PHYTOCHEMICAL AND ANTIOXIDANT POTENTIAL OF CRUDE METHANOLIC EXTRACT AND FRACTIONS OF CELTIS ERIOCARPA DECNE. LEAVES FROM LESSER HIMALAYA REGION OF PAKISTAN

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Abstract

Celtis eriocarpa Decne. belongs to family cannabaceae. It is commonly found in Indo-Pak subcontinent and is used in healthcare practices. Decoction of leaves is used against amenorrhoea, fruits are used against colic. Powdered bark is used to treat pimples, sprain, contusions and joint pain. Leaves of Celtis eriocarpa were collected from lesser Himalayan region of Pakistan (Murree and Galliyat) in April 2014, and subjected to proximate analysis, qualitative and quantitative phytochemicals and DPPH antioxidant determination after fractionation. Quantitative determination of total phenolic (TPC), total flavonoid (TFC) and DPPH antioxidant activity was carried out through spectrophotometric methods. Proximate analysis revealed low moisture content, higher protein, carbohydrate and nutritive values. Qualitative phytochemical analysis revealed presence of phenolics, tannins, flavonoids, terpenoids and saponins while absence of alkaloids. Higher TPC was found in crude methanolic extract (79.96±0.32 mg GAE/g) followed by ethyl acetate fraction (59.62±1.00 mg GAE/g) and lowest TPC was found in crude hexane fraction (24.97±0.67 mg GAE/g) at p<0.05. Higher TFC was found in crude methanolic extract (63.88±0.40 mg QE/g) followed by ethyl acetate fraction (55.49±1.22 mg QE/g) and lowest TFC was found in crude hexane fraction (6.01±0.66mg QE/g) at p<0.05. Ethyl acetate fraction showed higher DPPH EC50 value (324.81µg/ml) while hexane fraction showed lowest EC50 value (2981µg/ml) at p<0.05.

Key words: Celtis eriocarpa, DPPH antioxidant activity, Phytochemicals, Lesser Himalaya, Total phenolic content, Total flavonoid content, Proximate analysis.

Introduction

Despite the scientific advancement and uplift in global cultures, plants still play a crucial role in the wellbeing of man. Therapeutic role of plants in human cultures is evident from centuries (Cleber, 2012). In past few decades ethnobotany gathered a vast compendium of folk knowledge from indigenous cultures across the world. Ethnobotanically highlighted plant species served a key role in drug development and fueled pharmaceutical sector to meet the dynamic healthcare challenges of modern world (Houghton, 1995). Preliminary phytochemical screening and antioxidant activity determination is an important step for short listing medicinal plants for drug development (Karande et al., 2016).

A number of plant families contribute to healthcare practices of indigenous cultures due to their phytochemicals. Among them is family Cannabaceae, which is distributed in temperate zone. It is a smaller family consisting only of few genera, with Celtis and Cannabis the important one. A series of studies have been conducted on members of this family for their potential role in therapeutics. Cannabis genus includes plants with potentially valuable therapeutic properties. A recent study conducted on leaves of Celtis australis L. and Celtis occidentalis confirms their antioxidant potential (El-Alfy et al., 2011). Decoctions of leaves and fruits of Celtis australis L. is astrigent and used for peptic ulcers, dysentery, diarrhea and against heavy menstruation (Chiej, 1988; Chevallier, 1996). Celtis eriocarpa Decne. is a medium size deciduous tree. Previously genus Celtis was included in family Ulmaceae but recent studies supports its inclusion in family Cannabaceae (Angiosperm Phylogeny Group II, 2003). Its common name is “Batkarh or Khairik” in indo-pak subcontinent. Its English name is European nettle tree or Hackberry. Its leaves are ovate to lanceolate and serrate in the upper half. Leaves are shed in autumn and sprout again in spring with small greenish flowers in the axils. Fruits are densely tomentose, yellow colored (Kanchan et al., 2010).

