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Bioactivity profiling of four traditional medicinal plants leave extracts native to Alesaga Forest, Ethiopia

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ARTICLE INFO

Article type:

Research article

Article history:

Received February 2019

Accepted August 2019

October 2019 Issue

Keywords:

Phytochemicals

DPPH

Peroxide value

Antioxidant activities

IDF methods

ABSTRACT

Total content of secondary metabolites, free radical scavenging activities and peroxide value from *Cordia africana* Lam., *Croton macrostachyus* Hochst., *Vernonia amygdalina* Del. and *Justicia schimperiana* T. leave extracts were carried out using methanol as a solvent. (1,1-diphenyl-2-picryl-hydrazyl) DPPH and peroxide value (PV) using IDF methods were used for evaluating antioxidant potentials. The results confirmed the presence of secondary metabolites such as Coumarins, Leucoanthocyanths, proteins, alkaloids, flavonoids, phenols, terpenoids, saponins, tannins, cardenolides, phlobatannins, steroids and glycosides. Quantitatively, steroids showed highest amount (5.4±0.24%, 7.12±0.32%, 8.6±0.21%) for *Croton macrostachyus* Hochst., *Justicia schimperiana* T. and *Vernonia amygdalina* Del. leave extracts respectively. In *Cordia Africana* Lam. leave extracts saponins showed highest amount (8.1±0.17%). *Justicia schimperiana* T. leave extracts showed highest value DPPH scavenging activity ranged from (67.64±0.03% to 77.48±0.04%) and the lowest scavenging value recorded in *Cordia africana* Lam. ranged from 67.64±0.03% to 77.48±0.04%. In PV assay, the highest value was recorded in *Cordia africana* Lam. leave extracts (86.17±0.22meq/kg) and the lowest PV was recorded in *Justicia schimperiana* T. leave extracts (16.24±0.12meq/kg) using IDF modified method. Thus the leave extract of the plants possess antioxidants activities with the lowest antioxidant activities in *Cordia africana* lam. and highest antioxidant activities in *Justicia schimperiana* T. leave extracts with presence of different phytochemicals.

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Capsule Summary: Bioactivity profiling of four traditional medicinal plants leaves was performed i.e., flavonoids, phenols, terpenoids, saponins along with antioxidant value activity evaluation. *Justicia schimperiana* T. leave extracts showed highest antioxidant activities and *Cordia Africana* Lam. leave extracts showed the lowest antioxidant activities.

Cite This Article As: L. Abate. Bioactivity profiling of four traditional medicinal plants leave extracts native to Alesaga Forest, Ethiopia. Chemistry International 5(4) (2019) 281-290. <https://doi.org/10.5281/zenodo.3364191>

INTRODUCTION

Since time immemorial, mankind has used extracts from different plants to cure many diseases and thus relieve him

from physical agony (Refaz et al., 2017). Medicinal plant is an important element of indigenous medical systems in all over the world (Saleh et al., 2015). Nearly 80% of the world populations rely on traditional medicines for primary health care, most of which involve the use of plant extracts (Tarun et

al., 2014). The medicinal properties of these plants are due to their phytochemical constituents (Abdur et al., 2012). Phytochemicals are naturally occurring in the medicinal plants, especially in their leaves, fruits, stems and roots. They have defense mechanism, protecting plants from various diseases. Important phytochemicals include alkaloids, flavonoids, anthocyanins, tannins, terpenes, phenolics, vitamins (Angeline et al., 2015; Attia et al., 2019; Boulaaba et al., 2019; Chinnadurai et al., 2019; Kane et al., 2019; Maema et al., 2019; Mehwish et al., 2019; Shahid-Ud-Daula et al., 2019; Vasantharaja et al., 2019; Weli et al., 2018). Ethiopia is endowed with a diverse biological resources including about 6,500 species of higher plants, with approximately 12% endemic, hence making it one of the six plant biodiversity rich regions (Abera et al., 2014). *Cordia africana*, *Croton macroactachyus*, *Vernonia amygdalina* and *Justicia schimperiana* are medicinal plants found in Ethiopia (Mirutse et al., 2007), *Vernonia amygdalina* is a valuable medicinal plant that is widespread in West Africa, it is known as bitter leaf due to its characteristic bitter taste and flavour, and can be used as an active anticancer, antibacterial, antimalarial and antiparasitic agent (Udochukwu et al., 2015). *Croton macrostachyus* has widely utilized for the management of a large number of public health, different parts of this plant have been used as a remedy for malaria, abdominal pain, gonorrhoea, wounds, ringworm infestation, hemorrhoids, ascariasis, venereal diseases, cough and rheumatism (Asfaw et al., 2019). The pharmacological studies carried out with extracts and purified compounds indicates that the plants of *Cordia* species possess analgesic, anti-inflammatory, antimicrobial, antiviral and antifertility activities (Isa et al., 2016). *Justicia schimperiana* one of the traditional medicinal plants in Ethiopia for the treatment of diabetes mellitus (Andualem et al., 2016).

