



International Scientific Organization  
<http://iscientific.org/>  
 Chemistry International  
[www.bosaljournals.com/chemint/](http://www.bosaljournals.com/chemint/)



## Appraisal of solvent system effect on bioactivity profiling of *Cordia africana* stem bark extracts

Alemayehu Mekonnen<sup>1,\*</sup>, Yilkal Degu<sup>1</sup> and Rolf Carlson<sup>2,\*</sup>

<sup>1</sup>Department of Chemistry, Science College, Bahir Dar University, P.O. Box 79, Bahir Dar, Ethiopia

<sup>2</sup>Department of Chemistry, University of Tromsø, N-9037, Tromsø, Norway

\*Corresponding author's E. mail: [negaalex@yahoo.com](mailto:negaalex@yahoo.com) (A. Mekonnen), [rolf.carlson@uit.no](mailto:rolf.carlson@uit.no) (R. Carlson)

### ARTICLE INFO

#### Article type:

Research article

#### Article history:

Received September 2018

Accepted February 2019

January 2020 Issue

#### Keywords:

*Cordia Africana*

Antioxidant activity

Flavonoid

Polyphenols

Ferric reducing power

Antioxidant activity

Phytochemical screening

Free radicals

Antibacterial activity

### ABSTRACT

Phytochemicals and antioxidant and antibacterial activities of *Cordia africana* stem bark were evaluated. Four different solvent systems were used for extraction and the evaluation of all chemical components, antioxidant and antibacterial activities were made following established methodologies. The antioxidant activity was determined using free radical scavenging and 2,2-diphenyl-1-picrylhydrazyl (DPPH) reduction assays. The phytochemical screening study on the methanol extracts revealed the presence of different plant constituents such as alkaloids, phenolics, flavonoids, tannins terpenoids and saponins. All the extracts had significant ( $P < 0.05$ ) antioxidant activity. DPPH radical scavenging activity ranging from 87.76 % (90 % methanol) to 22.94 % (chloroform) and FRAP values varied between 75.42 (90 % methanol) to 31.22 mg AAE/100g (chloroform). Antibacterial activity evaluation of methanolic extract from *C. africana* bark was carried out using four different bacterial strains. However, the extracts tested did not show clinically relevant antibacterial activity. All crude extracts significantly inhibited oxidation. The results support the use of *C. africana* leaves in traditional medicine to treat several human ailments.

© 2020 International Scientific Organization: All rights reserved.

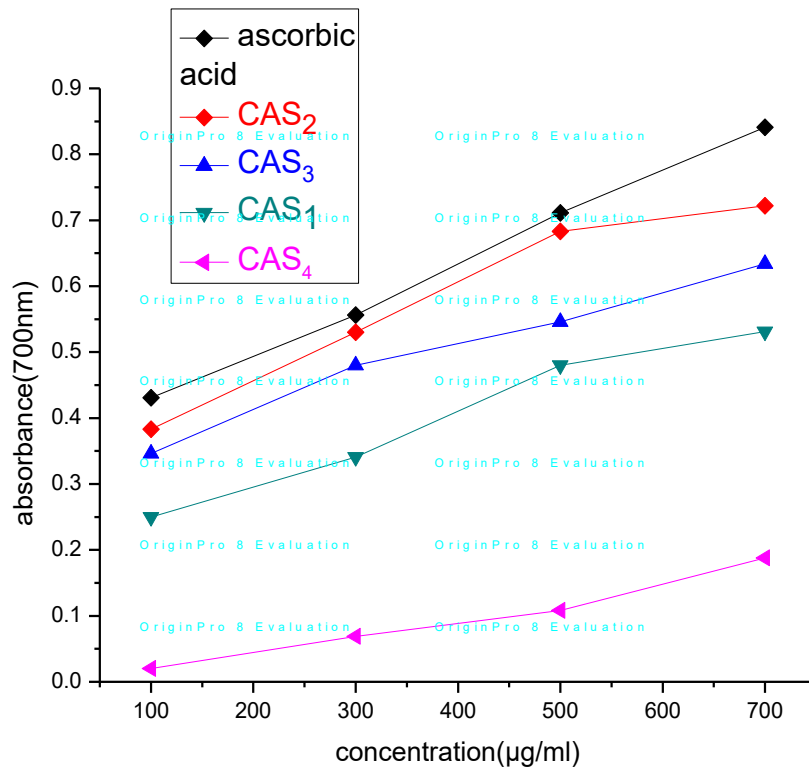
**Capsule Summary:** Phytochemical screening and antioxidant activities of the *Cordia africana* stem bark extracts have been examined using different test systems. The extracts showed effective activity in delaying oxidation as compared to the standard.

**Cite This Article As:** A. Mekonnen, Y. Degu and R. Carlson. Appraisal of solvent system effect on bioactivity profiling of *Cordia africana* stem bark extracts. Chemistry International 6(1) (2020) 1-10. <https://doi.org/10.5281/zenodo.2574105>

