climate and propagated by stem cuttings in the months of June and July.

Plant profile

It is a climbing herb, tendrils simple, opposite to the leaves, leafless when old. Leaves simple or lobed, cordate, broadly ovate or reniform, serrate, dentate, sometimes trifoliate and glabrous. Flowers small, greenish white, bisexual, tetramerous, in umbellate cymes, opposite to the leaves. Calyx is cup shaped. Fruit globose or obovoid fleshy berries, succulent, very acrid, dark purple to black, one seeded; seeds ellipsoid or pyriform. The stem is buff colored with a greenish tinge, dichotomously branched, sub- angular, glabrous, fibrous 'and smooth^{.[2]}

- Kingdom Plantae Plants
- Subkingdom Tracheobionta Vascular plants
- Super division Spermatophyta Seed plants
- Division Magnoliophytes Flowering plants
- Class Magnoliopsida Dicotyledons
- Subclass Rosidae
- Order Rhamnales
- Family Vitaceae Grape family
- Genus Cissus L. Treebine
- Species Cissus quadrangularis L.

Synonyms

Cissus succulent, Cissus tetragona, Vitis quadrangularis, Vitis succulent

Plant Parts Used

The whole plant used specially leaves, roots and stem.

Monographs

Bengali Names: Hadjod, Harbhanga English Names: Edible Stemmed VineSanskrit : Asthisamdhani Marathi : KandvelTamil: Perandi Scientific Name: Cissus quadrangularis L.Family: Vitaceae Duration: Perennial or annualGrowth habit: Herb







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REVIEW OF LITERATURE

- **P. Balasubramanian et al.** has evaluated the antiviral activity of olden system of ayurvedic medicinal plant Cissus quadrangularis L. (Vitaceae). Moderately purified methanolic extract of Cissus quadrangularis (belonging to Vitaceae member, South Indian medicinal plant) have been estimated for antiviral activity and their phytochemical description. In vitro antiviral activity against HSV type1 and 2, and Vero cells at non-cytotoxic concentration were detected. HSV1 and HSV2 showed additional sensitivity against the moderately purified compound. Phytochemical investigation showed the existence of the Steroids and Terpenoids^[3]
- Bhagath Kumar Potu et al. has studied that the petroleum ether extract of Cissus quadrangularis(Linn.) increases bone marrow mesenchymal stem cell proliferation and facilitates osteoblastogenesis. To estimate the efficiency of the petroleum ether extract of Cissus quadrangularis on the proliferation rate of bone marrow mesenchymal stem cells, the segregation of marrow mesenchymal stem cells into osteoblasts (osteoblastogenesis) and extracellular matrixcalcification. This research also aimed to find out the additive effect of osteogenic media and Cissus quadrangularis on proliferation, segregation and calcification. The results suggest that Cissus quadrangularis stimulates osteoblastogenesis and can be used as precautionary/substitute natural medicine for bone diseases such as osteoporosis.
- Sarath Babu K et al has estimated the antioxidant activity of Cissus quadrangularis was carriedout to calculate the antioxidant activity of flavonoid rich fraction from Cissus quadrangularis Linnon sodium perchlorate induced oxidative stress in rats. Male Albino rats were fed with 0.2% sodium perchlorate to induce oxidative stress. The flavonoid rich fraction of the plant (1mg/100gm, 2mg/100gm) was administered orally along with sodium perchlorate two groups ofanimals for 30 days. Animals showed improved antioxidant levels in serum, heart, liver, kidney compared with sodium perchlorate treated group. They suggested that a Cissus quadrangularis has potent antioxidant property if it possess more flavonoid rich fraction. Therefore, it could be used as a potential antioxidant agent in the healing of variety of diseases.
- **Krunal V et al.** have reported that the Pharmacognostical and Phytochemical Evaluation of Stemof Cissus quadrangularis L. The current exploration was therefore undertaken to verify therequisite pharmacognostic values for evaluating the plant material. Phytochemical analysisshowed the presence of many basic classes of phytoconstituents like alkaloids, flavonoids, cardiac glycosides and triterpenes. The purpose of these characteristics will aid future investigators in their pharmacological analyses of this species.
- **Bhagath Kumar Potu** et al has investigated the anti-osteoporotic activity of the petroleum etherextract of Cissus quadrangularis Linn. Was done to authenticate the anti-osteoporotic role of thepetroleum ether extract of Cissus quadrangularis on ovariectomy-induced osteoporosis in rats. The petroleum ether extract of Cissus quadrangularis stem seems to own anti-osteoporotic activity rats. On the basis of results obtained in this study, they concluded that the petroleum ether extract of Cissus quadrangularis stem seems to acquire ether extract of Cissus quadrangularis stem seems to acquire anti-osteoporotic activity in rats. The

results of biomechanical and histomorphometrical analysis of the femur bone seems to support the traditional use of this plant in bone related disorders.

