

## Phytochemical Screening, Determination of Total Phenol Content, Total Flavonoid Content and Quantitative Estimation of Rutin and Quercetin Using RP-HPLC in the Fruits of *Capparis decidua* (Forsk.) Edgew

Sonali Bhagat<sup>1\*</sup>, Mala Rathore<sup>2</sup>, Sumita Kachhwaha<sup>3</sup> and Hemant K. Sharma<sup>1</sup>

<sup>1</sup>Arid Forest Research Institute, Jodhpur

<sup>2</sup>Forest Research Institute, Dehradun

<sup>3</sup>Botany Department, University of Rajasthan, Jaipur

\*Corresponding Author E-mail: [sonali.cct@gmail.com](mailto:sonali.cct@gmail.com)

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### ABSTRACT

*Capparis decidua* fruits, commonly known as Kair in most parts of India, have been used in traditional herbal medicines since ancient times to cure numerous ailments and disorders. Medicinal properties of any herbal drug formulation are mainly due to the presence of various secondary metabolites like alkaloids, sterols, flavonoids, etc. The present study deals with the phytochemical screening of kair fruits for their qualitative assessment and determining Total Phenol Content (TPC) and Total Flavonoid Content (TFC) using spectrophotometric methods. The TPC and TFC was estimated to be  $38.25 \pm 1.04$  mg GAE/g and  $18.58 \pm 0.18$  mg QE/g respectively, in the methanolic fruit extract on DW (dry weight) basis. Quantitative estimation of flavonoids like Rutin and Quercetin was done by peak area measurement using RP-HPLC technique which gave the results as 0.32 and 0.16 ppm in the samples respectively. Our results give an idea about phytochemical profile of these wild edible fruits of western Rajasthan. A further deep probe into other bioactive principles responsible for their medicinal values can give an insight that can be more beneficial for exploring them to be used in Pharmaceutical industry.

**Keywords:** Kair fruits, Flavonoid content, Phenol content, Quercetin, Rutin, HPLC.

### INTRODUCTION

*Capparis decidua* (Forsk.) Edgew (Capparidaceae) is a wild, self-growing shrub mainly found in the Northern parts of India, mostly in the hot arid regions of western Rajasthan. Besides having many socioeconomic and ecological importance,

nearly all parts of this plant possess one or many medicinal properties. The green fruits, locally known as Kair, are used as a vegetable (in food preparations like ‘panchkuta’), in pickles and fully ripe fruits are also consumed fresh as they are sweet in taste (Baloda & Bangarwa, 2010).

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*Capparis decidua* is a part of many traditional herbal medicines to cure asthma, cough, toothache, malaria, inflammation, intermittent fevers, rheumatism and swelling (Singh & Singh, 2011; & Mann et al., 2013). *C. decidua* is also well known for its antibacterial, antifungal (Keymanesh et al., 2009; & Gull et al., 2015), antihemolytic (Vaishnav et al., 2015) and antioxidant properties (Zia-Ul-Haq et al., 2011). The fruits and the seeds are claimed to possess antidiabetic and diuretic properties as well as they are used to cure cholera, dysentery and urinary tract disorders (Rathee et al., 2010; & Zia-Ul-Haq et al., 2011). Antiarthritic potential of *Capparis decidua* stem, root and leaves on rats have been reported by Kumar et al. 2017. The green small sized immature fruits possess antihelminthic properties (kills intestinal worms) and used to cure many digestive tract disorders like constipation and piles acting as a laxative (Goyal & Grewal, 2003; Joseph & Jini, 2011; & Singh et al., 2011). *C. decidua* fruit extract possesses hypolipidemic (Yadav et al., 1997; & Chahlia, 2009) and antiatherosclerotic properties (Purohit & Vyas, 2006).

