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Research Article

# Phytochemical Analysis and *In vitro* Anti Obesity Activity of Different Fractions of Methanolic Extract of *Fagonia cretica* L.

**Divyang Patel[1*], Vimal Kumar[2]**

[1]*Department of Pharmacognosy, Institute of Pharmacy, Nirma University, S. G. Highway, Ahmedabad-382481, Gujarat, India*
[2]*ITM School of Pharmacy, ITM (SLS) Baroda University, Dhanora Tank Road, Village-Paldi, Nr. Jarod, Tal-Waghodia, Vadodara-391510, Gujarat, India*

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ABSTRACT

Obesity is one of the most prevalent health concerns among all age groups and populations worldwide, resulting in a significant increase in mortality and morbidity related to metabolic disorders. Targeting one or more enzymes involved in lipid metabolism can be selective for evaluation of anti-obesity action of drug. The present study was aimed to evaluate *in vitro* anti-obesity action by inhibiting pancreatic lipase and ɑ amylase enzyme by various fractions of methanolic extract of aerial parts *Fagonia cretica* L. along with their phytochemical analysis. The n-hexane (HFFC), chloroform (CFFC), ethyl acetate (EAFFC), n-butanol (BFFC), and aqueous fractions (AQFFC) were prepared from methanolic extracts of *F. cretica* L, and were analyzed for qualitative, as well as, quantitative phytochemical study using reported methods. The qualitative phytochemical studies of prepared extract and fractions showed presence of flavonoids, saponins, phenolics, alkaloids, and carbohydrates. All the fractions were then examined for their *in vitro* lipase inhibitory and ɑ amylase inhibitory activities at a concentration level of 50, 100, 150, and 200 µg/mL, and their percentage inhibitory effects were reported. Among the analyzed samples, BFFC showed highest lipase inhibitory action, i.e., 83.02 ± 2.47%, as compared to other fractions. EAFFC showed significantly higher ɑ amylase inhibitory action, i.e., 80.22 ± 1.18% as compared to other fractions.

## Introduction

Obesity is one of the non-communicable lifestyle disorders characterized by excess adipose tissue mass with body mass index (BMI) > 25 kg/m$^2$.[1] It can be considered as a cosmetic problem associated with various other lifestyle disorders, like diabetes, dyslipidemia, hypertension, cardiovascular diseases, musculoskeletal disorders, cancer, etc. Change in lifestyle along with regular exercise for obesity treatment is less effective for long term weight loss. Targeting the inhibition of one or more enzymes involved in lipid and carbohydrate metabolism is the best option for evaluation of anti-obesity action of drugs. Absorption of dietary triglycerides in small intestine involves their hydrolysis into free fatty acids by pancreatic lipase enzyme.[2] On the other side, absorption of carbohydrates in small intestine involves their hydrolysis into simple sugars by amylase enzymes. Inhibition of these enzymes could be beneficial in weight control and weight loss treatments.

*F. cretica* L. (family: Zygophyllaceae) is a short erect spiny undershrub with slender branches, terete striates, a glabrous or sparsely glandular puberulous growing almost throughout the year majorly in north-west India.[3] The plant is used as a bitter tonic, diuretic, astringent, and prophylactic against smallpox. It is also used for the treatment of dysentery, asthma, fever, thirst, vomiting, urinary discharges, liver trouble, dyslipidemia, typhoid, toothache, stomach troubles, and skin diseases.[4,5] Research reports available on *F. cretica* L. showed presence of alkaloids, saponins, flavonoids, phenolics, tannins, and vitamins in whole plant.[6] A number of *in vivo* and/or *in vitro* studies have explained a wide spectrum of pharmacological properties of crude extracts of

*Corresponding Author: Divyang Patel

**Address:** Department of Pharmacognosy, Institute of Pharmacy, Nirma University, S. G. Highway, Ahmedabad-382481, Gujarat, India

**Email** ✉: divspatel87@gmail.com

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plant, including anticancer,[7,8] anti-inflammatory, analgesic,[9] anti-microbial,[10] immunomodulatory,[11] hepatoprotective,[12] and antioxidant.[13] In *Ayurvedic* literature, this plant is mentioned for fat reduction property,[14] but to date, there is no scientific evidence available proving this claim. Therefore, the present study was designed to investigate *in vitro* anti-obesity action of different fractions of methanolic extracts of *F. cretica* L. by using lipase inhibitory and amylase inhibitory assays by reported methods.