- Vijayakumari P et al. has reported the studies on the Physico-Phytochemical and Anti-diabeticProperties of Cissus quadrangularis 1. and Solanum torvum The hydroalcoholic extracts of theseplants at dose levels of 200 mg/kg body weight showed promising anti-diabetic activity in the Alloxan- induced model in rats. The overall anti-diabetic activity exhibited by the extracts is found to be low as compared to standard drug Glibenclamide. The Cissus quadrangularis rhizome extract exhibited more beneficial anti-diabetic modulating effect on plasma glucose levelin Alloxan-induced diabetic rats than the fruits of Solanum torvum.
- Shah Unnati et al. has performed the pharmacognostical investigation on Cissus quadrangularisLinn revealed that it consists of β -carotene, c kaempferol and quercetin. The stem consists of twounsymmetric tetracyclic tri terpenoids, 21 β -diol and onocer 7-ene-3 β , onocer-7-ene-3 α , 21 α diol, two steroidal principles I and II, δ -amyrone, δ -amyrin, The plant is arranged in the prehistoricAyurvedic literature as a common tonic and analgesic, with definite bone fracture healing properties and the pharmacognostic analysis done on basis of microscopy, macroscopy and physicochemical parameters.
- Merinal S et al. The current research was intended to assess the antimicrobial activity of diethylether, ethanol and aqueous leaf extracts of Cissus quadrangularis L. against bacterial pathogens such as Escherichia coli, Klebsiella pneumonia, methicillin resistant Staphylococcus aureus (MRSA) and fungal pathogens such as Aspergillus flavus, Candida albicans and Fusarium solaniby in vitro agar well diffusion assay. They concluded that the ethanol extract of the plant was found to possess strong antimicrobial activity against tested pathogens.
- Deka D Ket al. has suggested that the preliminary study was undertaken to estimate the effect of methanolic extract of Cissusquadrangularis Linn on the curing process of experimentally fractured radius-ulna of dog. Cissus quadrangularis treated animals discovered faster initiation of healing process than the control animals on histopathological and radiological examination. The treated group also revealed a diminish in serum calcium level to a larger extent than the control group. Curing was almost complete on 21st day of fracture both the groups.
- Ashutosh M et al. has studied about the Indian Medicinal Plant Cissus quadrangularis Linn. an ethno botanical and ethnomedicinal assessment. The plant Cissus quadrangularis commonly known as 'Hadjodi' in Oriya belongs to family Vitaceae. The plant is medicinally important, specially stem which is used to cure various diseases in Indian traditional system of medicine particularly Ayurveda and Unani. All most all parts of the plant are utilized by tribal people. In this review, an attempt has been made to provide utmost information associated with plant Cissusquadrangularis to confirm its identity and it has been predicted that this information will be readyto lend a hand for pharmacognostical, phytochemical, pharmacological, toxicological and clinical research in near future.
- Viswanatha Swamy AHM et al. The current research reports some neuropharmacological activities of methanolic root extract of Cissus quadrangularis in mice. Results showed that the Cissus quadrangularis considerably reduced acetic acid induced writhings in mice and enhanced

in tail flick withdrawal response. The dose-dependent inhibition of acetic acid induced writhing indicated a peripheral effect, which was more effective than aspirin29. Tail flick analgesic testing is usually measured appropriate for centrally acting analgesic still clear cut dose response relationship.

- Ngo Bum1 et al. have been studied that the Sedative and anticonvulsant properties of stems of Cissus quadrangularis in mice. The aqueous extract of the stems of Cissus quadrangularis powerfully improved the entire sleep time induced by diazepam (50 mg/kg i.e.). It also confinedmice against strychnine, pentylenetetrazol, n-methyl-daspartate and maximal electroshock -induced seizures or turning behavior and delayed the onset time of seizures induced by isonicotinic hydrazid acid. The reports lead to the ending that the extract of Cissus quadrangularispossesses anticonvulsant and sedative properties in mice and could clarify its use in customary medicine in Africa, in the healing of insomnia and epilepsy.
- Sukij Panpimanmas MD et al. has reported the comparative experimental study of the efficiency and side effects of Cissus quadrangularisL. (Vitaceae) to Daflon (Servier) and placebo in the healing of acute hemorrhoids. To study efficiency and side effects of Cissus quadrangularis L. and micronised purified flavanoid fraction (MPFF) in healing of hemorrhoids. The remedial efficiency of flavanoid mixture, Cissus quadrangularis L. and placebo are not dissimilar indicating that they play no task in improving early hemorrhoidal symptoms. Long-term studies should be conducted for property in protective and therapeutic action.



PHARMACOGNOSTICAL STUDIES

1. <u>STEM</u> :-

The aqueous extract of C. quadrangularis was reported to have anticonvulsant and sedative property 7. Some other reports on justifies its effectiveness in management of obesity and complications associated with metabolic disorders 8 its antioxidant and free radical scavenging activity "in vitro" 9, 10. In currentscenario formulations now contain extracts of C. quadrangularis in combination with other activeingredients, used for the purpose of management of overweight and obesity, as well as complications resulting from these conditions, specifically metabolic syndrome (syndrome X).^[4]

• Microscopic Characters:

For microscopic studies, the leaves were cut and removed from the plant and fixed in FAA (formalin 5ml + acetic acid 5ml + 7.0% ethanol 90ml). After 24 hours of fixation, the epidermal peels and transverse sections of leaf was taken by free hand. The section was stained in safrain (1%) and mounted in glycerol.

Quantitative Microscopy:

The total number of stomata was calculated by stomatal index = No. of stomata x 100/ total no. of epidermal. The type of the stomata was recorded in the epidermal peeling. The quantitative microscopy was studies as per the procedure given by 11, 12.

• Organoleptic characters:

Organoleptic characterization of dried leaf of Cissus quadrangularis was carried out. The texture of the leaf, smell, color, taste was observed.

• Anatomical Study:

Free hand section of Stem of Cissus quadrangularis were taken, stained with Safranin and mountedin glycerol and observed under light microscope and photographed at 40x.

• Maceration:

The stems of Cissus quadrangularis were cut into small piece, boiled repeatedly in water to expelair, till the pieces settled down. Treated pieces of the plants was soaked in jeffery's fluid (equal volume of 10% of nitric acid and 10% chromic acid) for 24 hours at 30-40c, decanted washed andthen stored in 50% alcohol. Pieces of macerated stem treated with aqueous safranin overnight, dehydrated through alcohol series (50%, 60%, 70%, 80%, 90%,100%) for five minutes and passedthrough alcohol : xylol (1:1 ration) Series for five minutes. Then each material was macerated andobserved.

• Histochemical Test:

The plant section were treated with various reagent such as Wagners reagent (Potassium iodide and Iodine) for detection of alkaloid, Toludine blue 0 for lignin, ferric chloride in IN Hydrochloricacid for Tannin, Sulphuric acid for calcium oxalate Crystals, methylene blue for phenols.

• Phytochemical Screening:

The stem of Cissus quadrangularis was washed thoroughly, blotted dry and completely air dried. The dried powder was extracted with aqueous, petroleum ether and Dichloromethane. Chemical tests for various extracts were carried out according to the standard procedures.

• Powder Analysis:

The dried stem was powdered and sieved to obtain coarse powder. The powder thus obtained wasplaced on to a clean slide and observed under microscope.