The chemical makeup of a plant determines its uses in the medicinal or pharmacological field. Several phytochemical and pharmacological investigations have been carried out on *C. decidua* to evaluate various Sterols, alkaloids, fatty acids, flavones, oxygenated heterocyclic constituents etc. found in the plant (Sharma & Kumar, 2008; Rathee et al., 2010; & Shad et al., 2014). An isothiocyanate glucoside was also reported from different parts of this plant (Juneja et al., 1970). The nutritional value of flowers and fruits of *C. decidua* has also been reported (Chauhan et al., 1986; Dahot et al., 1993; & Alrasheid et al., 2018). Pharmacological properties have been depicted by different extracts of this plant (Chishty & Monika, 2016). The plant extract has also been reported for its activity as sedative and Central Nervous System depressant (Goyal et al., 2009). Anti-inflammatory activity of the extracts of the fruits of *C. decidua* has been experimentally

proven on mice (Zhou et al., 2010). Methanol and water extracts of *C. decidua* stems were reported to possess hepatoprotective activity (Ali et al., 2011). *C. decidua* extracts have been reported to be more potent than *C. spinosa* extracts in terms of their anti-microbial activity (Gull et al., 2015). Owing to the presence of a saga of phenomenal compounds present in *C. decidua*, its economic importance has increased proportionately and it can be regarded as a miracle tree (Nazar et al., 2020).

Zia-Ul-Haq et al. 2011 studied the proximate composition of tocopherols, sterols, glucosinolate and total phenolic content in the extract of aerial parts of *C. decidua* and have reported its antidiabetic and antioxidant activity. Work on changes in the total phenolic content and total antioxidant activity of kair fruits during different stages of maturity was reported by Kumar, 2018.

The present study emphasizes on preliminary phytochemical screening for qualitative examination, estimation of proximate values of total phenol content (TPC), total flavonoid content (TFC) and quantitative estimation of rutin and quercetin in the methanolic extract of fruits of *Capparis decidua* using RP-HPLC technique.

## MATERIAL AND METHODS

### Sample collection

Fruit samples were collected from the fully grown shrubs of *Capparis decidua* from the Forest Ecology Experimental Field of Arid Forest Research Institute, Jodhpur. Collection was done in the months of April and May (summer season) in the years 2016, 2017 and 2018.

### Chemicals used

All the chemicals used in the experiment were of Analytical grade. HPLC grade Methanol, Ortho-Phosphoric Acid, Triethylamine (TEA) and Pure water was procured from Merck. Standards (high-purity i.e. >95% pure) viz. Quercetin, Rutin, Gallic acid were procured from SIGMA.

### Sample extraction

Fruits of *C. decidua* were air-dried and ground using a mixer grinder. The crude powdered

fruits (100 g) were exhaustively extracted in methanol using Soxhlet apparatus for about 48 Hrs. Then they were dried and made solvent free by using Rotary Vacuum Evaporator (make- EYELA). This sample extract was ready for further analysis.

### Experimental

#### Phytochemical screening

Various standard methods (Harborne, 1998; Brunton, 1995; & Evans, 2009) were used to qualitatively detect the presence or absence of specific secondary metabolites in the fruit extract.

**Test for Terpenoids:** 5 ml of the sample extract was taken, to which 2 ml of  $\text{CHCl}_3$  was added in a test tube. Then 3 ml of concentrated sulfuric acid was carefully added to the reaction mixture. No interface of reddish-brown coloration confirmed the absence of terpenoids.

**Test for Sterols (Salkowski's test):** 2ml of extract was mixed with 2 ml of chloroform. Then, 2 ml of concentrated Sulphuric acid was added, which formed a layer and a reddish/brown color interface was observed which reflects positive results for the presence of sterols.

**Test for alkaloids:** A reddish-brown or orange red precipitation after the addition of few drops of Wagner's reagents or Dragendorff's reagent is considered as a positive test for alkaloids.

**Test for flavonoids:** Sodium hydroxide test and Shinoda test were used for the presence of flavonoid. Precipitation of yellow coloration formed after the addition of 2ml of 10% aqueous sodium hydroxide solution indicates the presence of flavonoids. This yellow color turns into colorless upon addition of dilute hydrochloric acid to it. In case of Shinoda test, when few pieces of magnesium chips are added along with 2 drops of concentrated HCl in sample extract appearance of a red or pink color marks the presence of flavonoids.