Scientifically there were no enough studies about the use of extracts of different parts of these plants as antioxidant. Also, quantitative phytochemical investigations have not been carried out intensely. Therefore the objectives of this research were to determine and compare the antioxidant activity of leaves of *Cordia africana*, *Croton macroactachyus*, *Vernonia amygdalina* and *Justicia schimperiana*, using peroxide value and DPPH free radical scavenging activities and to investigate the phytochemicals present to them both qualitatively and quantitatively.

MATERIAL AND METHODS

Plant material

The leaves of *Cordia africana*, *Croton macroactachyus*, *Vernonia amygdalina* and *Justicia schimperiana* were collected from Alemsaga forest near to Debre Tabor town in the month of March and authenticated by the taxonomist found in Debre Tabor University. After collection, the leaves were washed properly with tape water to avoid dust particles from the surface and cut into small portions and then placed at room temperature (23°C) without sun light. The dried leave were

powdered by electrical grinder and stored in a clean polyethylene bag until extraction.

Extraction procedure

Powdered leaves of *Cordia africana*, *Croton macroactachyus*, *Vernonia amygdalina* and *Justicia schimperiana* (20 grams from each) were added to 200 mL of methanol in separate conical flask and shaken with electrical shaker for 48 hours. Each solution was filtered using Whatman filter paper number 1 in a separate conical flask and concentrated in rotary evaporator at 35°C. After filtration and concentration at 35°C, extracts were labeled as MCA, MCM, MVA and MJS to represent methanol extract of leave *Cordia africana*, *Croton macroactachyus*, *Vernonia amygdalina* and *Justicia schimperiana* respectively. For all analyses we used analytical grade chemicals/reagents and solvents. For PV determination four different samples were prepared (sun flower oil plus MCA, MCM, MVA or MJS) and kept at room temperature (23°C) in open air conditions for a Month.

Preliminary phytochemical screening

The leaves of *Cordia africana*, *Croton macroactachyus*, *Vernonia amygdalina* and *Justicia schimperiana* extract was used for preliminary screening of phytochemicals such as leucoanthocyanins, emodins, anthocyanins, proteins, coumarins, carbohydrate, xanthoproteins, anthraquinones, phenols, flavonoids, tannins, alkaloids, saponins, steroid, glycosides, quinine, phlobatannin and carotenoids using standard methods (Abdur et al., 2012).

Determination of total alkaloid

Alkaloids in the samples of leave of *Cordia africana*, *Croton macroactachyus*, *Vernonia amygdalina* or *Justicia schimperiana* were determined alkaline precipitation gravimetric method. Powdered (5g) was soaked in 20 mL of 10% ethanoic acetic acid. Mixture kept for four hour at room temperature (23°C) and filtered using Whitman filter paper. The filtrate was concentrated by evaporation over a steam bath to ¼ of its original volume. To precipitate the alkaloid, concentrated ammonia solution was added drops wise until it was in excess. The resulting precipitate was recovered by filtration using previously weighed filter paper. After filtration, the precipitate was washed with 9% ammonia solution and dried in the oven at 60°C for 30 min, cooled in a desiccation and reweighed. The process repeated three more times and the average was taken. The weight of alkaloid was determined by the differences and expressed of weight of sample analyzed as shown in Eq. 1 (Okwulehie et al., 2015).

$$\text{Alkaloids (\%)} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100 \quad (1)$$