• Fluorescence Analysis: The dried stem powder was placed on a slide and treating with severaldrops of specified reagent like Hydrochloric acid, Sodium hydroxide, Nitric acid, Sulphuric acid, Ferric chloride, Iodine Acetic acid, HNO3 + Ammonia, Methanol, NaOH + Methanol. The slideswere observed under UV 265 nm and 365 nm and the emitted fluorescence was observed that helps in identifying the drug in powdered sample. Fluorescence analysis has been carried out according to the method of Kokoshi et al 15.



FIGURE 1: A - Raphide in cortical tissue; B- Fibers; C- Fiber scleride; D- Rays with pits; E- Raphides of calcium oxalate crystal; F-Macroscleride; G- Stephanocytic stomata; H- Epidermal cells; I- Acicular and sand crystals (C, D, E, F, G, H, I- Powder analysis) J and K- Histochemical test for Tannin and Alkaloid.

≻ <u>LEAF</u>:-

- Macroscopy of leaf:
- Size : 10-15 cm. x 7.5-12.5 cm.
- Shape : broadly ovate
- Margin : cordate
- Venation : Palmate
- Apex : Acute
- Surface : glabrous
- Colour : Dark green adaxially, light green abaxially
- Taste : Characteristic, bitter
- Odour : None

• MICROSCOPY

Transverse section of leaf through midrib, epidermis in surface view, lamina in surface view and lamina in transverse view show following characteristic. § Leaf is having single layer of epidermis on both the surface. Upper epidermis is covered by thin cuticle. Both the epidermis show anomocytic stomata. Simple covering trichomes are very rare on both epidermises. § Transverse view of lamina shows single layer of closely pack palisade cells below upper epidermis. § Midrib show 5-7 layered thick wall closely pack collenchyma on both surface. Spongy mesophyl and vascular bundle.

Parameters Studies	Variant I	Variant II
Stomal Index		
Upper Surface	3.05-3.79-5.08	2.08-3.04-4.34
Lower Surface	3.75-5.61-6.61	2.66-3.76-6.00
Vein islet number	2.56-2.65-3.50	2.00-2.48-3.70
Vein termination number	5.16-7.71-10.9	5.0-7.18-11.70

Table 1 : Quantitative microscopical values of leaves of Cissus quadrangularis

Table 2 : QC Parameter for cissus quadrangularis

Parameters Studies	Variant I	Variant II		
Total ash value (%)	11.88 ± 0.006	10.00 ± 0.20		
Water-soluble ash (%)	6.52 ± 0.04	6.62 ± 0.11		
Acid-insoluble ash (%)	5.70 ± 0.004	3.50 ± 0.04		
Vein islet number	16.89 ± 0.04	18.33 ± 0.004		
Vein termination number	91.00 ± 0.20	87.00 ± 0.20		
Solubility (%)				
Alcohol Water	6.00 ± 0.20	5.00 ± 0.16		
	36.60 ± 0.16	26.80 ± 0.008		
Successive extract values				
Petroleum ether	2.86 ± 0.002	3.30 ± 0.12		
Benzene	1.00 ± 0.001	1.24 ± 0.001		
Chloroform	0.37 ± 0.001	0.48 ± 0.0008		
Alcohol	18.64 ± 0.001	13.02 ± 0.007		
Water	11.80 ± 0.008	24.92 ± 0.002		
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Table 3 : Behaviour of the powder for variations of C. quadrangularis with various chemical regents.

Parameters Stu <mark>dies</mark>	Variant I	Variant II
Powder as such	Yellowish green	Green
Powder + Conc. H ₂ SO ₄	Dark brown	Blackish green
Powder + Conc. HCl	Yellowish red	Reddish brown
Powder + Conc. HNO3	Yellowish red	Reddish brown
Powder + Acetic acid	Brownish green	Dark green
Powder + 10% NaOH	Yellow	Grey

Chemical reagent	Visible Light		UV Light		
	Visible I	Visible II	Visible I	Visible II	
Powder as such	Dark grey	Grey	Yellowish	Green	
			green		
Powder + 1N HCL	Yellowish green	Green	Brown	Grey	
Powder + 50% HCL	Dark green	Grey	Light brown	Grey	
Powder + 50% H2SO4	Brown	Grey	Brown	Grey	
Powder + 50% HNO3	Dark yellowish	Green	Dark brown	Yellowish brown	
	Green				

Table 4 : Flourescent behaviour of powdered roots of two variants of Cissus quadrangularis



Stomatal Number

Stomatal Number is the average number of stomata per sq.mm of the epidermis of the leaf. For the study of epidermal tissues and stomatal index, the epidermal peelings were prepared by partial maceration technique by employing Jeffery's maceration fluid. Mechanical pulling by forceps was useful forobtaining peelings. Stomatal Index Stomatal Index is the percentage which the number of stomata forms to the total number of epidermal cells, each stoma being counted as one cell.^[5]

It can be calculated by the formula.

I = S/(E+S) * 100

Where I = Stomatal Index

S = Number of stomata per unit area E = Epidermal cells in the same area.

<u>Vein islet number</u>

Vein islet number is defined as the number of vein islets per square mm of the leaf surface midway between the midrib and margin. The numbers of vein islets were calculated by counting vein islets / mm2from different pieces of lamina of different leaves.

Vein termination number

Vein termination number is defined as the number of vein terminations per square mm of the leaf surfacemidway between the midrib and margin. For the study of vein islet and vein termination number, the leafmaterial was cut into small bits and boiled in 70 % alcohol for 10 min to remove chlorophyll pigments. Then the bits were immersed in 10 % sodium hydroxide and kept in thermostat at 40°C for 2 to 3 days. The materials were then washed in distilled water, stained with safranin and mounted in glycerin forobservation and photography. Palisade ratio it is the average number of palisade cells beneath each upperepidermal cell of the leaf.

PHYTOCHEMICAL STUDIES

PHYTOCHEMICAL STUDIES:-

Qualitative Studies

Following standard protocols were used for qualitative analysis of samples to check for the presence of Alkaloids, Carbohydrates, and Cardiac glycosides, Flavonoids, Phenols, Saponins, Tannins, Terpenoids, Quinones and Proteins.

