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Research Article

Evaluation of the *in vitro* anti-inflammatory activity of different fractions of the aqueous extract of *Curcuma zedoaria* Roscoe rhizome and formulation of a cream with anti-inflammatory potential

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Introduction: *Curcuma zedoaria* Roscoe (*C. zedoaria*) is used in traditional medicine for various diseases, including inflammation. This study aimed to evaluate the *in vitro* anti-inflammatory activity of the aqueous extract of *C. zedoaria* rhizome and its fractions and develop an anti-inflammatory cream incorporating its aqueous extract.

Methods: The crude aqueous extract of *C. zedoaria* rhizome was prepared by the decoction method. A portion of it was freeze-dried and the other part was subjected to sequential fractionation using hexane, dichloromethane and ethyl acetate. All the fractions were dried using a rotary vacuum evaporator. The dry powders of the above extracts were assessed for their *in vitro* anti-inflammatory activity using heat-induced egg albumin denaturation assay and compared with the reference drug, diclofenac sodium. A cream was formulated by incorporating the most effective fraction; the aqueous extract and its anti-inflammatory activity was compared against a commercially available diclofenac sodium cream.

Results: The aqueous extract of *C. zedoaria* rhizome and its hexane, dichloromethane, ethyl acetate fractions and the remaining aqueous extract showed anti-inflammatory activity against heat-induced protein denaturation with the IC_{50} values; 94.45 µg/mL, 3822 µg/mL, 373.2 µg/mL, 337.7 µg/mL and 565.4 µg/mL respectively. The IC_{50} value obtained for diclofenac sodium powder was 915.7µg/mL. The IC_{50} value obtained for the formulated cream (containing 3% w/w aqueous extract of *C. zedoaria* rhizome) was 1894 µg/mL. The observed IC_{50} value of the commercially available diclofenac sodium 1% w/w cream was 1227 µg/mL.

Conclusion: The cream formulated with the aqueous extract of *C. zedoaria* rhizome possesses *in vitro* anti-inflammatory efficacy. Further studies are required to improve its drug-releasing capacity.

Keywords

Anti-inflammatory cream, *Curcuma zedoaria,* Egg albumin denaturation assay, Sequential extraction

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INTRODUCTION

Inflammation is a complex biological reaction that occurs as a response to various noxious conditions (1,2) and commonly expressed by pain, heat, redness, swelling and loss of function at the site of inflammation. (1,3) Currently, several anti-inflammatory drugs are used against inflammatory conditions. Among them, glucocorticoids and non-steroidal anti-inflammatory drugs (NSAIDs) are the main classes of drugs. (4) However, these drug therapies produce many drawbacks, such as unwanted effects on the gastrointestinal tract and the renal system. In addition, toxicity and the reappearance of symptoms after discontinuation are also experienced with recently available potent synthetic drugs. (4) Therefore, new anti-inflammatory substances are urgently needed to overcome such drawbacks.

In history, plants were the first source of treatment against diseases. Since then, chemical characterization and identification of herbal bioactive compounds have powered drug development. (5) *Curcuma zedoaria* Roscoe (*C. zedoria*) is an annual, fragrant, rhizomatous, and perennial herb that belongs to the family Zingiberaceae (6). This plant is native to Bangladesh, Sri Lanka and India. It is also broadly cultivated in China, Japan, Brazil, Nepal and Thailand. (7)

The rhizome of *C. zedoaria* can be considered as a good herbal source to be incorporated into pharmaceutical dosage forms developed as anti-inflammatory drugs considering its traditional use during inflammatory conditions. (8,9) In addition, thrombolytic, antimicrobial, antifungal, antioxidant, neuroprotective and antivenom activities have also been reported with the *C. zedoaria* plant. (10-14) In this study, an attempt was made to evaluate the anti-inflammatory potential of the aqueous extract of the rhizome of *C. zedoaria* and its different fractions and then formulate an anti-inflammatory cream incorporating the most effective extract against inflammatory conditions.

