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EVALUATION OF *CISSUS VITIGINEA* (KUNDUPIRANDAI) MUCILAGE AS SUSPENDING AGENT

Bindu Reddy S*, Ramya K, Ravindra Reddy K

*Department of Pharmaceutics, PRRM College of Pharmacy,
Prakruthi Nagar, Utukur, Nagarajpeta, Kadapa Dist, Andhra Pradesh, India-516003.

ABSTRACT

The present study was undertaken to evaluate the mucilage isolated from *Cissus vitiginea*, commonly known as 'Virginia snakeroot' (Family- Vitaceae) as an innovative suspending agent. The purpose of this study is to search for a natural excipient which is cost effective that can be used as an alternative for the formulation of pharmaceutical suspensions. Characterization studies like solubility, swelling index, loss on drying, ash value, pH, viscosity along with microbial load and acute toxicity studies were carried out on the mucilage. Aluminium hydroxide gel suspension was prepared using different concentration of *Cissus vitiginea* mucilage and its properties were compared with standard suspending agents like sodium carboxymethylcellulose and Acacia. The evaluation parameters included the sedimentation profile, redispersability, rheology and particle size analysis. The results suggested that the mucilage was found to be a superior suspending agent than acacia indicating that it may be a good source as pharmaceutical adjuvant.

Key Words:- *Cissus vitiginea*, Mucilage, Suspending agent.

INTRODUCTION

Mucilages are polysaccharide macro molecules that dissolve more or less upon contact with water and form colloidal solutions (Bruneton *et al*., 1999). Mucilages and gums are well known since ancient times for their medicinal value. In recent years, plant gums and mucilages have evoked tremendous interest due to their diverse application in pharmacy in the formulation of both solid and liquid dosage forms as thickeners, water retention agents, and emulsion stabilizers, suspending agents, binders and film formers (Anroop *et al*., 2005). Apart from its use in finished medicines, newer uses have been found in the preparation of cosmetics, textiles and paint paper. Hence the demand for these substances is increasing and new sources are getting tapped. Vast application of plant mucilages and gums in various industries is because of low cost, ready availability and important properties which they confer on products. With the increase in demand for natural mucilages, it has become necessary to explore the newer sources of mucilage to meet the industrial demands (Bummer *et al*., 2005). A pharmaceutical suspension may be defined as coarse dispersion containing finely divided insoluble material suspended in a liquid medium (Martin *et al*., 1993). Most pharmaceutical suspensions, are thermodynamically unstable, thus, making it necessary to include in the dosage form, a stabilizer or suspending agent which reduces the rate of settling and permits easy redispersion of any settled particulate matter both by protective colloidal action and by increasing the consistency of the suspending medium (Banker and Rhodes 1998).

Corresponding Author

Bindu Reddy S
Email:- reddy.bindu639@gmail.com

Suspending agents are
(i) Inorganic materials,
(ii) Synthetic compounds
(iii) Polysaccharides

Natural gums like Acacia, Tragacanth, karaya and *Cissus vitiginea* mucilage belong to the latter group. *Cissus vitiginea* is medicinally important; specially stem which is used to cure various diseases in Indian traditional system of medicine particularly Ayurveda and Unani (Trease and Evans 2008). All most all parts of the plant are utilized by tribal people (Ayurvedic Pharmacopoeia 2008). It is useful in piles, bone fracture, pain in joints, swelling and asthma (Chopra *et al*., 1992). Stem juice is used for the treatment of Scurvy, irregular menstruation, disease of ear and nose-bleeding (Kirtikar *et al*., 1992). Stem paste is also useful in bone fracture, swelling, muscular pain, asthma, burns, wounds and bites of poisonous insects (Kirtikar *et al* 2007).

MATERIALS AND METHODS

The materials used include Aluminium hydroxide gel, Acacia, Sodium carboxymethylcellulose, Sorbitol. All the chemicals used were of analytical grade. The stems of *Cissus vitiginea* were collected from Tirupati foot hills, Tirupati. It was authenticated by Prof. Madhava Chetty, and a Voucher specimen was assigned.

Extraction of Mucilage

The mucilage was isolated from freshly, dried and coarsely powdered stems of *Cissus vitiginea*. The materials were homogenized with water in the ratio of 1:7 and kept aside for 8 hrs to release the mucilage into water. The material was filtered through muslin cloth and the filtrate centrifuged at 5000 rpm for 10 minutes to obtain clear viscous solution. Mucilage was precipitated from water using acetone. Precipitated mucilage was dried in a vacuum oven at a temperature of 45^0C and passed through sieve no.80 and stored in desiccator.

Phytochemical Examination

Preliminary test namely ruthenium red test, Molisch test, test for the reducing sugars was performed to confirm the nature of mucilage obtained (Sepulveda *et al*., 2007).