• Test for Flavonoids:

2 ml of each extract was added with few drops of 20% sodium hydroxide, formation of intenseyellow color is observed. To this, few drops of 70% dilute hydrochloric acid were added and yellow color was disappeared. Formation and disappearance of yellow color indicates the presence of flavonoids in the sample extract.

• Test for Alkaloids: To 1 ml of each extract, 1 ml of marquis reagent, 2ml of concentrated sulphuric acid and few drops of 40% formaldehyde were added and mixed, appearance of darkorange or purple colour indicates the presence of alkaloids.

• Test for Saponins:

To 2 ml of each extract, 6 ml of distilled water were added and shaken vigorously; formation of bubbles or persistent foam indicates the presence of saponins.

• Test for Tannins:

To 2 ml of each extract, 10% of alcoholic ferric chloride was added; formation of brownish blueor black colour indicates the presence of tannins.

• Test for Phenols:

To 2 ml of each extract, 2 ml of 5% aqueous ferric chloride were added; formation of blue colourindicates the presence of phenols in the sample extract.

• Test for Proteins:

To 2 ml of each extract, 1 ml of 40% sodium hydroxide and few drops of 1% copper sulphate were added; formation of violet colour indicates the presence of peptide linkage molecules in thesample extract.

• Test for Cardiac Glycosides:

To 1 ml of each extract, 0.5ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride solution were added, formation of brown ring at the interface indicates the presence of cardiacglycosides in the sample extract.

• Test for Terpenoids:

Take 1 ml of extract of each solvent and add 0.5 ml of chloroform followed by a few drops of concentrated sulphuric acid, formation of reddish brown precipitate indicates the presence of terpenoids in the extract.

• Test for Carbohydrates:

Take 1 ml of extract, add few drops of Molisch's reagent and then add 1 ml of concentrated sulphuric acid at the side of the tubes. The mixture was then allowed to stand for 2 to 3 minutes. Formation of red or dull violet colour indicates the presence of carbohydrates in the sample extract.

> Quantative Studies:-

Depending on the above qualitative results the quantitative assay is carried out for Alkaloids, Tannins, Phenols, Proteins and Carbohydrates.

• Total Tannins Content Determination:

The tannins were determined by slightly modified Folin and Ciocalteu method. Briefly, 0.5 ml ofsample extract is added with 3.75 ml of distilled water and added 0.25 ml of Folin Phenol reagent, 0.5 ml of 35% sodium carbonate solution. The absorbance was measured at 725 nm.

Tannic acid dilutions (0 to 0.5mg/ml) were used as standard solutions. The results of tannins are expressed in terms of tannic acid in mg/ml of extract.

• Total Phenol Content Determination:

The phenols were determined by slightly modified Folin and Ciocalteu method. Briefly, to the 200 μ l of the sample extract, 800 μ l of Folin Ciocalteu reagent mixture and 2 ml of 7.5% sodium carbonate added. The total content is diluted to 7 volumes with distilled water and finallykept the tubes for 2 hrs incubation in dark. The absorbance was measured at 765 nm. Gallic acid dilutions were used as standard solutions. The results of phenols are expressed in terms of Gallicacid in mg/ml of extract.

• Total Protein Content Determination:

The total proteins content was determined by using Bradford's method. Briefly, to the 100 μ l of the sample extract add 3 ml of Bradford's reagent and incubate in dark for 5 minutes. The absorbance was measured at 595nm. Bovine serum albumin dilutions (0.1mg/ml to 0.5mg/ml) are used as standard solutions.

Total Alkaloid Content Determination:

40 ml of 10% acetic acid in ethanol was added to 1g of powdered sample, covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath to get 1/4th of its original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and collected precipitate was washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

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• Total Carbohydrate determination:

For estimating the polysaccharide content, take 1ml of sample solution and add 1 ml of 5% phenol and then add 5 ml of concentrated sulphuric acid mix well and leave for 10 minutes. Measure the absorbance at 488 nm against blank. Then compare it with standard solution of glucose. To prepare blank, 1 ml of distilled water added to1 ml of 5% phenol followed by 5 mlof concentrated sulphuric acid.

Phytochemistry:-

The plant contains potassium, calcium, zinc, sodium, iron, lead, cadmium, copper, calcium oxalate and magnesium. Other constituents of the plant are resveratrol, piceatannol, pallidol, parthenocissus, 31 methyl triacontanoic acid, taraxeryl acetate, taraxerol, iso-pentadecanoic acid, phenol, tannin, carotene and vitamin. It also contains 31 methyl tritiacontanoic acid and 7 –Oxo onocer-8-ene-3 β 21 α diol. The chemical constituents of Cissus quadrangularis are shown inbelow table.

Part used		Constituents
Steam		Calcium ions and phosphorus
Steam		Calcium oxalate, 31 methyl tritiacontanoic acid, erol and iso-pentadecanoicacid
Steam	International	d β -amyrins, β -sitosterol, ketosetosterol, phenols, tannins, vitamin, carotene
Steam		Saponins and phenol
Aerial parts		7-Oxo-O <mark>noc</mark> er-8-en <mark>e-3</mark> β21αdiol
Root powder		Potassium, calcium, zinc, sodium, iron, lead, cadmium, copper and magnesium
Ash of plant		Sodium, potassium, magnesium and calcium, potassium tartrate
Leaves	Research Th	atanon, pallidol, parthenocissus, alicyclic lipids

Table - Chemical constituents of Cissus quadrangularis.

The stem of plant Cissus quadrangularis, purchased from local market of Jabalpur, were identified and authenticated by Dr. AB Tiwari, Professor and Head, Department of crop and herbal physiology, JNKVV, Jabalpur (M.P.). C. quadrangularis were collected in the month of November and dried in shade. C. quadrangularis were coarsely powdered and used for preparation of extract. The powder of Cissus quadrangularis was subjected to successive solvent extraction using sohxlet apparatus the powder was extracted with petroleum ether, benzene, chloroform, acetone, methanol, ethanol and water extract was filtered through No. 4 Whatman filter paper extract was then evaporated at 40^{-C} to dryness, and stored at

40.Cfor further use.