## Materials and Methods

### Collection and Identification of Plant Material

Drug sample of F. cretica L. was procured from local market of Vadodara, Gujarat, India, in the month of June 2015. The drug sample was identified by comparing its morphological characters described in various standard texts.[14] It was further authenticated by botanist Dr. P K Patel, Head of Department, Sheth P. T. Arts and Science College, Godhra, Gujarat bearing voucher specimen number PPDC/COG/2015/001 as F. cretica L. belongs to the family Zygophyllaceae. It was dried under sunlight for 2 days to minimize moisture content and then powdered, and the powder sample was passed through 60# sieve and stored in an airtight container at room temperature for further use.

### Extraction and Fractionation

Powdered aerial parts of *F. cretica* L. (1 kg) were extracted with methanol (2.5 L) by maceration method at room temperature for 7 days, followed by filtration using filter paper. Dried marc was again extracted with methanol (2.5 L) by same method for 7 days. Two percolates were mixed, concentrated and evaporated to dryness to give methanolic extracts of *Fagonia cretica* (MEFC) (yield: 4.87% w/w). The dried extract was suspended in water (250 mL), and subsequently extracted with n-hexane, chloroform, ethyl acetate, and n-butanol (250 mL each) by liquid-liquid partition in separating funnel with respective solvent. All solvent fractions were evaporated to dryness under reduced pressure and designated as HFFC (0.59% w/w), CFFC (0.67% w/w), EAFFC (0.53% w/w), BFFC (0.82% w/w), and remaining AQFFC (1.69% w/w).

### Preliminary Phytochemical Screening and Quantitative Estimation of Phytoconstituents

Preliminary phytochemical screening of methanolic extracts, along with all the fractions, was performed using standard reported procedures for the detection of various secondary metabolites.[15-17] Total flavonoids and saponins contents of EAFFC and BFFC were determined by using standard reported methods.[18,19]

### *In vitro* Lipase Inhibitory Activity

Lipase inhibitory activity of prepared fractions of methanolic extracts of *F. cretica* L. was determined by using a method described by Etoundi CB *et al.*[20] The rate of release of oleic acid from triolein was determined for measuring lipase inhibitory action. A suspension containing 1% (v/v) of triolein, and 1% (v/v) tween 40 in 0.1 M phosphate buffer (pH 8) was prepared and emulsified. Porcine pancreatic lipase (0.5 gm) was dissolved in 15 mL 0.1 M phosphate buffer (pH 8). 800 µL of the triolein emulsion was added to 200 µL of porcine pancreatic lipase and to those different concentrations of fractions of methanolic extracts (50, 100, 150, and 200 µg/mL) were added. Orlistat, a potent pancreatic lipase inhibitor was taken as reference standard drug. Immediately after mixing the contents the absorbance was measured at 450 nm and designated as $T_1$. The test tubes were incubated at 37°C for 30 minutes, and at the end of the incubation, the absorbance at 450 nm was recorded and designated as $T_2$.

The variation in absorbance = [A450 ($T_1$) - A450 ($T_2$)] was calculated for both control and the treatment, and the % inhibition was calculated using the formula:

$$\% \text{ inhibition} = [(\Delta A450 \text{ control} - \Delta A450 \text{ extract})/\Delta A450 \text{ control}] \times 100$$

### *In vitro* α-Amylase Inhibitory Activity[21]

Soluble starch (500 mg) was dissolved in 25 mL of 0.4 M NaOH and heated for 5 minutes at 100°C. The pH of solution was adjusted to 7 with 2 M HCl cooling in ice $H_2O$, and water was added to adjust the volume to 100 mL. The substrate (40 µL) and fractions (20 µL) solutions (50, 100, 150, and 200 µg/mL) were mixed in a microplate well, and the mixtures were pre-incubated at 37°C for 3 minutes, followed by addition of 20 µL of α-amylase solution (50 µg/mL) to each well, and incubation of plate for 15 minutes. At last 80 µL of 0.1 M HCl and 200 µL of 1 mM iodine solution were added to terminate the reaction. Acarbose, a potent α-amylase inhibitor, was selected as reference standard drug. The absorbance (Abs) was measured at 650 nm.