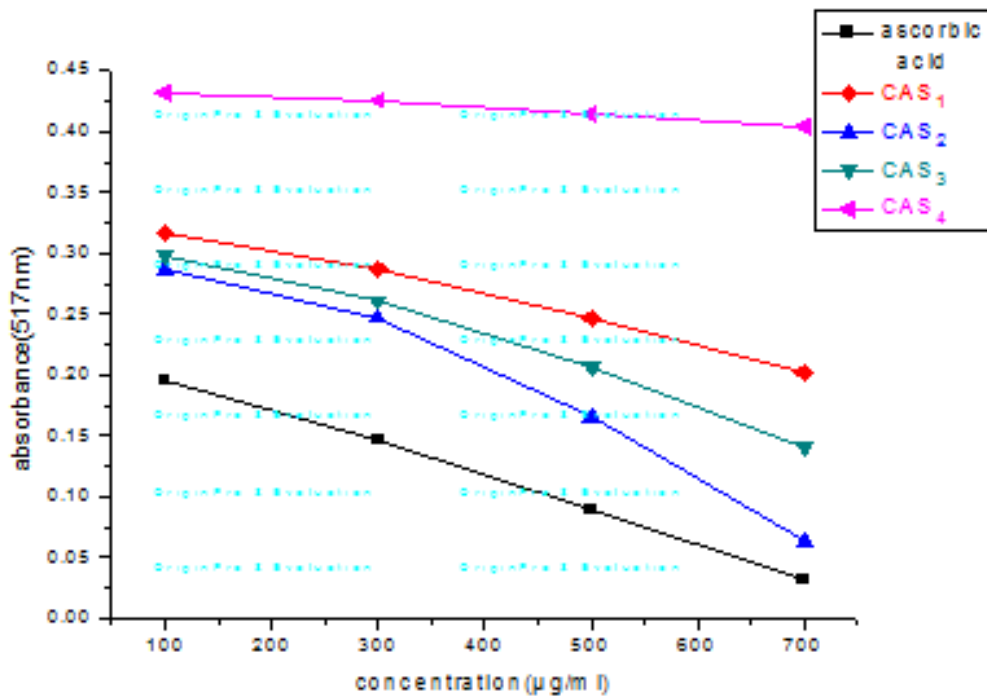
### INTRODUCTION

Medicinal plants have been used nearly all cultures as a source of medicine. It is believed that a major part of traditional therapy involves the use of plant extracts or their active principles (Kesatebrhan, 2013). According to the WHO, due to their poverty and lack of access to modern medicine, 65-80% of the world's population in developing countries

depends on plants for their primary health care need (Kalayu et al., 2013). In Africa, most of the population uses traditional medicine for primary health care (Kebede et al., 2006). Accordingly, in Ethiopia nearly all of the population in the country use plant based traditional medicine as their major primary health care system. Ethnobotanical knowledge (knowledge has been passed from one generation to another) which still exists on remote areas is the main reason for the widespread use of herbs as source of traditional medicine.



**Fig. 1:** Absorbance of test solutions and the standard versus different concentrations of extracts on the reducing power assay (ascorbic acid at the top and CAS<sub>4</sub> at the bottom).



**Fig. 2:** Comparison of DPPH radical scavenging activity of stem bark extracts and ascorbic acid at different concentration (ascorbic acid < CAS<sub>2</sub> < CAS<sub>3</sub> < CAS<sub>1</sub> < CAS<sub>4</sub>).

**Table 1:** The results of phytochemical screening of the various crude extracts of *C. africana* bark

Phytochemicals	80% methanol	90% methanol	100% methanol	Chloroform
Alkaloids	+	+	+	-
Phenolics	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	-
Tannins	+	+	+	+
Cardiac glycoside	-	-	-	-
Terpenoids	+	+	+	++

- : absent    +: present

**Table 2:** Ferric reducing antioxidant power of different solvent extracts of *C. africana* bark and the standard, ascorbic acid, at 700 nm

Concentration ( $\mu\text{g/mL}$ )	Samples and standard absorbance at 700 nm				
	CAS <sub>1</sub>	CAS <sub>2</sub>	CAS <sub>3</sub>	CAS <sub>4</sub>	Ascorbic acid
100	0.250 $\pm$ 0.006	0.383 $\pm$ 0.002	0.346 $\pm$ 0.002	0.020 $\pm$ 0.001	0.194 $\pm$ 0.002
300	0.341 $\pm$ 0.002	0.530 $\pm$ 0.003	0.480 $\pm$ 0.002	0.069 $\pm$ 0.001	0.444 $\pm$ 0.002
500	0.480 $\pm$ 0.004	0.683 $\pm$ 0.003	0.546 $\pm$ 0.002	0.108 $\pm$ 0.001	0.694 $\pm$ 0.002
700	0.531 $\pm$ 0.002	0.722 $\pm$ 0.007	0.634 $\pm$ 0.001	0.188 $\pm$ 0.002	0.944 $\pm$ 0.002

Sample for three independent measurements (triplicate; n = 3, mean  $\pm$  SD). There is a significant difference in the mean mg equivalent per g dry weight of the samples for  $P < 0.05$  by paired t-test

**Table 3:** Absorbance of DPPH solution at different concentration of samples and ascorbic acid

Concentration ( $\mu\text{g/mL}$ )	Absorbance of the samples and AA				Absorbance
	CAS <sub>1</sub>	CAS <sub>2</sub>	CAS <sub>3</sub>	CAS <sub>4</sub>	
100	0.317 $\pm$ 0.001	0.287 $\pm$ 0.002	0.298 $\pm$ 0.002	0.431 $\pm$ 0.004	0.196 $\pm$ 0.002
300	0.286 $\pm$ 0.002	0.247 $\pm$ 0.001	0.261 $\pm$ 0.001	0.426 $\pm$ 0.003	0.147 $\pm$ 0.002
500	0.247 $\pm$ 0.002	0.165 $\pm$ 0.003	0.207 $\pm$ 0.017	0.414 $\pm$ 0.005	0.090 $\pm$ 0.001
700	0.201 $\pm$ 0.002	0.064 $\pm$ 0.003	0.141 $\pm$ 0.001	0.403 $\pm$ 0.003	0.031 $\pm$ 0.002

Sample for three independent measurements (triplicate; n=3, mean  $\pm$  SD). There is a significant difference in the mean mg equivalent per g dry weight of the samples for  $P < 0.05$ .

**Table 4:** Percentage DPPH radical scavenging activity of *C. africana* bark extracts at different concentration

Concentration ( $\mu\text{g/mL}$ )	Samples and AA% inhibition				Absorbance
	CAS <sub>1</sub>	CAS <sub>2</sub>	CAS <sub>3</sub>	CAS <sub>4</sub>	
100	39.39 $\pm$ 0.100	45.12 $\pm$ 0.390	43.02 $\pm$ 0.330	17.59 $\pm$ 0.190	62.52 $\pm$ 0.280
300	45.31 $\pm$ 0.400	52.77 $\pm$ 0.190	50.09 $\pm$ 0.190	18.55 $\pm$ 1.150	71.89 $\pm$ 0.240
500	52.77 $\pm$ 0.330	68.06 $\pm$ 0.500	60.12 $\pm$ 0.330	20.84 $\pm$ 0.190	82.79 $\pm$ 1.200
700	61.56 $\pm$ 0.390	87.76 $\pm$ 0.510	73.04 $\pm$ 0.500	22.94 $\pm$ 0.580	94.07 $\pm$ 0.290

Data is expressed as mean of three determinations  $\pm$  SD. There is a significant difference in the mean mg equivalent per g dry weight of the samples for  $P < 0.05$ .