S.No.	Name of extract	Nature	Colour	% Yield(w/w gm)
1	Petroleum ether	Sticky	Dark green	4.8
2	Benzene	Powder	Black	4.1
3	Chloroform	Sticky	Green	3.2
4	Acetone	Sticky	Green	4.9
5	Ethanol	Sticky	Green	5.6
6	Methanol	Sticky	Green	4.4
7	Water	Sticky	Brown	5.2

Table - Nature and percentage yield of the extracts

S.No.	Tests	Petroleum Ether extract	Benzene Extract	Chlorofo rm Extract	Acetone Extract	Ethanol Extract	Aethanol Extract	Water Extract
1	Alkaloid Dragendroff's test Hager's test	present	absent	present	absent	present	absent	present
2	Carbohydrates Salvino' s test Molish test	absent	absent	absent	absent	present	absent	
3	Amino acid Millons test	absent	absent	absent	absent	present	present	
4	Tannins Aq. FeCl ₃ Gelatine test	absent	absent	present	-absent		present	
5	Flavonoids Zinc HCl test Alkaline test	absent	absent	present	present	present		
6	Protein Heat test Biuret test	- absent	absent	present	absent	present	present	
7	Saponin Foam test	absent	absent	absent	- absent	absent	present	present
8	Volatile oil	present	- absent	absent	present	- absent	absent	absent

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9	Glycoside Saponin glycoside Legal test	absent	absent	absent		absent	absent	
10	Fat and Fixed oil Copper sulphate		present	absent	absent	absent	absent	present
11	Steroid Salkwoski test	absent	0	sent	absent	absent	absent	absent



PHARMACOLOGICAL ACTIVITY

• Bone fracture healing activity:

Cissus quadrangularis (Vitaceae), a rambling shrub, characterized by a thick quadrangular fleshystout stem, commonly known as the "Bone Setter," the plant is referred to as "Asthisamdhani" inSanskrit and "Hadjod" in Hindi because of its ability to join bones. A phytogenic isolated steroidis believed to be the main constituent in Cissus quadrangularis. Studies on fracture healing

suggest that this unidentified anabolic steroid may act on estrogenic receptors of the bone. Efficacyof Cissus quadrangularis on early ossification and remodeling of bones have been reported and it has been observed that Cissus quadrangularis acts by stimulation of metabolism and increased uptake of the mineral's calcium, sulpher and strontium by the osteoblasts in fracture healing. Cissus quadrangularis is found to contain vitamins and steroids, which are found to have specific effect on bone fracture healing. The anabolic steroidal principles from Cissus quadrangularis showed a marked influence in the rate of fracture healing by influencing early regeneration of all connectivetissues involved in the healing and quicker mineralization of callus. Systemic use of Cissus quadrangularis in rats caused complete restoration of normal composition of bone, after fracture in four weeks while the controls required six weeks. There was a shortening of about two weeks in the bone healing duration. The total weight of the Garima Mishra et al /Int.J. PharmTech Res.2010,2(2) 1301 fractured bone also came down towards normal much earlier than the controlsindicating quickest bone remodeling. All the events namely fibroblastic phase (first week), collagen phase (second week) and osteochondroital phase (third and fourth weeks) were hastened by about 10 to 14 days in the treated group. This hastening in the fracture healing was attributed to the stimulation of all the cells of mesenchyma origin, namely the fibroblasts, the chondroblasts and osteoblasts by Cissus quadrangularis. It has greater impact on osteoblastic proliferation than other cellular responses. In both the models the mucopolysaccharide and collagen levels of the bones in the treated group came down to normal at the end of only four weeks while the control required 6 weeks as confirmed with histological and histochemical observations. Radioactive calcium (Ca45) studies indicated that Cissus quadrangularis causes less lowering of calcium (Ca45) uptake in the treated animals while in the control animals there was a greater decrease in the calcium (Ca45) uptake in the first week followed by a gradual increase in the subsequent weeks which reached its maximum in the 4th weeks the calcium (Ca45) uptake in the treated group cameto normal at the end of 5th week as compared to 6 - 8 weeks in controls. Thus it was concluded that Cissus quadrangularis caused less amount of tissue reaction in the fractured region leading tooptimum decalcification in the early stage with minimum of callus formations. Hence deposition of calcium was just enough to join the two broken segments of bones so that it's remodeling takesmuch faster in the treated group as compared with controls. This early completion of calcification process and earlier remodeling phenomenon lead to early recovery of animals. The tensile strengthstudies indicated much early gain in the tensile strength in Cissus quadrangularis treated group, leading to 90 percent of gain of its normal strength at the end of 6th week in comparison to 60 percent of gain in strength in the controls. Thus Cissus quadrangularis builds up the chemical

composition of the fractured bone namely its mucopolysaccharides, collagen, calcium, phosphorusand others as well as its functional efficiency. Healing of the fractured bone is delayed considerably by the administration of Cortisone. The periosteal reaction is reduced and the amountand density of callus is lowered. The mortality rate of the treated subjects is very high due to severebody wasting, atrophy of muscles and gastric perforation. Cissus quadrangularis treatment in thesecortisone treated rabbits caused a significant increase in mucopolysaccharides level and also caused proliferation of osteoblastic, chondroblastic and cartilage proliferation. It also led to increased mineralization in the callus. Thus the parenteral administration of the total extract of Cissus quadrangularis not only neutralized the anti-anabolic effect of cortisone in healing of fractures but also enhances the mineralization of cortisone possibly due toits vitamin contents. A clinical study was planned to evaluate the effect of the Cissus quadrangularis in the healing of fractures.

