Extraction and identification of flavonoids from *Prosopis farcta* plant and study anti-inflammatory properties

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Abstract

Prosopis farcta, a member of the Fabaceae family, contains a diverse range of bioactive and medicinal compounds and is considered one of the most important medicinal plants. Various studies have investigated different chromatographic methods to isolate flavonoids from Prosopis. This particular research utilized two different extraction methods. *Prosopis farcta* as a suitable source of powerful flavonoids, with various pharmacological properties. leaves and floweres was done as per the standard method. Different concentrations of the extract was used for anti-inflammatory activity by inhibition of albumin denaturation. The results shown the plant extract had anti-inflammatory activity at 500 µg/ml similar to that standard drug aspirin.

1. Introduction

Prosopis farcta is a herbaceous plant that is commonly consumed in Asia and the Middle East [1]. Prosopis are often spiny trees, 2 to 3 m or taller or small shrubs, *Prosopis farcta* present in a highly incomprehensible or broken way through dense clusters of shrubs helped grow fields. And also well designed to warm weather and time period with not enough rain [2]. *Prosopis fracta* is found in northern Africa, Southwestern Asia, West to the Middle East, and in the USA [3].

Figure 1: *Prosopis farcta* plant

Scientific classification:

Kingdom: Plantae

Order: Fabales

Family: Fabaceae

Subfamily: Caesalpinioideae

Genus: Prosopis

Species: *Prosopis farcta*

Prosopis fracta is a valuable and useful medicinal plant for the purpose of extracting flavonoids and it is one of the most important plants with medicinal properties. *Prosopis farcta* has various pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and anti-diabetic, and anti-cancer properties. The medicinal value of these plants can be observed from the chemical agents they possess which may alter certain physiologic actions in the human body. We note that this plant uses *P. farcta* as a known and available treatment for angina and as a therapeutic agent in the treatment of cardiovascular disorders, relieving heart or chest pain [3] .

Epidemiological studies have suggested an inverse association between the consumption of phytochemicals (such as carotenoids and phenolics) and a reduced risk of certain diseases, namely chronic disorders Medicinal plants, and specifically *Prosopis* plants, are rich sources of phytochemicals, among them alkaloids, phenolic compounds, particularly flavonoids [4].

1.2. Luteolin

Luteolin a bioflavonoid is known to be present in many types of plants and possesses diverse biological properties including antioxidant, anti-inflammatory, and anticancer activities[5]. It is also proven to attenuate anxiety and ameliorate amnesia. It has been demonstrated that the administration of luteolin prevented ischemia-reperfusion injury through a rebalancing of pro-oxidant/antioxidant status [6].

1.3. Rutin

Rutin has one of the most widespread uses as an antioxidant and as an antitumor agent among the flavonoids because of its abundance in the human diet such as fruits [8]

1.4.Hesperidin

Due to its antioxidant and anti-inflammatory properties, hesperidin possesses a variety of biological effects in models of cardiovascular disease and diabetes as well for the prevention of cancer [9]

1.5. Flavonoids

Flavonoids or bioflavonoid, are type of secondary metabolites which are known as Vitamin P and citrin. They are the pigments that color most flowers, fruits, and seeds. Are widely distributed in plants with different metabolic functions. Flavonoids have polyphenolic structure, for that they become responsible for different pharmacological activities (figure2). Generaly flavonoid includes monomeric flavanols, flavanones, anthocyanidins, flavones, isoflavonoid and flavonols. 2-phenyl-g-benzopyrones is the main constituent of flavonoid. Flavonoids are synthesised via phenylpropanoid pathway [10,11]

