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Research Article

PREPARATION, STANDARDIZATION AND EVALUATION OF PRELIMINARY ANTI-INFLAMMATORY ACTIVITY OF HERBAL FORMULATION OF *CITRULLUS COLOCYNTHIS*

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ABSTRACT

Present study involves preparation and standardization of topical herbal cream followed by evaluation of anti-inflammatory potential of active extract using albumin denaturation method. Topical herbal cream formulation was prepared using extracts of *Citrullus colocynthis*. The plant is traditionally known for its anti-arthritic potential therefore selected for the study. Crude raw material of plant was collected, dried, grinded and sieved through 100 mesh sizes. The physiochemical constants like extractive values and ash values were determined followed by preparation of plant extract using maceration method. Formulation was prepared using typical ingredient required for topical formulation. The standardization of plant constituent was performed using modern analytical technique that after inhibition of bovine serum albumin denaturation method was utilized to perform anti-inflammatory activity. Study observed appreciable anti-inflammatory activity of test compound comparable to the standard drug Diclofenac sodium. The results of study suggested future possibility of anti-inflammatory topical formulation of *Citrullus colocynthis*.

Keywords: Citrullus colocynthis, Formulations, Anti-inflammatory, Standardization, HPTLC.

1. INTRODUCTION

The traditional Indian society is considered as mother hub of ancient medical sciences such as; Ayurveda and Siddha. The belief of Indian society in natural medicine is very high hence therefore market of natural medicines growing day by day not only in India but also in other Asian countries. Ayurvedic formulations, nutraceuticals, cosmoceutical, health drinks, dietary supplements, crude extracts and poly-herbal formulations, etc. are major products originated from natural sources. These products are utilized for therapeutic, rejuvenating and health restoration purposes since many natural drugs offer preventive effect against various types of pathological conditions. The utility and historical background of herbal products is vast but their quality standardization yet to be elaborated [1-3].

The term standardization related to the identity, quality and purity of drug substances including its shelf-life, stability and toxicity profiling. The standardization of herbal formulation is a cumbersome issue due to the lack of guidelines and availability of feasible and cost effective techniques of standardization. The regulatory agencies paid huge attention towards the qualitative, quantitative characterization and fingerprint profiling of herbal medicines. The principle active component of herbal formulation can be utilized for standardization purpose; similarly marker substance can also be employed for analytical purpose when active ingredient is unknown [2-4].

The term standardization in relation to the natural compounds itself represents organoleptic and pharma-cognostic evaluation, evaluation of ash and extractive values, phytochemical evaluation, testing for microbial load and phytochemical profiling using modern techniques of standardization. Determination of moisture content, heavy metals, pesticide residues, radioactive contamination and quantification of chemical groups, etc. are also performed to standardize raw material obtained from natural sources [4-6]. The development of modern techniques for quality estimation of herbal product is a need of current scenario not only to ensure quality and safety of herbal medicines but herbal drug standardization is also required to enhance global acceptance of natural medicines.

Citrullus colocynthis is one of the herbal medicines used for its therapeutic values including laxative, purgative, antibacterial, anti-diabetic and analgesic activities [8]. It also exhibited anti-arthritic activity thus useful in case of autoimmune disease like rheumatoid arthritis [8]. Present study involves preparation and standardization of topical herbal cream followed by evaluation of anti-inflammatory potential of active extract using albumin denaturation method. Topical herbal cream formulation was prepared using extracts of *Citrullus colocynthis*. Presence of quercetin and cucurbitacin glycoside are considered responsible for different biologic activities of *Citrullus colocynthis* [7-9].

2. MATERIAL AND METHODS

2.1. Collection and Identification of Crude drugs

Crude raw material of *Citrullus colocynthis* was collected from local forest area, and indentified in department of plant science *Central Ayurveda Research Institute* (CARI) Jhansi UP. Plant materials dried in shade, grinded, sieved (mesh size 100) and stored in tight containers for further use.

2.2. Determination of Physiochemical Constants [10]

2.2.1. Ash values

Ash values are useful to ascertain contents like carbonates, phosphates and silicates of potassium, sodium, calcium and magnesium. The total ash, acid-insoluble ash and water-soluble ash values were determined after ignition of plant material.

2.2.2. Extractive values

Extractive values related to the nature of chemical constituents present in drug, it gives idea about the solubility of plant material in specific solvents used for the extraction purpose. The extractive values of selected plant material were determined using water and alcohol as solvent.

2.3. Preparation of Extract

The dried plant material was extracted using aqueous maceration method continued for seven days; decoction was filtered finally, concentrated and stored in desiccators for further uses [11].

2.4. HPTLC fingerprinting

High Performance Thin Layer Chromatography (HPTLC) method was employed for quality standardization of herbal extract [12]. In this method, 10 µL diluted sample solution was employed on HPTLC plate. Chromatographic analysis was performed on HPTLC glass plates pre-coated with layers of silica gel 60F254. Samples were applied as bands 6 mm using CAMAG Linomat IV sample applicator equipped with a syringe able to deliver sample in µL range. Solvent system was developed on trial and error basis and finally solvent system comprises ethyl acetate: formic acid: glacial acetic acid: water (80:05:05:10) was selected for study. The chromatographic plate was developed using selected solvent system as mobile phase and developed plate was dried in hot air oven to allow evaporation of solvent. The spots were detected in photo chamber under UV light.