All the sixteen patients with various types of fractures were treated with external application of the paste prepared from the Cissus quadrangularis. This treatment was given in addition to the standard treatment of fractures, e.g. complete immobilization. As per radiological observations there sults were excellent in 6 cases with the 40% reduction in the healing time, good in 8 cases with 53% reduction in the healing time and poor in 1 case with 7% reduction in healing time. Clinically in about 80% of the cases, excellent results were observed and in the remaining 14% of the cases the results were good. Only in one case there was no demonstrable effect. In few of the treated cases although radiologically only an early callus formation was observed but clinically the symptoms of fracture such as pain, tenderness and swelling were significantly absent. It was also beeved that the injured bones surrounded by muscles showed a greater beneficial effect of this herb than those that are subcutaneous. It was hypothesized that Cissus quadrangularis helps in theearlier formation of collagen fibers leading to earlier calcification and callus formation. A study was undertaken to evaluate the effect of Cissus quadrangularis extract on the healing process of experimentally fractured radius-ulna of dog. Histopathological and radiological investigations on 11th day revealed faster initiation of the healing process and a greater decrease in serum calcium level in the treated group than the control group. On 11th day, the treated group exhibited initiation of osteogenesis, which was absent in the control group. Fracture was completely healed in 21 days in the treated group and remained incomplete in the control group. Radiograph of the treated group revealed almost complete bridging of the fractured ends with extensive bony deposition and peristoneal reaction compared to that of control group. The treated group also revealed replacement of Garima Mishra et al /Int.J. PharmTech Res.2010,2(2) 1302

cartilaginous cells by osteoblastic cells and union of the fractured gap at several places with the formation of new bony trabeculae whereas bony trabeculae were absent in the control group. Alkaline phosphates are involved in bone formation and healing of fractures. The enzyme, secretedby the osteoblasts accelerates the process of mineralization either by increasing the local concentration of inorganic phosphate or activating the collagen fibers to induce deposition of calcium salts. Cissus quadrangularis has caused an increase in alkaline phosphate levels during fracture healing in adult dogs. A study was conducted using albino rats to explore whether the beneficial effect of Cissus quadrangularis in the healing of fractures is due to its vitamin C content. The animals receiving Cissus quadrangularis showed rapid accumulation of larger quantity of mucopolysaccharides in the first week followed by more rapid fall and its earlier disappearance from the fractured area and both of these actions have beneficial effect on the healing of the fractures. At the end of the third week the Skiagram showed greater amount of calcification in theCissus quadrangularis treated group in which one could hardly see a gap at the site of the fracture, while the control and vitamin C treated group showed some gap. At the end of 5th week the unionat the fractured site was more firm in the Cissus quadrangularis treated group than the others. Earlier disappearance of mucopolysaccharides from the fractured area is associated with the earlier calcification and firmer callus formation. Mucopolysaccharides play an important role in the healing by supplying raw materials for repairs. Therefore, it seems that in the early period the greater the accumulation of these materials more rapid will be the rate of healing. In the later period when the mucopolysaccharides content decline in the fractured area is an indicative of rapidutilization of these raw materials leading to earlier completion of healing process. This effect of the Cissus quadrangularis is not due to its vitamin C content alone, since the administration of thevitamin C to normal animals did not produce such a beneficial effect. Its action is more systemic, which is responsible for the greater mobilization of mucopolysaccharides from the tissues

in to the blood and earlier utilization of the substances required in the healing process. **[55, 56]** Phosphorus (P32) is a useful isotope to study the rate of mineralization during healing of fracturessince it is readily incorporated in the area where the calcium phosphate complex is deposited during the latter part of healing. In control animals, such a mineralization process takes place at the site of fracture during the latter half of the third week. This is accompanied with the fall of mucopolysaccharides in the region. In animals treated with Cissus quadrangularis such a mineralization process takes place much earlier, roughly in the second half of the second week. Not only the healing has been faster but also the quality of the callus seems to be better in terms of the enormous deposition of the mineralization so that the callus becomes remodeled to take a normal shape of the bone. These findings further confirmed that the Cissus quadrangularis has favorable action in the rate of healing of fracture in experimental animals.

• Analgesic activity:

The analgesic effect of the drug as observed by Haffner's tail flip and Eddy's hot plate methods were dose related. There was increase in reaction time even with such small dose as 1/40 th of theLD50. The effect lasted for about 4 hrs. Cissus quadrangularis exhibited significant analgesic activity compared to that of Aspirin when tested using Haffner's clip and Eddy's hot plate methods. The extract was found to be effective by both oral and i.p. routes significantly (P<0.001) and reaction time was found to be increased by both methods. The duration of analgesic activity was from 2 to 4 hr and optimum effect was observed at 1/20th-1/10th of LD50 dose. The extract compared well with Acetylsalicylic acid. The analgesic effect of this plant when used in bone fractures may be of great value in relief of pain which is a constant feature in these cases. As it compared well with acetyl salicylic acid in its analgesic response the nature of its chemically active constituents needs to be explored.

• Antiosteoporotic activity:

Osteoporosis, a silent epidemic, has become a major health hazard in the recent years afflicting over 2000 million people worldwide. It is a chronic, progressive condition associated with micro-architectural deterioration of bone tissue that results in low bone mass. The leading cause of osteoporosis is the lack of certain hormones, particularly estrogen in women and androgen in menas well as Imbalance in the activities osteoblasts and osteoclasts cells lead to osteoporosis in postmenopausal women. In osteoporosis the bones begin to deteriorate due to calcium deficiency.