However, cultural systems are dynamic, the skills are fragile and easily forgettable since most of the indigenous knowledge transfer in the country based on oral transmission (Kalayu et al., 2013).

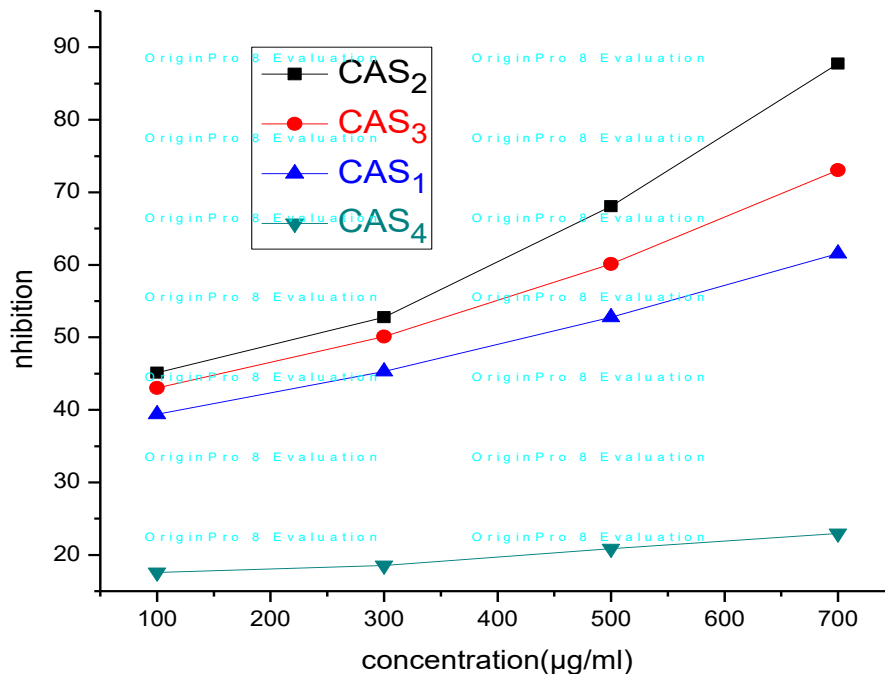
A variety of chemical constituents derived from plants have been used for the prevention and treatment of diseases virtually in all cultures. Plant constituents have

become an important source of active natural products which differ widely in terms of their structure and biological properties (Cao et al., 2019; Elhidar et al., 2019; Khan et al., 2019; Pyrzynska and Sentkowska, 2019; Roberts et al., 2019; Šircelj et al., 2019; Yan et al., 2019). In recent years, the prevention of many disorders such as cancer and cardiovascular diseases has been found associated with the

ingestion of plants that are rich in natural antioxidants. The bioactivity for plant products is due to the presence enzymes and proteins, vitamins, carotenoids, flavonoids, anthocyanins and other phenolic compounds (Muhammad et al., 2011).

2013). Therefore, attention has been paid to extraction of biologically active compounds from plants.

## MATERIAL AND METHODS



**Fig. 3:** The percentage inhibition of DPPH radical scavenging activity of *Cordia africana* bark extracts at different concentration (CAS<sub>2</sub> > CAS<sub>3</sub> > CAS<sub>1</sub> > CAS<sub>4</sub>).

Dietary antioxidant compounds can protect the organism against oxidation damage (Marit, 2008). When the mechanisms of antioxidant protection become unbalanced by some factors, progressive deterioration and loss of normal body functions may result in symptom or disease such as aging, cancer, coronary heart diseases, etc. (Ebrahim and Mekonnen., 2018). Bioactive components from medicinal plants are said to be responsible for the antimicrobial effects of plant extracts *in vitro*. Many pharmaceuticals currently available and physicians have a long history of use of herbal remedies, including opium, aspirin, digitalis and quinine (Mariata, 2010). *Cordia africana* is one of the African *Boraginaceae* families. The genus *Cordia* consists of about 250 species in the tropical and subtropical regions of all continents, 10 species are found in Ethiopia. This plant is locally known as “Wanza” and used for firewood, timber (furniture, beehives, boxes, mortars, church, and drums), food (fruit), medicine (bark, roots), fodder (leaves), bee forage, soil conservation, ornamental, and shade (Alberto, 2007). In recent years, antibiotic resistance has become a global concern and this problem is more in developing country because the infectious diseases are still an important cause of morbidity and mortality among humans (Resat et al.,

### Sample collection and preparation

The fresh barks of *C. africana* were collected from forest area, around Dangila. *C. africana* bark was collected, washed carefully with tap water, air dried for two weeks under shed. The dry barks were ground to obtain a fine powder. The extract of the samples were prepared by soaking 40 g of dried powder in 400 mL of different solvents (80% methanol, 90% methanol, 100% methanol and chloroform) at room temperature. The mixtures were extracted upon shaking with electrical shaker for 48 h and filtered using Whatman filter paper. The extracts were filtered and concentrated using rotary evaporator under reduced pressure at 40°C to obtain the crude extracts. The yields were calculated and the crude extracts obtained were labeled as CAS<sub>1</sub>, CAS<sub>2</sub>, CAS<sub>3</sub> and CAS<sub>4</sub> for 80% methanol, 90% methanol, 100% methanol and chloroform solvents, respectively.

### Phytochemical screening

Phytochemical tests were performed for all the extracts of *C. africana* following published procedure with slight modifications for alkaloids, polyphenols, flavonoids,

saponins, tannins, glycosides and terpenoids (Oyaizu, 1986). All these tests were performed in triplicates.

### Antioxidant activities evaluation

The antioxidant activity (AOA) was determined by two methods (Mekonnen et al., 2018), using the free radicals 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and reducing power, FRAP assay compared with a standard antioxidant (ascorbic acid) in a dose response curve being expressed as mg AAE per gram of sample.