Inhibitory activity was calculated as follows:

$$\text{Inhibition (\%)} = [1 - (\text{Abs 2} - \text{Abs 1})/(\text{Abs 4} - \text{Abs 3}) \times 100]$$

Where, Abs 1 is the absorbance of incubated solution containing fractions, starch, and amylase; Abs 2 is the absorbance of incubated solution containing fractions and starch; Abs 3 is the absorbance of incubated solution containing starch and amylase; Abs 4 is the absorbance of incubated solution containing starch.

### Data Analysis

All the experimental results were expressed as mean ± standard error mean (SEM) of three parallel measurements. The $IC_{50}$ values of all test samples were calculated from concentration-inhibition curves. Statistical significance of differences between means was calculated by analysis of variance (ANOVA), followed

**Table 1:** Preliminary phytochemical screening of methanolic extracts and different fractions of *Fagonia cretica* L.

| | *Extract and fractions* | | | | | |
|---|---|---|---|---|---|---|
| *Phytoconstituent* | *MEFC* | *HFFC* | *CFFC* | *EAFFC* | *BFFC* | *AQFFC* |
| Flavonoids | + | - | - | + | + | + |
| Saponins | + | - | + | + | + | + |
| Phenolics | + | - | - | + | + | + |
| Alkaloids | + | - | + | + | - | - |
| Terpenoids | + | + | - | + | - | - |
| Anthraquinone glycosides | - | - | - | - | - | - |
| Cardiac glycosides | + | - | - | + | - | + |
| Carbohydrates | + | - | - | - | + | + |

+ = present; - = absent

**Table 2:** Effects of different concentrations of various fractions of methanolic extracts of *Fagonia cretica* L. on pancreatic lipase activity

| *Test sample* | *Concentration (µg/mL)* | *Inhibition (%)*[*] |
|---|---|---|
| HFFC | 50 | 12.34 ± 0.32 |
| | 100 | 14.76 ± 1.16 |
| | 150 | 16.12 ± 0.65 |
| | 200 | 20.33 ± 0.42 |
| CFFC | 50 | 23.12 ± 0.87 |
| | 100 | 29.33 ± 1.34 |
| | 150 | 32.45 ± 2.41 |
| | 200 | 34.72 ± 1.32 |
| EAFFC | 50 | 27.62 ± 1.75 |
| | 100 | 34.26 ± 1.43 |
| | 150 | 41.17 ± 0.22 |
| | 200 | 48.22 ± 1.74 |
| BFFFC | 50 | 43.32 ± 2.37 |
| | 100 | 57.76 ± 1.56 |
| | 150 | 72.33 ± 0.28 |
| | 200 | 83.02 ± 2.47 |
| AQFFC | 50 | 5.23 ± 0.41 |
| | 100 | 7.76 ± 1.76 |
| | 150 | 11.87 ± 1.82 |
| | 200 | 13.45 ± 2.16 |
| Orlistat | 50 | 64.22 ± 1.45 |
| | 100 | 79.87 ± 2.25 |
| | 150 | 88.23 ± 1.63 |
| | 200 | 92.54 ± 3.36 |

[*]n = 3; values are represented as mean ± SEM

by Dunnett's multiple comparisons test. $p < 0.05$ was considered statistically significant.

## Results

### Preliminary Phytochemical Screening and Quantitative Estimation of Phytoconstituents

Different chemical tests were performed to detect presence of different classes of phytoconstituents. Results of the preliminary phytochemical screening of methanolic extracts and prepared fractions are shown in Table 1. The qualitative phytochemical studies of prepared extract and fractions showed the presence of flavonoids, saponins, phenolics, alkaloids, and carbohydrates. The total flavonoid content of EAFFC and BFFC was found to be 12.28 ± 0.37 mg and 2.43 ± 0.83 mg of quercetin equivalents/ grams of fraction, respectively. Total saponin content of EAFFC and BFFC was found to be 9.76 ± 0.33 mg and 36.12 ± 1.04 mg of diosgenin equivalent/grams of fraction, respectively.