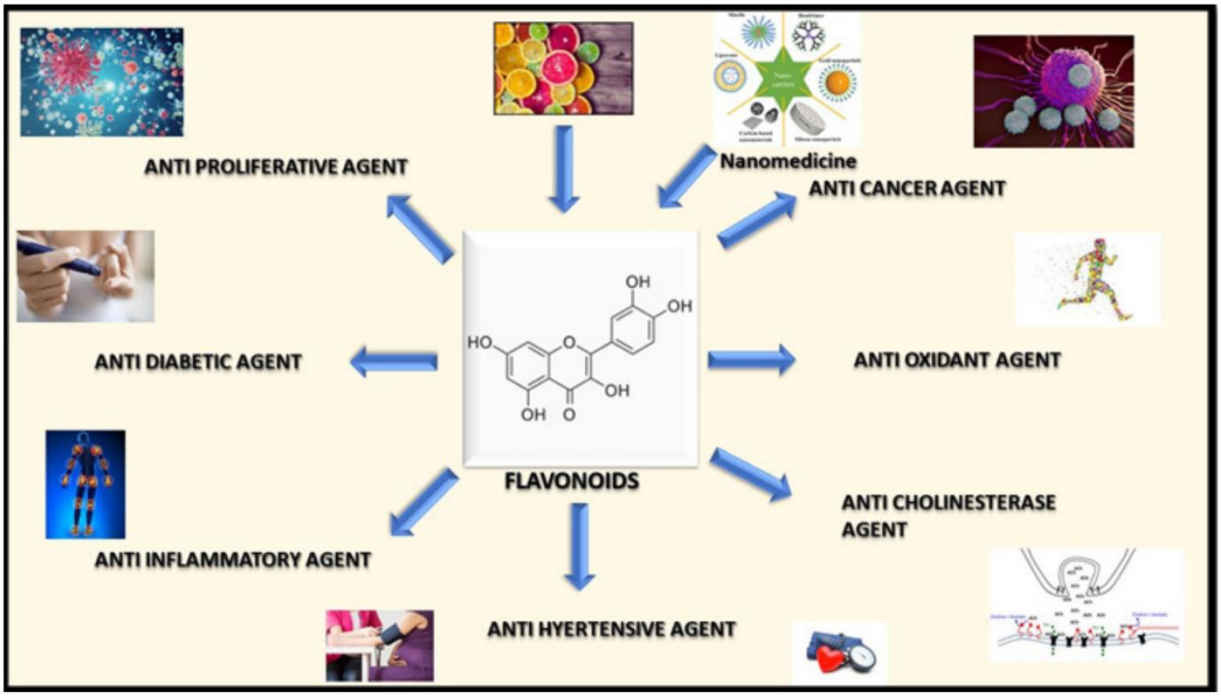

Figure 2: flavonoid different pharmacological activities

1.6. Anti-inflammatory properties for flavonoids

Inflammation plays a key role in diseases such as diabetes, asthma, cardiovascular diseases and cancer. Diet can influence different stages of inflammation and can have an important impact on several inflammatory diseases. Increasing scientific evidence has shown that polyphenolic compounds, such as flavonoids, which are found in fruits, vegetables, legumes, or cocoa, can have anti-inflammatory properties. Recent studies have demonstrated that flavonoids can inhibit regulatory enzymes or transcription factors important for controlling mediators involved in inflammation. Flavonoids are also known as potent antioxidants with the potential to attenuate tissue damage or fibrosis. Consequently, numerous studies in vitro and in animal models have found that

flavonoids have the potential to inhibit the onset and development of inflammatory diseases [12]. In the present study, we focused in type of flavonoids present in *Prosopis farcta* plant and determine the anti-inflammatory activity of their extract.

2. Materials and methods

2.1. Plant material

Prosopis farcta leaves were procured from Al-garma town in Basrah , Iraq. At the Pharmaceutical University in Basrah, Iraq, Assistant Professor Dr. Ula Almousawi identified and verified the plant species.

2.2. Extraction Methods

To get rid of any adherent foreign material, the leaves of *P. farcta* were thoroughly cleaned. The leaves were partially ground using a household grinder after being air dried in the shade. Two distinct extraction techniques being utilized to extract flavonoids [13].

2.2.1. Method 1: Maceration method

In total, 15 g of leaf powder was soaked in 120 mL of 90% methanol solvent for 72 h at room temperature. The menstruum was then filtered and the filtrate was evaporated to obtain the residue. The residue was quantitative estimation of flavonoids in the extract.

2.2.2. Method 3: reflux method

Hot solvent extraction was done using a reflux apparatus for 2 h at 50 °C using 120 ml of 80% methanol. The supernatant was taken and filtered through a

0.45 µm membrane filter. After partitioning with 80 mL of n-hexane to remove lipids.

2.3. Qualitative assessment of phytochemicals *Prosopis* extracts

2.3.1. Flavonoids

According to favonoids Shinoda's assay, the test was carried out by adding conc. hydrochloric acid dropwise to 1 mL of methanolic extract containing a fragment of magnesium ribbon where positive result gives pinkish color [14].

2.4. Thin layer chromatography (TLC)

The TLC was performing used conventional techniques. The substances were solubilized in their corresponding solvents in very small amounts (2 mg/ml). The screening procedure included a variety of mobile phases with varied concentrations, as in (table.1), all plates were viewed in the UV TLC viewer using ultaviolet at 254 nm and 366 nm. Using different flavonoid standards (table.2), the Rf value of the several point that were seen was determined.

$$R_f \text{ value} = \frac{\text{distance moved by analyte}}{\text{distance moved by solvent front}}$$

Table1: mobile phases were tested

Mobile phase	Percent
n-butanol: acetic acid**:** water	6 **:** 1.5 **:**7.5
Tolune **:** ethylacetate **:** methanol **:**acetic acid	4 **:** 3 **:** 2 **:** 0.5
Ethylacetate **:** methanol: water	15:3:2