Determination of reducing power: Reducing power assay was determined according to the method (Oyaizu, 1986). 2 mL of different concentrations of different bark extracts (100, 300, 500, and 700 µg/mL) were mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide followed by incubation at 50 °C for 20 min. After adding 2.5 mL of 10% trichloroacetic acid, the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (2 mL) was taken and mixed with 2 mL of distilled water and 0.5 mL of 1% ferric chloride. After incubation for 10 min, formation of green colour was observed and the absorbance of this solution was measured at 700 nm. All these tests were performed in triplicates and ascorbic acid was used as standard.

Determination of DPPH radical scavenging activity: The antioxidant activities of the extracts were evaluated spectrophotometrically following DPPH method with little modification (Mekonnen et al., 2018). From previously prepared diluted sample, 1.0 mL crude extracts having different concentrations (100, 300, 500, and 700 µg/mL) were transferred into four separated test tubes. Then to each of the extracts, 1 mL of 0.1 mM DPPH solution was added. The volume was adjusted to 4 mL with water. The mixture was vigorously shaken for the purpose of homogenizing the solution and left for 30 min until the reaction was completed. Finally, the absorbance was recorded at 517 nm and ascorbic acid was used as the standard.

### Antibacterial activity evaluation

The antimicrobial activity of the methanol extracts from *C. africana* bark were evaluated using agar well diffusion method (Mekonnen et al., 2018, Rahman and Gray, 2002) against two gram-positive bacteria (*Staphylococcus aureus*, *Sa* and *Streptococcus pyogenes*, *Sp* and two gram-negative bacteria (*Escherichia coli*, *Ec* and *Klebsiella pneumonia*, *Kp*). The bacterial cultures were inoculated at 37 °C into the Muller Hinton Agar (MHA). Auxofloxacin and Ampicillin were used as positive and negative control, respectively. 1.0 mL of standardized bacterial stock suspension ( $1 \times 10^8$  CFU/mL) was mixed with 100 mL of molten sterile nutrient agar which was maintained at 45 °C. 20 mL aliquots of the inoculated nutrient agar were distributed into sterile Petri-dishes. The agar was left to set and the plates (6 mm in diameter) were cut using a sterile cork borer. A standard solution of 100 µg/mL concentration of the extracts was prepared. From this

stock solution, 6.25-50 µg/mL solution was prepared by serial dilution. The wells were filled with 100 µL sample of extracts using automatic micro-liter pipette and the plates were incubated at 37 °C for 24 h. Zone of inhibition around the wells were observed and measured after 24 h.

### Statistical analysis

All values are presented as mean and corresponding standard deviation based on the different replicas made. The statistical analyses were performed using one way analysis of variance (ANOVA) by Origin 6.0 software. Linear regression with the least squares method was used to fit the calibration curves. For all tests, differences with values of  $P < 0.05$  were considered significant. Correlations between variables were established by regression analysis.

## RESULTS AND DISCUSSION

### Qualitative phytochemical analysis

The preliminary phytochemical tests are helpful in finding information about chemical constituents present in the plant material. This information could be used to facilitate quantitative estimation and qualitative separation of pharmacologically active compounds from plant extracts. Phytochemical screening of the different *C. africana* bark extracts demonstrated the presence of polyphenols, flavonoids, saponins, alkaloids, tannins and terpenoids with varying concentrations (Table 1). No anthraquinones, cardiac and cyanophoric glycoside were detected in all extracts. Particularly, the test revealed the presence of polyphenols, flavonoids, tannins and terpenoids in all solvent extracts with high degree of precipitation but alkaloids and saponins were not detected in the chloroform extracts. However, chloroform is an ideal solvent to extract terpenoids since high degree of precipitation was observed. These findings are consistent with previous reports (Abebe et al., 2017, Abate et al., 2017).

### Reducing power (FRAP)

All extracts were analyzed for their FRAP reducing activity (Tables 2). Aqueous solutions of ascorbic acid standard at different concentrations (100, 300, 500, 700 µg/mL) were used as reference solution to measure the reducing power of the extracts by FRAP method. The reducing power of the extracts calculated from the Ascorbic acid calibration curve ( $y = 0.00125x + 0.0693$ ,  $R^2$  of 0.99754) shown in Figure 1, were found to be  $112.41 \pm 1.56$  mg AAE/g (milligram of Ascorbic acid equivalent per gram of dry extract). But the FRAP of the crude extract was found to be 70.28 mg AAE/g extract. Out of the different extracts, the highest antioxidant activity was observed in the 90 % methanol extract ( $75.42 \pm 0.364$ ) while the chloroform showed comparably low activity ( $31.22 \pm 0.119$ ).

**Table 5:**  $IC_{50}$  values for antioxidant activity of different solvent extracts of *C. africana* stem bark extracts and the standard, ascorbic acid

Samples	CAS <sub>1</sub>	CAS <sub>2</sub>	CAS <sub>3</sub>	CAS <sub>4</sub>	Ascorbic Acid
$IC_{50}$ ( $\mu\text{g/mL}$ )	460.74 $\pm$ 0.010	322.27 $\pm$ 0.10	438.86 $\pm$ 0.070	636.56 $\pm$ 0.050	34.16 $\pm$ 0.072

Data is expressed as mean of three determinations  $\pm$  SD

**Table 6:** Antibacterial activity of *C. africana* bark extract and standard antibiotics against two gram-positive and two gram-negative bacteria

Extract and Antibiotics	Concentration ( $\mu\text{g/mL}$ )	Zone of inhibition of different bacteria in mm			
		<i>E. coli</i>	<i>K. pneumonia</i>	<i>S. pyogenes</i>	<i>S. aureus</i>
Methanol	6.250- 50.000	NI	NI	NI	NI
	50.000	NI	NI	19.25 $\pm$ 0.600	19.64 $\pm$ 1.800
Ampicillin	25.000	NI	NI	17.43 $\pm$ 2.600	18.41 $\pm$ 0.100
	12.500	NI	NI	13.76 $\pm$ 0.200	15.34 $\pm$ 0.500
	6.250	NI	NI	12.24 $\pm$ 1.200	11.74 $\pm$ 0.200
	50.000	NI	NI	22.41 $\pm$ 0.100	20.21 $\pm$ 0.800
Auxofloxacin	25.000	NI	NI	18.21 $\pm$ 2.100	14.26 $\pm$ 2.100
	12.500	NI	NI	14.46 $\pm$ 1.200	13.46 $\pm$ 1.200
	6.250	NI	NI	11.21 $\pm$ 1.500	11.26 $\pm$ 1.500