Leaves of Celtis eriocarpa are used as fodder for cattle (Jabeen et al., 2009). Fruits are edible (Sher et al., 2011; Badshah et al., 2012; Dangwal et al., 2014). Bark is dried and ground to form powder, which is used for treatment of tumor, scabies and many other skin problems. Dry seeds are ground and powder is used against dysentery (Ajaib & Khan, 2014). Fruits are used against amenorrhoea and colic (Singh, 1982; Sher et al., 2011). Ground powder of bark is used to treat pimples, sprain, contusions and joint pain (Adhikari et al., 2010). Decoction of the leaves is used against amenorrhoea (Saeed et al., 2013). Celtis. eriocarpa is reported to have laxative, astringent and antioxidant activities (Amjad, 2015).

A thorough literature review demonstrates that a number of studies have been conducted on the related species of Celtis genus (Sher et al., 2011; Ahmed et al., 2014), but no evidence exists on publication of phytochemical and antioxidant aspect of Celtis eriocarpa especially from lesser Himalaya region of Pakistan. Keeping in view the ethnobotanical importance of Celtis eriocarpa, the present study was therefore aimed to determine the qualitative
phytochemical analysis, proximate analysis, antioxidant activity, total phenolic contents and total flavonoid contents of crude methanolic extract and its fractions. This study will be a benchmark for detailed evaluation of therapeutic potential of *Celtis eriocarpa*.

**Materials and Methods**

**Collection and processing of plant material:** Leaves of *Celtis eriocarpa* were collected from Lesser Himalayan region of Pakistan (Murree and Galliyat) in April 2014. Collected samples were rinsed with tap water to remove dirt. They were shade dried and ground to make fine powder which was stored in sterile polythene bags until extraction. Identification of plant was done by Dr. Mushtaq Ahmed, Associate Professor from Herbarium of Quaid-e-Azam University, Islamabad and voucher specimen (129779) was deposited there as a reference (Fig. 1).

**Extract preparation and fractionation:** Extraction of plant material was done using cold maceration technique (Ewansiha et al., 2012). 150g powder of plant material was soaked in 450ml methanol and placed on a mechanical shaker for continuous stirring, after a week of soaking, filtration was done by using Whatman filter paper No. 1, the residue was reprocessed in the same way and filtered. Both the filtrates were then combined and solvent was evaporated to get concentrated extract via rotavap at 40°C under vacuum. The crude methanolic extract was 18.25g with 12.17% extraction yield. The extract was fractionated on the basis of increasing order of polarity, with *n*-hexane, chloroform, ethyl acetate and remaining aqueous fraction by solvent-solvent partitioning using the scheme given below (Fig. 2).

**Chemicals and reagents:** Analytical grade chemicals were used. Ascorbic acid, Quercetin trihydrate, Gallic acid, Aluminium chloride, Folin-ciocalteu phenol reagent, Ferric chloride, Hydrochloric acid (37%), Ammonia.

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**Fig. 1. Celtis eriocarpa** Decne.

**Fig. 2. Scheme for fractionation of plant extract.**

<table>
<thead>
<tr>
<th>Extracts/fraction</th>
<th>Extract obtained from 150g of dry powder</th>
<th>Extraction yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Methanolic</td>
<td>18.25g</td>
<td>12.17%</td>
</tr>
<tr>
<td><em>n</em>-Hexane fraction</td>
<td>4.25g</td>
<td>2.83%</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>1.7g</td>
<td>1.13%</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>0.8g</td>
<td>0.53%</td>
</tr>
<tr>
<td>Aqueous fraction</td>
<td>11.5g</td>
<td>7.67%</td>
</tr>
</tbody>
</table>

All the fractions were concentrated at rotary evaporator and then air dried. The extraction yield of each fraction is represented (Table 1). All the fractions were stored at 4°C for further *In vitro* investigations.

**Phytochemical analysis:** Plant samples were subjected to proximate, qualitative and quantitative analysis to determine their phytochemical contents.

