REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 2 (2024)
http://www.veterinaria.org
Article received- Revised- Accepted-

Preparation And Evaluation Of Polyherbal Formulation For Inflammation Management

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ABSTRACT
Trigonellafoenum-graecum seed (TFG), *Zingiber officinale* rhizome (ZO) and *Cissus quadrangularis* stem (CQ) were extracted using purified water and mixed in equal ratio to obtain a polyherbal formulation. The extraction yield for TFG, ZO and CQ were found to be 11.8, 10.2 and 13.4% respectively. The total phenolic content in TFG, ZO and CQ extracts were found to be 36.2137.91 and 30.26 GAE mg/100g respectively. The angle of repose of the polyherbal formulation was found to be 26°18' with compressibility index of 18.07% and Hausner ratio of 1.22. The formulation was able to inhibit 54.81% denaturation of albumin at 1000mM concentration suggesting significant anti-inflammatory action.

Keywords: Polyherbal, total phenolic, albumin denaturation, anti-inflammatory, flow property

INTRODUCTION

Inflammation is normal and necessary protective response to the harmful stimuli such as infectious agents, antigen-antibody reactions, thermal, chemical, physical agents, and ischemia (Bhugra and Tyagi, 2021). All inflammatory diseases have almost a common pathway of generation of disease which involves generation of various inflammatory mediators at various stages due to initial stimulation by one or various etiological factors which may be an infection, an injury or even an allergic stimulus. Recent studies have revealed the advantages and benefits of using natural products in human health issues. Indeed, the use of plants, parts of plants and isolated phytochemicals for the prevention and treatment of various diseases have been practiced since ancient times (Grosser et al., 2016). *Cissus quadrangularis* is a medicinal plant which belongs to the Vitaceae family usually cultivated in India and Ceylon. In Ayurvedic system of medicine, *Cissus quadrangularis* is used for the treatment of sexually contracted diseases, gout, piles, leucorrhoea and syphilis (yoganarsimhan SN, 2000)[3] In Siddha traditional medicine this plant is believed to heal broken bones, as also act as an analgesic and a tonic ()Mishra et al., 2010.*Trigonella foenum-graecum* has been known to contain flavonoids and the extracts are reported to possess potential against acute and chronic inflammatory conditions (Goyal et al., 2016). *Zingiber officinale* has also been reported to produce xanthine oxidase inhibitory potential and can be utilized for the management of inflammation (Ghasaemzadeh et al., 2010).
It was therefore decided upon to prepare a polyherbal formulation containing the extracts of *Trigonella foenum-graecum, Zingiber officinale and Cissus quadrangularis* for management of inflammation.

MATERIAL AND METHODS

Cissus quadrangularis (CQ) and *Zingiber officinale* (ZO) plants were procured from Shubham Nursery Bhopal. Seeds of *Trigonella foenum-Graecum* (TFG) were purchased from local market. Water was freshly distilled and used for extraction.

Extraction and phytochemical screening of plant material (SahiraBanu et al., 2015)
The shade dried; powdered plant material was used for the extraction process. 100 g of plant powder (of individual plants) was evenly packed in the extractor of the Soxhlet apparatus and extracted with ethanol by hot continuous extraction process for about 13 h. The extracts were filtered while hot through Whatman filter paper to remove any impurity. The extract was allowed to dry in air and then transferred to lyophillizer for complete drying of the extracts. The dried extracts were stored in air tight containers until further processing. The phytochemical screening of the extracts was done as per reported methods.

Total Phenolic content in the extracts (Chanthasri et al., 2018)
For total phenolic content determination, 200µL of each sample was mixed with 1.4mL purified water and 100µL of Folin-Ciocalteu reagent. After at least 30 s (but not exceeding 8 min), 300µL of 20% Na_2CO_3 aqueous solution was added and the mixture was allowed to stand for 2 h. The absorbance was measured at 765 nm with a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-60 ppm) were similarly treated to plot the analytical curve. The control solution contained 200µL of ethanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

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Preparation and evaluation of the polyherbal formulation

The extracts were mixed in equal ratio by tumbling action and sifted through sieve no.40 to obtain a uniform blend. This polyherbal blend (PHF) was evaluated for total phenolic content as per the previously reported method. The micromeritic properties (Ahmed et al., 2021) and anti-inflammatory potential of the PHB1 was also evaluated.