Where NI- no inhibition

All extracts possessed the ability (either strong or weak) to reduce  $\text{Fe}^{3+}$ /ferric cyanide complex to the ferrous form. The reducing power of all the extracts gradually increased with increasing concentration of the extracts. The reductive capability of the extracts decreases in the following order: 90% methanol (75.42 $\pm$ 0.364) > absolute methanol (57.85 $\pm$ 0.222) > 80% methanol (37.28 $\pm$ 0.25) > chloroform extract (31.22 $\pm$ 0.119) per mg AAE/100g extract. This revealed that the 90 % methanol extract has the highest electron donating capacity possibly due to high concentration of reductones such as phenols and flavonoids. Contrary, lower reduction power was exhibited in 80 % methanol extract compared with 90 % methanol extracts. This might be due to increase in rate of hydrolysis and decomposition of active polar components when the amount of water increases (Lih et al., 2001). It is also highly probable that components having lone pair of electrons on nitrogen (alkaloids) and oxygen of the carbonyl group other than, phenols and flavonoids, can easily be donated to the ferric ions. However, ascorbic acid demonstrated higher reducing power compared to the crude extracts.

The FRAP of the methanol stem bark extract of *C. africana* was previously evaluated and similar results to our findings have been reported (Tewolde-Berhan et al., 2013). The FRAP from the bark extract is higher (93.84) compared with that found from the bark extract in a similar species, *Cordia dichotoma* bark (22.8 mg mL<sup>-1</sup>) on a dry weight basis (Ganjare et al., 2011). The value reported by (Kumar et al., 2015, Gebremariam et al., 2011) from the methanolic leaf extract was significantly higher (160  $\mu\text{g/mL}$ ). This variation could be attributed to harvesting time of the plant, climatic and agronomic conditions and vegetative development of the plant, the solvent and the extraction protocol. In the reducing power assay, the yellow color of the test solutions

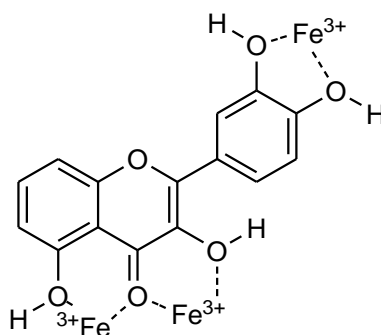
changed to various shades of green and blue depending upon the reducing power of each extract. The presence of antiradicals in the test extracts result in the reduction of the  $\text{Fe}^{3+}$  cyanide complex to the  $\text{Fe}^{2+}$  form. The  $\text{Fe}^{2+}$  ions can, therefore, be monitored by measuring the formation of Pearl's Prussian blue at 700 nm (Yeshiwas and Mekonnen, 2018).

Most phenols, specially, flavonoids are very effective scavengers of free radicals. Flavonoids are chelators of metals and inhibit the Fenton and Haber-Weiss reactions, which are important sources of active oxygen radicals. In addition, flavonoids retain their free radical scavenging capacity after forming complexes with metal ions (Bendary et al. 2013). The electron donating capacities of flavonoids seem to contribute to the termination of oxidation chain reaction based on their reducing power (Scheme 1).

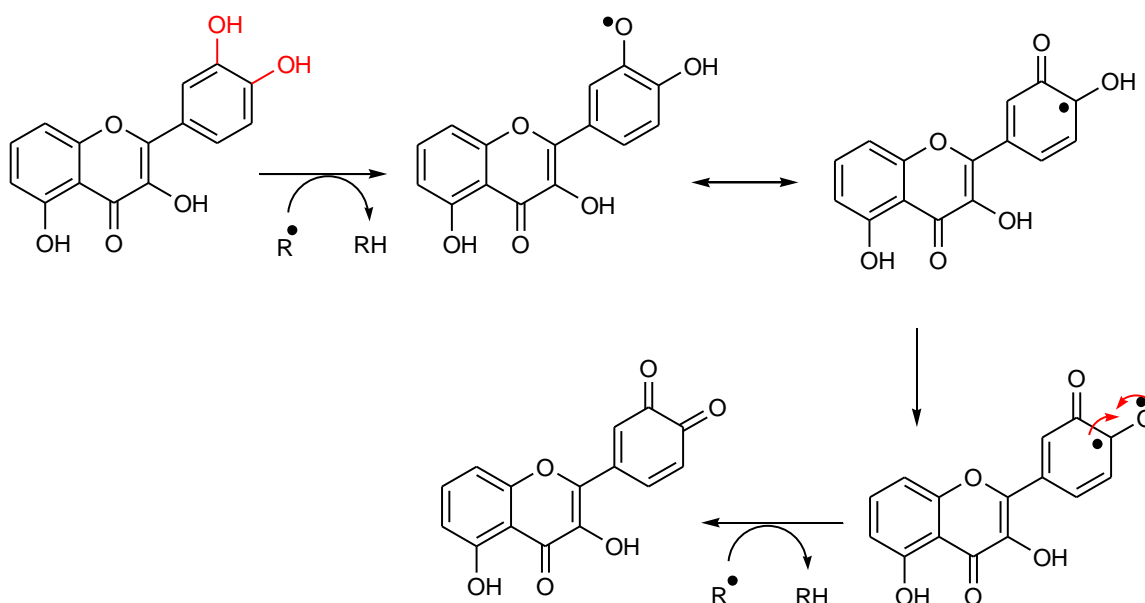
### Radical scavenging activity (DPPH')

DPPH is a nitrogen-centered free radical, stable at room temperature and produces a purple solution in methanol. In its radical form, DPPH has an absorbance maximum at 517 nm which disappears upon reduction by antioxidants. The determination of the antioxidant activity of *C. africana* bark extracts by DPPH radical scavenging methods was given in terms of ascorbic acid equivalent (Table 3) as a standard. The concentration versus mean percent inhibition curve is depicted in Figure 2. DPPH radical scavenging activity of the *C. africana* bark extracts varied depending on the concentration of the extracts and type of solvents used for extraction (Abera et al., 2015, Stratil et al., 2006) (equation 1).