**Proximate analysis:** Proximate analysis of powdered plant material of *Celtis eriocarpa* was carried out in research laboratory of “Poultry Research Institute Punjab, Rawalpindi”. Determination of dry matter, moisture content, crude fats, crude proteins, crude fiber and total ash contents were carried out using official methods of analysis (Anon., 2000). 30g plant powder was weighed and placed in a hot oven at 105°C upto constant weight. Difference in weight was calculated as moisture content, remaining is the dry matter. In a pre-weighed porcelain crucible 5g plant powder was placed and ignited in an ashing furnace which was maintained at 600°C. Upon formation of white ash and a constant weight, ash content

![Fig. 1. Celtis eriocarpa Decne.](image1)

![Fig. 2. Scheme for fractionation of plant extract.](image2)
was calculated (Anon., 2000). Total carbohydrates and available carbohydrates were calculated from the formulas given below following Shukla et al. (2014).

\[
\text{Total carbohydrates} = 100 - (\% \text{ Moisture} + \% \text{ Ash} + \% \text{ Crude fat})
\]

Available carbohydrates = % Total carbohydrate - % Crude fiber

**Nutritive value:** Nutritive value of aerial parts was calculated by the formula given below and was expressed as Kilocalories/100 gram of dry weight following Shukla et al. (2012).

\[
\text{Nutritive value} = \{4 \times \% \text{ Protein} + (9 \times \% \text{ Crude fat}) + (4 \times \% \text{ Total carbohydrates})\}
\]

**Qualitative analysis for phytochemicals:** Preliminary phytochemical analysis using standard protocols was carried out for alkaloids, flavonoids, tannins, saponins, phenolics and terpenoids.

**Test for alkaloids:** Determination of alkaloids in crude methanolic extract and various fractions of *Celtis eriocarpa* was carried out following Harborne (1973). 500mg of each extract was dissolved in 8ml of 1%HCl, slightly heated and then filtered. Then 2ml of filtrate was treated with Dragendorff’s reagent. Bright red precipitates indicate the presence of alkaloids.

**Test for phenolics:** Determination of phenolics was determined by following Trease & Evans (1989). 5ml of plant material was dissolved in distilled water (5mL). 2ml of ferric chloride solution was poured in the mixture and shaken. Blue color indicates the presence of phenolics.

**Test for flavonoids:** Determination of flavonoids was carried out following method of Harborne, (1973) and Sofowora, (1993). Each fraction (50mg) was dissolved in 100ml distilled water and filtrate was obtained. To 10mL of filtrate, 5mL dilute ammonia solution was added, then 2mL concentrated H$_2$SO$_4$ was added. Yellow color indicates the presence of flavonoids.

**Test for tannins:** Detection of tannins in each fraction was determined according to Harborne (1973) and Sofowora (1993). Each sample (50mg) was boiled in distilled water (20mL) and then filtered. 2mL of 0.1% ferric chloride was added in each mixture. Brownish green or blue-black color indicates tannins.

**Test for saponins:** Screening of Saponin was done according to Harborne (1973). Each sample (20mg) was boiled in (1:1 ratio) distilled water for 5min and then filtered. Each sample (10ml) was then mixed with distilled water (5mL) and vigorously shaken for froth formation, than Olive oil (3ml) was added for emulsion formation, which indicated presence of saponins.

**Test for terpenoids:** Determination of terpenoids was done using standard procedure of Harborne, (1973). 5ml (1mg/ml) extract was dissolved in 2ml Chloroform, carefully added 3ml of concentrated H$_2$SO$_4$ to form a layer. Development of reddish brown color at the interface represents terpenoids.

**Quantitative determination of phytochemicals**

**Total phenolic contents:** Determination of total phenolic contents of crude methanolic extract and fractions of *C. eriocarpa* was determined by Folin-Ciocalteu calorimetric method (Cai et al., 2004; Chlopicka et al., 2012; Iqbal et al., 2015) with some modifications. 1mg plant extract was dissolved in 1ml methanol to make 1000µg/ml solution. Standard used was Gallic acid. 4000 µg/ml stock solution of Gallic acid was prepared, which was then used to make dilutions, 12.5, 25, 50, 100, 150, 200 and 250 µg/ml. 300 µl of each aliquot was taken into a vial and mixed with 2.25ml of Folin-Ciocalteu phenol reagent. 6% Sodium Carbonate solution (2.25ml) was added in it after 5 Min. The mixture was placed for 90Min at room temperature to incubate. Absorbance was measured at 725nm. Standard calibration curve for 0-250µg/ml for Gallic acid was prepared and results were represented as mg Gallic acid equivalent (GAE) per gram of extract.