Acute Toxicity Study

The *Cissus vitiginea* mucilage was subjected to acute oral toxicity studies in rats according to OECD guidelines (no.423) to evaluate its toxicity and median lethal dose (LD50). The albino rats of wistar strain (160-200 gms, either sex) were taken and a dose of 2000 mg/kg, p.o. was administered to them. Test and control rats were observed for behavioral changes, toxicity and mortality up to 48 hrs. Permission from Animal Ethics Committee was obtained for the studies done (Ghosh 1984; Shetty 2004).

Physicochemical Characterization of the Mucilage

The separated mucilage was evaluated for solubility, swelling index, loss on drying, ash value, pH, viscosity, microbial load, density, compressibility index and angle of repose (Indian Pharmacopoeia 2007).

Preparation of Suspensions

4gm of Aluminium hydroxide gel was levigated with glycerin (2gm) and dispersed in 10ml of water. Hydrated suspending agent was added gradually to the dispersed aluminium hydroxide gel, triturated well and finally the volume was made upto 100ml with distilled water. The formulation also contains Sorbitol at 7% concentration, Methyl paraben (0.1%) as preservative and peppermint oil as flavoring agent. This procedure was used for the preparation of suspensions using different concentration of suspending agents' viz. Curculigo orchioidesmucilage, Acacia and sodium CMC in the concentration 0.5%, 1% and 2.0%w/v. The suspensions were stored in stoppered glass bottles. All the prepared suspensions were deflocculated to determine the degree of flocculation, flocculated suspensions were made using magnesium aluminium silicate (0.04mol) as flocculating agent (Lachman *et al*., 2007).

Evaluation of Suspensions

i. Sedimentation volume

The sedimentation volume is ratio of the ultimate height (Hu) of the sediment to the initial height (Ho) of the total suspension before settling. Each suspension (100 ml) was stored in a 100 ml measuring cylinder for 45 days at room temperature. The sedimentation volume of different suspensions was calculated by the equation

$$F = Hu / Ho$$

Where F is the sedimentation volume.

ii. Redispersibility

Suspension produces a sediment on storage, it is essential that it should be readily dispersible so that uniformity of the dose is assured. The method essentially consisted of holding the sample tube straight in upright position between two fingers with thumb at the bottom and the middle finger at the top, followed by almost uniform rotation through 180˚and brought back to same path. The pair of successive upward and down ward movement each of approximately equal force, constituted one complete shake. The number of shakes required for

complete elimination of sediment from the bottom of the tube was recorded (Mann *et al*., 2007).

iii. Rheology

The time required for each suspension sample to flow through a 10 ml pipette was determined and the apparent viscosity (ηαin ml-1) was calculated using the equation

Flow rate = ηα= Volume of pipette (ml)/ Flow time (seconds)

The viscosity (in poise) of the samples was determined at 25^0C using Brookfield dial viscometer (model-RVT) at 50 rpm using spindle no. 3 for 1 minute. The dial readings were recorded and experiment was repeated for three times and the results obtained were expressed as the mean values.

iv. pH

The pH of the suspensions were determined at intervals of one week for 21 days using pH meter (Elico- LI 127)

v. Particle size analysis

It was done by microscopy using Eye piece micrometer which was calibrated using stage micrometer. A slide of above suspension was prepared, placed under microscope and size of the particles was measured using ocular micrometer in terms of eye piece divisions. The number of eyepiece divisions when multiplied with the calibrated value gave the diameter of the particle which was further classified into size ranges and the frequency of particles was arranged in terms of number distributions (Jafar *et al*., 2007).

vi. Degree of Flocculation

The degree of flocculation was determined using the equation

$$\beta = F/F\infty$$

Where, F is ultimate sedimentation volume in flocculated suspension. F∞ is ultimate sedimentation volume in deflocculated suspension. The sedimentation volume gives only a qualitative account of flocculation since it lacks a meaningful reference point. The degree of flocculation is more fundamental parameter than F since it relates the volume of flocculated sediment to that in a deflocculated system (Kumar *et al*., 2007).

RESULTS AND DISCUSSION

The average yield of dried *Cissus vitiginea* mucilage obtained was found to be 1.3 %. The mucilage obtained was subjected to physicochemical characterization, the results of which, is summarized in table 1. Phytochemical tests carried out on *Cissus vitiginea* mucilage confirmed the absence of alkaloids, glycosides and tannins. On treatment of mucilage with ruthenium red, it showed red colour confirming the obtained product as mucilage. A positive test with Molisch's reagent and red colour with Fehling's solution after hydrolysis was obtained. Acute toxicity study of mucilage showed no manifestations of toxic syndromes.