**Scheme 1:** Binding sites in flavonoids for trace metals



**Scheme 2:** Scavenging of radicals by flavonoids and formation of stable structure

$$\text{Scavenging rate (\%)} = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100$$

$$= 1 - \frac{\text{Abs (sample at 517nm)}}{\text{Abs (control at 517 nm)}} \times 100 \quad (1)$$

Where “A<sub>0</sub>” was the absorbance of blank control without any sample, “A<sub>1</sub>” was the absorbance of the reaction solution, and “A<sub>2</sub>” was the absorbance of solution without DPPH and “Abs” is representing absorbance.

As indicated in Table 3 and Fig 2, the absorbance value for CAS<sub>2</sub> was the least (0.064±0.003) compared with CAS<sub>1</sub> (0.201±0.0021), CAS<sub>3</sub> (0.141±0.001) and CAS<sub>4</sub> (0.403±0.003) at a concentration of 700 µg/mL. When the concentration of the extract increases, absorbance decreases for each extract. This means absorbance and concentration have inverse relationship. The results of *C. africana* bark extracts were also expressed in terms of

percentage inhibition of DPPH radical scavenging activity (equation 2).

$$\text{DPPH inhibition (\%)} = \frac{A_c - A_s}{A_c} \times 100 \quad (2)$$

Where, A<sub>c</sub> is the absorbance of the black reaction and A<sub>s</sub> is the absorbance of test extract. The calibration curve was constructed by drawing percent inhibition versus concentration of ascorbic acid (y= 0.0528x + 56.708, R<sup>2</sup>=0.99737, where y is percent of inhibition and x is concentration of ascorbic acid). It was observed that the percentage of DPPH scavenging activity increased with increasing concentration of the extracts (Table 4). Thus, as indicated in Fig 3, the percentage radical scavenging activity of the extracts from *C. africana* bark increases in order: CAS<sub>4</sub> (bottom) < CAS<sub>1</sub> < CAS<sub>3</sub> < CAS<sub>2</sub> at same concentration. The highest percent inhibition observed for

CAS<sub>2</sub> (87.76±0.51) might be due to nature of the solvent which extracts more polar components. This means there is high concentration of polyphenols, flavonoids, etc, in CAS<sub>2</sub> extract which results an increase in scavenging activity. As the concentration of polyphenols in the extract increases, the rate of consumption of free radicals increases. This leads to the rapid decrease in radical concentration measured by the spectrometer (Torres et al., 2006).

In addition, the activities of the extracts were calculated in terms of *IC*<sub>50</sub> value. The lower the *IC*<sub>50</sub> value, the higher is the scavenging potential (Table 5). The *IC*<sub>50</sub> value was calculated by using concentration versus mean percent inhibition of DPPH radical curve ( $y = 0.097x + 8.296$ ,  $R^2 = 0.993$ ). In the present study, the CAS<sub>2</sub> extract exhibited the lowest *IC*<sub>50</sub> value (322.27±0.10 µg/mL) as compared to the other extracts. As shown in Table 5, the highest *IC*<sub>50</sub> value was found in CAS<sub>4</sub> which exhibited the weakest scavenging effect. Indeed the chloroform extract presented lower antioxidant potential (*IC*<sub>50</sub> 636.56±0.050 µg/mL), followed by the pure methanol extract (*IC*<sub>50</sub> 460.74±0.010 µg/mL), respectively. The results are expressed relative to ascorbic acid having *IC*<sub>50</sub> of 34.16±0.07 µg/mL. In terms of *IC*<sub>50</sub> value, upon comparison of our findings, we could emphasize the activity of *C. africana* stem bark lie in slightly lower than those reported by (Emtinan et al., 2015), (37±0.10 to 95±0.00%) but considerably higher than those reported by (Isa et al., 2015, Tijjani et al., 2015), (6.79±0.07 to 331.98±0.07 and 20.12 to 40.38 µg/mL). For that reason, several factors including harvesting time of the plant, genetic factors, variety of the plant, climatic conditions, vegetative development of the plant, the plant part used, the solvent and the extraction technique can be considered as responsible for fluctuations in the chemical compositions and activities of the plant.

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups and may contribute directly to antioxidative action. In the DPPH assay, the key mechanism of the chain-breaking action was attributed to hydrogen atom transfer (HAT) from the phenolic OH to the free radicals. The H-atom from phenols will be abstracted by free radicals, resulting in the formation of stable molecule. In this mechanism, the bond dissociation energies of the O–H bonds is an important parameter in evaluating the antioxidant action, because the weaker the OH bond the easier will be the reaction of free radical inactivation. The position of the OH groups also plays an important role of structure–antioxidant relationship activity. The *ortho*-position was found to be the more active one, due to its ability to form intramolecular hydrogen bonding, followed by *para*-position and then *meta*-position of compounds. Flavonoids usually give rise to semi-quinone free radical in alkaline solution. The semi-quinone free radicals or aroxyl radicals may react with the second radical acquiring a stable quinone structure (Scheme 2). The activities of the antioxidants are related to the stability of the free radicals

formed after they react with active radicals (Bendary et al., 2013).

### Antimicrobial activity

Phenolics have general antimicrobial activity and have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable to them. As shown in Table 6, two gram-positive bacterial strains and two gram-negative bacterial strains were used. Different concentrations of (6.25, 12.5, 25 and 50 µg/mL) the bark of *C. africana* methanol extracts were used and no zones of inhibition against all tested bacteria were observed at all concentrations. Antimicrobial activity is mostly related with the concentration of glycoside. As the phytochemical screening test revealed, glycosides were not detected in all extracts. Similar result was reported by (Aboaba et al., 2006) from *C. africana* leaf extracts. However, (Emtinan et al., 2015) from Sudan reported the presence of antimicrobial activity from methanolic leaf, stem and fruit extracts of *C. Africana*. Methanolic extracts from *C. africana* bark was showed no any activity against standard bacteria. The *C. africana* was found to be potent source of bioactive compounds for the ailment of human being diseases (Alsherbiny et al., 2019; Banzragchgarav et al., 2019; Chianese et al., 2019; Lei et al., 2019; Miranda Pedroso et al., 2019; Xie et al., 2019).

