

Extraction and isolation of Ketosterone from *Cissus quadrangularis*

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Abstract

Cissus quadrangularis, an important medicinal plant belonging to the family of Vitaceae, which has been valued for centuries in Ayurvedic medicine used as anthelmintic, laxative, chronic ulcer, analgesics and in Bone healing activity. Different extract of *Cissus quadrangularis* (water, Methanol (90%) & Ethyl acetate) were prepared and tested of ketosterone. The maximum ketosterone content was observed in 90% methanolic extract followed by ethyl acetate and water extracts. It was 6.2%, 2.46% and 0.10% in 90% methanol, ethyl acetate and water extract respectively. The physiochemical properties such as loss on drying (LOD), Ash value and pH of hadjod ext were carried out. The determination of these characteristics will aid future investigators in their pharmacological analysis of this species. The different qualitative chemical test were performed on the extract to detect the various constituents. The Phytochemical screening reveals the presence of many therapeutically important compounds such as ketosterone, phenol and flavaonoid. *Cissus quadrangularis* has been used by common man in india for promotion of fracture healing and well known as “Hadjod”.

Key Words: *Cissus quadrangularis*, Ketosterone, Phytochemical screening

Introduction

Many Pharmaceutical companies continue to invest enormous resources to identify agents that could be used to fracture healing and Bone setter deterioration afflicting numerous people around the world. Therefore several plants have been selected based on their use in traditional systems of medicine. This plant that has been used as fracture healing condition is *Cissus quadrangularis*, family vitaceae, used by Ayurvedic medicinal practitioners in India for almost 2000 Years and is classified as a medhyarasayana, a drug used to improve body pain and join bone.

Cissus quadrangularis – the plant

Cissus quadrangularis Linn.(Syn. *Vitis quadrangularis* L.) is belongs to vitaceae family. It is referred as “Asthisanhari” in Sanskrit, “Hadjod” in Hindi because of its ability to join bones and commonly known as the “bone setter” in English. 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 3- foliate. Flowers small, greenish white, bisexual, tetramerous, in umbellate cymes, opposite to leaves. Calyx is cup shaped. Fruit globose or obovoid fleshy berries, succulent, very acrid, dark purple to black, one seeded; seed ellipsoids or pyriform. Stem is buff coloured with greenish ting, dichotomously branched, sub angular, glabrous, fibrous and smooth.

Medicinal Uses

It has been prescribed in Ayurveda as an alternative, anthelmintic, dyspeptic, digestive, tonic, analgesic in eye and ear diseases and in the treatment of irregular menstruation and asthma. In some part of world, the whole plant is used in oral re-hydration, while the leaf, stem, and root extracts of this plant are important in the management of various ailments. Some other reports on *Cissus quadrangularis* justifies its effectiveness in management of obesity and complications associated with metabolic disorders.

Bone Fracture Healing Activity

Cissus quadrangularis is commonly as the “Bone setter” the plant is referred to as “Hadjod” in Hindi because of its ability to join bones. A bioactive steroid is believed to be the main constituent in *Cissus quadrangularis*. Studies on fracture healing suggest that the steroid may act on estrogenic receptors of the bone. Also it has observed that *Cissus quadrangularis* acts by stimulation of metabolism and increased uptake of the minerals calcium, sulphur and strontium. *Cissus quadrangularis* is useful not only in building up bones but in improving functional efficiency. The extract also neutralizes the antianabolic effect of cortisone in healing of fractures, possibly due to high vitamin C content.

Material and Methods

Plant Material

The herb of *Cissus quadrangularis* were purchased from Orissa, Kamataka and Dehradun India and identified by our Taxonomist. A voucher specimen has been maintained at R&D Department, Phyto Ingredients Biopharma Pvt. Ltd. Yamunanagar, Haryana, India. All other reagents were LR Grade as per requirement. The active compound 3-Ketosterone was tested by gravimetric method.

Extraction Method

Preparation of Water Extract

The air dried plant of hadjod were powdered and passed through 20mesh sieve. The sieved material (100g) was extracted with 1200ml of water in a well sealed, flat bottomed glass container for overnight accompanying occasional shaking and stirring. The whole mixture was filtered and concentrated on water bath then dry.

Preparation of Methanol Extract

The air-dried plant of Hadjod were powdered and passed through 20 mesh sieve. The sieved material (100g) was extracted with 400ml 90% methanol at the temperature of 60-70°C for 1-2 Hr on a water bath. The material was filtered and marc was further refluxed three times. Following this all the extracts were pooled together. Concentrated up to 40% under vacuum using rota-vac (Heidolph, schwalbach, Germany) And wash with Hexane to remove oil part. The material was air-dried and use for assay.

Preparation of Ethyl Acetate Extract

The air-dried plant of Hadjod were powdered and passed through 20 mesh sieve. The sieve material (100g) was extracted with 400ml ethyl acetate at the temperature of 60-70°C for 1-2 hr on a water bath. The material was filtered and marc was further refluxed three times. Following this all the extracts were pooled together, concentrated up to 40% under vacuum using rota-vac (Heidolph,schwalbach, Germany).The material was air-dried and use for assay.