**Total flavonoid contents:** Determination of total flavonoid contents was done by aluminium chloride calorimetric method (Stankovic, 2011) with some modifications. 1mg plant extract was dissolved in 1ml methanol to make 1000µg/ml solution. Standard used was Quercetin. 4mg of quercetin was dissolved in 1ml of methanol to make 4000 µg/ml stock solution, which is then used to make dilutions, 12.5, 25, 50, 100, 150, 200 and 250 µg/ml. 500 µl of each aliquot was taken into a vial and dissolved in 1.5ml of methanol. Then added 100 µl of 10% aluminium chloride solution, 100 µl of 1M Potassium acetate solution and distilled water (2.8ml) to make the total volume 5ml. The mixture was placed at room temperature for 30Min to incubate. Absorbance was taken at 415nm. Standard calibration curve for 0-250µg/ml for Quercetin was prepared and results were represented as mg Quercetin equivalent (QE) per gram of extract.

**DPPH antioxidant method:** DPPH radical scavenging activity of crude methanolic extract and fractions of *C. eriocarpa* was determined following Abdulwahab et al. (2011) with some modifications. 2.4 mg DPPH reagent was dissolved in 100ml methanol to make 1M stock solution and stored at -20°C till analysis was carried out. Standard used was Ascorbic acid. 4mg each of ascorbic acid, crude methanolic extract of *C. eriocarpa* and its fractions were dissolved in 1ml methanol to form 4000µg/ml stock solution. Dilutions used were 25, 50, 100, 150, 200, 250µg/ml. Scavenging activity of samples was expressed as E$_{C50}$ (Effective concentration of sample which causes 50% reduction in DPPH absorption, when compared against negative control).

200µl each of plant extract and standard solution was placed in separate test tubes and 2ml of DPPH solution
(Absorbance 0.7) was added in each test tube. After vertex mixing test tubes were incubated in dark for 35Min. Absorbance was measured at 517nm using spectrophotometer. Experiment was performed in triplicate. The % inhibition of each concentration of all the samples and standard was calculated and graph was plotted (% Scavenging Vs Concentration). EC50 for each sample and standard was calculated by using the graph.

Data analysis: Data analysis in each experiment was carried out for triplicate independent pool of each sample and results were presented as means ± standard error of mean. SPSS version 16.0 was used for statistical analysis of data. Analysis of variance was calculated under factorial design (5 x 3 x 3) to know the effect of different extracts, their concentrations and interactions on DPPH percentage scavenging activity. Standard used was Ascorbic acid. p<0.05 was considered significant. EC50 was measured using regression line equation. Means and Standard errors were calculated by descriptive statistics. Post Hoc LSD was used for multiple comparisons between various concentrations of CM and fractions of Celtis eriocarpa. Bivariate correlation was also determined at p<0.05 using SPSS 16.0 between total phenolic contents (TPC), Total flavonoid content (TPC) and EC50 of CM and all fractions of Celtis eriocarpa.

Results and Discussion

Proximate analysis and nutritive value: Results for proximate analysis of Celtis eriocarpa are represented below (Table 2). The analysis reveals that percentage moisture content is 12.40±0.21, which is within the acceptable limit of 6-15% for crude drugs (Shellard, 1958; Kunle, 2012). Low moisture content reduces hydrolysis of crude drug by reducing the activity of hydrolytic enzymes and therefore minimizes the deterioration of bioactive constituents. It also reduces chances of bacterial or fungal colonization in crude drug. It also aids in precise quantification of crude drug for various bioactivity analysis. The results reveal that leaves of Celtis eriocarpa are good source of proteins, fat and fibers. High fiber content of 12.50±0.07, reveals increased digestibility of leaves. The higher percentage carbohydrates content 61.96±0.29 and higher nutritive value 326.91 Kcal/100g suggests that Celtis eriocarpa leaves can be used in dietary supplements and can be a good source of food for cattle.