### CONCLUSIONS

The results of the present work brought to light some important chemical components present in *C. africana* bark, namely phenols, flavonoids, alkaloids, tannins, terpenoids and saponins. The results from DPPH and FRAP revealed that the *C. africana* bark extracts showed significant antioxidant activities. The findings of the study appear to suggest that *C. africana* bark methanolic extract could be used as a natural supplement in the management and control of human ailments caused by free radicals. Therefore, the traditional practices of using the *C. africana* plant to treat several health problems that could be attributed to the antioxidant activity of *C. africana*. However, the methanol bark extracts were found inactive against several bacterial strains.

### ACKNOWLEDGEMENT

The authors are thankful to Bahir Dar University for the provision of laboratory facility and financial support.

### AVAILABILITY OF MATERIAL

A herbarium voucher (Yilkaal-001) was identified by Dr Ali Seid and deposited in the mini-herbarium of a Bahir Dar University, (Bahir Dar, Ethiopia). All data generated or analyzed during this study are included in this published article.



## REFERENCES

- Abate, L., Abebe, A., Mekonnen, A., 2017. Studies on antioxidant and antibacterial activities of crude extracts of *Plantago lanceolata* leaves. *Chemistry International* 3(3), 277-287.
- Abebe, M., Abebe, A., Mekonnen, A., 2017. Assessment of antioxidant and antibacterial activities of crude extracts of *verbena officinalis linn* root or atuch (Amharic). *Chemistry International*, 3(2), 172-184.
- Abera, A., Mekonnen, A., Tebeka, T., 2015. Studies on Antioxidant and Antimicrobial Activities of *Plumbago zeylanica* Linn. Traditionally Used for the Treatments of Intestinal Worms and Skin Diseases in Ethiopia. *Research Journal of Medicinal Plant* 9(6), 252-263.
- Aboaba, O.O., Smith, S.I., Olude, O., 2006. Antibacterial effect of edible plant extract on *Escherichia coli*. *Pakistan Journal of Nutrition* 5(4), 325-327.
- Alberto, Z., 2007. Evaluation of plants used for the control of animal ecto-parasitoses in Southern Ethiopia (Oromiya and some other regions). MSc Thesis Pretoria University.
- Alsherbiny, M.A., Abd-Elsalam, W.H., El badawy, S.A., Taher, E., Fares, M., Torres, A., Chang, D., Li, C.G., 2019. Ameliorative and protective effects of ginger and its main constituents against natural, chemical and radiation-induced toxicities: A comprehensive review. *Food and Chemical Toxicology* 123, 72-97.
- Banzragchgarav, O., Murata, T., Tuvshintulga, B., Suganuma, K., Igarashi, I., Inoue, N., Batkhuu, J., Sasaki, K., 2019. Chemical constituents of *Bergenia crassifolia* roots and their growth inhibitory activity against *Babesia bovis* and *B. bigemina*. *Phytochemistry Letters* 29, 79-83.
- Bendary, E., Francis, R.R., Ali, H.M.G., Sarwat, M.I., Hady, S.El., 20013. Antioxidant and structure-activity relationships (SARs) of some phenolic and anilines compounds *Annals of Agricultural Science* 58(2), 173-181.
- Cao, D.-H., Sun, P., Liao, S.-G., Gan, L.-S., Yang, L., Yao, J.-N., Zhang, Z.-Y., Li, J.-F., Zheng, X.-L., Xiao, Y.-D., Xiao, C.-F., Zhang, P., Hu, H.-B., Xu, Y.-K., 2019. Chemical constituents from the twigs and leaves of *Trichilia sinensis* and their biological activities. *Phytochemistry Letters* 29, 142-147.
- Chianese, G., Golin-Pacheco, S.D., Tagliatalata-Scafati, O., Collado, J.A., Munoz, E., Appendino, G., Pollastro, F., 2019. Bioactive triterpenoids from the caffeine-rich plants guayusa and maté. *Food Research International* 115, 504-510.
- Ebrahim, K., Mekonnen, A., 2018. Studies on Antioxidant and Antimicrobial Activities of *Salvadora persica*. *Journal of Medicinal Plants Research* 12(1), 26-32.
- Elhidar, N., Nafis, A., Kasrati, A., Goehler, A., Bohnert, J.A., Abbad, A., Hassani, L., Mezrioui, N.-E., 2019. Chemical composition, antimicrobial activities and synergistic effects of essential oil from *Senecio anteuphorbium*, a Moroccan endemic plant. *Industrial Crops and Products* 130, 310-315.
- Emtinan, A., Alhadi, H.S., Khalid, M.S., Alhassan, A.A., Ali, S.G., Babiker, E.M., Zain, A., Ahmed, S.K., 2015. Antioxidant and cytotoxicity activity of *Cordia Africana* in Sudan. *Advancement in Medicinal Plant Research* 3(2), 29-32.
- Ganjare, B.A., Nirmal, A.S., Rub, A.R., Patil, N.A., Patten, R.S., 2011. Use of *Cordia dichotoma* bark in the treatment of ulcerative colitis. *Pharmaceutical Biology* 49, 850-855.
- Gebremariam, T., Alehegne, W., Tadess, E., 2011. *In vitro* antibacterial activity of four Ethiopian medicinal plants against some bacterial of veterinary and public health importance. ICOPHI Addis Ababa, Ethiopia.
- Isa, A.L., Saleh, M.I., Alhaji1, A.A., Dzoyem, J.P., Adebayo, S.A., Musa, I., Sani, U.F., Daru, P.A., 2015. Evaluation of anti-inflammatory, antibacterial and cytotoxic activities of *cordia africana* leaf and stem bark extracts. *Bayero Journal of Pure and Applied Sciences* 9(1), 228-235.
- Kalayu, M., Gebru, T., Teklemichael, T., 2013. Ethnobotanical study of traditional medicinal plants used by Indigenous people of Gemad District, Northern Ethiopia. *Journal of Medicinal Plants Studies* 1(4), 32-37.
- Kebede, D.K., Alemayehu, A., Binyam, G., Yunis, M., 2006. A historical overview of traditional medicine practices and policy in Ethiopia. *Ethiopian Journal of Health Development* 20(2), 127-134.
- Kesatebrhan, H.A., 2013. Antimicrobial activity and Phytochemical Screening of Medicinal plants grown in Eastern Ethiopia. *International Journal of Pharma and Bio Sciences* 4(4), 326-333.
- Khan, H., Marya, Belwal, T., Mohd, T., Atanasov, A.G., Devkota, H.P., 2019. Genus *Vanda*: A review on traditional uses, bioactive chemical constituents and pharmacological activities. *Journal of Ethnopharmacology* 229, 46-53.
- Kumar, G., Neethu, L., Suresh, K.P.N., Sharmila, B.G., 2015. Evaluation of phytochemical screening and *In-Vitro* antioxidant activity of *Cordia Africana* Lam. (Family: *Boraginaceae*), a native African Herb. *International Journal for Pharmaceutical Research Scholars* 4, 1-4.
- Lei, Z.-Y., Chen, J.-J., Cao, Z.-J., Ao, M.-Z., Yu, L.-J., 2019. Efficacy of *Aeschynomene indica* L. leaves for wound healing and isolation of active constituent. *Journal of Ethnopharmacology* 228, 156-163.
- Lih, S., Su, T., Wen, W., 2001. Studies on the antioxidative activities of *Hsian tsao* (*Mesona procumbens* Hems) leaf Gum. *Journal of Agricultural and Food Chemistry* 49, 963-968.
- Mariata, MR., 2010. Efficacy of medicinal plants used communities around Lake Victoria region and the Samburu against *Mycobacteria*, selected bacteria and *Candida Albicans*. Plant and Microbial Sciences department. M. Sc Thesis Kenyatta University, Kenya.
- Marit, K., 2008 Additive and synergistic modulations of nuclear *factor-kB* by dietary plant extract department of nutrition, Faculty of Medicine. M. Sc Thesis, Oslo University.
- Mekonnen, A., Atlabachew, M., Kassie, B., 2018. Investigation of antioxidant and antimicrobial activities of *Euclea schimperii* leaf extracts. *Chemical and Biological Technologies in Agriculture* 5, 16.