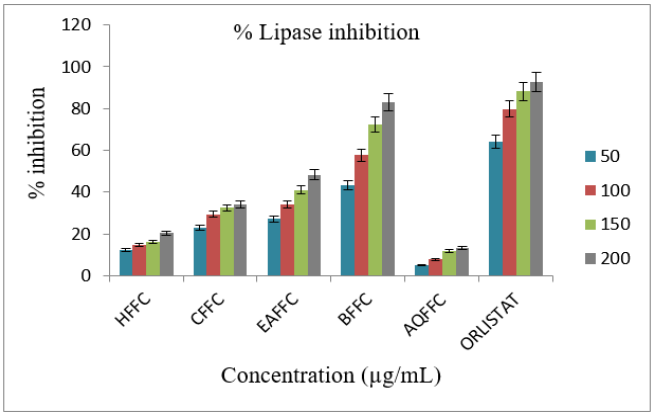


**Fig. 1:** % lipase inhibition of different fractions of MEFC

### *In vitro* Lipase Inhibitory Activity

In the present study, *in vitro* lipase inhibitory effect of different fractions of methanolic extracts of *F. cretica* L. was evaluated using rate of release of oleic acid from triolein. Results of % lipase inhibitory activity of all fractions and standard drug orlistat are shown in Table 2. The present findings revealed statistically significant ($p < 0.05$) lipase inhibitory action of BFFC and standard drug orlistat as compared to other fractions (Fig. 1). $IC_{50}$ value of BFFC was found to be 72.32 ± 0.52 µg/mL, which was comparable with standard drug orlistat having $IC_{50}$ value 42.36 ± 1.43 µg/mL (Fig. 2).

### *In vitro* α-Amylase Inhibitory Activity

Results of % α-amylase inhibitory of all fractions and standard drug acarbose are shown in Table 3. The present studies state statistically significant ($p < 0.05$) α-amylase inhibitory action of EAFFC and standard drug acarbose as compared to other fractions (Fig. 3). $IC_{50}$ value of

