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Antioxidant Activity Of The Leaf Extracts of *Boscia coriacea* Graells And *Uvaria leptocladon* Oliv

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Abstract

***Background**: Synthetic antioxidants used for the management of oxidative stress have been shown to have severe side effects. Some medicinal plants that belong to the genus Uvaria and Boscia contain chemicals with antioxidant properties such as flavonoids and alkaloids.*

***Objective:** This study was aimed to evaluate the antioxidant activities of the leaf extracts of Boscia coriacea Graells and Uvaria leptocladon Oliv.*

***Method**: Fresh leaves of B. coriacea and U. leptocladon were collected in April 2021 from Alie and Konso, located in Southern Ethiopia. First, the leaves of the medicinal plants were dried in dark conditions. Maceration extraction method was used to extract the powdered leaf of Boscia coriacea and Uvaria leptocladon. The crude extract of B. coriacea was fractionated using chloroform ($CHCl_3$), methanol (CH_3OH), $CHCl_3$: CH_3OH(1:1 v/v) and petroleum ether. The crude extract of U. leptocladon was fractionated using $CHCl_3$, ethanol (C_2H_6OH), and ethyl acetate ($C_4H_8O_2$). Evaluation of the antioxidant activity was done using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), and ferric reducing antioxidant power (FRAP) assays. Data analysis was done using one-way analysis of variance.*

***Result**: The antioxidant evaluation demonstrated that the leaf extracts of B. coriacea and U. leptocladon have 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid radical scavenging activity. Based on IC_{50} value, the CH_3OH: $CHCl_3$ (1:1) fraction of B. coriacea (IC_{50}=38.2 µg/mL) and the C_2H_6OH fraction of U. leptocladon (IC_{50}=30.5 µg/mL) are highly active in 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity. The total flavonoid content in the CH_3OH:$CHCl_3$ fraction of B.coricea and in the C_2H_6OH fraction of U.leptocladon were calculated to be 136.8±0.04 mg/g of extract and 172.9±0.41 mg/g of extract, respectively. The total phenol content in the CH_3OH: $CHCl_3$ fraction of B.coricea and in the C_2H_6OH fraction of U.leptocladon were calculated to be 145 ± 0.025 mg/g and 187.7 ± 0.055 mg/g, respectively. The CH_3OH: $CHCl_3$ (1:1) fraction of B. coriacea and the C_2H_6OH fraction of U. leptocladon showed the highest ferric reducing antioxidant power. The antioxidant effects of the leaf extract of B. coriacea are due to β-sitosterol and lucidine-type compound. The antioxidant effects of the leaf extract of U. leptocladon are attributed to β-sitosterol, β-sitosterol glucoside, and α-humulene.*

***Conclusion**: The leaf extracts of B. coriacea and U. leptocladon have high antioxidant activity. Further study is needed to determine the mechanism of action of compounds isolated from the leaf extracts* of *B. coriacea* and *U. leptocladon.*

Introduction

Oxidative stress refers to condition when an imbalance occurs between formations of free radicals and antioxidants (1). Several studies demonstrated that that oxidative stress can be involved in the onset and/or progression of several diseases (i.e., cancer, diabetes, metabolic disorders, atherosclerosis, and cardiovascular disease (2). A free radical is a molecule which possesses an unpaired electron that makes it unstable. The unstable free radicals become stable when electron pairing occurs with biological macromolecules such as proteins and lipids, in healthy human cells (3). Antioxidants are chemicals that prevent and stabilize the damage caused by free radicals by supplying electrons and converting them into waste by-products (3). Synthetic antioxidants that are used for the management of oxidative stress have been shown to have severe side effects (4). For this reason, searching for safe and effective natural antioxidants is needed.

Medicinal plants are major sources of chemicals with bioactive compounds. Some medicinal plants that belong to the genus *Uvaria* and *Boscia* have antioxidant properties (5, 6). The commonly mentioned phytochemicals that are found in leaf extracts of plants that belongs to the genus *Boscia* include flavonoids, alkaloids, saponins, steroids, and cardiac glycosides (6-9). The major phytochemicals in the leaf extracts of *Uvaria* species include: phenol, steroid, alkaloids, saponins, flavonoids and terpenoids (9-14).

The genus Boscia belongs to the family Capparidaceae and the genus *Uvaria* belongs to the family Annonaceae, which are widely distributed in Ethiopia (15). For instance *Boscia coricea* is found in Bale, Sidamo, Kefa, Konso, Gamo Gofa and Hararge regions of Ethiopia. *Uvaria leptocladon* is found in Sidamo, Kefa, Konso (Alie), and Gamo Gofa regions of Ethiopia (15). Thus, the objective of this study was to evaluate the antioxidant activities of the leaf extracts of *B. coriacea* Graells and *U. leptocladon* Oliv.