Table 2. Proximate analysis and nutritive value of Celtis eriocarpa (Mean ± SEM, n=3).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Percentage contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>87.60 ± 0.21</td>
</tr>
<tr>
<td>Moisture</td>
<td>12.40 ± 0.21</td>
</tr>
<tr>
<td>Crude proteins</td>
<td>8.75 ± 0.17</td>
</tr>
<tr>
<td>Crude fats</td>
<td>4.90 ± 0.04</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>12.50 ± 0.07</td>
</tr>
<tr>
<td>Total ash</td>
<td>12.0 ± 0.12</td>
</tr>
<tr>
<td>Total Carbohydrates</td>
<td>61.96 ± 0.29</td>
</tr>
<tr>
<td>Available Carbohydrates</td>
<td>49.46 ± 0.23</td>
</tr>
<tr>
<td>Nutritive value (Kcal/100gm)</td>
<td>326.91 ± 0.46</td>
</tr>
</tbody>
</table>

Qualitative phytochemical analysis: Results for qualitative analysis of phytochemicals for crude methanolic extract and fractions of Celtis eriocarpa are represented below (Table 3). The results show that phenolics, flavonoids, tannins, and terpenoids are present in more quantity, saponins in less quantity and absence of alkaloids. Presence of aforementioned phytochemicals is suggestive of therapeutic role of this plant. For example, phenolics and flavonoids are well known for their chemoprotective properties like, anti-inflammatory, antioxidant and anticancer (Huang et al., 2010; Ghasemezadeh & Ghasemzadeh, 2011). Tannins have the ability to precipitate microbial proteins thus protecting the host. They also impart antiviral, antimutor (Kumari & Jain, 2012), and antibacterial activity (Hisanori et al., 2001). Saponins are anti-cancer (Man et al., 2010) antispasmodic (Corea et al., 2005), and anti-inflammatory (Lee et al., 2012).

Table 3. Qualitative phytochemical test results of crude methanolic extract and fractions of Celtis eriocarpa.

<table>
<thead>
<tr>
<th>Extracts/Phytochemicals</th>
<th>Phenolics</th>
<th>Flavonoid</th>
<th>Alkaloid</th>
<th>Tannins</th>
<th>Terpenoids</th>
<th>Saponins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Methanolic extract</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>n-Hexane fraction</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Aqueous fraction</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Abundantly present (+++), moderately present (+), Absent (-)

Quantitative Phytochemical analysis

Total Phenolic contents (TPC): Standard calibration curve primed with Gallic acid yielded following linear equation (Y=0.006x - 0.249, R² = 0.992), which was used for determination of total phenolic contents, in terms of Gallic acid equivalent (mg GAE/g) calculated by Folin-Ciocalteu reagent.

The present study indicates that crude methanolic extract of Celtis eriocarpa contains significantly (p<0.05) higher total phenolic contents i.e., 79.96±0.32mg GAE/g of extract. For fractions total phenolic contents was found significantly (p<0.05) higher for ethyl acetate fraction (59.62±1.00mg GAE/g of extract) and n-Hexane fraction exhibited significantly (p<0.05) lower value as 24.97±0.67mg GAE/g of extract. For fractions total phenolic contents was found in higher concentration in young leaves of Celtis australis, while their concentration declines with their age. Total phenolic contents found in plants generally acts as free
radical scavengers and also chelate metal ions responsible for their production (Iqbal et al., 2015). Therefore antioxidant role of crude methanolic extract and fraction with high total phenolic content may be presumed accordingly. Plant phenolic also impart their therapeutic role against skin diseases (Dzialo et al., 2016), neurodegenerative diseases, cardiovascular and cancer (Del Rio et al., 2013).

**Total flavonoid contents (TFC):** Standard calibration curve primed with Quercetin yielded following linear equation (Y = 0.004x+0.408, R² = 0.988), which was used for determination of total flavonoid contents. Quercetin equivalent (mg QE/g) total flavonoid content of crude methanolic extract and fractions were calculated by Aluminium chloride (AlCl₃) method (Table 4).

The present study indicates that crude methanolic extract of *C. eriocarpa* contains significantly (p<0.05) higher i.e. 63.88±0.40 mg QE/g of extract. For fractions total flavonoid contents were found significantly (p<0.05) higher for ethyl acetate fraction (55.49±1.22mg QE/g of extract) and n-hexane fraction exhibited significantly (p<0.05) lower value as 6.01±0.66mg QE/g of extract (Table 4). Spitaler et al. (2009), conducted a study and also confirmed the presence of flavonoid in *C. australis* leaves. Flavonoid play therapeutic role against cancer, aging and many other human diseases (Ghasemzadeh & Ghasemzadeh, 2011). Flavonoids impart antioxidant, antiviral, hepato-protective, anti-inflammatory and coronary heart disease prevention (Kumar & Pandey, 2013). Therefore total flavonoid content of crude methanolic extract or fractions of *C. eriocarpa* may account for any of aforementioned role of flavonoids.

