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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 *n*-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 *n*-Hexane fraction (6.01±0.66mg QE /g) at $p<0.05$. Ethyl acetate fraction showed higher DPPH EC_{50} value (324.81µg/ml) while *n*-hexane fraction showed lowest EC_{50} value (2981µg/ml) at $p<0.05$. The mean EC_{50} value of ascorbic acid at $p<0.05$ was 10.86µg/ml. As DPPH EC_{50} value, total phenolic content and total flavonoid content in the leaves of *C. eriocarpa* is considerably high therefore this plant could be a source of bioactive compounds. This study will be a benchmark for detailed evaluation of therapeutic potential of *Celtis eriocarpa.*

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 astringent 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 Kharik" 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 grinded 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).

Fig. 1. *Celtis eriocarpa* Decne.

Table 1. Extraction yield of crude methanolic extract and fractions of *Celtis eriocarpa*.

Extracts/fraction	Extract obtained from 150g of dry powder	Extraction yield
Crude Methanolic	18.25g	12.17%
n-Hexane fraction	4.25g	2.83%
Chloroform fraction	1.7g	1.13%
Ethyl acetate fraction	0.8g	0.53%
Aqueous fraction	11.5g	7.67%

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.

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, Sulphuric acid (98%), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Sodium carbonate, Chloroform, *n*-Hexane, Ethyl acetate, Methanol, Potassium acetate, Bismuth nitrate and Potassium Iodide.

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 than 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).

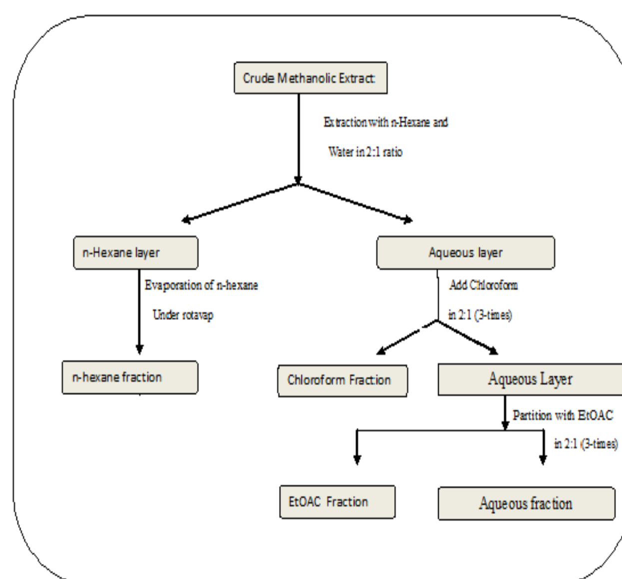

Fig. 2. Scheme for fractionation of plant extract.

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

was calculated (Anon., 2000). Total carbohydrates and available carbohydrates were calculated from the formulas given below following Shukla *et al.* (2014).

Total carbohydrates = 100 – (% Moisture + % Ash + % Crude proteins + % 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).

Nutritive value = {(4× % Protein) + (9× % Crude fat) + (4× % 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 phenols.

Test for flavonoid: Determination of flavonoid 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, than 2mL concentrated H_2SO_4 was added. Yellow color indicates the presence of flavonoids.

Test for tannin: Detection of tannins in each fraction was determined according to Sofowora (1993). Each sample (50mg) was boiled in distilled water (20mL) and then filtered. 2mL 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_2SO_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 -20ºC 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 EC_{50} (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). $EC_{50}$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. EC_{50} 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 *C. eriocarpa.* Bivariate correlation was also determined at $p<0.05$ using SPSS 16.0 between total phenolic contents (TPC), Total flavonoid content (TPC) and EC_{50} of CM and all fractions of *Celtis eriocarpa.*

Results and Discussion

Proximate analysis and nutritive value: Results for proximate analysis of *C. 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 *C. 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 *C. 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).

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

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; Ghasemzadeh & Ghasemzadeh, 2011). Tannins have the ability to precipitate microbial proteins thus protecting the host. They also impart antiviral, antitumor (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*.

Extracts/Phytochmicals	Phenolics	Flavonoid	Alkaloid	Tannins	Terpenoids	Saponins
Crude Methanolic extract	++	++	-	++	++	+
n-Hexane fraction	+	-	-	-	+	-
Chloroform fraction	++	++	-	-	-	-
Ethyl acetate fraction	++	++	-	++	+	-
Aqueous fraction	+	+	-	++	+	+

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

Quantitative Phytochemical analysis

Total Phenolic contents (TPC): Standard calibration curve primed with Gallic acid yielded following linear equation ($Y=0.006x - 0.249$, $R^2 = 0.992$), 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 (Table 4). Sommavilla *et al.* (2012), reported that total phenolic contents are found in higher concentration in young leaves of *C. australis*, while their concentration declines with their age. Total phenolic contents found in plants generally acts as free

radical scavengers and also chellate 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 ($Y = 0.004x+0.408$, $R^2 = 0.988$), 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 ($AlCl_3$) 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 EC_{50} value was calculated using regression line equation and compared with standard (Ascorbic acid). The mean EC_{50} value of ascorbic acid was 10.86µg/ml, while significantly ($p<0.05$) highest EC_{50}value is of ethyl acetate fraction i.e., 324.81µg/ml and significantly ($p<0.05$) lowest EC_{50} value is of *n*-Hexane fraction i.e. 2981.03µg/ml. The comparative results indicate that EC_{50} 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).