**Test for Tannins:** Brownish green or a blue-black coloration obtained from the addition of 2-3 drops of 5% ferric chloride solution to the extract confirms the presence of tannin.

**Test for Phenolic compound:** Three drops from a freshly prepared mixture containing equal amount of 1% ferric chloride solution and 1% potassium ferrocyanide was added to fruit extract. After filtering this solution, presence of a bluish-green color of the solution indicate positive results.

**Test for Saponins (Froth test):** 2.5 ml of extract was added to 10 ml of distilled water in a test tube. Then, this test tube was closed with cap and shaken vigorously for 30 second and allowed to stand still for about half an hour. No froth formation observed indicates the absence of saponins.

**Test for Anthraquinones:** To the extract added 1ml benzene, mixed with 1ml of 10% ammonia. No pink, red or violet color develops, which indicates the absence of anthraquinones.

**Test for Quinones and Coumarins:** Addition of 10% NaOH into the test sample did not give blue green or red precipitation that confirms the absence of quinones whereas no yellow color developed from the same test indicates the absence of coumarin.

#### Total Phenol Content (TPC)

The total phenol content was determined using the Folin-Ciocalteu's assay (Donald Mc et al., 2001) with Gallic acid as a reference standard. In this method, 0.5 ml of the plant extract was taken in a 10 ml volumetric flask, to which 1.5 ml of Folin- Ciocalteu's reagent (FCR) which was pre-diluted to 1:10 v/v was added, five minutes later 1.5 ml of 7% sodium carbonate solution was added. The final volume was made up to 10 ml with distilled water and allowed to stand for 90 minutes at room temperature. Absorbance of the sample as well as standard was measured against the blank at 750 nm using a UV-Vis spectrophotometer (make- SPEKOL, 2000). The experiments were performed in triplicates to give more precise results. The values were expressed as mean  $\pm$  standard deviation in terms of phenol content, mg GAE (Gallic acid equivalent) per g of dry weight (DW basis) of sample extract using a linear equation based on the calibration curve of standard Gallic acid with  $R^2$  (Regression coefficient) value of 0.9941.

**Total Flavonoid Content (TFC)**

Total flavonoid content was determined using Aluminium chloride method (Olajire et al., 2011), quercetin was taken as a reference standard. 1ml of the test sample and 4 ml of distilled water was added to a volumetric flask of 10 ml volume. To this, 0.3 ml of 5 % Sodium nitrite was added. After 5 minutes, 0.3 ml of 10% Aluminium chloride was added. Then, after 6 minutes, 1ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the test solution was filled with distilled water to make final volume to 10 ml. Absorbance of the sample was measured against the blank using a UV-Vis spectrophotometer at 510 nm. Values were expressed as mean  $\pm$  standard deviation in terms of flavonoid content, mg QE (Quercetin equivalent) per g of dry weight (DW basis) of sample extract based on the calibration curve of standard Quercetin with  $R^2$  (Regression coefficient) value of 0.9939.

**Quantitative estimation of Quercetin and Rutin**

A Waters HPLC system equipped with a Waters 2998 PDA detector and Gradient Pump Control Module with two pumps (WATERS, 515) was used for RP-HPLC analysis. Chromatographic separation was managed, chromatograms were recorded, and data were processed with the EMPOWER V.2.0 software. Chromatographic separations were carried out by using a Spherisorb ODS2 column (250 mm, 5  $\mu$ m particle size). The column was operated at a constant temperature of 25°C. The volume of the extract being injected in the manual Rheodyne injector was 20  $\mu$ L. The flow rate was 1 mL/min, and gradient elution was used. The mobile phase consisted of Methanol, o-phosphoric acid, TEA, Water. Standard Quercetin and Rutin solutions of concentration 100 ppm each separately were prepared in HPLC grade methanol and filtered through 0.45 micron HPLC filters before injecting into the WATERS HPLC system.