Table2: types of flavonoid standards were tested

Number	Type
1	Luteolin
2	Quercetin
3	Hesperidin
4	Rutin

2.4. Anti-inflammatory bioassay in vitro

Anti-inflammatory bioassay in vitro of crude extract was done according to [15]. Serial dilution from (250 µg/ml, 500 µg/ml, and 1000µg/ml) was performed for plant extract and for reference drug (Aspirin). All samples contained 5.0 ml of total volume. Reaction mixtures were prepared using 2.8 ml of phosphate buffered saline (pH 6.4) and 0.2 ml of Bovine Serum albumin. Then 2 ml of extract from each different concentration were mixed gently with reaction mixtures. A similar procedure was used for reference drugs (Aspirin). In addition, distilled water was used as negative control. Reaction mixtures were incubated in a water bath at 37°C for 20 min, and later, it was heated at 70°C at which the reaction mixture was maintained for 5 min. Then, the reaction mixture was allowed to cool down at room temperature for 15 min. Absorbance of reaction mixture after denaturation was measured for each concentration at 680 nm. The percentage of inhibition of

protein was determined on a percentage basis with respect to control using the following formula.

% inhibition = 100 × ([Vt/Vc] –1).

Where, Vt = absorbance of test sample, Vc = absorbance of control.

3. Results and Discussion:

3.1. extraction of flavonoids

In the present study, flavonoids were extracted from the leaves by two different extraction techniques such as maceration, and reflux using methanol, as solvents. (figure3). Varying amount of extract yields was obtained with different extraction techniques. It is important to mention that define the oils from plant powder before methanol production process.

Figure3: extraction method of flavonoids from leaves plant powder

3.2. Flavonoid identification test

All flavonoid identification with different extraction method was showed positive results (figure4). But with different in resulting color the reflux method of extract give the best result with Shinoda test. Shinoda test was detected the presence of flavan-3,4-diol groups, flavanones, or isoflavones. In the Shinoda test, strong acid was hydrolyzed the glycoside-flavonoid to aglycone-flavonoid, then forma red or orange complex with magnesium. Shinoda's test results for reflux method were orange, which indicates the flavones or isoflavones [16].

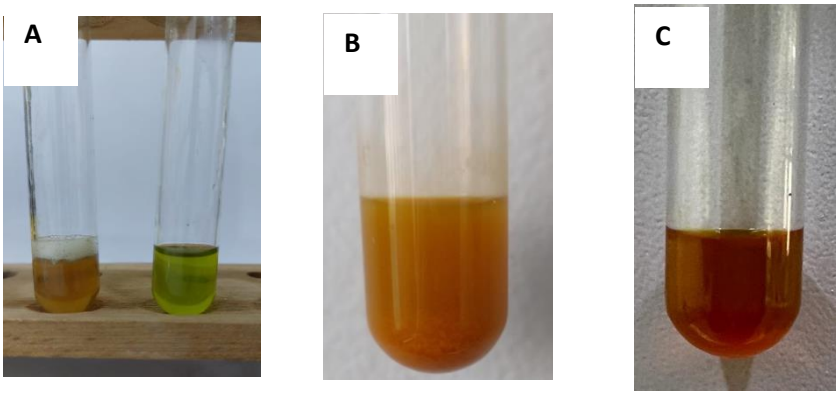

Figure4: Shinoda test result A: sample B: reflux method C: maceration method:

3.3. Thin layer chromatography (TLC)

Thin layer chromatography (TLC) was used to investigate the composition of the various extracts. TLC can be used for qualitative as well as semi-quantitative analysis of crude extracts for identification of constituents. Qualitative analysis is done by comparing the retardation factor (Rr value) on the TLC against a reference value of a standard [17]. The preparation of the extract by TLC using different

mobile phase as developing solvent gave spots for different flavonoids present in extract which had Rf value as in (table 3 and figure 5).

Table3: Rf value of flavonoid in extract and standards were tested

Mobile phase	Type of flavonoid	Rf value of sample	Rf value of standard
n-butanol: acetic acid**:** water	Luteolin	0.83	0.84
Ethylacetate **:** methanol: water	Hesperidin	0.4	0.45
Ethylacetate **:** methanol: water	Rutin	0.56	0.53

