

## In-Vitro Evaluation of Anti-Inflammatory and Anti-Microbial Properties of Ethanolic Extract of *Cydonia Oblonga* Seeds

Bushra Shaida<sup>1,2</sup>, N B Singh<sup>3\*</sup> and Karuna Singh<sup>4</sup>

<sup>1,4</sup>Amity institute of food technology, Amity University, Noida, India

<sup>2</sup>School of Allied Health Sciences, Sharda University, Greater Noida, India

<sup>3</sup>Department of Chemistry, SBSR, Research and technology department centre, Sharda University, Greater Noida, India

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Phytochemical, antimicrobial, antioxidant and anti-inflammatory properties of ethanol extract of *Cydonia oblonga* were studied. The antioxidant and anti-inflammatory efficacy of the extract was assessed through different methods using DPPH and bovine albumin respectively. Total phenolic content (TPC) was estimated using Folin-Ciocalteu reagent. Antimicrobial effect of seeds was studied using disc diffusion method against gram positive and negative bacteria viz; *S.aureus*, *P.aeruginosa* and fungi *C. albicans*. Phyto-chemical analysis showed the presence of alkaloids, phenols, tannins, amino acids, carbohydrate, fats/oils and glycosides. TPC of the seed extract was found to be 37.46±0.10 mg GAE/gm, extract also showed significant antioxidant and anti-inflammatory activity with IC<sub>50</sub> value of 299.98µg/ml and 300µg/ml respectively. Anti-microbial results indicated that *Cydonia oblonga* seeds extract is effective against gram positive bacteria.

**Keywords:** *Cydonia oblonga*, Anti-oxidant, Anti-inflammatory, Antimicrobial, Minimum Inhibitory concentration (MIC)

### Introduction

Oxidative stress and inflammation can lead to many health problems<sup>1</sup>. Researchers have worked on quantification of anti-inflammatory and antioxidants potential of plant constituents<sup>2</sup>. Antioxidants are the compounds that inhibit or delay oxidation reaction and many naturally occurring plants have good antioxidant properties. Quince fruit peel and pulp extract has shown a significant antioxidant property against DPPH<sup>3</sup>. It is reported that Quince leaf has anti-inflammatory activity<sup>4</sup>. Similarly, studies on antimicrobial activity of different aerial parts of quince plant demonstrated that its leaf and fruit is effective against gram positive and gram-negative bacteria<sup>5</sup>. However, experiments on seeds are limited. In the present study antioxidant, anti-microbial and anti-inflammatory activity of *C oblonga* seed extract has been carried out and results discussed.

### Material and methods

#### Materials

*Cydonia oblonga* seeds were obtained from Srinagar, Jammu & Kashmir India. Seeds were washed several times with water and air dried. The

dried seeds were packed under vacuum and stored at 18°C in dark till taken out for experiments.

#### Methods

##### Preparation of extract

The seeds as stored above were ground to fine powder and 100 g was taken in Soxhlet apparatus for solvent extraction by using ethanol as solvent at 60-80 °C for 9-10 h. The solvent of the extract was allowed to evaporate at room temperature and the material left was stored at 4 °C for analysis<sup>6</sup>.

##### Phytochemical analysis

Quantitative phytochemical analysis was done on freshly prepared crude ethanolic extracts of *Cydonia oblonga* seeds to identify different phyto constituents such as phenolic, alkaloids, phytosterols, tannins, flavonoids, proteins, terpenoids and glycosides etc. by the method used by Amin and Thakur<sup>7</sup>.

##### Quantitative estimation of Phenolic content:

Total phenolic content of *Cydonia oblonga* seed extract, was estimated by the method as suggested by Andressa Blainski<sup>3</sup>. 1.0 ml of distilled water was added to 20 µg of ethanolic extract, and 500 µl of diluted Folin-Ciocalteu reagent (1:1 ratio with water) and 2.5 mL of sodium carbonate Na<sub>2</sub>CO<sub>3</sub> (20%) was also added. Colour was developed after incubating the

\*Author for Correspondence  
E-mail: nbsingh43@gmail.com

well shaken mixture in dark for 40 min. Total phenolic content was determined by using UV-visible spectrophotometer. Concentration was determined with the help of a calibration curve by taking Gallic acid as a standard.