**Programming Gradient method for HPLC:**

Time (in mins.)	Methanol (%)	Water+ 0.5% o-phos.acid+ 0.1% TEA
0-10	30	70
11-20	45	55
21-30	60	40
31-35	80	20
36-45	100	0

**RESULTS AND DISCUSSION****Phytochemical screening of *Capparis decidua* fruits**

The methanolic extract yield obtained was 32.52 $\pm$ 2.34 g per 100 g of crude powdered fruit sample. Qualitative analysis was done for

phytochemical screening using the standard methods. The presence of Phenolic compounds, alkaloids, flavonoids, sterols and tannin was detected in the fruit extract of *C. decidua*.

**Table 1: Phytochemical screening of *Capparis decidua* fruits**

Secondary metabolite	Phytochemical Screening of MeOH extract
Phenolic compounds	+
Alkaloids	+
Flavonoids	+
Tannin	+
Saponin	-
Triterpenoids	-
Sterols	+
Anthraquinones	-
Quinones & Coumarins	-

+ shows presence of compound; - shows absence of compound

### Total Phenol Content (TPC) and Total Flavonoid Content (TFC) determination

The Total Phenol Content was estimated to be  $38.25 \pm 1.04$  mg GAE/g of extract on dry

weight (DW) basis whereas Total Flavonoid Content was calculated to be  $18.58 \pm 0.18$  mg QE/g of extract on DW basis in the methanolic extract of fruits of *C. decidua*.

**Table 2: Total Phenol content (TPC) and Total Flavonoid content (TFC) in the fruit extract of *C. decidua* (values as mean  $\pm$  standard deviation).**

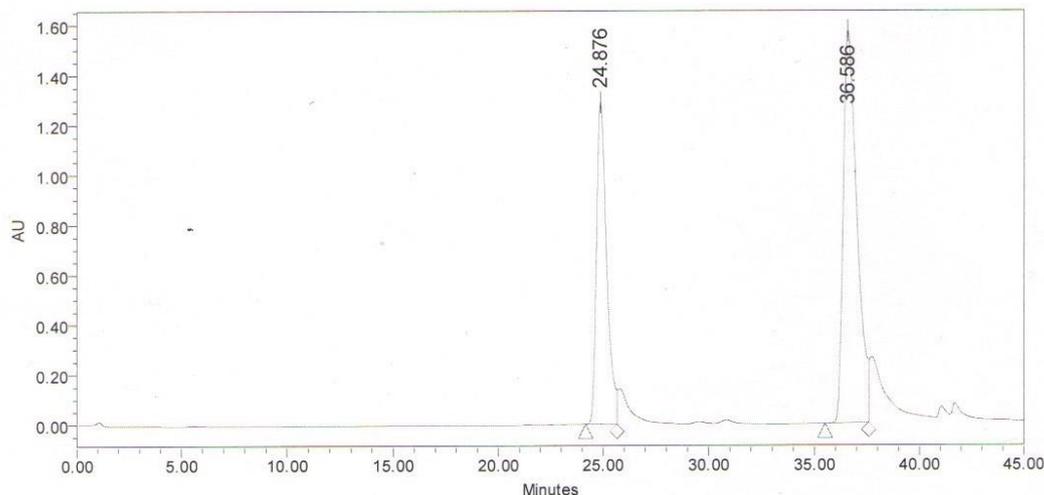
Analysis	Value (Mean $\pm$ SD)
TPC (mg GAE/g)*	$38.25 \pm 1.04$
TFC (mg QE/g)*	$18.58 \pm 0.18$