Material and Method

Plant collection

Fresh leaves of *B. coriacea* and *U. leptocladon* were collected from Konso and Alie, located in the Southern Nation, Nationalities, and People Region, Ethiopia, in April 2021. The plant materials were authenticated by one of the authors of this manuscript (Mr. Melaku Wondafrash a Botanist at the Department of Plant Biology and Biodiversity Management, Addis Ababa University), and a voucher specimen of each plant (ST001 and ST002, representing *B. coriacea* and *U. leptocladon*, respectively) was deposited at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University (AAU).

Extraction

First, the leaves of the plants dried in dark condition. Then, the extraction of the powdered leaf of *B. coriacea* (1 kg) and *U. leptocladon* (1 kg) was done using 10 liters of 80% methanol by maceration extraction method. Filtration of the mixture was performed using Whatmann no.1 filter paper. Rotary evaporator (PHOENIX instrument, RE-100D) was used to evaporate the CH_3OH from the filtrate. A lyophilizer equipment (Christ, ALPHA 2-4-LD plus, Germany) was used to remove the water component of the mixture.

The crude leaf extracts of the two plants were fractionated in the Department of Chemistry, CNCS, AAU. The crude extract of *B. coriacea* was fractionated using chloroform ($CHCl_3$), methanol (CH_3OH), $CHCl_3$: CH_3OH (1:1 v/v) and petroleum ether. The crude extract of *U.leptocladon,* was fractionated using $CHCl_3$, ethanol (C_2H_6OH), and ethyl acetate ($C_4H_8O_2$). All the extracts were stored at -20°C until the experiments were conducted. The isolation and characterization of compounds were done at the Department of Chemistry, CNCS, AAU. The characterization of compounds was done using nuclear magnetic resonance spectrometry (1H NMR and 13C NMR).

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was carried out according to Brand-Williams et al (1995) (16) and Molyneux (2004) (17) with modification.

Stock solutions of the plant extracts, 1mg/mL diluted to different concentrations ranging from 0.02 to 0.20 mg/mL (0.02, 0.04, 0.08, 0.12, 0.16 and 0.20 mg/mL). Five mL of 1 mM DPPH in CH_3OH was added to the different concentrations of extracts. Then, the mixture was shaken and allowed to stand in dark condition. After 30 minutes, absorbance was read at 517 nm against a blank containing CH_3OH using spectrophotome-

ter (UV-UV/Vis/NIR spectrophotometer, Perkin Elemer, Lambda 950). Ascorbic acid was used as positive control with similar concentrations of the leaf extracts. The experiment was done in triplicate.

The following formula was used to calculate the percent (%) inhibition of DPPH (17):-

Scavenging effect (%) = $[(A_0 - A/A_0)] \times 100$

Where,

A_0 = absorbance of the blank solution (DPPH without extract)

A = absorbance of the extract + DPPH

2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)

The 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging activity of the leaf extracts of *B. coriacea* and *U. leptocladon* was determined using the method described by Re et al (1999) (18). First ABTS radical was produced by reaction of 7 mM solution of ABTS in water with 2.45 mM potassium persulphate ($K_2O_8S_2$) (1:1). The mixture was held in darkness at 27°C for 16 h. After 16 h, the ABTS radical solution was further diluted with distilled water until the initial absorbance was reached 0.7 at 734 nm. Stock solutions of 1mg/ml of the extract was diluted to different concentrations ranging from 0.025 to 0.2 mg/mL (0.025, 0.05, 0.1, 0.15, and 0.2 mg/mL). Then, 1.9 ml of ABTS working solution was mixed with 0.1 ml of the extract or standard. Ascorbic acid was used as standard. Absorbance was taken at 734 nm using spectrophotometer (UV-UV/Vis/NIR spectrophotometer, Perkin Elemer, Lambda 950). The experiment was done in triplicates.