MATERIALS AND METHODS

General equipment

Ultraviolet spectrophotometer (Spectrum-SP-UV-5000DB, England), analytical balance (ACZET, CY 224C, S. No: 17409873, Japan), water bath (EQUITRON, model No-#8428, S. No-8428.AHE.062, Germany), rotary vacuum evaporator (HAHNSHIN, model No: HS-2005VN, S. No: V00449, Japan), refrigerator (WRN 25LX, SG66CY1, S. No: INA 200806593, India), laboratory drying oven (Schutzart DIN 40050, UM 400, Germany), microscope (DMO636, S. No: LT60000014, Japan), pH meter, (EUTECH, S. No: 02433, Poland) and a freeze dryer (Labconco, freezone205, USA) were used for the study. Diclofenac sodium B.P powder was received as a gift pack from the State Pharmaceuticals Manufacturing Corporation of Sri Lanka. Analytical grade solvents (Sigma-Aldrich, India); hexane, dichloromethane, ethyl acetate, and dimethyl sulfoxide were used for the study. White soft paraffin, emulsifying wax, tween 80, and liquid paraffin were purchased from the local market.

Plant materials

The fresh rhizomes from the mature plant of *C. zedoaria* were collected from the Galle District in the Southern Province, Sri Lanka. The plant was collected from November to December 2020. The whole plant was

identified, and its authenticity was confirmed at the Bandaranayake Memorial Ayurvedic Research Institute, Nawinna, Maharagama, Sri Lanka.

Preparation of the aqueous extract of *C. zedoaria*

Thoroughly cleaned, fresh rhizomes were cut into small pieces. As per the traditional preparation method of decoctions, 480 g of *C. zedoaria* rhizome were boiled in 1920 mL of distilled water until the total volume was reduced approximately to 1/8 of the original volume. (15) The resulting aqueous extract was filtered using a double-layered muslin cloth and with Whatman no. 01 filter papers. The resultant filtrates were combined and freeze-dried.

Preparation of fractions of the aqueous extract of *C. zedoaria*

The aqueous extract of *C. zedoaria* rhizome obtained as per the aforementioned method was sequentially extracted into hexane, dichloromethane and ethyl acetate, using a separatory funnel. (16) The solvents of all the extracts and the residual aqueous extract were evaporated using a rotary vacuum evaporator. The resulting sludge was further dried using a water bath at 45ºC for one hour to obtain dry powders.

Investigation of the *in vitro* anti-inflammatory activity of the aqueous extracts of *C. zedoaria* and its fractions

The *in vitro* anti-inflammatory potencies of the test samples were evaluated by the inhibition of egg albumin denaturation assay. A volume of 5 mL reaction mixtures was prepared by adding 0.2 mL of egg albumin and 2.8 mL of phosphate buffer (pH 6.4) to 2.0 mL of various concentrations (from 31.25 µg/mL to 4000.00 µg/mL) of dried samples of the aqueous extract of *C. zedoaria* and its fractions (hexane, dichloromethane, ethyl acetate and remaining water fraction). For reference, 5 mL of the reaction mixtures were made by adding 0.2 mL of egg albumin, 2.8 mL of phosphate buffer (pH 6.4), to 2.0 mL of various concentrations (from 78.125 µg/mL to 5000.00 µg/mL) of diclofenac sodium. A 5 mL reaction mixture was made for the control by adding 0.2 mL of egg albumin, 2.8 mL of phosphate buffer (pH 6.4), to 2.0 mL of distilled water. (17,18) The above mixtures were incubated at 37ºC for 15 minutes. After the incubation period, the temperature was gradually increased up to 70ºC and kept at 70ºC for a further 5 minutes. Then the samples were cooled, and the absorbance values of the resulting samples were measured at 660 nm using the UV-visible spectrophotometer. The percentage inhibition of protein denaturation was calculated. (8,17,18) The IC_{50} (concentration providing 50% of inhibition of albumin thermal denaturation) values were calculated by plotting the graphs, percentage inhibition of albumin denaturation versus log concentration of the respective sample. (8,17, 18) The anti-inflammatory activities of *C. zedoaria* extracts were compared with the diclofenac sodium reference drug. The milligrams of diclofenac sodium equivalents to different extracts of *C. zedoaria* were calculated.