**Free Radical scavenging activity:** The DPPH antioxidant potential of crude methanolic extracts and fractions of *C. eriocarpa* are expressed in Table 4. The EC₅₀ value was calculated using regression line equation and compared with standard (Ascorbic acid). The mean EC₅₀ value of ascorbic acid was 10.86µg/ml, while significantly (p<0.05) highest EC₅₀ value is of ethyl acetate fraction i.e., 324.81µg/ml and significantly (p<0.05) lowest EC₅₀ value is of n-Hexane fraction i.e. 2981.03µg/ml. The comparative results indicate that EC₅₀ of crude methanolic extract and fractions is EF>CM>CF>AF>HF. The DPPH percentage scavenging of crude methanolic extract and fractions is also compared with Ascorbic acid (standard) in Fig. 3. Because of unavailability of any previous work on antioxidant activity of *C. eriocarpa*, no comparison can be made.

### Table 4. DPPH radical scavenging, total phenolic content and total flavonoid content of *Celtis eriocarpa* (Mean ± SEM, n = 3).

<table>
<thead>
<tr>
<th>Extracts</th>
<th>DPPH EC₅₀ (µg/mL)</th>
<th>TPC (mg GAE/g)</th>
<th>TFC (mg QE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude methanolic</td>
<td>593.68 ± 2.34ᵃ</td>
<td>79.96 ± 0.32ᵃ</td>
<td>63.88 ± 0.40ᵃ</td>
</tr>
<tr>
<td>n-Hexane fraction</td>
<td>2981.03 ± 4.44ᵃ</td>
<td>24.97 ± 0.67ᵃ</td>
<td>6.01 ± 0.66ᵃ</td>
</tr>
<tr>
<td>Chloroform fraction</td>
<td>1058.18 ± 4.41ᵇ</td>
<td>51.86 ± 0.69ᵇ</td>
<td>23.72 ± 0.66ᵇ</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>324.81 ± 2.60ᵃ</td>
<td>59.62 ± 1.00ᵇ</td>
<td>55.49 ± 1.22ᵇ</td>
</tr>
<tr>
<td>Aqueous fraction</td>
<td>1155.0 ± 1.64ᵃ</td>
<td>31.02 ± 1.08ᵃ</td>
<td>11.36 ± 1.15ᵃ</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>10.86 ± 1.02ᵃ</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Within each column values followed by one or more of same letters are not significantly different at p <0.05

The results reveal that DPPH EC₅₀ values of chloroform fraction, aqueous fraction and n-hexane fractions are significantly (p<0.05) low, while crude methanolic extract and ethyl acetate fraction shows significantly (p<0.05) higher DPPH EC₅₀ value as compared to standard (ascorbic acid), the pure compound. This significant (p<0.05) increase in DPPH EC₅₀ value of ethyl acetate fraction may be due to antagonistic effect of other groups of phytochemicals, which cause a significant (p<0.05) decrease in crude methanolic extract when fractions are combined. While comparing with qualitative and quantitative phytochemical results, it is clear that the significantly (p<0.05) higher DPPH EC₅₀ value of ethyl acetate fraction and crude methanolic extract of *C. eriocarpa* is due to their higher total phenolic content and total flavonoid contents. It is because phenolics and flavonoids are well known for their anti-inflammatory and antioxidant activities (Huang et al., 2010; Ghasemzadeh & Ghasemzadeh, 2011). Determination of DPPH EC₅₀ value at fraction level also suggests the polarity level of biologically active compounds.