The following formula was used to calculate the percent (%) inhibition of ABTS (18):-

$$\% \text{ ABTS inhibition} = \frac{[(Ab_{blank} - Ab_{sample})]}{Ab\ blank} \times 100$$

Where,

Ab_{blank}=the absorbance of the blank solution (ABTS without the extract)

Ab_{sample}=the absorbance of the extract + ABTS

Ferric Reducing Antioxidant Power (FRAP) assay

The ferric reducing antioxidant power of the leaf extracts of *B. coriacea* and *U. leptocladon* was determined using the method described by Benzie and Strain (1996) (19). First, stock solutions of 300 mM acetate buffer, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution were prepared. Then, fresh FRAP solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 mL$FeCl_3 \cdot 6H_2O$. Then, the FRAP solution was incubated at 37°C for 30 min. After 30 min of incubation at 37^0C, 100 µL of the plant extract (0.1 mg/mL) was allowed to react with 2900 µL of the FRAP solution for 30 min in the dark. Finally, absorbance was read at 593 nm using spectrophotometer (UV-UV/Vis/NIR spectrophotometer, Perkin Elemer, Lambda 950). Ferrous sulfate ($FeSO_{4)}$ was used as standard (different concentrations ranging from 10 to 80 µg/mL). The experiment was performed in triplicates. Results are expressed in µM Fe (II)/g dry mass. The following formula was used to calculate the FRAP of the crude extracts and fractions of the leaf extracts of *B. coriacea* and *U. leptocladon* (20):-

FRAP value (µM Fe (II)/g dry mass) = $c \times V \times t/m$

Where, FRAP value (c) is the $FeSO_4$ concentration (µmol/mL) calculated from the $FeSO_4$ calibration curve, V is the extract volume (mL), t is the dilution factor, and m is the mass of the extract (g).

Determination of total phenolic content

Total phenolic content of the leaf extracts of *B. coriacea* and *U. leptocladon* was determined by using the Folin-Ciocalteau method (21). The reaction mixture was prepared by mixing 1.25 mL of 10% Folin-Ciocalteau reagent (diluted with distilled water 1:10 v/v), 1.25 mL of sodium carbonate (7.5%) and 0.25 mL of extract in CH_3OH (0.1 mg/mL). Then, the mixture was incubated for 45 min under dark condition. The absorbance was measured at 760 nm using spectrophotometer (UV-UV/Vis/NIR spectrophotometer, Perkin Elemer, Lambda 950). Gallic acid was used as standard (different concentrations ranging from 20 to 140 µg/mL). The experiment was done in triplicates. Results were expressed in mg Gallic acid equivalents (GAE) per gram dry extract weight. The following formula was used to calculate the total phenol content in the crude extracts and fractions of the leaf extracts of *B. coriacea* and *U. leptocladon* (21):-

C=c.V/m

Where, C=total phenolic content mg GAE/g dry extract, c=concentration of Gallic acid calculated from the Gallic acid calibration curve in mg/mL, V=volume of extract (mL), and m=mass of extract (g).

Determination of total flavonoid content

Total flavonoid content in the leaf extracts of *B. coriacea* and *U. leptocladon* was determined by using aluminum chloride colorimetric assay (22). Sodium nitrate (0.15 mL of 5 %) was added to 0.25 ml extract in CH_3OH (0.1 mg/mL). After 5 min,

0.15 mL of 10 % $AlCl_3$ was added and left for 5 min. Then, 1 mL of 1 M NaOH was added into the solution and filled with CH_3OH to make final volume to 5 mL. Finally, the solutions were allowed to stand for 30 minutes in the dark at room temperature. Absorbance of the mixture was measured at 510 nm using spectrophotometer (UV-UV/Vis/NIR spectrophotometer, Perkin Elemer, Lambda 950). Quercetin was used as standard (different concentrations in MeOH ranging from 25 to 150 µg/mL). The experiment was performed in triplicates. Total flavonoid content was expressed in mg quercetin equivalent (QE) per gram dry extract weight (mg/g). The following formula was used to calculate the total flavonoid content in the crude extracts and fractions of the leaf extracts of *B. coriacea* and *U. leptocladon* (22):-

C=c.V/m

Where, C=total flavonoid content mg QE/g dry extract, c=concentration of quercetin calculated from the quercetin calibration curve (mg/mL), V=volume of extract (mL), and m=mass of extract (g).

Data analysis

The DPPH and ABTS radical scavenging activities of the leaf extracts of *B. coriacea* Graells and *U. leptocladon* Oliv were expressed as IC_{50} and mean percent inhibition ± SD (mean ± SD). The FRAP of the leaf extracts was expressed as µM Fe (II)/g dry extract (mean ± SD). Total phenol content was expressed as mg Gallic acid equivalent per gram of dry extract (mean ± SD). Total flavonoid content was expressed as mg quercetin equivalent per gram of dry extract (mean ± SD). $P<0.05$ was considered statistically significant. Analysis of the difference between the antioxidant activities of the different groups was performed using one-way analysis of variance (ANOVA) with post hoc comparison (Tukey's test).