Table 1. Results of physicochemical characterization of mucilage

Test	*Cissus vitiginea*
Solubility	Slightly soluble in water, practically insoluble in ethanol, acetone, ether and chloroform.
Swelling index (ml) In 0.1 N Hydrochloric acid In phosphate buffer pH 7.4 In distilled water	 3.2 ± .310 3.1 ± 0.020 3.4± 0.264
Loss on drying (% w/w)	8.12 ± 0.234
pH of 1% solution	6.24 ± 0.021
Viscosity of 1% solution (cp)	1.54 ± 0.016
Total ash (% w/w)	8 ± 0.110
Acid insoluble ash (% w/w)	1.1 ± 0.02
Bulk density (gm/ml)	0.600 ± 0.009
Tapped density (gm/ml)	0.723 ± 0.00
Compressibility index (%)	15.44± 0.263
Hausner ratio	1.39 ± 0.11
Angle of repose (in degrees)	24.17 ± 0.321

Table 2. Determination of flow rate and viscosity of suspension

Suspending agent	Concentration (%w/v)	Flow rate ml/sec	Viscosity (Poise)
Acacia	0.5	1.55	0.55
	1.0	1.37	0.70
	2.0	1.00	0.90
Na CMC	0.5	1.00	0.95
	1.0	0.83	1.30
	2.0	0.67	1.45
Cissus vitiginea	0.5	1.14	0.71
	1.0	1.05	0.91
	2.0	0.95	1.10

Table 3. Determination of sedimentation volume (%) using different concentration of suspending agents

Time	Acacia			Sodium CMC			*Cissus vitiginea*		
	0.5%	1%	2.0%	0.5%	1%	2%	0.5%	1%	2.0%
1/2hr	100	100	100	100	100	100	100	100	100
1st day	88	90	94	92	96	98	91	92	94
2nd day	80	84	87	88	93	96	86	89	92
3rd day	74	80	84	85	91	95	81	85	89
4th day	69	75	80	83	90	93	77	81	87
5th day	65	71	77	81	88	93	74	78	85
6th day	62	69	75	80	87	91	72	75	83
7th day	60	67	73	79	86	90	70	73	82
14th day	55	62	69	76	82	88	66	70	80
21st day	52	57	66	74	80	85	63	69	77
45th day	30	34	40	59	64	72	45	52	74

Table 4. Determination of redispersibility and pH

Suspending agent	Concentration	Rate of redispersibility (cycles)			pH after storage for			
		5 Days	15 Days	25 Days	0th Day	7th Day	14th Day	21st Day
Acacia	0.5	7	9	11	6.15	6.10	6.03	6.00
	1.0	6	8	9	6.20	6.16	6.09	6.04
	2.0	5	6	7	6.34	6.28	6.20	6.15
Sodium CMC	0.5	6	8	9	7.32	7.25	7.14	7.10
	1.0	5	6	8	7.48	7.42	7.33	7.26
	2.0	4	5	7	7.60	7.51	7.42	7.35
Cissus vitiginea	0.5	7	8	9	6.80	6.70	6.66	6.60
	1.0	6	7	7	6.89	6.85	6.78	6.71
	2.0	5	5	6	7.06	7.00	6.95	6.92

Table 5. Degree of flocculation of various suspending agent

Concentration (%w/v)	Concentration (%w/v)		
	Acacia	Sodium CMC	*Cissus vitiginea*
0.5	1.066	1.101	1.07
1	1.088	1.110	1.096
2	1.10	1.152	1.11

The *Cissus vitiginea* mucilage was found to be safe (LD_{50} >2000 mg /kg, p.o). To evaluate the suspending properties of the mucilage, an Aluminium hydroxide suspension formulation was prepared in different batches containing the suspending agent: either *Cissus vitiginea* mucilage, Na CMC or Acacia in the concentration range of 0.5 to 2% w/v. The preparations were evaluated based on their sedimentation profile, redispersability, rheology, pH and particle size analysis. The sedimentation volume and viscosity of the suspensions increased with increasing concentration of the suspending agent (Table 2 and 3). The reverse was the case for flow rate (Table 2).

As observed in table 3, the sedimentation volume of 2% *Cissus vitiginea* mucilage at the end of 45 days was comparable with 1% sodium CMC at a value of 64%. Since the suspension produces sediment on storage it must be readily dispersible so as to ensure the uniformity of the dose. If sediment remains even after shaking vigorously for specified time, the system is described as caked. The redispersion ability of the suspendants (Table 4) was found to be uniform with respect to the entire suspending agent. The pH of all the suspensions was found to be between 6- 7.6 .The particle size of the suspensions was in range of 9-15μm. Sedimentation volume provides only a qualitative account of flocculation.

The degree of flocculation (β) is more useful parameter, which is the ratio of ultimate sedimentation volume in the flocculated and deflocculated system. The degree of flocculation for 2% *Cissus vitiginea* mucilage was found to be 1.11 which was comparable with Acacia (Table 5). The suspensions prepared with *Cissus vitiginea* mucilage had good sedimentation volume, redispersibility abilities, viscosity and flow rate when compared with suspensions prepared with Na CMC and Acacia as standard. Thus, it can be concluded that the extracted mucilage from roots of *Cissus vitiginea* the potential of a suspending agent and it can be used as a pharmaceutical adjuvant.

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