

**FORMULATION AND EVALUATION OF ANTI-INFLAMMATORY
HERBAL TOPICAL FORMULATION OF *CISSUS QUADRANGULARIS*
L.**

**Biresh Kumar Sarkar*¹, Ravi Kumar¹, Prince Kumar¹, Palash Mandal¹, Vidya Bhusan
Pal¹, M. Devgan²**

¹National Institute of Ayurvedic Pharmaceutical Research, Patiala, India.

²R.P. Educational Trust Group of Institutions, Karnal, Haryana, India.

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***Correspondence for
Author**

Dr. Biresh Kumar Sarkar
National Institute of
Ayurvedic Pharmaceutical
Research, Patiala, India.

ABSTRACT

Herbal medicine now has been gained global acceptance due to their therapeutic values. Many researchers investigated herbal formulations recently to explore their medicinal value as well as quality compliances. Considering these facts present study involves formulation and evaluation of topical formulation of *Cissus quadrangularis*. Dispersion method was used for the preparation of formulation with varying ratio of ingredients. The various instrumental techniques were used for drug analysis like; Ultraviolet Spectroscopy, Fourier-transform Infrared Spectroscopy and HPTLC etc. The formulation was also evaluated for various quality parameters like; pH, viscosity, spreadability, *in vitro* drug release, etc. The formulations C2 was found to possess satisfactory results in terms of quality parameters like; viscosity, spreadability and *in vitro* drug release. FT-IR studies suggested no chemical interactions between the drugs and ingredient.

KEYWORDS: *Cissus quadrangularis*, herbal medicine, topical formulation, quality control.

INTRODUCTION

Inflammation is a major problem since ancient times affecting older population specially. The joints are most susceptible towards the inflammation. Inflammation may occur due to the consequences of various diseases; there are many diseases through which inflammation associated as a major symptom.^[1] Herbal drugs are becoming popular day by day for their

therapeutic values with less toxic effects. Plants and their derived material have been widely employed as medicine for the prevention and treatment of diseases anciently. The acceptance towards herbal medicine increases day by day due to their patient compliances and safety profile. The development cost of herbal medicine also low which increases economic benefits.^[2] Recently medicinal plants are extensively explored scientifically for their therapeutic and safety benefits. The medicinal values of plant material may be attributed to the chemical constituent present in them.^[3] The drug development through medicinal plants involves isolation and identification of active constituent. The identification and isolation of plant material utilizes various analytical techniques including spectroscopy like; Ultraviolet & Infrared Spectroscopy and chromatographic techniques like; HPLC & HPTLC. The choice of technique depends largely on the solubility properties and volatilities of the compounds to be separated. The complexity of plant extracts can be simplified by isolating and separating them using different techniques based on their solubility pattern.^[4] *Cissus quadrangularis* belongs to the family *Vitaceae*, an edible plant found in India, Malaya, Java, West Africa and also found throughout Thailand.^[5] It is commonly known as “bone setter”; help in bone empowerment and also reduces pain, the plant is also referred as “*Hadjod*” because of its ability to join bones.^[6] Traditionally plant has been reported to possess various medicinal uses in gout, piles, tumors, peptic ulcers and leucorrhoea.^[7] Looking towards the importance of plant; the aim of the present study was to prepare and evaluated topical herbal formulation of *Cissus quadrangularis* as anti-inflammatory agent especially in gout to support traditional claim of plant.

MATERIALS AND METHOD

The plant material *Cissus quadrangularis* was collected then shade dried, homogenized to fine powder and stored in airtight bottles. All chemicals and reagents used were of analytical grade.

Extraction

Dried powder of plant material was extracted methanol using Soxhlet apparatus. The extract was poured into evaporating dishes and the solvent was evaporated under reduced pressure.

Identification Tests

Identification tests such as Ultraviolet (UV) spectroscopy, Fourier Transform Infrared (FT-IR) spectroscopy and HPTLC were conducted.^[8]

Ultraviolet visible absorption (UV)

The methanol extract of plant was analyzed in UV-Visible range between 200-780 nm using UV-Visible Spectrophotometer (UV-1800, Shimadzu).

Infra-red spectroscopy (IR)

This technique is based on the fact that chemical substance shows specific absorption in infrared region. Under the influence of IR radiations the molecules shows various mode of vibration, which gives different absorption spectrum selective to the functional group present in molecule. The spectrum of compound is unique feature of molecular framework. The IR spectra of methanol extract of *Cissus quadrangularis* stem were scanned on FT-IR spectrophotometer over the frequency range from 4000-400 cm^{-1} .