Extracts	DPPH EC_{50} (µg/mL)	TPC (mg GAE/g)	TFC (mg QE/g)
Crude methanolic	593.68 ± 2.34^a	79.96 ± 0.32^a	63.88 ± 0.40^a
n-Hexane fraction	2981.03 ± 4.44^a	24.97 ± 0.67^a	6.01 ± 0.66^a
Chloroform fraction	1058.18 ± 4.41^b	51.86 ± 0.69^a	23.72 ± 0.66^a
Ethyl acetate fraction	324.81 ± 2.60^a	59.62 ± 1.00^a	55.49 ± 1.22^a
Aqueous fraction	1155.0 ± 1.64^c	31.02 ± 1.08^a	11.36 ± 1.15^a
Ascorbic acid	10.86 ± 1.02^a	---	---

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 EC_{50} 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 EC_{50} value as compared to standard (ascorbic acid), the pure compound. This significant ($p<0.05$) increase in DPPH EC_{50} 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 EC_{50} 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 EC_{50}value at fraction level also suggests the polarity level of biologically active compounds.

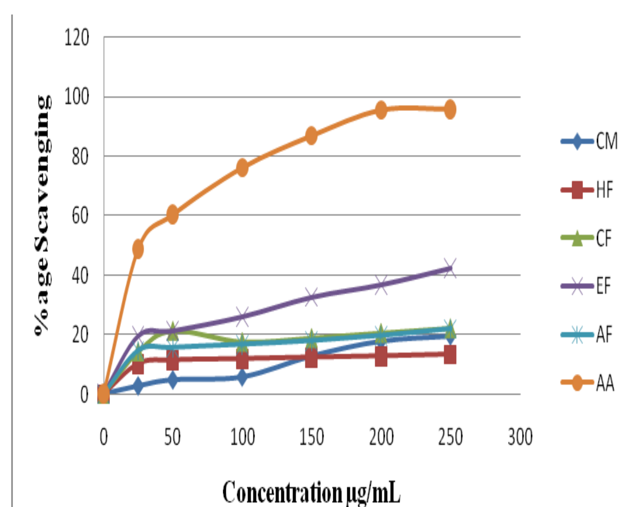

Fig. 3. DPPH antioxidant activity of *Celtis eriocarpa*.
CM = Crude Methanolic extract, HF= n-Hexane fraction, CF= Chloroform fraction, EF= Ethyl acetate fraction, AF=Aquous fraction, AA= Ascorbic acid (standard)

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, $F(4,60) = 4.798$, $MS_e = 0.212$, $p = 0.00$, $\alpha = 0.05$. 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 EC_{50}, total phenolic and total flavonoid contents: Pearson's correlation co-efficient demonstrates a strong correlation between DPPH EC_{50}, total phenolic and total flavonoid contents. Pearson's correlation coefficient calculated between DPPH EC_{50} and total phenolic is -0.541 ($p<0.05$), DPPH EC_{50} 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 EC_{50}, suggests that an increase in total phenolics and flavonoids contents decreases the DPPH EC_{50} value of extracts. There also exists a strong positive correlation between total phenolics and total flavonoid contents.

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

Source	Dependent variable:% scavenging				
	Type III sum of squares	d_f	Mean square	F	Sig.
Corrected model	6342.560[a]	29	218.709	1.030E3	.000
Intercept	28038.084	1	28038.084	1.320E5	.000
Extracts	4076.074	4	1019.018	4.798E3	.000
Conc.	1566.979	5	313.396	1.476E3	.000
Extracts * Conc.	699.507	20	34.975	164.669	.000
Error	12.744	60	0.212		
Total	34393.388	90			
Corrected total	6355.304	89			

$R^2 = 0.998$ (Adjusted $R^2 = 0.997$), (df= Degree of freedom, F= F statistics, Sig.= Significance probability, Conc.=Concentration)

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 EC_{50} value of ethyl acetate fraction is significantly high. By comparing DPPH EC_{50} with qualitative and quantitative results, it is evident that higher DPPH EC_{50} of ethyl acetate fraction may be due to higher phenolic and flavonoid contents. As DPPH EC_{50}value, total phenolic and flavonoid contents of leaves of *C. 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.

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Conflict of interest

The authors declare no conflict of interest.

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