![Fig. 3. DPPH antioxidant activity of *Celtis eriocarpa*. CM = Crude Methanolic extract, HF= n-Hexane fraction, CF= Chloroform fraction, EF= Ethyl acetate fraction, AF=Aqueous fraction, AA= Ascorbic acid (standard)](image-url)
Statistical analysis was conducted under factorial design (5 x 6 x 3), revealed that the effect of extracts, concentrations and their interactions remained significant at p<0.05 (Table 5). The 5 x 6 between-subjects analysis of variance (ANOVA) revealed a main effect of extracts, F(4,60) = 4.798, MS_e = 0.212, p = 0.00, α = 0.05. The ANOVA revealed a main effect of concentrations, F(5,60) = 1.476, p = 0.00. The ANOVA also revealed an interaction of extracts and concentrations, F(5,60) = 164.669, p = 0.00. The analysis reveals that highest DPPH radical scavenging was exhibited by ethyl acetate fraction at 250µg/ml concentration followed by crude methanolic extract. The lowest DPPH radicle scavenging was exhibited by n-hexane fraction at 25 µg/ml concentration.

Correlation between DPPH EC₅₀, total phenolic and total flavonoid contents: Pearson’s correlation coefficient demonstrates a strong correlation between DPPH EC₅₀, total phenolic and total flavonoid contents. Pearson’s correlation coefficient calculated between DPPH EC₅₀ and total phenolic is -0.541 (p<0.05), DPPH EC₅₀ and total flavonoids is -0.724 (p<0.05), and between total phenolics and total flavonoids is 0.949 (p<0.05). The negative Pearson’s correlation coefficient between total phenolics, total flavonoids and DPPH EC₅₀, suggests that an increase in total phenolics and flavonoids contents decreases the DPPH EC₅₀ value of extracts. There also exists a strong positive correlation between total phenolics and total flavonoid contents.

Conclusion

The results of this study are very informative about Celtis eriocarpa. The proximate analysis reveals that leaves are good source of proteins, fat and fibers. High fiber content reveals increased digestibility of leaves. Higher percentage carbohydrates content and higher nutritive value suggests its use as a good source of food for cattle. Preliminary phytochemical screening showed presence of phenolics, flavonoids, tannins, terpenoids and saponins, while absence of alkaloids. As all these groups of phytochemicals are very well known for their pharmacological and therapeutic effects, therefore this plant can contribute impressive role in healthcare. The present study indicated that crude methanolic extract and ethyl acetate fraction contain high total phenolic and total flavonoid contents.

The results also reveal that DPPH EC₅₀ value of ethyl acetate fraction is significantly high. By comparing DPPH EC₅₀ with qualitative and quantitative results, it is evident that higher DPPH EC₅₀ of ethyl acetate fraction may be due to higher phenolic and flavonoid contents. As DPPH EC₅₀ value, total phenolic and flavonoid contents of leaves of Celtis eriocarpa is considerably high, therefore this plant could be a source of bioactive compounds. This preliminary study will act as a benchmark for detailed evaluation of therapeutic potential of this plant. Further work on bioactivities, phytochemical profiling and compound isolation of Celtis eriocarpa leaves may lead to isolation of potential bioactive markers for future use in healthcare.

Table 5. Tests of between-subjects effects for Celtis eriocarpa.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected model</td>
<td>6342.560</td>
<td>29</td>
<td>218.709</td>
<td>1.03</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>28038.084</td>
<td>1</td>
<td>28038.084</td>
<td>1.32</td>
<td>.000</td>
</tr>
<tr>
<td>Extracts</td>
<td>4076.074</td>
<td>4</td>
<td>1019.018</td>
<td>4.79</td>
<td>.000</td>
</tr>
<tr>
<td>Concentration</td>
<td>1566.979</td>
<td>5</td>
<td>313.396</td>
<td>1.47</td>
<td>.000</td>
</tr>
<tr>
<td>Extracts * Conc.</td>
<td>699.507</td>
<td>20</td>
<td>34.975</td>
<td>164.66</td>
<td>.000</td>
</tr>
<tr>
<td>Error</td>
<td>12.744</td>
<td>60</td>
<td>0.212</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>34393.388</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected total</td>
<td>6355.304</td>
<td>89</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R² = 0.998 (Adjusted R² = 0.997), (df= Degree of freedom, F= F statistics, Sig.= Significance probability, Conc.=Concentration)

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Conflict of interest

The authors declare no conflict of interest.

References