- Miranda Pedroso, T.F.d., Bonamigo, T.R., da Silva, J., Vasconcelos, P., Félix, J.M., Cardoso, C.A.L., Souza, R.I.C., dos Santos, A.C., Volobuff, C.R.F., Formagio, A.S.N., Trichez, V.D.K., 2019. Chemical constituents of *Cochlospermum regium* (Schrank) Pilg. root and its antioxidant, antidiabetic, antiglycation, and anticholinesterase effects in Wistar rats. *Biomedicine & Pharmacotherapy* 111, 1383-1392.
- Muhammad, A.Z., Nasrullah, K., Muhammad, A., Durre, S., Muhammad, W., Muhammad, F.S., 2011. Antibacterial and Antioxidant activities of an Ethno botanically important plant *Sauromatum Venosum* (Ait.), Schott, of district kotli, Azadjammu and Kashmir. *Pakistan Journal of Botany* 43(1), 579-585.
- Oyaizu, M., 1986. Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition* 44, 307-315.
- Pyrzynska, K., Sentkowska, A., 2019. Liquid chromatographic analysis of selenium species in plant materials. *TrAC Trends in Analytical Chemistry* 111, 128-138.
- Rahman, M.M., Gray, A.I., 2002. Antimicrobial constituents from the stem bark of *Feronia limonia*. *Phytochemistry* 59(1), 73-77.
- Resat, A., Kubilay, G., Birsen, D., Mustafa, Ö., Saliha, E.C., Burcu, B.K., Isil, B., Dilek, O., 2013. Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC Technical Reports). *Pure and Applied Chemistry* 85(5), 957-998.
- Roberts, G.K., Gardner, D., Foster, P.M., Howard, P.C., Lui, E., Walker, L., van Breemen, R.B., Auerbach, S.S., Rider, C., 2019. Finding the bad actor: Challenges in identifying toxic constituents in botanical dietary supplements. *Food and Chemical Toxicology* 124, 431-438.
- Šircelj, H., Vidrih, R., Veberič, R., Mikulic-Petkovsek, M., 2019. Evaluation of bioactive constituents in European bladdernut (*Staphylea pinnata* L.) seed kernels. *Journal of Food Composition and Analysis* 78, 33-41.
- Stratil, P., Klejudus, B., Kuban, V., 2006. Determination of total content of phenolic compounds and their antioxidant activity in vegetable-evaluation of spectrophotometric method. *Journal of Agricultural and Food Chemistry* 54, 607-616.
- Tewelde-Berhan, S., Remberg, S.F., Abegaz, K., Narvhus, J., Abay, F., Wicklund, T., 2013. Ferric reducing antioxidant power and total phenols in *Cordia africana*. *African Journal of Biochemistry Research* 7(11), 215-224.
- Tijjani, R.G., Zezi, A.U., Shafiu, R., Umar, M.L., 2015. Anti-inflammatory and antioxidants properties of the ethanolic stem bark extract of *Cordia africana* Lam. *Annals of Phytomedicine* 4(2), 83-87.
- Torres, R., Faini, F., Modac, B., 2006. Antioxidant activity of coumarin and flavonols from the resinous exudates of *Haplopappus multitolius*. *Phytochemistry* 67, 984-998.
- Xie, G., Xu, Q., Li, R., Shi, L., Han, Y., Zhu, Y., Wu, G., Qin, M., 2019. Chemical profiles and quality evaluation of *Buddleja officinalis* flowers by HPLC-DAD and HPLC-Q-TOF-MS/MS. *Journal of Pharmaceutical and Biomedical Analysis* 164, 283-295.
- Yan, M., Chen, M., Zhou, F., Cai, D., Bai, H., Wang, P., Lei, H., Ma, Q., 2019. Separation and analysis of flavonoid chemical constituents in flowers of *Juglans regia* L. by ultra-high-performance liquid chromatography-hybrid quadrupole time-of-flight mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis* 164, 734-741.
- Yeshiwas, D., Mekonnen, A., 2018. Comparative study of the antioxidant and antibacterial activities of two guava (*Psidium guajava*) fruit varieties cultivated in Andasa Horticulture Site, Ethiopia. *Chemistry International* 4(3), 154-162.

---

Visit us at: <http://bosajournals.com/chemint/>

Submissions are accepted at: [editorci@bosajournals.com](mailto:editorci@bosajournals.com)

---