Ethical consideration

Ethical clearance (IRB/03/14/2022) was obtained from the Institutional Review Board (IRB) of the College of Natural and Computational Sciences, Addis Ababa University.

Result

DPPH radical scavenging activity of the leaf extract of *B. coriacea*

The different concentrations of *B. coriacea* (the crude extract and fractions of *B. coriacea*) had dose dependent DPPH antioxidant activities. The CH_3OH:$CHCl_3$ fraction of *B. coriacea* leaf extract (IC_{50}=38.2 µg/mL) demonstrated the highest DPPH radical scavenging activity as compared with the other three fractions of *B. coriacea* leaf extract ($CHCl_3$, CH_3OH, and Petroleum ether fractions) (Table 1). The Petroleum ether fraction of *B. coriacea* showed the lowest DPPH radical scavenging activity (IC_{50}=1149 µg/mL).

Table 1: DPPH free radical scavenging activity by the crude extract and fractions of the leaf extract of *B. coriacea*

Concentrations (µg/mL)	Percent inhibition of DPPH (%) (mean±SEM)					
	Crude extract of *B. coriacea*	CH_3OH: $CHCl_3$ fraction of *B.coriacea*	CH_3OH fraction of *B.coriacea*	$CHCl_3$ fraction of *B.coriacea*	Petroleum ether fraction of *B.coriacea*	Ascorbic acid
20	38±0.63 bcdef*	47±0.005 acdef*	23±0.22 abdef*	21±0.01 abcef*	16±0.01 abcdf*	45±0.005 abcde*
40	45±0.37 bcdef*	51±0.04 acdef*	25±0.01 abdef*	23±0.16 abcef*	17±0.05 abcdf*	55±0.003 abcde*
80	52±0.61 bcdef*	55 ±0.04 acdef*	27±0.72 abdef*	25±0.01 abcef*	18 ±0.05 abcdf*	65±0.01 abcde*
120	58±0.021 bcdef*	60±0.006 acdef*	30±0.01 abdef*	27±0.03 abcef*	20 ±0.05 abcdf*	75±0.02 abcde*
160	65±0.6 cdef*	65±0.005 cdef*	32±0.01 abdef*	29±0.14 abcef*	21 ±0.05 abcd*	85 ± 0.02 abcde*
200	71.4±0.63 bcdef*	69±0.01 acdef*	34±0.02 abdef*	31±0.03 abcef*	22 ±0.6 abcd*	98.9±0.02 abcde*
IC_{50} (µg/mL)	74.2 bcdef*	38.2 acdef*	462 abdef*	590 abcef*	1149 abcdf*	30.5 abcde*

Note: SEM stands for standard error of the mean. * indicates the mean difference is significant at $p<0.05$. a: compared with crude extract of *B. coriacea,* b: compared with CH_3OH: $CHCl_3$ fraction, c: compared with CH_3OH fraction, d: compared with $CHCl_3$ fraction, e: compared with Petroleum ether fraction, f: compared with ascorbic acid.

DPPH radical scavenging activity of the leaf extract of *U. leptocladon*

The different concentrations of *U. leptocladon* (the crude extract and fractions of *U. leptocladon*) had dose dependent DPPH antioxidant activities. The C_2H_6OH fraction of *U. leptocladon* leaf extract (IC_{50}=30.5 µg/mL) demonstrated the highest DPPH radical scavenging activity as compared with the other two fractions of *U. leptocladon* leaf extract ($CHCl_3$ and $C_4H_8O_2$ fractions) (Table 2). The $C_4H_8O_2$ fraction of *U. leptocladon* showed the lowest DPPH radical scavenging activity (IC_{50}=1175 µg/mL).

Table 2: DPPH free radical scavenging activity by the crude extract and fractions of the leaf extract of *U. leptocladon*