In menopause, the decrease in hormones affects the body's ability to maintain calcium levels resulting in an increased loss of minerals from the bone. Postmenopausal women are at particularrisk to Garima Mishra et al /Int.J. PharmTech Res.2010,2(2) 1303 osteoporosis because the loss of estrogen associated with the menopause leads to bone loss of much greater magnitude than expected on the basis of age alone. Treatment focuses on slowing down or stopping the mineralloss thereby preventing bone fractures and controlling the pain associated with the disease. Manysynthetic agents such as estrogens in hormone replacement therapy, selective estrogen receptor modulators like raloxifen and droloxifen, bisphosphonates and calcitonin have been developed to treat osteoporosis but each one of them is associated with side effects such as hypercalcemia, hypercalciuria, increased risk of endometrial and breast cancer, breast tenderness, menstruation, thromboembolic events, vaginal bleeding and hot flushes. Cissus quadrangularis significantlyinhibits antianabolic effects and exerts some beneficial effects on recovery of bone mineral densityin postmenopausal osteoporosis. A study was conducted in which ethanol extract of Cissusquadrangularis was evaluated for its anti-osteoporotic activity in ovariectomized rat model of osteoporosis at two different dose levels of 500 and 750mg/kg per day. Healthy female albino ratswere divided into five groups of six animals each. The first group served as control. All the remaining groups were ovariectomized. Group 2 was fed with equivolume of saline and served as ovariectomized control. Group 3-5 were orally treated with Raloxifen (5.4mg/kg) and ethanolextract of Cissus quadrangularis (500 and 750mg/kg), respectively. The findings assessed on the basis of biomechanical, biochemical and histopathological parameters showed that the ethanolextract of the plant had a definite Antiosteoporotic effect.

Antiulcer activity:

Cissus quadrangularis is an indigenous plant commonly mentioned in Ayurveda for treatment of gastric ulcers. The ulcer-protective effect of a methanolic extract of Cissus quadrangularis was comparable to that of the reference drug sucralfate. Further, gastric juice and mucosal studiesshowed that Cissus at a dose of 500 mg/kg given for 10 days significantly increased the mucosal defensive factors like mucin secretion, mucosal cell proliferation, glycoproteins and life span ofcells. The present investigation suggests that Cissus not only strengthens mucosal resistance against ulcerogens but also promotes healing by inducing cellular proliferation. Thus, Cissusquadrangularis has potential usefulness for treatment of peptic ulcer disease.

Antioxidant activity:

Extracts of Cissus quadrangularis Linn were tested for antioxidant activity by β -carotene linoleicacid model and also by 1, 1-diphenyl-2-picrylhydrazyl model. The ethyl acetate fraction of both fresh and dry stem extracts at a concentration of 100 ppm showed 64.8% antioxidant activity in the β -carotene linoleic acid system and 61.6% in the 1, 1-diphenyl-2- picrylhydrazyl systems.

Another study was performed to evaluate the effect of the methanolic extract of Cissus quadrangularis against free radical damage. The test extract exhibited significant inhibition in DPPH free radical formation, superoxide radical production and lipid peroxide production in erythrocytes. The activities of liver marker enzymes and antioxidant defense enzymes in rat liverhomogenate were assessed in control and experimental animals. Carbon tetrachloride (CCl4) caused a significant increase in aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT), alkaline phosphatase (ALP) and decrease in superoxide dismutase

(SOD), catalase (CAT), glutathione. peroxidase (GPx) and reduced glutathione (GSH), which wasreverted by Cissus quadrangularis pretreatment. The results obtained suggest that Cissus showed inhibition of lipid peroxidation, free radical production and increase in antioxidant enzymes activities, which reveal its antioxidant property. It can be concluded that the free radical scavenging activity of the plant extract may be responsible for the therapeutic action against tissue damage. The stem part of Cissus quadrangularis contains vitamin C, carotenoids, calcium, steroidal and these are known to be excellent antioxidants and numerous studies suggest that dietary intake of plant polyphenol antioxidants may have positive effects in oxidative stress related pathologies. These antioxidative constituents present in Cissus might be responsible for the free radical scavenging activity, antilipid peroxidative and ant superoxide formation.

• Parasympathomimetic activity:

Aqueous extract showed acetylcholine like activity on isolated ileum of rabbit and rat, uterus of rat, dog tracheal muscle and ileum in situ of dog. The responses on dog blood pressure were analogous to Muscarinic and Nicotinic actions of Acetylcholine. It was ineffective on frog rectusmuscle. Garima Mishra et al /Int.J. PharmTech Res.2010,2(2) 1304

Anabolic and Androgenic activity:

In addition to speeding the remodeling process of the bone, Cissus also leads to a much faster increase in bone tensile strength. In clinical trials Cissus has led to a fracture healing time in the order of 55 to 33 percent of that of controls. Cissus exerts antiglucocorticoid properties is suggested by a number of studies where bones were weakened by treatment with Cortisol and upon administration of Cissus extract the Cortisol induced weakening was halted and the healingprocess begun. Glucorticoids including the body's endogenous hormone Cortisol activate pathways that degrade not only bone, but skeletal muscle tissue as well. Glucocorticoids induce muscle breakdown. They activate the so-called Ubiquitin-Proteasome pathway of proteolysis. This pathway of tissue breakdown is important for removing damaged and non-functional proteins. By exerting an anabolic, antiglucorticoid effect Cissus preserve muscle tissue during times of physicaland emotional stress, which is of more interest to the average bodybuilder or athlete.

• Anti-inflammatory activity:

Studies have revealed that oral administration of phenidone produced marked inhibition of the paw edematous response induced by arachidonic acid injection. CQ exerted inhibitory effect on the edema formation in this animal model. It has been observed that flavonoids, several flavones, flavanols, flavanols and flavanonols are inhibitors of lipoxygenase, especially luteolin which is one of the compounds found in C. quadrangularis. In this regard, it has been previously shown thatat least flavonoid, one of the major components of C. quadrangularis, inhibits the inflammatory process. Furthermore, the anti-inflammatory activity of β sitosterol was also demonstrated which is another active constituent of C. quadrangularis. The result of the study confirmed the finding that C. quadrangularis have an inhibitory effect on edema induced by both carrageenin and arachidonic acid. Taken together, it is evident that both cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism are inhibited by C. quadrangularis. It is therefore

suggested that C. quadrangularis is a dual inhibitor of arachidonic acid metabolism. Cissus also posses antiinflammatory activity on a mg per mg basis comparable to aspirin or ibuprofen. Cissusquadrangularis constitutes one of the ingredients of an Ayurvedic preparation, 'Laksha Gogglu', which has been proved to be highly effective in relieving pain, reduction of swelling andpromoting the process of healing of the simple fractures as well as in curing the allied disorders associated with fractures. It acts by preventing the conversion of arachidonic acid to inflammatoryprostaglandins. Recently anti-inflammatory activity assay of Cissus extract was performed. Cyclooxygenase is a key enzyme in the prostaglandin biosynthetic pathway, which is important in the inflammatory process. The ability to inhibit the COX-1 activity was used to evaluate the anti-inflammatory activity of Cissus extract. The anti-inflammatory activity of the extract was expressed as the percentage of inhibition of prostaglandin synthesis using a COX-1 assay. The amount of (14C)-labelled prostaglandin synthesized was measured using a scintillation counter after removing the unmetabolized (14C)-Arachidonic acid substrate by column chromatography. The percent inhibition of prostaglandin synthesis was calculated.

