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

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- 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.

**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.

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**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 CAS4 at the bottom).

**Fig. 2:** Comparison of DPPH radical scavenging activity of stem bark extracts and ascorbic acid at different concentration (ascorbic acid < CAS2 < CAS3 < CAS1 < CAS4).
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

<p>| Table 1: The results of phytochemical screening of the various crude extracts of C. africana bark |</p>
<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>80% methanol</th>
<th>90% methanol</th>
<th>100% methanol</th>
<th>Chloroform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Phenolics</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>- : absent</td>
<td>+ : present</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<p>| Table 2: Ferric reducing antioxidant power of different solvent extracts of C. africana bark and the standard, ascorbic acid, at 700 nm |</p>
<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Samples and standard absorbance at 700 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAS₁</td>
</tr>
<tr>
<td>100</td>
<td>0.250±0.006</td>
</tr>
<tr>
<td>300</td>
<td>0.341±0.002</td>
</tr>
<tr>
<td>500</td>
<td>0.480±0.004</td>
</tr>
<tr>
<td>700</td>
<td>0.531±0.002</td>
</tr>
</tbody>
</table>

Sample for three independent measurements (triplicate; n = 3, mean ± 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

<p>| Table 3: Absorbance of DPPH solution at different concentration of samples and ascorbic acid |</p>
<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Absorbance of the samples and AA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAS₁</td>
</tr>
<tr>
<td>100</td>
<td>0.317±0.001</td>
</tr>
<tr>
<td>300</td>
<td>0.286±0.002</td>
</tr>
<tr>
<td>500</td>
<td>0.247±0.002</td>
</tr>
<tr>
<td>700</td>
<td>0.201±0.002</td>
</tr>
</tbody>
</table>

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

<p>| Table 4: Percentage DPPH radical scavenging activity of C. africana bark extracts at different concentration |</p>
<table>
<thead>
<tr>
<th>Concentration (µg/mL)</th>
<th>Samples and AA% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAS₁</td>
</tr>
<tr>
<td>100</td>
<td>39.39±0.100</td>
</tr>
<tr>
<td>300</td>
<td>45.31±0.400</td>
</tr>
<tr>
<td>500</td>
<td>52.77±0.330</td>
</tr>
<tr>
<td>700</td>
<td>61.56±0.390</td>
</tr>
</tbody>
</table>

Data is expressed as mean of three determinations ± SD. There is a significant difference in the mean mg equivalent per g dry weight of the samples for P < 0.05.
Ingestion of plants that are rich in natural antioxidants. The bioactivity for plant products is due to the presence of enzymes and proteins, vitamins, carotenoids, flavonoids, anthocyanins and other phenolic compounds (Muhammad et al., 2011). 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 (Mariiata, 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., 2013). Therefore, attention has been paid to extraction of biologically active compounds from plants.

**MATERIAL AND METHODS**

**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 CAS1, CAS2, CAS3 and CAS4 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 x 10⁸ 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 finding are consistent with previous reports (Abbe 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 ± 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±0.364) while the chloroform showed comparably low activity (31.22±0.119).
Table 5: IC\textsubscript{50} values for antioxidant activity of different solvent extracts of \textit{C. africana} stem bark extracts and the standard, ascorbic acid

<table>
<thead>
<tr>
<th>Samples</th>
<th>CAS\textsubscript{1} (µg/mL)</th>
<th>CAS\textsubscript{2} (µg/mL)</th>
<th>CAS\textsubscript{3} (µg/mL)</th>
<th>CAS\textsubscript{4} (µg/mL)</th>
<th>Ascorbic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC\textsubscript{50}</td>
<td>460.74±0.010</td>
<td>322.27±0.10</td>
<td>438.86±0.070</td>
<td>636.56±0.050</td>
<td>34.16±0.072</td>
</tr>
</tbody>
</table>

Data is expressed as mean of three determinations ± SD

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

<table>
<thead>
<tr>
<th>Extract and Antibiotics</th>
<th>Concentration (µg/mL)</th>
<th>Zone of inhibition of different bacteria in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>\textit{E. coli}</td>
</tr>
<tr>
<td>Methanol</td>
<td>6.250-50.000</td>
<td>NI</td>
</tr>
<tr>
<td></td>
<td>50.000</td>
<td>NI</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>25.000</td>
<td>NI</td>
</tr>
<tr>
<td></td>
<td>12.500</td>
<td>NI</td>
</tr>
<tr>
<td></td>
<td>6.250</td>
<td>NI</td>
</tr>
<tr>
<td>Auxofloxacin</td>
<td>25.000</td>
<td>NI</td>
</tr>
<tr>
<td></td>
<td>12.500</td>
<td>NI</td>
</tr>
<tr>
<td></td>
<td>6.250</td>
<td>NI</td>
</tr>
</tbody>
</table>

Where NI - no inhibition

All extracts possessed the ability (either strong or weak) to reduce Fe\textsuperscript{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±0.364) > absolute methanol (57.85±0.222) > 80% methanol (37.28±0.25) > chloroform extract (31.22±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 (Li 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 \textit{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, \textit{Cordia dichotoma} bark (22.8 mg mL\textsuperscript{-1}) 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 µg/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 Fe\textsuperscript{3+} cyanide complex to the Fe\textsuperscript{2+} form. The Fe\textsuperscript{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 \textit{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 \textit{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).
Scavenging rate (%) = \left(1 - \frac{A_1 - A_2}{A_0}\right) \times 100 \hspace{1cm} (1)

Where \(A_0\) was the absorbance of blank control without any sample, \(A_1\) was the absorbance of the reaction solution, and \(A_2\) was the absorbance of solution without DPPH and "Abs" is representing absorbance.

As indicated in Table 3 and Fig 2, the absorbance value for CAS2 was the least (0.064±0.003) compared with CAS1 (0.201±0.0021), CAS3 (0.141±0.001) and CAS4 (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).

DPPH inhibition (%) = \frac{A_c - A_s}{A_c} \times 100 \hspace{1cm} (2)

Where, \(A_c\) is the absorbance of the black reaction and \(A_s\) is the absorbance of test extract. The calibration curve was constructed by drawing percent inhibition verses concentration of ascorbic acid (y= 0.0528x + 56.708, \(R^2=0.99737\), 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: CAS4 (bottom) < CAS1 < CAS3 < CAS2 at same concentration. The highest percent inhibition observed for
Chemical composition and activities of the plant. 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 (Yilikal-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.

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