% yield of the extract was calculated using the following equation.

$$\% \text{ Yield} = (\text{Wt. of curde extract} / \text{Wt. of dried plant}) \times 100$$

$$\text{Wt. of curde extract} = \text{Weight of crude extract}$$

$$\text{Wt. of dried plant} = \text{Weight of dried plant material}$$

Estimation of Physio-chemical Parameters

The total ketosterone content in *Cissus quadrangularis* L. extract was determined by Gravimetric method. pH, Ash content(%) & LOD(Loss on Drying) were estimated by the method of Ayurvedic Pharmacopoeia of India(API,1999).

Table 1 : Physiochemical Analysis of *Cissus quadrangularis* L extract.

SNo.	PARAMETERS	OBSERVATIONS
1	Description	Dark brown colour powder
2	Total Ketosterons(%w/w)	6%w/w
3	pH of 1% aqueous solution	3.78
4	Loss on drying (% at 105°C)	4.2%w/w
5	Ash Content	7.86%w/w

Table 2 : Comparison data of different solvent extraction

S.No.	PLANT EXTRACTION	YIELD	ASSAY
1	Water extracts	32.83 % w/w	0.10 % w/w
2	Methanol Extracts	6.00 % w/w	6.2 % w/w
3	Ethyl acetate extracts	4.38 % w/w	2.46 % w/w

Preparation of Sample Solution and Testing

Take sample and dissolved in 50ml methanol(80%) and heating on a water bath and filter the solution and rinse with 5ml methanol, combine filtrate and evaporate methanol solution till 20ml and add 15ml distilled water, extract the above solution 4 times with diethyl ether 25ml each in separating funnel. Take ether layer and discard the lower layer (aqueous) and wash the ether layer with 20ml of 5% NaOH solution twice then discard the alkaline layer and then ether layer wash with water till neutral pH and filter ether layer with anhydrous sodium sulphate. Combine filtrate and evaporate to dryness at 80°C on water bath and calculate weight and percentage of Ketosterone.

Extract was calculated using the following equation

$$\% \text{ Ketosterone} = (\text{Weight of residue after evaporation} / \text{Weight of sample taken}) \times 100$$

Recent Research on the Medicinal uses of *Cissus quadrangularis*

Recent studies on extracts of *Cissus quadrangularis* suggest that its treatment causes bone healing. It is able to speed bone healing when they showed it acts as glucocorticoid antagonist. Since anabolic/androgenic compound are well known to act as antagonists to the glucocorticoid receptor as well as to promote bone growth and fracture healing, several group have postulated that *Cissus quadrangularis* may possess anabolic and /or androgenic properties.

More recent research investigated the anti-inflammatory activity of methanolic extract of *Cissus quadrangularis* brought about 55% may lead to a much faster increase in bone tensile strength in particular, *Cissus quadrangularis* has been shown to be highly effective in the relief of pain, the reduction of swelling and the promotion of the process of healing of sample fractures as well as in the cure of allied disorders associated with bone fractures. However, the mechanism through which *Cissus quadrangularis* is analgesic and anti inflammatory properties has not been well characterized.

It reported that plant extract of *Cissus quadrangularis* have therapeutic efficacy and are known to possess antioxidant antimicrobial activity, and are routinely used to accelerate the process of bone fracture healing. The plant is considered as a versatile medicinal plant in both Ayurvedic and modern drug development area for its valuable medicinal uses it is a very rich sources of some minerals, which are necessary for proper functioning of human body. In the present overview the Emphasis has been laid on the phytochemical constituents and pharmacological activity of the plant *Cissus quadrangularis* Linn.

It was also reported that standardized *Cissus quadrangularis* extract showed anti inflammatory activity in vitro. *Cissus quadrangularis* extract may promote an increase in lean body mass since glucocorticoids induce the breakdown of skeletal muscle tissue by antagonizing the catabolic action glucocorticoids on muscle tissue.

Results and Discussion

Cissus quadrangularis L, a traditional Ayurvedic medicinal plant shows osteoporosis, anti inflammatory, Anabolic and Androgenic activity, antihemorrhoidal activity. From the recent researches, it is concluded that further studies are being conducted to find out the impact of the extract on the other mediators of inflammation and its ultimate effect on the settlement of bones. Recent studies concluded that extract of *Cissus quadrangularis* L. may be reduce heart disease risk factor.

More recently preclinical studies have reported metabolic effect with various extract of *Cissus quadrangularis* L. but the exact mechanism of its action is still uncertain. It has been observed the *Cissus quadrangularis* acts by stimulation of metabolism and increase uptake of the mineral, calcium, sulphur and strontium. *Cissus* may be useful not only in building of bones but in improving functional efficiency and extract also neutralized the anti anabolic effect of cortisol in healing of fracture, possibly due to high vitamin C content and *Cissus* an effective herbal medicine for the treatment of obesity and diabetes.

To optimize the extraction method for hadjore, different methods were performed. The yield of the extract and percent amount of total Ketosterones calculated from each method were compared (Table 2). In order to keep the extraction process as simple as possible, methanolic extraction method was chosen in most experiment we mainly used methanol as an extraction solvent as it has been seen in many studies that the biological activities of Hadjore was found better in methanol extract of the plant. The results showed that water extract yield is 32.83% & assay is 0.10% & in ethyl acetate yield is 4.38% & assay is 2.46% when compared with methanol yield is 6.00% & assay is 6.2%. However the amount of the total ketosteroids in all extract were significantly different & methanol extract showing better results as compare to all.

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