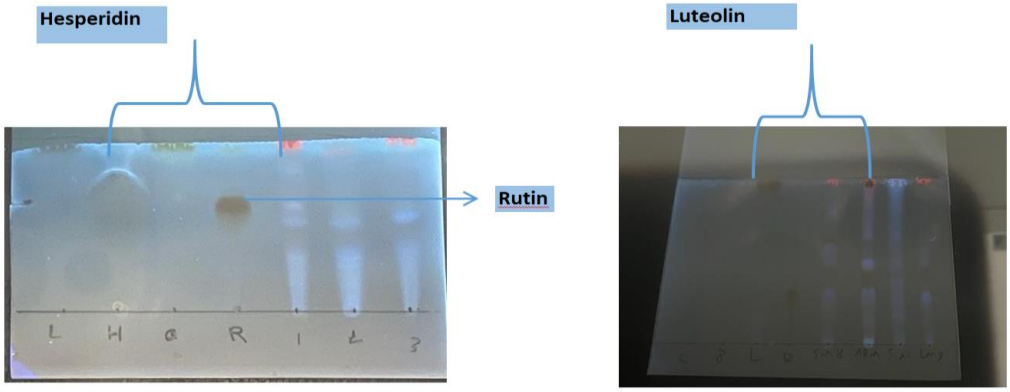

Figure5: TLC plate for the identification of flavonoids in the extracts from leaves of four *P. farcta*. UV 365nm .

3.5. antinfamatory activity

Figure 6 and figure 7 represent the effect of different concentration of methanol extract on inhibition of albumin denaturation. Protein denaturation is a well established cause of inflammation. Leaf extract showed the inhibitory activity of 30,60, and 80% respectively while aspirin showed a inhibitory activity of 40,60, and 96 % respectively. which could comparable with the commercially available synthetic anti inflammatory drug aspirin plant extract has the same inhibitor activity to aspirin at concentraion 500 µg/ml.

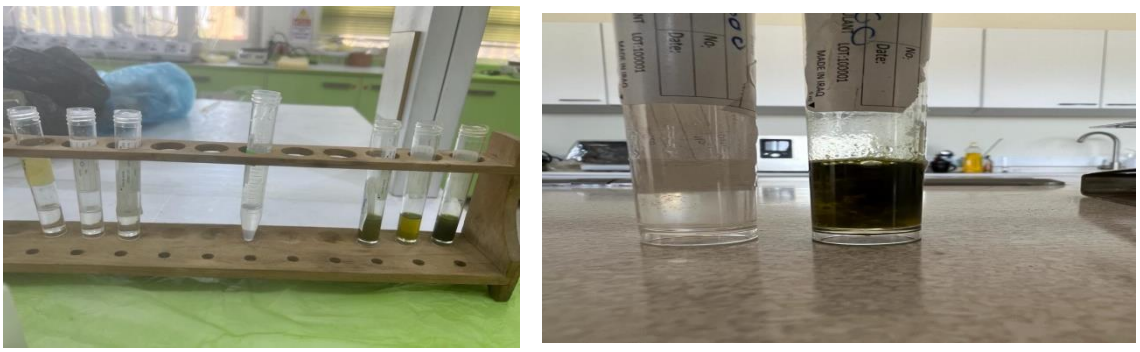

Figure 6: tubes after and before protein denaturation, white tube: protein with aspirin brown tube: protein with plant extract

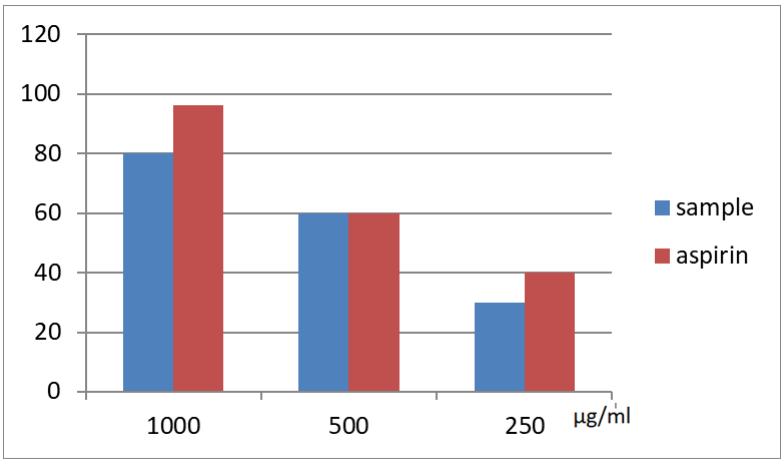

Figure 7: *P. farcta* leaf extract % of denaturation inhibition.

Protein denaturation is the most common cause of prolonged inflammation. Therefore, inhibition of such denaturation can have a clinically favorable effect on inflammation. Moreover, stabilizing the regenerative molecules can yield better clinical results. Mizushima and Kobayashi (1968) have shown that when these phytochemicals inhibit protein denaturation and they have anti-inflammatory activity [18].

Conclusion Our present study concules that *Prosopis farcta* possess anti-inflammatory properties which could be due to presence of active constitutents presen in the plant extract such as luteolin, rutin and hesperidin flavonoids .

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