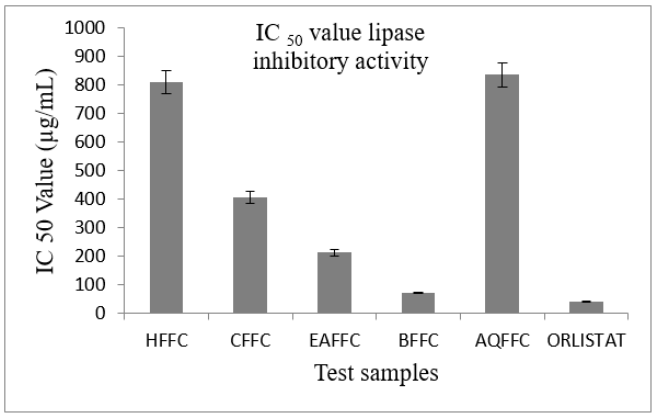


**Fig. 2:** $IC_{50}$ values of different fractions MEFC for lipase inhibitory activity

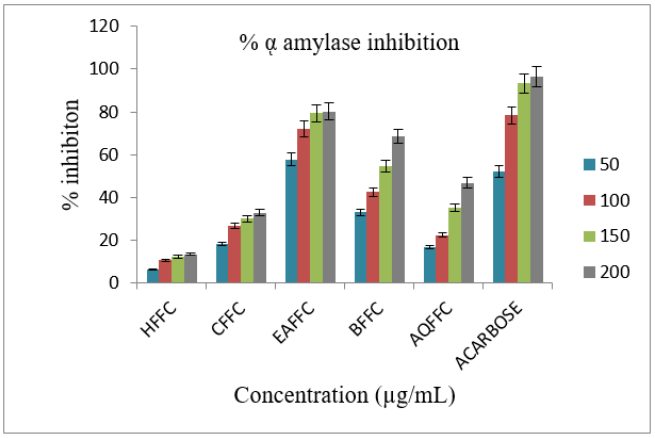


**Fig. 3:** % ɑ amylase inhibition of different fractions of MEFC

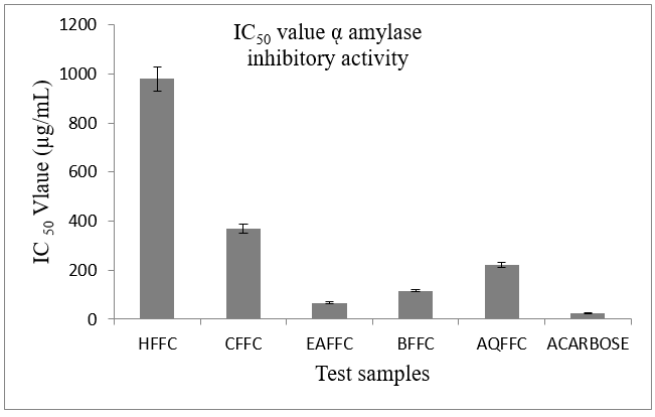


**Fig. 4:** $IC_{50}$ values of different fractions MEFC for ɑ amylase inhibitory activity

EAFFC was found to be 66.52 ± 2.48 µg/mL, which was comparable with standard drug acarbose having $IC_{50}$ value 23.35 ± 0.23 (Fig. 4).

## Discussion

Obesity is now dramatically on the rise in low- and middle-income countries, particularly in urban settings. It is now considered a chronic disease that is reaching epidemic proportions in the developed world.[22] Development of nutrient digestion and absorption inhibitors is one of the most important strategy in the treatment of obesity. Inhibition of digestive enzymes is one of the most widely studied mechanisms used to determine the potential efficacy of natural products as anti-obesity agents. In present study, the methanolic extracts were prepared by using the maceration method of extraction. Fractionation of prepared methanolic extracts was done to separate different classes of phytoconstituents as per their solubility in different solvents. Preliminary phytochemical screening showed the presence of flavonoids, saponins, phenolics, alkaloids, and carbohydrates in different fractions, while anthraquinone and cardiac glycosides were found absent. Quantitative estimation of BFFC and EAFFC showed higher contents of saponins (36.12 ± 1.04 mg/gm) and flavonoids (12.28 ± 0.37 mg/gm), respectively. BFFC showed highest (83.02 ± 2.47%) inhibition of pancreatic lipase with $IC_{50}$ value of 72.32 ± 0.52 µg/mL. EAFFC showed highest (80.22 ± 1.18%) inhibition of ɑ amylase with $IC_{50}$ value of 66.52 ± 2.48 µg/mL. The higher % inhibition of pancreatic lipase by BFFC and ɑ amylase by EAFFC is attributed to the higher saponins and flavonoids contents, respectively, as determined by quantitative phytochemical analysis. Any prior report on *in vitro* anti-obesity activity of methanolic extracts and all different fractions of this plant has not been reported so far. The present study provided valuable preliminary data through demonstration of its probable use in weight loss therapy.

**Table 3:** Effects of different concentrations of various fractions of methanolic extracts of *Fagonia cretica* L. on α-amylase activity

| *Test sample* | *Concentration (µg/mL)* | *Inhibition (%)** |
|---|---|---|
| HFFC | 50 | 6.34 ± 0.09 |
| | 100 | 10.76 ± 0.56 |
| | 150 | 12.32 ± 0.31 |
| | 200 | 13.58 ± 1.98 |
| CFFC | 50 | 18.41 ± 0.49 |
| | 100 | 26.89 ± 1.52 |
| | 150 | 30.24 ± 1.83 |
| | 200 | 32.98 ± 2.46 |
| EAFFC | 50 | 57.88 ± 2.54 |
| | 100 | 72.15 ± 2.69 |
| | 150 | 79.44 ± 1.66 |
| | 200 | 80.22 ± 1.18 |
| BFFFC | 50 | 33.06 ± 1.03 |
| | 100 | 42.56 ± 1.51 |
| | 150 | 54.57 ± 0.94 |
| | 200 | 68.56 ± 1.24 |
| AQFFC | 50 | 16.76 ± 0.31 |
| | 100 | 22.47 ± 1.72 |
| | 150 | 35.06 ± 1.91 |
| | 200 | 46.88 ± 1.06 |
| Acarbose | 50 | 52.17 ± 2.56 |
| | 100 | 78.42 ± 1.16 |
| | 150 | 93.23 ± 0.77 |
| | 200 | 96.54 ± 1.72 |

*n = 3; values are represented as mean ± SEM

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