HPTLC fingerprinting

HPTLC systems equipped with Linomat-5 applicator with WinCATS-4 software were used. All the solvents used for HPTLC analysis were of selective grade. Extract was dissolved in methanol and used for HPTLC analysis. The samples (10 μl) spotted in the bands of width 8 mm with a Camag microlitre syringe on pre-coated silica gel glass plate. The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase and the plate was developed up to optimum level. The Toluene: Ethyl acetate: Glacial acetic acid (75:24:01) were employed as mobile phase for separation. Linear ascending development was carried out in glass chamber saturated with the mobile phase. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was scanned by UV 366 nm. Densitometric scanning was performed on Camag scanner and operated by CATS software.

Topical Formulation of *Cissus quadrangularis*

Gel was formulated by dispersion method. Aerosil was dispersed in sesame oil using mechanical stirrer and stand for a day. Then BHT & BHA were added as per requirement. Then plant extract was added to the formulation with continuous stirring; that after mixture of remaining ingredients were added properly to the gel formulation drop wise with continuous stirring along with tri-ethanolamine. Finally gel like consistency was obtained.^[9]

Evaluation of Topical Formulation^[10, 11]

Physical Evaluation: The colour & appearance of the prepared herbal gel formulations were observed physically.

pH measurement: The pH of the gel was determined using digital pH meter. Gel formulation was dissolved in water and pH was determined by dipping the glass electrode completely into gel solution. Then instrument reading was recorded as a pH of solution.

Spreadability

It was determined by wooden block. Spreadability was measured on the basis of slip and drag characteristics of gels. An excess of gel was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide. The upper slide was then pulled apart horizontally with a string, then weight was applied. The time required separating the two slides was measured as spreadability using following formulae

$$S = M \times L / T$$

Where,

S = Spreadability

M = Weight in the pan (tied to the upper slide)

L = Length moved by the glass slide

T = Time (in sec.) taken to separate the slide completely each other.

Homogeneity

Gel formulations were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

Viscosity

Viscosity was measured by Brookfield Viscometer which measures the shearing stress on a spindle rotating at a definite, constant speed while immersed in the sample.

In-vitro Drug Release Study

The dissolution studies were performed USP rotating basket method in 6.8 phosphate buffer solution. The compartment containing 900 ml of 6.8 phosphate buffer solution. The sample of 5 ml each was withdrawn at predetermined time interval and were replenished immediately with the same volume of phosphate buffer maintaining sink condition throughout the experiment. The aliquots following suitable dilution with phosphate buffer were analyzed spectrophotometrically at 262 nm. The concentrations of test samples were calculated using regression equation.

RESULTS AND DISCUSSION

Table 1: The % compositions of formulations

Ingredients	C1	C2	C3
Plant extract	25	25	25
Methyl salicylate	15	14	13
Menthol	7	6	5
Aerosil	6	5	4
BHA	0.5		
BHT			
Sesame oil	Q.S.		

Table 2: Results of quality control evaluation

Quality Parameters	Results		
	C1	C2	C3
Appearance	Translucent		
pH	5.85	6.8	5.5
Homogeneity	Homogenous		
Spreadability (g.cm/sec)	26.04	22.73	24.86
Viscosity (cps)	10814	9476	8151

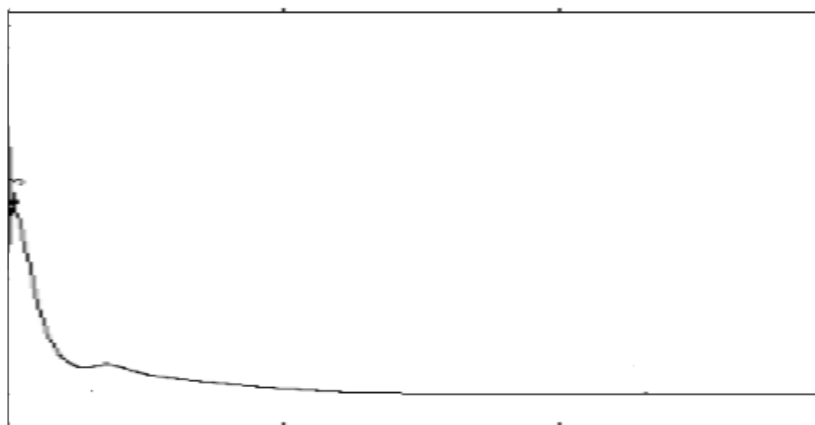


Figure 1. UV spectra.