Concentrations (µg/mL)	Percent inhibition of DPPH (%) (mean±SEM)				
	Crude extract of *U.leptocladon*	$CHCl_3$ fraction of *U. leptocladon*	$C_4H_8O_2$ fraction of *U. leptocladon*	C_2H_6OH fraction of *U. leptocladon*	Ascorbic acid
20	45±0.01 bcd*	23 ±0.02 acde*	15±0.24 abde*	48±0.01 abce*	45± 0.005 bcd*
40	53±0.04 bcde*	27 ±0.02 acde*	16±0 abde*	52±0.02 abce*	55±0.003 abcd*
80	63±0.01 bcde*	32 ±0.005 acde*	17±0.006 abde*	56±0.03 abce*	65±0.01 abcd*
120	71±0.08 bcde*	36±0.01 acde*	18±0.03 abde*	60±0.11 abce*	75±0.02 abcd*
160	80±0.01 bcde*	41 ±0.02 acde*	19±0.005 abde*	65±0.008 abce*	85 ±0.02 abcd*
200	91±0.005 bcde*	46 ±0.02 acde*	20±0.04 abde*	69±0.39 abce*	98.9±0.02 abcd*
IC_{50} (µg/mL)	33.5 bcde*	238 acde*	1175 abde*	30.5 abc*	30.5 abc*

Note: SEM stands for standard error of the mean. * indicates the mean difference is significant at $p<0.05$. a: compared with crude extract of *U.leptocladon.* b: $CHCl_3$ fraction, c: compared with $C_4H_8O_2$ fraction, d: compared with C_2H_6OH fraction, e: compared with ascorbic acid.

DPPH radical scavenging activity of Isolated compounds

The antioxidant activity of the compounds isolated from leaf extracts of *B. coriacea* and *U. leptocladon* was shown in Table 3. The antioxidant effects of the leaf extract of *B. coriacea* is due to β-sitosterol and lucidine-type compound. The antioxidant effects of the leaf extract of *U. leptocladon* is attributed to β-sitosterol, β-sitosterol glucoside, and α-humulene.

The highest DPPH antioxidant activity was shown by the lucidine type compound (IC_{50}=48.5 µg/mL). 1-tricontanol demonstrated the lowest antioxidant activity (IC_{50}=572.2 µg/mL).

ABTS radical scavenging activity

ABTS radical scavenging activity by the leaf extract of *B. coriacea*

Table 4 shows ABTS radical scavenging activity of the crude extract and fractions of the leaf extract of *B. coriacea.* The findings revealed that the different concentrations of *B. coriacea* (the crude extract and fractions of *B. coriacea*) had dose dependent ABTS antioxidant activity.

The CH_3OH: $CHCl_3$ fraction of *B. coriacea* leaf extract (IC50=70 µg/mL) demonstrated the highest ABTS radical scavenging activity as compared with the other three fractions of *B. coriacea* ($CHCl_3$, CH_3OH, and Petroleum ether fractions) (Table 4). The Petroleum ether fraction of *B. coriacea* showed the lowest ABTS radical scavenging activity (IC50=382.6 µg/mL).

Table 3: Antioxidant activity by the compounds isolated from leaf extracts of *B. coriacea* and *U. leptocladon* using DPPH radical scavenging assay

Concentrations (µg/mL)	Percent inhibition of DPPH (%) (mean±SEM)					
	β-sitosterol	**β-sitosterol glucoside**	**α-humulene**	**Lucidine type compound**	**1-tricontanol**	**Ascorbic acid**
20	26±0.06 bcdef*	30 ±0.03 acdef*	23±0 abdef*	40±0.005 abcef*	5±0.17 abcdf*	45±0.008 abcde*
40	31±0.01 bcdef*	35 ±0.01 acdef*	28±0.08 abdef*	48±0.003 abcef*	8±0.003 abcdf*	55±0.008 abcde*
80	41±0.005 bcdef*	50 ±0.05 acdef*	38±0.003 abdef*	61±0.33 abcef*	11±0.02 abcdf*	65.7±0.005 abcde*
120	55 ±0.05 bcdef*	65 ±0.003 acdef*	53±0.008 abdef*	72±0.006 abcef*	14± 0.003 abcdf*	76.8±0.008 abcde*
160	70±0.005 bcdef*	80 ±0.01 acdef*	68±0.01 abdef*	85±0.003 abcef*	17±0.003 abcdf*	90 ±0.006 abcde*
200	85±0.01 bcdef*	95±0.003 acdef*	83±0.006 abdef*	96.7±0.008 abcef*	20±0.01 abcdf*	98.6±0.005 abcde*
IC_{50} (µg/mL)	99 bcdef*	77.6 acdef*	105.6 abdef*	48.5 abcef*	572.2 abcdf*	29.3 abcde*

Note: a: compared with β-sitosterol, b: compared with β-sitosterol glucoside; c: compared with α-Humelene; d: compared with lucidine type compound; e: compared with 1-tricontanol; f: compared with ascorbic acid. SEM stands for standard error of the mean. * indicates the mean difference is significant at $p<0.05$.