Preparation of the base of the cream and stability studies

Different base formulas (S_1, S_2, and S_3) for the preparation of the cream, were prepared by changing the ratios of oil (white soft paraffin, liquid paraffin), water (distilled water) and surfactant (tween 80, emulsifying wax). The aqueous phase and the oil phase were weighed separately according to the relevant ratios and heated up to 65±2ºC using a water bath. When the emulsifying wax and

other surfactants were dissolved, the heated aqueous phase was added drop-wise to the mixture of the oil and emulsifying agents. Then it was stirred until the cream was formed. (19) The formulated bases were subjected to long term stability studies (60 days) and characterization studies. The dye test (using methylene blue) was used to evaluate the nature of the cream base. The pH was measured after dissolving 0.1 g of the cream in 30 mL of distilled water. In addition, the appearance of base samples was judged by their consistency, colour, odour and texture. (20)

Formulation of creams incorporating the plant extract of *C. zedoaria*

According to the stability studies, the two best bases (named S_1 and S_2) were selected to incorporate the *C. zedoaria* plant extracts. Initially, weighed and freeze-dried aqueous extract was dissolved in distilled water. Then the weighed oil phase, emulsifying agents and aqueous phase were heated using a water bath to around 65 ± 2°C. Then aqueous phase with plant extract mixture was added dropwise to the oil phase containing the emulsifying agent using a glass dropper. Then the mixture was stirred until the cream was formed. (19) Three creams with different strengths, 0.5%, 1% and 3% (w/w) dry powder of aqueous extract of *C. zedoaria,* were formulated using the above procedure with both S_1 and S_2 base ratios.

Characterization studies were done on all the formulated cream samples. The dye test (microscopic analysis), texture, visual appearance, pH measurement, colour changes and odour changes were tested over two months. The observations were made on 1^{st}, 3^{rd}, 5^{th}, 7^{th}, 10^{th}, 15^{th}, 20^{th}, 25^{th}, 30^{th}, 35^{th}, 40^{th}, 45^{th}, 50^{th}, 55^{th} and 60^{th} days at different storage conditions. (8ºC, room temperature and 40ºC).(20)

Evaluation of inhibition of protein denaturation potency of the cream developed with *C. zedoaria*

Anti-inflammatory activity of 3% w/w (S_2 cream) of *C. zedoaria* was investigated using the *in vitro* egg albumin denaturation method, and results were compared with a commercially available diclofenac sodium cream 1% (w/w). Before the test, the cream containing 3% (w/w) *C. zedoaria* was dissolved in 5% dimethyl sulfoxide with distilled water to prepare a 5000 µg/mL stock solution. The mixture was sonicated for 5 minutes. The resultant solution was filtered using a cotton swab, and it was further filtered by using a microfilter with a pore size of 0.45µm. A dilution series was prepared from the filtered stock solutions. The stock solution for the reference cream (positive control), diclofenac sodium cream 1% (w/w), was also prepared in the same manner. Then, the egg albumin denaturation assay was performed for both creams as mentioned above.

Statistical analysis

All the results were triplicated to determine the mean and the standard error of the mean. The half-maximal inhibitory concentration (IC_{50}) value and concentration dependencies were calculated using nonlinear regression with GraphPad Prism 9 software.

RESULTS

Appearance of the extracted powders

The freeze-dried aqueous extract of *C. zedoaria* appeared as a fine brown powder (Figure 1.a) with a fragrant odour. The rotary evaporated extracts of *C. zedoaria;* hexane extract, dichloromethane extract, and ethyl acetate extract appeared as a dark brown fine

powder (Figure 1.b), yellowish-brown powder (Figure 1.c), and yellow crystal-like powder (Figure 1.d). The rotary evaporated remaining aqueous extract of *C. zedoaria* appeared as a light brown powder (Figure 1.e). All the fractions had a fragrant odour

Inhibition of protein denaturation potency of aqueous extracts of C. zedoaria and its fractions

According to the results of the albumin denaturation assay (Figure 2), reference drug (R=0.9815, p<0.001), aqueous extract (R= 0.9814, p<0.001), hexane extract (R= 0.9405, p<0.001), dichloromethane extract (R= 0.9438, p<0.001), ethyl acetate extract (R=0.9718, p<0.001) and, remaining aqueous extract (R= 0.9803, p <0.001) of *C. zedoaria* showed positive and statistically significant correlation between log concentration and the percentage of inhibition of albumin denaturation.