\* on dry weight basis of extract

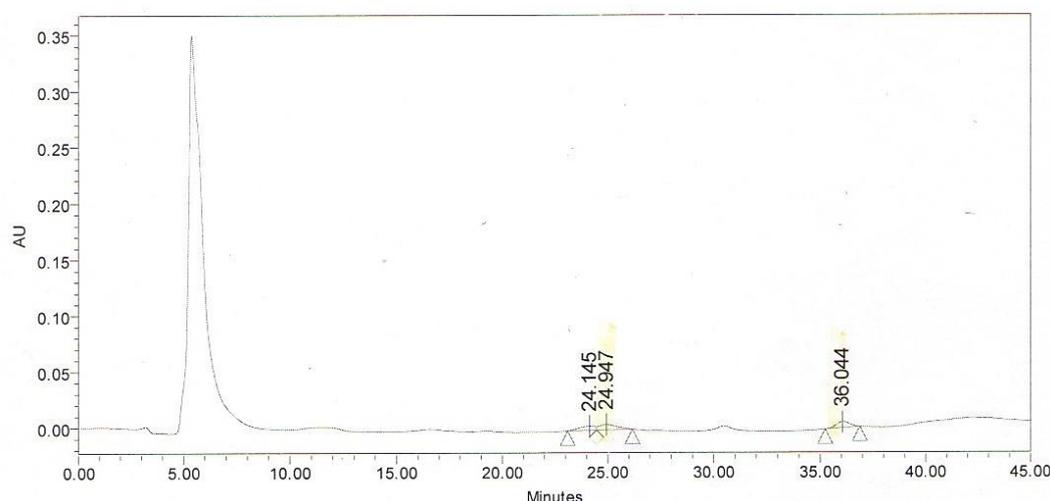
### Quantitative estimation of Quercetin and Rutin using RP-HPLC

Retention time (Rt.) for standard Quercetin was found at 24 minutes and Rt. for Rutin was obtained at 36 minutes. The sample prepared from fruit extract also gave the Rt. at 24 and

36 minutes for Rutin and Quercetin, respectively. The quantitative estimation of Rutin and Quercetin was done using the peak area measurement and comparison from that of standard and was found to be 0.32 ppm and 0.16 ppm, respectively.



**Fig. 1: HPLC chromatogram of standard Rutin (Rt. at 24.436 minute) & Quercetin (Rt. at 36.0026 minute)**



**Fig. 2: HPLC chromatogram of methanolic extract of fruits of *C. decidua* (Rt. at 24.947 & 36.044 minute for Rutin and Quercetin, respectively)**

The phenolic and flavonoid content in the methanolic extract of fruits of *Capparis decidua* has been estimated in the present study. The values indicated that the fruits of *C. decidua* are rich in phenolics and flavonoids, which are the compounds primarily responsible for antioxidant, antibacterial and antidiabetic potential of kair fruits (Zia-ul-Haq et al., 2011). Proestos et al., 2006 studied the correlation between phenolic content and antimicrobial activity of plant extracts, which revealed that plant extracts with higher phenolic and flavonoid contents show upsurged antibacterial activity.

Studies on the phenolic compounds in *Capparis* fruits were also reported by Zia-Ul-Haq et al. 2011 and the reported values are quite different from the ones obtained in present study. This difference might be due to divergence in extraction method, environmental conditions or standard compounds used by them. Contrary to this Shad et al., 2014 evaluated phenolic and flavonoid content in the fruits of *C. decidua* and documented values were in a range near to the present study. Tlili et al. 2010 studied phenolic content and Ghafoor et al. 2020 evaluated TPC & TFC values in the *C. spinosa* fruits, the values of which were somewhat similar to the values of the current study. Recently Neeraj et al. 2019 also identified the presence of six polyphenols from extract of *C. decidua*, out of which two were Rutin and Quercetin but they did not evaluate these compounds quantitatively. Gull et al. 2018 have documented from their studies that it is the repercussions of the season in which fruits are collected that determines the amount of phenols present in the fruit of *C. decidua* and they obtained similar values of TPC in comparison to the present study while TFC value was higher than ours. This could be attributed to the fact that they have expressed in Catechin Equivalent (mg CE/g) whereas we have expressed the values in Quercetin Equivalent (mg QE/g) in the present study, so this difference in standard compound used might be the basis for difference obtained.

## CONCLUSION

Present study is an attempt to determine various phytochemicals from the fruits of *C. decidua* growing in western Rajasthan. There is need of exhaustive phytochemical analytical studies for further exploration of the bioactive principles present in these fruits and elucidation of their role in the effectiveness against various ailments, thereby making it beneficial for herbal drug/pharmaceutical industry.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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