Table 4: ABTS radical scavenging activity by crude extract and fractions of the leaf extract of *B. coriacea*

Concentration (µg/mL)	Percent ABTS inhibition (mean±SEM)					
	Crude extract of *B. coriacea*	CH_3OH: $CHCl_3$ fraction of *B. coriacea*	CH_3OH fraction of *B. coriacea*	$CHCl_3$ fraction of *B. coriacea*	Petroleum ether fraction of *B. coriacea*	Ascorbic acid
25	38±0.03 bcdef*	40±0.05 acdef*	34±0.01 abdef*	22±0.02 abcef*	21±0.02 abcdf*	49±0.03 abcde*
50	45±0.03 bcdef*	46±0.08 acdef*	41±0.01 abdef*	25±0.01 abcef*	24±0.04 abcdf*	58±0.01 abcde*
100	56±0.03 cdef*	56±0.02 cdef*	49±0.01 abdef*	29±0.006 abcef*	28±0.01 abcdf*	72±0.02 abcde*
150	68±0.03 bcdef*	66±0.02 acdef*	57±0.01 abdef*	33±0.03 abcef*	32±0.01 abcdf*	89±0.3 abcde*
200	81.3±0.05 bcdef*	76.7±0.02 acdef*	66±0.05 abdef*	37±0.01 abcef*	36±0.01 abcdf*	99±0.08 abcde*
IC_{50} (µg/mL)	74 bcdef*	70 acdef*	106.2 abdef*	370 abcef*	382.6 abcdf*	24 abcde*

Note: SEM stands for standard error of the mean. * indicates the mean difference is significant at $p<0.05$. a: compared with crude extract of *B. coriacea,* b: compared with CH_3OH: $CHCl_3$ fraction, c: compared with CH_3OH fraction, d: compared with $CHCl_3$ fraction, e: compared with Petroleum ether fraction, f: compared with ascorbic acid.

ABTS radical scavenging activity the leaf extract of *U. leptocladon*

Both the crude extract and fractions of the leaf extract of *U. leptocladon* showed ABTS radical scavenging activity (Table 5). The findings revealed that the different concentrations of *U. leptocladon* (the crude extract and fractions of *U. leptocladon*) had dose dependent ABTS antioxidant activities.

The C_2H_6OH fraction of *U. leptocladon* leaf extract (IC50=24.4 µg/mL) demonstrated the highest ABTS radical scavenging activity as compared with the other two fractions ($CHCl_3$ and $C_4H_8O_2$ fractions) (Table 5). The $C_4H_8O_2$ fraction of *U. leptocladon* showed the lowest ABTS radical scavenging activity (IC50=658.6 µg/mL).

Table 5: ABTS radical scavenging activity by crude extract and fractions of the leaf extract of *U. leptocladon*

Concentrations (µg/mL)	**Percent inhibition of ABTS (%) (mean±SEM)**				
	Crude extract of *U. leptocladon*	$CHCl_3$ fraction of *U. leptocladon*	$C_4H_8O_2$ fraction of *U. leptocladon*	C_2H_6OH fraction of *U. leptocladon*	Ascorbic acid
25	49±0.01 bcd*	41±0.02 acde*	18±0.02 abde*	48±0.03 abce*	49±0.03 bcd*
50	57±0.03 bcde*	51±0.006 acde*	20±0.01 abde*	58±0.01 abc*	58±0.01 abc*
100	71±0.3 bcde*	61±0.01 acde*	22±0.6 abde*	68±0.005 abce*	72±0.02 abcd*
150	87±0.04 bcde*	71±0.02 acde*	25±0.03 abde*	78±0.05 abce*	89±0.3 Abcd*
200	96±0.04 bcde*	83±0.03 acde*	27±0.01 abde*	90±0.14 abce*	99±0.08 abcd*
IC_{50} (µg/mL)	25.2 Bcde	54.9 acde*	658.6 abde*	24.4 abc*	24 abc*

Note: SEM stands for standard error of the mean. * indicates the mean difference is significant at $p<0.05$. a: compared with crude extract of *U.leptocladon.* b: $CHCl_3$ fraction, c: compared with $C_4H_8O_2$ fraction, d: compared with C_2H_6OH fraction, e: compared with ascorbic acid.