2.5. Formulation Development

The cream formulation was prepared using extract and other ingredient listed in the table 1. Stearic acid, liquid paraffin, cetosteryl alcohol and petroleum jelly were melted using steam bath and other ingredients were dissolved in water and boiled gently. This aqueous solution was added to the oily phase prepared earlier and agitated for a while, that after extract was mixed to the cream base with constant stirring and glycerin was added finally to the cream formulation [13].

Table 1: Composition of topical herbal formulation base

S. No.	Ingredients	Quantity (% w/w)
1	Stearic acid	15
2	Cetosteryl alcohol	5
3	Glycerin	7
4	Liquid paraffin	3
5	Potassium hydroxide	2
6	Petroleum jelly	4

2.6. Evaluation of Formulation

The prepared topical formulation was evaluated for different parameters like; appearance, consistency, extrudability and pH, etc [13].

2.6.1. Physical appearance

The formulated cream was observed for their appearance, transparency, consistency and colour, etc as characteristic features of topical cream formulation.

2.6.2. Determination of pH

The pH of prepared formulation was determined using a pH meter by dissolving 1 gm cream in 100 ml of distilled water and maintaining temperature 25°C. The calibrated pH meter was employed to determine pH of formulation.

2.6.3. Determination of Extrudability

Extrudability is useful test to measure the required force essential to extrude material from the packing tube. The quantity of cream extruded from a collapsible tube when applied certain load is considered as extrudability. The prepared formulation was filled in a standard collapsible tube, the weight of tube was recorded and tube was placed between two glass slides of equal dimensions. The weight of 500 g was applied over the glass slide and cap of tube was opened. The amount of formulation extruded from tube was collected, weighed and percentage of cream extruded from the tube was calculated.

2.7. *In vitro* anti-inflammatory activity (Anti-denaturation assay)

In vitro anti-inflammatory activity was performed using anti-denaturation assay in which inhibition of bovine serum albumin denaturation was estimated as a measure of *in vitro* anti-inflammatory activity [14]. Different aliquots of the test compound were incubated with 0.5% w/v solution of bovine serum albumin for 20 min at 37°C that after temperature was raised to 57°C for 30 min, mixture was cooled and turbidity was measured using UV-Visible spectrophotometer (660 nm) following addition of phosphate buffered saline. Diclofenac sodium was used as standard drug and % inhibition of protein denaturation was calculated using following formulae:

% Inhibition =100 - [(optical density of test solution-optical density of product control)/(optical density of test control)] × 100

3. RESULTS AND DISCUSSION

3.1. Determination of Physiochemical Constants

3.1.1. Ash values

The different ash values *i.e;* total ash, acid insoluble ash, sulphated ash and water soluble ash were determined and reported in table 2. The crude drug showed more percentage of water soluble ash value compared to acid insoluble ash and slightly less amount of sulphated ash value.

3.1.2. Extractive values

The water and alcohol soluble extractives values were determined and study observed alcohol soluble extractive value more as compared to water soluble, however both values not differ too much as depicted in table 2 and this indicate abundant of polar constituent in tested sample.

3.2. HPTLC fingerprinting

The HPTLC chromatogram is presented in fig. 1; presence of several peaks indicates diversified composition of *Citrullus colocynthis*. These constitutes can be considered responsible for biological activities of *Citrullus colocynthis* especially its well known anti-inflammatory activity. These HPTLC fingerprinting can be used for quality estimation and identification of extract of *Citrullus colocynthis*.

Table 2: Results of physiochemical constants

S. No.	Parameters	Observed Value (% W/W)
	Ash Values	
1	Water soluble ash	4.75
2	Acid insoluble ash value	2.8
3	Sulphated ash value	0.09
	Extractive Values	
1	Alcohol soluble extractive values	15.33
2	Water soluble extractive values	11.74

Fig. 1: Chromatograms of extract in TLC analysis

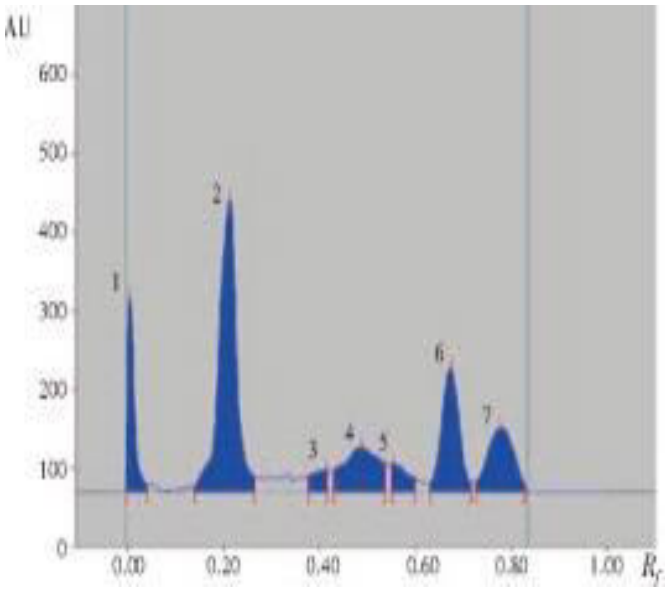

Fig. 2: Chromatograms of extract in HPTLC analysis.