The IC_{50} values obtained for different extracts of *C. zedoaria* by albumin denaturation assay and the calculated diclofenac sodium equivalents/gram values are given in Table 1. According to the results, the highest IC_{50} value (IC_{50} = 3822.0 µg/mL) was observed for the hexane extract, and the lowest IC_{50} value (IC_{50} = 94.4µg/mL) was observed for the aqueous extract of *C. zedoaria*. Also, IC_{50} values observed for the aqueous extract, dichloromethane fraction, ethyl acetate fraction, and residual aqueous fraction were lower than the IC_{50} value obtained for the reference drug, diclofenac sodium. According to the results, the highest diclofenac equivalents/gram value was observed for the aqueous extract, and the lowest diclofenac equivalents/gram value was observed for hexane extract of *C. zedoaria*.

Figure 1. Appearance of dry powder extracts of *C. zedoaria* (a: Aqueous extract, b: Hexane extract, c: Dichloromethane extract, d: Ethyl acetate extract and e: Remaining aqueous extract)

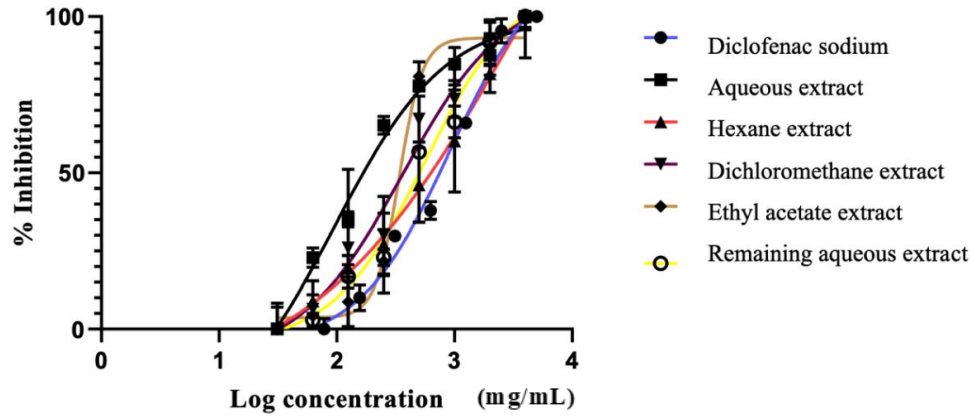

Figure 2. Percentage inhibition of egg albumin denaturation by aqueous extract of *C. zedoria*, its fractions and the reference drug

Table 1. IC_{50} values and grams of diclofenac sodium equivalents of aqueous extract of *Curcuma zedoaria* rhizome and its fractions

Plant extracts/drug	IC_{50} values (µg/mL)	Values of diclofenac sodium equivalents (grams)
Aqueous extract	94.4	9.51
Hexane fraction	3822.0	0.25
Dichloromethane fraction	373.2	2.41
Ethyl acetate fraction	337.4	2.70
Remaining aqueous fraction	565.4	1.61
Diclofenac sodium	915.7	-

Characterization and stability of the cream bases

When considering the different base samples (S_1, S_2, and S_3) of the cream without the plant extract as mentioned above, all base samples were smooth in texture and white in appearance. The dye test indicated that the formed base samples were as oil in water emulsions. According to pH measurement data, all the base samples showed minor pH variations in different temperatures (8ºC, room temperature and 40ºC) for 60 days. According to the visual and odour observations, among the prepared base samples, two samples (S_1 and S_2) were stable at different temperatures (8℃, room temperature, 40℃) for 60 days without any discolouration, odour change and phase separation (Table 2).