FRAP of the leaf extracts of *B. coriacea* and *U. leptocladon*

The FRAP of the leaf extracts of *B. coriacea* and *U. leptocladon* was calculated based on the standard curve of FeSO4. The ability of the crude leaf extracts of *B. coriacea* and *U. leptocladon* to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II)) were found to be 887.6 ± 0.41 µM Fe(II)/g dry mass and 1680 ± 0.13 µM Fe(II)/g dry mass, respectively. The MeOH:$CHCl_3$ fraction of *B. coriacea* (940.1 ± 2.87µM Fe (II)/g dry mass) and the C_2H_6OH fraction of *U. leptocladon* (1842.6 ± 0.85 µM Fe(II)/g dry mass) had the highest ferric reducing potential (Table 6).

Table 6: FRAP of the leaf extract of *B. coriacea* and *U. leptocladon*

Plant name	Plant extract/fractions	µM Fe(II)/g dry mass (mean ± SEM)
B. coriacea	Crude extract	815.3±0.24 bcdefghi*
	CH_3OH:$CHCl_3$ fraction	940.1±1.65 acdefghi*
	CH_3OH fraction	389.4±0.04 abdefgh*
	$CHCl_3$ fraction	374.9± 0.04 abcfghi*
	Petroleum ether fraction	374.8±0.04 abcfghi*
U. leptocladon	Crude extract	1680± 0.07 abcdeghi*
	C_2H_6OH fraction	1842.6±0.07 abcdefhi*
	$CHCl_3$ fraction	809.6±0.05 abcdefgi*
	$C_4H_8O_2$ fraction	388.8± 0.04 abdefgh*

Note: a: compared with crude extract of *B. coriacea;* b: compared with CH_3OH: $CHCl_3$ fraction of *B. coriacea;* c: CH_3OH fraction of *B. coriacea;* d: $CHCl_3$ fraction of *B. coriacea;* e: Petroleum ether fraction of *B. coriacea;* f: crude extract of *U. leptocladon;* g: C_2H_6OH fraction of *U. leptocladon;* h: $CHCl_3$ fraction of *U. leptocladon;* i: $C_4H_8O_2$ fraction of *U. leptocladon.* * indicates the mean difference is significant at $p< 0.05$. SEM stands for standard error of mean.

Determination of total phenol and flavonoid content

The crude extract of *B. coriacea* and *U leptocladon* had total phenol contents of 137.7 and 147.7 mg/g of dry extract, respectively (Table 7). The highest total phenol content was obtained in the CH_3OH: $CHCl_3$ (145.00 mg/g of dry extract) and C_2H_6OH (187.70 mg/g of dry extract) fractions in the *B. coriacea* and *U leptocladon*, respectively. Meanwhile the least concentrations of total phenol were found in the petroleum ether (11.60 mg/g of dry extract) and $C_4H_8O_2$ (10.90 mg/g of dry extract) fractions, respectively.

The total flavonoid content was highest in the crude extracts of both *B. coriacea* (111.70 mg/g of dry extract) and *U. leptocladon* (138.80 mg/g of dry extract). Among the fractions, the highest total flavonoid content was obtained in the CH_3OH:$CHCl_3$ (136.80 mg/g of dry extract) and C_2H_6OH (172.90 mg/g of dry extract) fractions in the *B. coriacea* and *U leptocladon,* respectively. In contrast, the least concentrations were found in the petroleum ether (0.75 mg/g of dry extract) and $C_4H_8O_2$ (4.24 mg/g of dry extract) fractions, respectively (Table 7).

Table 7: Total phenolic and total flavonoid content of the leaf extracts of *B. coriacea* and of *U. leptocladon*

Plant name	Test sample	Total phenolic content (mg /g dry extract) (mean ± SEM)	Total flavonoid content (mg/g dry extract) (mean ± SEM)
B. coricea	Crude extract	137.7± 0.34 bcdefghi*	111.7±0.051 bcdefghi*
	CH_3OH: $CHCl_3$ fraction	145± 0.01 acdefghi*	136.8±0.03 acdefghi*
	CH_3OH fraction	24.8± 0.01 abdefghi*	9.75±0.01 abdefghi*
	$CHCl_3$ fraction	12.3±0.01 abcfghi*	3.8±0.03 abcefgh*
	Petroleum ether fraction	11.6± 0.03 abcfgh*	0.75±0.005 abcdfghi*
U. leptocladon	Crude extract	147.7± 0.01 abcdeghi*	138.8± 0.05 abcdeghi*
	C_2H_6OH fraction	187.7±0.03 abcdefhi*	172.9±0.23 abcdefhi*
	$CHCl_3$ fraction	41.3±0.06 abcdefgi*	22.05±0.03 abcdefgi*
	$C_4H_8O_2$ fraction	10.9 ±0.005 abcdfgh*	4.24 ± 0.15 abcefgh*