#### Determination of Anti- Oxidant Property DPPH method:

Anti-oxidant property of *Cydonia oblonga* seed extract was assessed using 1, 1- diphenyl-2-picrylhydrazyl (DPPH) by the method given by Blois<sup>4</sup>. 0.2mmol/l of DPPH was prepared in ethanol and the solution was treated as control solution, 500 ml of the control was added to the solution of the extract of different concentration (100-500µg/ml). Each mixture was shaken vigorously and allowed to stand for 30 min at room temperature. Absorbance of each solution was noted at 517 nm with the help of UV-Visible spectrophotometer and the concentrations were determined for comparison of the absorbance of ascorbic acid under similar conditions. The antioxidant activity was also calculated by using Eq.1.

$$I \% = (Ac - As) / Ac \times 100 \quad \dots (1)$$

where, I is inhibition, Ac is the absorbance of control and As is the absorbance of extract solution, respectively.

#### Inhibition of albumin denaturation

Anti- inflammatory activity was determined by using the procedure described by Padmanabhan and Jangle<sup>8</sup> with minor modifications. Different concentration of test extract (100-500µg/ml) was prepared in water and 1% water solution of bovine albumin was added in each solution. 1.0 N HCl was used to adjust the pH of the solution to get better results. Incubation of samples was done at 37°C for 25 min and after that heated at 60°C for 25 min. Samples were cooled and absorbance was measured 660 nm using spectrophotometer. Inhibition percentage of albumin denaturation was calculated using Eq.2

$$I \% = (Ac - As) / Ac \times 100 \quad \dots (2)$$

where, I is inhibition, Ac is the absorbance of control and As is the absorbance of extract solution, respectively.

#### Antimicrobial Tests

Antimicrobial activity of the *Cydonia oblonga* ethanolic seeds extract was done using disc diffusion method. The micro-organism used for the study were:

*Staphylococcus aureus* (MTCC87) for Gram-(+) bacteria, *Escherichia coli* (MTCC 68), *Pseudomonas aeruginosa* (MTCC424) for Gram-(-) bacteria, the yeast *Candida albicans* (MTCC183). The extract susceptibility for the test of organism was determined by employing standard disc diffusion technique<sup>9</sup>. Different concentration of extract was prepared (100 µg/ml to 500µg/ml) in DMSO (Dimethylsulfoxide). The test microorganism was inoculated in nutrient agar medium by spread plate method (10<sup>6</sup> cells/ml) for 24 h. 5 mm diameter filter paper disc plates were prepared and infused with the seed extract, after that it was placed on test organism seeded plates. Ampicillin and Clotrimazole were used as positive control for anti-bacterial and anti-fungal activity. Plates were incubated for 24 h at 37°C, zone of inhibition was measured and compared with control.

#### Statistical analysis

All the readings were taken in triplicate and results were given with standard deviation. For anti-oxidant and anti-inflammatory correlation analysis was performed and P values take as ≤0.05.

#### Results and Discussion

Phytochemical analysis of *C oblonga* seed extract showed the presence of carbohydrate, glycosides, Phyto-sterols, flavonoids, phenolic compounds, alkaloids, amino acids as summarized in table 1. Phenolic content of the seed extract was calculated using standard calibration curve of Gallic acid, the phenolic content of ethanolic extract of *Cydonia oblonga* was estimated as 37.46±0.10 mg of Gallic acid equivalent (GAE) per gram of extract. This value represents a respectable content of phenolic compounds in the seeds which can further be used for medicinal purpose. As compared with other parts of

Table 1 — Preliminary phytochemical analysis of ethanolic extract of *Cydonia oblonga* seeds

Phyto constituents	Present/absent
Alkaloids	+
Phenols	+
Tannins	+
Flavonoids	-
Proteins/amino acids	+
Carbohydrates	+
Fats/oils	+
Steroids	-
Terpenoids	-
Glycosides	+
Anthoquinones	-

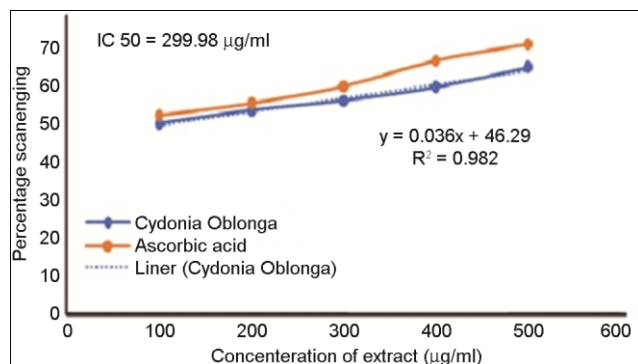


Fig. 1 — Percentage Scavenging of DPPH Radical by Ethanolic Extract of various seeds

Table 2 — In-vitro anti-inflammatory activity of ethanolic extract of *Cydonia oblonga*