Angle of Repose

The powder mixture was allowed to flow through the funnel fixed to a stand at definite height (h). The angle of repose (θ) was then calculated by measuring the height and radius (r) of the heap of powder formed using the following formula

$$\tan\theta = \frac{h}{r}$$

Bulk and Tapped Density

A weighed quantity of blend (10g) was taken into a graduated cylinder (50 mL) and measuring the volume of this weight. The bulk density (ρ bulk) was calculated by the formula

ρ bulk = weight of the powder/initial volume

The above cylinder containing the powder blend was tapped until no further volume change occurs. The tapped density (ρ tap) was calculated by the formula

ρ tap = weight of the powder/finial volume

Hausner's ratio and Carr's Index

Hausner's ratio is the ratio of tapped density to bulk density and is calculated by the following formula

HR = ρ tap/ ρ bulk

The Compressibility index is also known as Carr's Index and is calculated using the values of bulk and tapped density using the formula

$$\text{Carr's Index} = \frac{\rho\,\text{tap} - \rho\,\text{bulk}}{\rho\,\text{tap}} \text{ X } 100$$

Albumin denaturation assay

The PHF was prepared as solution of various concentrations (62.5, 125, 250, 500 and 1000 mM) by dissolving in purified water and used for albumin denaturation assay. A solution of 1% BSA in deionized water was prepared for the test. Ibuprofen solution of concentration 1 µg/mL was used as the positive control. The reaction vessel was filled with 200 µL of BSA, 1400 µL of PBS and 1000 µL of the extract solution. Ibuprofen solution was used in the positive control and distilled water was used in the negative control vessels instead of the extract solution. The reaction mixtures were incubated at 37°C for 15 min and then heated at 70°C for 5 min. The mixtures were then allowed to cool to room temperature and the absorbance of constituent of each vessel were analyzed in UV-Visible spectrophotometer at 660 nm (Singh and Mishra, 2020). The inhibition of percent denaturation of albumin was determined using the following formula:

% Denaturation inhibition = (1−D/C) × 100;

Where D is the absorbance reading of the test sample, and C is the absorbance reading without test sample (negative control).

RESULTS AND DISCUSSION

Extraction and phytochemical screening

The extraction yield for TFG, ZO and CQ were found to be 11.8, 10.2 and 13.4% respectively. The phytochemical screening revealed phenolic, tannin and flavonoids in each plant (Table 1).

Table 1: Observation of phytochemical screening of extracts

Chemical Tests	Observation	*TFG*	*ZO*	*CQ*
Alkaloids				
Mayer's reagent	cream colour precipitate	-	-	-
Hager's reagent	yellow colour precipitate	-	-	-
Wagner's reagent	reddish brown precipitate	-	-	-
Dragendorff's reagent	reddish brown precipitate	-	-	-
Glycosides				
Froth test	Frothing is seen	+	+	-
Kedde's Test	No color	-	-	-
Bontrager's Test	Rose pink or red color in the ammonical layer not found	-	-	-
Keller-Kiliani	No color in acetic acid layer	-	-	-
Phenols/Tannins				
Ferric chloride	Blue green color	+	+	+
Gelatin Solution	White precipitate	-	+	+
Alkaline reagent test	Yellow to red precipitate	+	+	+
Vanillin HCl test	Purplish red color	-	+	+

Flavonoids				
Shinoda test	red color	+	+	+
Alkaline reagent test	Yellow color that turns red on acidification	+	+	+
Zinc HClreductino test	red color	+	+	+
Proteins				
Millon's Test	white precipitate, turns red on heating	-	-	+
Ninhydrin Test	Voilet color	-	-	-
Sterols/triterpenoids				
Salkowski Test	Yellow color in lower layer	+	-	+

Total Phenolic Content

The extracts of TFG, ZO and CQ were evaluated for quantification of the total phenolic content in them. The total phenolic content is said to be responsible for the neutralization of the free radicals and other mediators of several diseases including gout. The calibration curve of gallic acid was constructed using distilled water for obtaining the absorption data. The results of the total phenolic content of the extracts examined, using Folin-Ciocalteu method, are depicted in Table 2. The total phenolic content in extracts, expressed as gallic acid equivalents (Table 2). The mixture of the extracts demonstrated an increase in the total phenolic content.