Note: a: compared with crude extract of *B.coriacea;* b: compared with CH_3OH: $CHCl_3$ fraction of *B.coriacea;* c: CH_3OH fraction of *B. coriacea;* d: $CHCl_3$ fraction of *B.coriacea;* e: Petroleum ether fraction of *B. coriacea;* f: crude extract of *U. leptocladon;* g: C_2H_6OH fraction of *U. leptocladon;* h: $CHCl_3$ fraction of *U. leptocladon;* i: $C_4H_8O_2$ fraction of *U. leptocladon.* * indicates the mean difference is significant at $p<0.05$. SEM stands for standard error of mean.

Discussion

This study showed that the leaf extracts of *B. coriacea* and *U. leptocladon* have potent DPPH and ABTS radicals scavenging effects. Based on the IC_{50} value, the ability of DPPH radical scavenging activity of antioxidants can be classified in to five groups:- highly active (IC_{50} < 50 µg/mL); active (IC_{50} 50-100 µg/mL); moderate (IC_{50} 101-250 µg/mL); weak (IC_{50} 250 to 500 µg/mL) and inactive (IC_{50} >500 µg/mL) (23). Accordingly, the crude extract of *U. leptocladon* (IC_{50}=33.5 µg/mL), the CH_3OH:$CHCl_3$ fraction of *B. coriacea* (IC_{50}=38.2 µg/mL), the C_2H_6OH fraction of *U. leptocladon* (IC_{50}=30.5 µg/mL) and the lucidine type compound (IC50=48.5 µg/mL) are highly active in their DPPH radical scavenging activity. Whereas, β-sitosterol glucoside (IC_{50}=77.6 µg/mL) and the crude extract of *B. coricea* (IC_{50}=74.2 µg/mL) demonstrated to be active against the DPPH radical. β-sitosterol (IC_{50}=99 µg/mL) and α-humulene (IC_{50}=105.6 µg/mL) had moderate DPPH scavenging activity. In contrast to the other compounds, 1-Tricontanol was inactive against DPPH radical (IC_{50}=572.25 µg/mL). The results of this study substantiate previous studies that investigated the antioxidant activities of the leaf extracts of other *Boscia* species such as *B. senegalensis* and *B. Arabica* (6, 8) and *Uvaria* species such as *U. chamae* (14).

Free radicals scavenging activity of plants is mainly related with their total phenol content (24). Therefore, the high inhibition of ABTS and DPPH by the leaf extract of *B. coriacea* and *U. leptocladon* noted in this study could be due the high

total phenol and flavonoid contents of the plants. Similarly, the highest inhibition of ABTS and DPPH by the CH_3OH:$CHCl_3$ fraction of *B. coriacea* and the C_2H_6OH fraction of *U. leptocladon* could be due the highest total phenol and flavonoid contents.

Antioxidant activity of medicinal plants is associated with steroids, alkaloids, phenols, flavonoids, and tannins that they possess (25-27). Therefore, the antioxidant activities of the crude leaf extracts of *B. coriacea* Graells and *U. leptocladon* Oliv observed in this study might be due to the presence of alkaloids, phenols, flavonoids, and tannins in the leaf extracts (9).

The ability of FRAP of antioxidants can be classified into five groups as follows (28):- a) Very low: with FRAP value <10 µmol/g; (b) Low: with FRAP value from 10 to 50 µmol/g; (c) Good: with FRAP value from 50 to 100 µmol/g; (d) High: with FRAP value from 100 to 400 µmol/g; Very high: with FRAP value >400 µmol/g. Accordingly, the crude extract of *B. coriacea* (FRAP value=887.6 µmol/g); the crude extract of *U. leptocladon* (FRAP value=1735.6 µmol/g); the CH_3OH:$CHCl_3$ fraction of *B. coriacea* (FRAP value=940.1 µmol/g); the C_2H_6OH (FRAP value=1828.2 µmol/g) and $CHCl_3$ fractions of *U. leptocladon* (FRAP value= 809.6 µmol/g) have very high FRAP. Taken together, the leaves extracts of the medicinal plants can be source of potent antioxidant drugs.

Conclusion and recommendation

The study demonstrated that the leaf extracts of *B. coriacea* and *U. leptocladon* have strong antioxidant activity. Further study is needed to determine the mechanism of action of the compounds isolated from the leaves extracts of *B. coriacea* and *U. leptocladon*.

Conflict of interest

There is no conflict of interest in this work.

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