Test Samples	Concentration (µg/ml)	Percent Inhibition Albumin Denaturation
Ethanolic Extract of <i>Cydonia Oblonga</i>	100	34.42 ± 1.11
	200	41.24 ± 1.21
	300	44.29 ± 0.76
	400	52.28 ± 0.69
	500	58.61 ± 0.43
Aspirin	100	62.38 ± 0.38
	200	70.50 ± 0.53

quince plant leaves have higher phenolic content<sup>10</sup>. Most widely used procedure employed to assess antioxidant potency of plant and biological samples were DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical scavenging activity<sup>4</sup>. The ethanolic extract of *Cydonia oblonga* showed DPPH radical scavenging activity in a concentration dependent manner, results were revealed in figure 1. IC<sub>50</sub> value indicates that concentration required to inhibit half of DPPH. IC<sub>50</sub> value of the ethanolic extract of quince seeds is 299.98 µg/ml which indicates that 299.98 µg/ml concentration is required to reduce 50% of DPPH. Denaturation is a process involving destruction of tertiary and secondary structure of protein which can further lead to loss of biological activity of molecule, thus causes inflammation. In the present study, anti-inflammatory activity of quince seed extract was investigated. Results are given in Table 2. Maximum inhibition, (58.61 ± 0.43%) was observed at 500 µg/ml, this conclude that albumin denaturation by the extract is concentration dependent. IC<sub>50</sub> value was found to be 300 µg/ml. Aspirin, used as standard anti-inflammatory drug and showed maximum inhibition, (62.85 ± 0.59%) at the concentration of 200 µg/ml. *Cydonia oblonga* seed extract exhibit

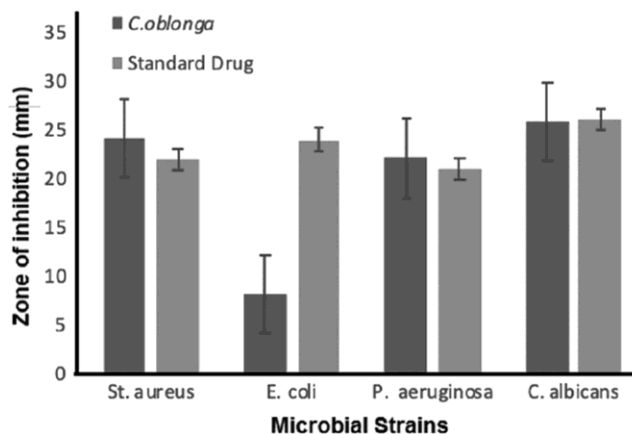


Fig. 2 — Zone of inhibition by ethanolic extract of *Cydonia oblonga* seeds

significant concentration dependent albumin protein denaturation compared with standard drug aspirin. Phenolic content of the seed was responsible for anti-inflammatory activity as these compounds has a capacity to inhibit either the production or the action of pro-inflammatory mediators resulting in anti-inflammatory capacity<sup>8</sup>. Anti-microbial activity of *Cydonia oblonga* seed extract against different micro-organism strains was investigated and results were revealed. These results indicate that quince seeds extract is effective against gram positive bacteria i.e *Staphylococcus aureus* and gram-negative bacteria i.e *E. coli*, *P. aeruginosa* and fungi *Candida albicans* with zone of inhibition 24.01 ± 0.31 mm, 8.15 ± 0.25 mm, 22.12 ± 0.23 mm and 25.82 ± 0.39 mm respectively, while when compared with standard drug, ampicillin zone of inhibition was found to be effective against *Staphylococcus aureus*, *P. aeruginosa* and for anti-fungal activity standard drug used was clotrimazole, the zone of inhibition was found to be effective for test extract. The diameters of zone of inhibition increases with increase in concentration of the extract, the result was in line with those reported by Sami Fattouch<sup>9</sup> *et al.* using peel and pulp quince extract on *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*. Minimum inhibitory concentration (MIC) was found to be 500 µg/ml for micro-organisms.

## Conclusions

From the results, it is found that *Cydonia oblonga* seeds are good source of important phytochemical compounds and have significant antioxidant activity. Anti-inflammatory activity of seed is also studied and found to be effective when compared to standard drug

aspirin. Results also indicated that *Cydonia oblonga* seed extract has an antimicrobial activity on organism viz; *S.aureus* and *P. aeruginosa* and fungi *Candida albicans* as these organism are most pathogenic and need to be cured at earliest. However, detailed investigations are needed to ascertain the mechanism and constituents behind all these activities of *Cydonia oblonga* seeds.

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