3.3. Evaluation of formulation

The evaluation of prepared topical formulation was performed on standard parameters and results presented in table 3. Characteristic appearance of topical cream was observed when formulation tested for physical appearance with desired consistency. The pH of formulation was observed suitable for topical preparation and compatible with skin layers. The optimum extrudability confirmed desired consistency and uniformity of prepared topical formulation.

Table 3: Evaluation of formulation

S. No.	Parameters	Observed Value (%W/W)
	Physical evaluations	
1	Appearance	Light brown
2	Consistency	Easily spreadable/Uniform Consistency
	Other Parameter	
1	Extrudability	+++
2	pH	6.7

+++ *Indicating optimum extrudability*

3.4. *In vitro* anti-inflammatory activity (Anti-denaturation assay)

The prepared cream formulation was evaluated for various standard parameters and all parameters were observed within specified limits. The formulation was then subjected to pharmacological evaluation using "inhibition of protein denaturation assay". The formulation showed appreciable activity in dose dependent manner. Tested compound exhibited anti inflammatory activity comparable to the standard drug diclofenac sodium. The activity increases with the concentration of sample as depicted in fig. 2. The presence of constituent such as such as flavonoids can be considered responsible for anti-inflammatory activity of compound.

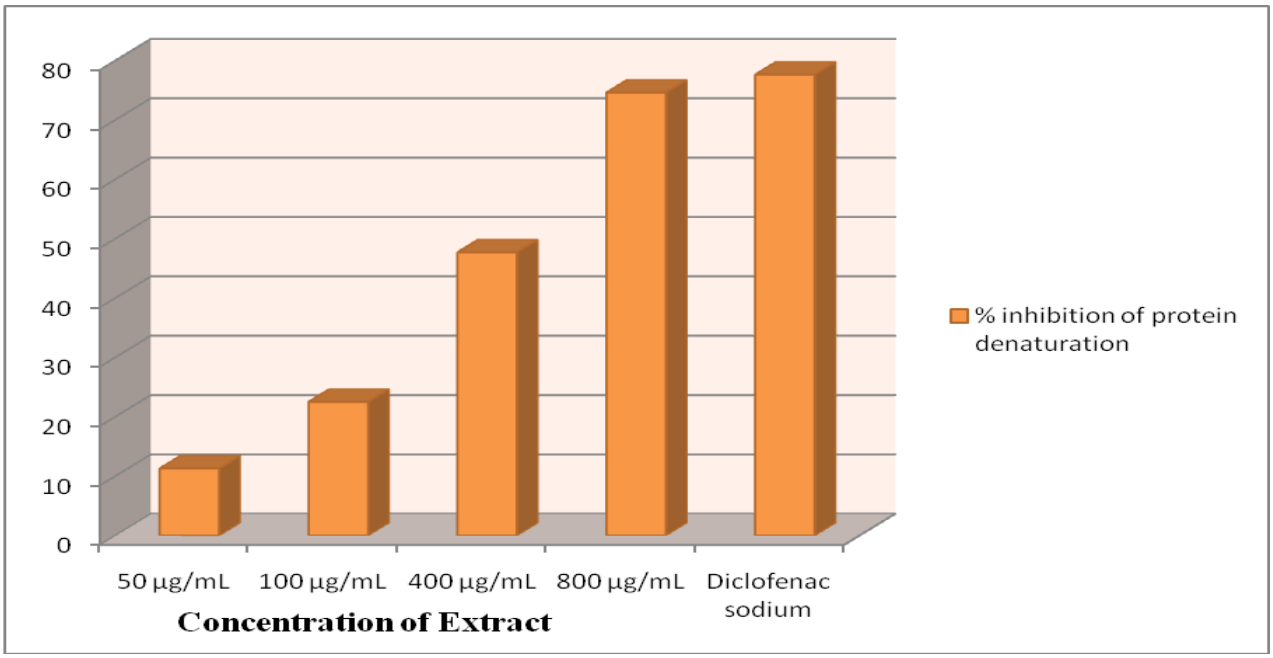

Fig. 3: Results of anti-inflammatory activity of extract at different concentration levels

4. CONCLUSION

Present study involves preparation, standardization and evaluation for anti-inflammatory activity of topical herbal cream of *Citrullus colocynthis* extract. The presence of important phyto-constituents observed during HPTLC analysis particularly flavonoids responsible for biological activity. Study observed remarkable anti-inflammatory activity of test compound which was also found comparable to the standard drug Diclofenac sodium. Results of study suggested future possibility to develop anti-inflammatory topical formulation of *Citrullus colocynthis*. However this study advises further investigation on animal model to ensure pharmacological and pharmacokinetic characteristics of topical herbal formulation.

Conflict of interest

None declared

5. REFERENCES

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