Characterization and stability of creams incorporating *Curcuma zedoaria* rhizome extracts

When considering the prepared creams incorporating 0.5%, 1%, 3% w/w (with S_1 and S_2 bases) of the dry powder of the aqueous extract of *C. zedoaria,* all creams showed minor pH variations in different temperatures (8℃, room temperature, 40℃) during the stability period (60 days).

According to visual observation data (Table 3), 1% of S_1 and 0.5% of S_2 creams kept at 40℃ showed colour changes during 60 days but all the other samples showed no change during the stability study period. Among S_1 and S_2 cream samples, the S_1 cream series were moderately smooth, and the S_2 cream series were classified as being smooth. The 3%w/w strength S_1 and S_2 creams had a light brown colour, while 0.5% and 1% w/w creams had a white appearance.

Comparison of percentage inhibition of egg albumin denaturation assay of anti-inflammatory cream with the reference drug

A positive, statistically significant correlation between log concentration and percentage inhibition of albumin denaturation by the reference drug (1% w/w diclofenac sodium cream) and the cream formulated with *C. zedoaria* (3% w/w) is given in Figure 3. According to the results, a positive correlation was observed with both the reference (R= 0.9770, <0.0001) and the 3% w/w S_2 cream (R= 0.9674, P <0.0001). The IC_{50} value detected for diclofenac sodium cream (1% w/w) was 1227 µg/mL while, the IC_{50} value of 1894 µg/mL was detected for the *C. zedoaria* cream (3% w/w).

Table 2. Visual observations of three trial base samples at different temperatures (8ºC, Room temperature and, 40ºC)

Day	Appearance									Colour									Odour								
	8ºC			RT			40ºC			8ºC			RT			40ºC			8ºC			RT			40ºC		
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
1	S	S	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	S	S	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	S	S	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	S	S	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
10	S	S	S	S	S	S	S	S	S	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
15	S	S	S	S	S	S	S	S	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
20	S	S	S	S	S	S	S	S	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
25	S	S	S	S	S	U	S	S	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
30	S	S	S	S	S	U	S	S	U	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
35	S	S	S	S	S	U	S	S	U	N	N	N	N	N	N	N	N	D	N	N	N	N	N	N	N	N	N
40	S	S	U	S	S	U	S	S	U	N	N	N	N	N	N	N	N	D	N	N	N	N	N	N	N	N	N
45	S	S	U	S	S	U	S	S	U	N	N	N	N	N	D	N	N	D	N	N	N	N	N	N	N	N	N
50	S	S	U	S	S	U	S	S	U	N	N	N	N	N	D	N	N	D	N	N	N	N	N	N	N	N	N
55	S	S	U	S	S	U	S	S	U	N	N	D	N	N	D	N	N	D	N	N	N	N	N	N	N	N	N
60	S	S	U	S	S	U	S	S	U	N	N	D	N	N	D	N	N	D	N	N	N	N	N	N	N	N	N

S-Stable, U-Unstable, N-No Change, D-Dis-coloured, RT- Room Temperature,

S1- Base Sample 1, S2- Base Sample 2, S3- Base Sample 3

Table 3. Visual observations of the prepared cream samples containing 0.5%, 1%, 3% w/w of the aqueous extract of *Curcuma zedoaria* rhizome at different temperatures

Day	Visual observations																	
	8ºC						RT						40ºC					
	S1			S2			S1			S2			S1			S2		
	0.5 %	1 %	3 %	0.5 %	1 %	3 %	0.5 %	1 %	3 %	0.5 %	1 %	3 %	0.5 %	1 %	3 %	0.5 %	1 %	3 %
1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
20	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
25	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
30	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
35	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
40	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
45	N	N	N	N	N	N	N	N	N	N	N	N	N	D	N	N	N	N
50	N	N	N	N	N	N	N	N	N	N	N	N	N	D	N	N	N	N
55	N	N	N	N	N	N	N	N	N	N	N	N	N	D	N	D	N	N
60	N	N	N	N	N	N	N	N	N	N	N	N	N	D	N	D	N	N