• Antihemorrhoidal Activity:

As the combination of flavonoids (90% diosmin and 10% hesperidin) used clinically for thetreatment of hemorrhoid was reported to have anti-inflammatory and analgesic activities as wellas venotonic effect which is not reported previously. Phytochemical study of C. quadrangularisrevealed that its major compounds are flavonoids. The bioflavonoids, particularly diosmin,hesperidin and oligomeric proanthocyanidin complexes have demonstrated potential in thetreatment of hemorrhoids and varicose veins. These bioflavonoids exhibit phlebotonic, vasculoprotective effects and antagonistic effect on the biochemical mediators of inflammation. The anti-inflammatory effect which is already been observed from the crude extract of C.quadrangularis could be produced by the flavonoids especially luteolin, and by β -sitosterol. The venotonic effect of C. quadrangularis may also be postulated to be due to the effect of flavonoidspresent in the extract which act in the same way as that of diosmin and hesperidin. As diosmin andhesperidin are used in combination (Daflon®) to treat hemorrhoidal drug. Besides these effects, C. quadrangularis also possesses analgesic effect, which can be very useful in painful hemorrhoid. The present study proved the traditional use of C. quadrangularis as anantihemorrhoidal drug in Thai folk medicine. Garima Mishra et al /Int.J. PharmTech Res.2010,2(2)1305.

• Gastroprotective Activity:

Cissus quadrangularis is well known for the treatment of gastric disorders in traditional medicine, owing to its rich source of carotenoids, triterpenoids and ascorbic acid, and has received considerable attention regarding its role in human nutrition. A number of studies have analyzed and revealed the effect against gastric toxicity and the gastroprotective effect of Cissus quadrangularis extract (CQE) along with its mechanism underlying the therapeutic action against gastric mucosal damage induced by aspirin. The studies have investigated the effect of CQE on the course of experimentally induced gastric ulcer by analyzing the levels of tumor necrosis factor- α (TNF- α), interleukines, microvascular permeability, activity of nitric oxide synthase-2 (NOS-2), mitochondrial antioxidants, lipid peroxidation and DNA damage. The investigational findings have shown significant increase in vascular permeability, NOS-2 activity. of TNF- α and

interleukines were monitored and oxidative damage were noted in aspirin administered rats. The optimum protective dose of 500 mg/kg of extract was given for the pretreatment of gastric ulcers with different doses of CQE (250, 500 and 750 mg/kg) for 7 days which significantly attenuated these biochemical changes caused by aspirin in rats. **[69, 70]** The results showed ulcer protectionby 40, 71.2 and 72.6%, respectively, as compared to ranitidine (RTD) (30 mg/kg) by 71.9% in theaspirin model. In addition, the findings of the studies have shown that administration of aspirin increases lipid peroxidation status, xanthine oxidase (XO), myeloperoxidase and decrease in superoxide dismutase (SOD), catalase (CAT) and selenium–glutathione peroxidase activities in the gastric mucosa, resulting in mucosal damage at both cellular and subcellular level which werereversed by CQE In addition, CQE prevents oxidative damage of DNA by reducing DNA fragmentation indicating its block on cell death. Pretreatment with CQE ameliorated the observed effect significantly in the gastric mucosa of ulcerated rats. Ulcer protection in CQE treated rats was also confirmed by histoarchitecture, which was comprised of reduced size of ulcer crater andrestoration of mucosal epithelium. findings suggest that the gastroprotective activity of CQE couldbe mediated possibly through its antioxidant, antiapoptotic effects as well as by the attenuation of the oxidative mechanism and neutrophil infiltration.

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THERAPEUTIC USES

The stout, fleshy quadrangular stem is traditionally used for the treatment of gastritis, bone fractures, ski infections, constipations, eye diseases, piles, anemia, asthma, irregular menstruation, burns and wounds. Th leaves and young shoots are powerful alteratives. is administered in treatment of hemorrhoid andcertain bowl infections. The juice of stem is useful in scurvy and in irregular menstruation whereas thestem paste boiled in lime water is given in asthma. It I also used as a powerful stomachic. quadrangularis Linn. has potent fracture healin property and antimicrobial, antiulcer, antioxidative, antiosteoporotic, gastroprotective, cholinergic activate as well as beneficial effects on cardiovasculardiseases. It has been investigated that methanolic extract of Cissus quadrangularis possesses antiulcer and cytoprotective property in indomethacin induced gastric mucosal injury. The aqueous extract also showsacetylcholine like activity on isolated ileum of rabbit and uterus of rat. The action on dog blood pressure analogous to muscarinic and nicotinic actions of acetylcholine. Ethyl acetate fractions of both fresh anddry stem extracts show antioxidant activity. The ethyl acetate extract and methanol extract of both fresh and dry stem further exhibit antimicrobial activity against gram-positive bacteria including Bacillussubtilis, Bacillus cereus, Staphylococcus aureus and Streptococcus species. Due to its widespread andversatile therapeutic uses as well as pharmacological actions, the present study highlighted the health promoting and therapeutic properties of Cissus quadrangular.^[8]

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CONC LUSION

The above discussion shows that cissus quadrangularis is an important plant and it is used in different activity such as Bone fracture healing activity, analgesic activity, antiosteoporotic activity, antiulcer activity antioxidant activity, parasympathomimetic activity, anabolic & androgenic activity, anti __inflammatory activity, antihememorrhoidal activity, gastroprotective activity. The plant shows the presence of many chemical constituent which are responsible for various pharmacological medicinal properties.



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