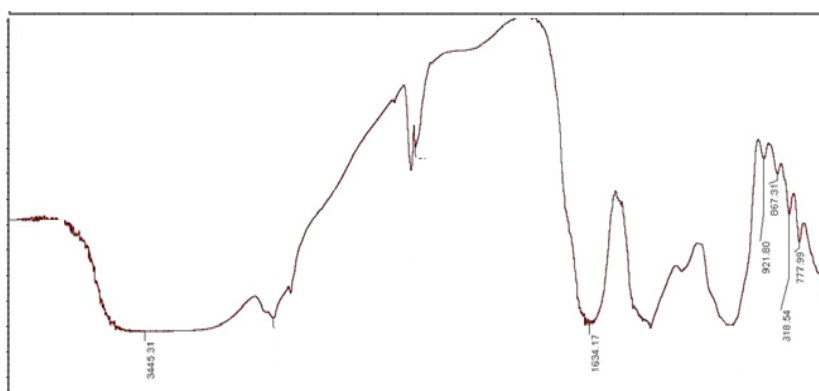


Figure 2. IR spectra.



Figure 3. HPTLC chromatogram.

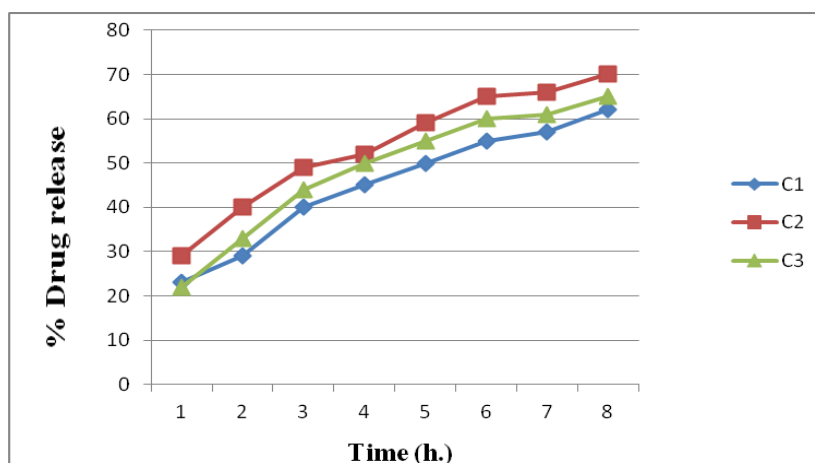


Figure 4. Comparative drug release profile of various formulations.

Identification

Ultraviolet visible absorption (UV)

UV-Visible spectra of extract are shown in **Fig. 1**. The UV spectrum of *Cissus quadrangularis* showed absorption maxima at 663, 275, 204 and 255 nm.

IR spectroscopy

IR spectra of plant extract are shown in **Fig. 2** the infrared region approximately 4000–400 cm^{-1} was used to study the different modes of vibration spectrum. Study confirmed characteristic peaks of plant material.

HPTLC fingerprinting

High Performance Thin Layer Chromatography (HPTLC) is one of the efficient techniques for separation and identification of plant material which gives better precision and accuracy.

The HPTLC fingerprinting of study are shown in **Fig. 3**. There are various characteristics peaks were observed in HPTLC fingerprint of extract.

Formulation and Evaluation

The topical formulations of *Cissus quadrangularis* were prepared using different combinations of ingredients as per **Table 1** and evaluated for various quality control parameters. The results of quality parameters are summarized in **Table 2**.

Evaluation of Gel

Physical Evaluation

The colour and appearance of the prepared herbal gel formulations was observed physically and it was found to be characteristic of gel formulation.

pH measurement

The pH of the gel was determined by using a digital pH meter. The pH of formulations were found to be optimum for skin application.

Spreadability

It was determined by wooden block which was provided by a pulley at one and spreadability was found to be 22.73 g.cm/sec for formulation C2.

Homogeneity

All developed gels were tested for homogeneity by visual inspection and all formulations were found to be homogenous.

Viscosity

Viscosity was measured by Brookfield Viscometer which measures the shearing stress on a spindle rotating at a definite, constant speed while immersed in the sample and it was found to be 9476 cps for formulation C2.

In-vitro Drug Release Study

The dissolution studies were using the USP rotating basket method in 6.8 phosphate buffer. The **Fig 4** suggested that the formulation C2 acquire satisfactory topical drug release profile as compared to other formulation. Study also confirmed that the ratio of ingredients affect release profile greatly.

CONCLUSION

The present study revealed that the optimized herbal formulation C2 consisting of plant extract shows comparatively better result than other formulations. FT-IR study revealed that there is no possible drug interaction with other components present in extract and *in vitro* drug release study shows that C2 releases the drug with desired profile. The release of formulation increased when the concentration of aerosil decreased. Thus the study concluded that the formulation containing *Cissus quadrangularis* extract may possess efficient anti-inflammatory profile.

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