RT= Room Temperature, N=No Change, D=Dis-coloured

S1- Base sample 1, S2- Base sample 2

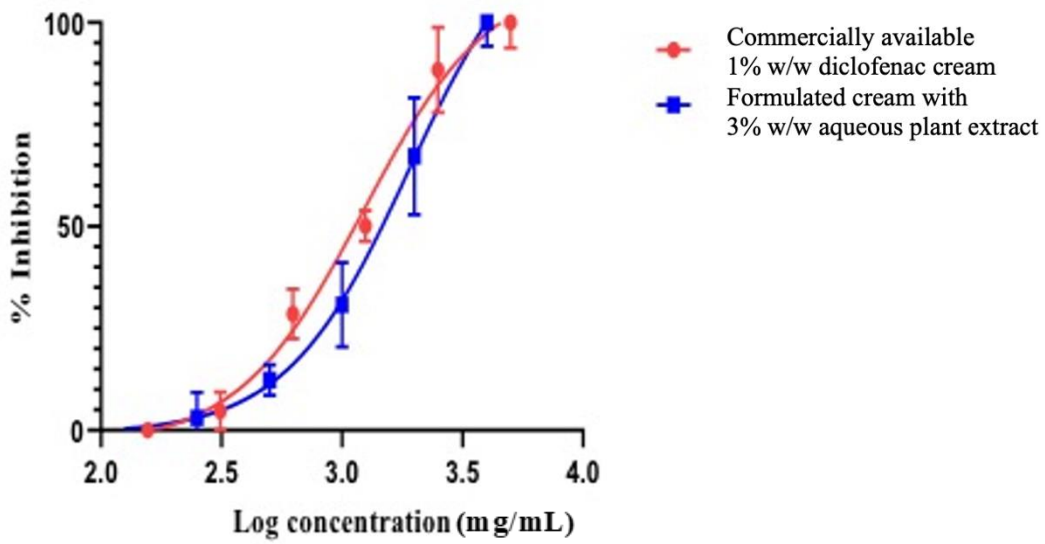

Figure 3. Percentage inhibition of egg albumin denaturation by 3% w/w S2 cream and the reference drug

DISCUSSION

In this research, studies were conducted to identify the anti-inflammatory potency of the aqueous and different organic fractions of the rhizome *C. zedoaria*, a native plant to Sri Lanka. In addition, an attempt was made to develop an anti-inflammatory cream incorporating the most effective extract of *C. zedoaria*, which was not previously attempted by any other research group.

The aqueous extract of *C. zedoaria* was prepared by the decoction method because it is employed in the traditional Ayurvedic system in the same manner. (15) According to the egg albumin denaturation assay, a promising protein denaturation activity was observed with the aqueous extract of *C. zedoaria* rhizome compared to the reference drug, diclofenac sodium. Therefore, the aqueous extract was further fractionated using the solvents; hexane, dichloromethane, and ethyl acetate according to the polarity gradient to evaluate the inflammatory activity in different fractions of the water extract. (21)

A dose-dependent anti-inflammatory effect was observed with the tested *C. zedoaria* extracts. The study results indicated a higher percentage inhibition of protein denaturation by the aqueous extract of *C. zedoaria*, thus it might offer significant relief of inflammation than all the other organic extracts of *C. zedoaria.* The higher activity detected with the aqueous extract may have resulted due to synergetic effect of chemical constituents of the aqueous extracts than the individual chemical constituents moved into the organic fractions.

The anti-inflammatory activity of ethanol extract of *C. zedoaria* rhizome (doses: 100 µg/mL-500 µg/mL) using *in vitro* protein denaturation assay had also been evaluated.(22) Moreover, anti-inflammatory activity of *C. zedoaria* rhizome has been evaluated using carrageenan induced inflammatory rat model using different solvent extracts such as the ethanolic and Aqueous extracts of the rhizome. (22,23) Therefore, these studies further prove the anti-inflammatory activity of the *C. zedoaria* rhizome.