Table 2: Total Phenolic content of TFG, ZO, CQ and PHF

Extract	Total phenolic content (GAE mg/100g)
Trigonellafoenum-graecum	36.21
Zingiber officinale	37.91
Cissus quandrangularis	30.26
PHF	53.25

Data expressed as gallic acid equivalent (GAE) mg per gm of the extract, Values are mean ± SEM of triplicate determinations

Evaluation of polyherbal blend

The results of prefill evaluation of the formulation blends are presented in Table 5. From the results it is evident that all the blends possessed the capability to flow freely and may present no hindrance in capsule filling process. The values of Hausner's ratio and Carr's Index are found to be within the specifications of good flow property of powders (Table 3).

Table 3: Micromeritic features of the polyherbalformulation

Formulation Code	Bulk density (g/cm^3)	Tap density (g/cm^3)	Angle of repose (θ^o)	Carr's Index (%)	Hausner's Ratio
PHF	0.322	0.393	26°18'	18.07	1.22

Albumin denaturation assay

Protein denaturation has been significantly correlated with the occurrence of the inflammatory response and may lead to various inflammatory diseases including arthritis. Tissue injury during life might be due to denaturation of the protein constituents of cells or of intercellular substance. Hence, the ability of a substance to inhibit the denaturation of protein signifies obvious potential for anti-inflammatory activity. The PHF exhibited the inhibition of albumin denaturation at all doses in a dose dependent manner (Table 4, Figure 1). The 1000mM concentration of the extract had shown the greatest inhibition capacity (54.81%).

Table 4. Albumin denaturation inhibition

S. No.	Test Group	Concentration	Absorbance	% Inhibition
1	Control	-	0.416	0
2	PHF	62.5 mM	0.306	26.44
3	PHF	125 mM	0.282	32.21
4	PHF	250 mM	0.249	40.14
5	PHF	500 mM	0.213	48.79
6	PHF	1000 mM	0.188	54.81

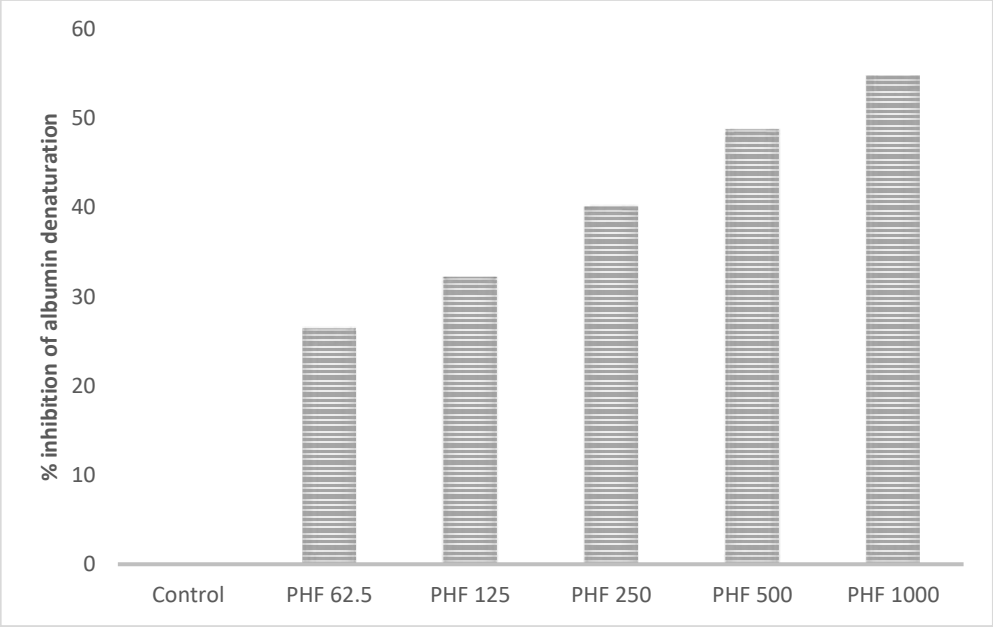

Figure 1: % inhibition of albumin denaturation by PHF

CONCLUSION

The use of liposomes in oral drug delivery has been an area of investigation in the recent times. Herbal drugs have been used since ages for the treatment of otherwise unmanageable ailments like arthritis and gout. The present work was undertaken with an aim to develop polyherbal formulation for the management of gout. Aqueous extracts of *Trigonella foenum-graecum, Zingiber officinale* and *Cissus quadrangularis* were mixed in equal ratios and tested for anti-inflammatory potential. Preparation and standardization of the capsule containing the polyherbal mixture would be undertaken according the guidelines for standardization of polyherbal formulations.

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