There are several *in vitro* assays available to investigate the anti-inflammatory activity of compounds. (24) Among them, *in vitro* protein denaturation test is a widely used, reliable and convenient method to evaluate the anti-inflammatory activity of natural products. (24) In the present study, potential for inhibition of denaturation of albumin protein is studied. Protein denaturation has been identified as a cause of inflammation. The denatured protein expresses antigens related to the Type III hypersensitivity reaction. The conventional NSAID's (indomethacin *etc.*) produce its anti-inflammatory action by inhibiting endogenous prostaglandins production by blocking the COX enzyme. It has been also proven that the mechanism is exerted via preventing denaturation of proteins. (25) Therefore, an *in vitro* protein denaturation assay has been adapted to evaluate the anti-inflammatory effect of *C. zedoaria* rhizome and the effect was compared with the reference drug diclofenac sodium. Moreover, as evident by the study, the aqueous extract of *C. zedoaria* rhizome would be able to control autoantigen production and thereby inhibit the denaturation of proteins. (26,27) Hence, its anti-inflammatory properties are indicated by inhibition of its denaturation process. Among the different *in vitro* assays available to investigate the anti-inflammatory activity of compounds, in this study, only the egg albumin denaturation assay was used. It is a limitation of this study. Hence, it is recommended to evaluate the anti-inflammatory potential of the study plant extract and its fractions using the other *in vitro* methods in future studies.

According to the results of the present study, the aqueous extract of *C. zedoaria* rhizome showed a higher percentage inhibition of protein denaturation than its ethyl acetate, dichloromethane and hexane fractions; thus, its aqueous extract may have the potency to offer significant relief of inflammation than all other organic extracts. Moreover, studies have shown the anti-inflammatory activity of proteins isolated from the aqueous extract of *C. zedoaria* rhizome against carrageen induced inflammation in rats.(28) Therefore, the findings of the present study support the use of the aqueous extract of the rhizome in traditional medicine for the treatment of inflammatory conditions. Hence, the cream was formulated by incorporating the aqueous extract of *C. zedoaria* rhizome.

Distilled water, oil and emulsifying agents were the main constituents of the cream formulation. When two immiscible liquids such as liquid paraffin and distilled are shaken together, they form a temporary emulsion. This technique is thermodynamically unstable, causing phase separation. Therefore, tween 80 and emulsifying wax were the emulsifying agents used in the cream bases prepared in this study. They have the potential to promote emulsification by stabilisation of oil droplets and also maintain the stability of the cream during storage (29).

A limited number of attempts have been made to develop dosage forms incorporating the extracts of *C. zedoria*. A study has reported that 2% white turmeric gel prepared with the ethanolic extract of *C. zedoria* rhizomes is effective in decreasing the interleukin-6 concentrations in patients after scaling and root planning treatment for chronic periodontitis.(30) Microspheres developed with Zedoary turmeric oil (ZTO), the extract from the dry rhizome of *C. zedoaria* have resulted in the inhibition of walker-256 cells transplanted solid tumours. (31) In addition, an attempt has been made to develop ZTO microspheres with improved bioavailability.(32) Even though the aqueous extract *C. Zedoria* is widely used in

traditional medicine, no pharmaceutical dosage forms incorporating the aqueous extract of *C. zedoria* has been developed before. Therefore, the findings of the current study can be used as an initiative for future formulation development with *C. zedoria*. However, the efficacy of this formulation needs to be further studied by *in vitro* methods such as skin permeation assays (Franz-type diffusion cells, cutaneous retention and tape-stripping methods), *in vivo* evaluations such as pre-clinical pharmacokinetic studies in animal models (33) and phase separation studies.

CONCLUSION

The anti-inflammatory potential of the aqueous extract of *C zedoaria* is the highest than its fractions; ethyl acetate, dichloromethane, hexane, and residual aqueous extract and the reference drug diclofenac sodium. The cream containing 3% (w/w) *C. zedoaria* exhibits a promising *in vitro* anti-inflammatory potential which could be further developed as a dosage form against inflammatory conditions.

Author contribution:

First, second and third authors contributed to study design, experimental studies, data analysis and manuscript preparation. Fourth, fifth and sixth authors contributed to concept development, study design, data analysis and manuscript preparation.

Author's declaration:

The authors declare that all persons listed as authors have read and given approval for the submission of this manuscript.

Competing interests:

The authors declare that they have no competing interests to disclose.

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