Where, W_1 = Weight of filtrate, W_2 = weight of filter paper + alkaloids precipitate

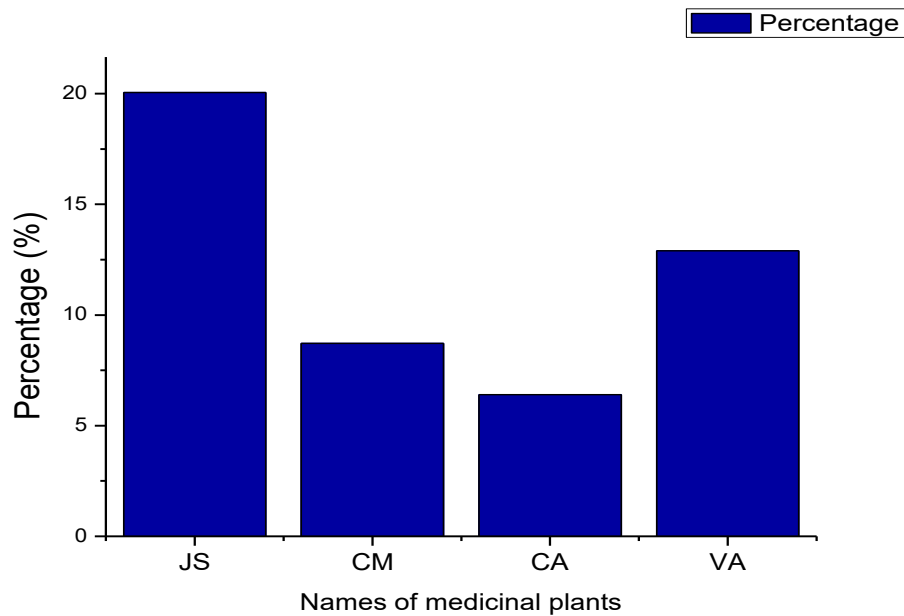


Fig. 1: Percentage of crude extracts from the leaf of four traditional medicinal

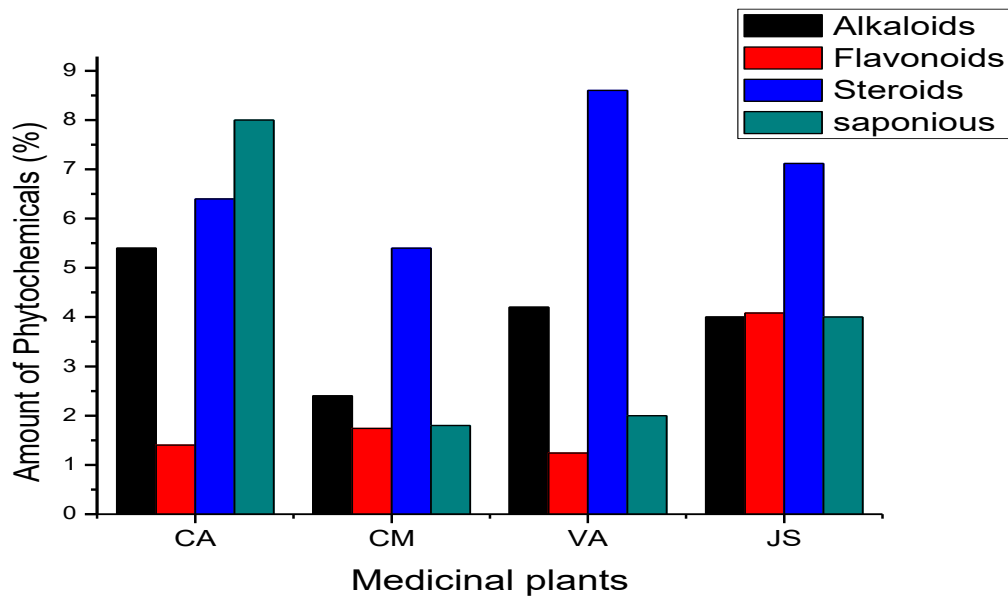


Fig. 2: Amount of phytochemical present in leaves of four traditional medicinal plants

Determination of total flavonoid

The flavonoid content of the sample leaf of *Cordia africana*, *Croton macroactachyus*, *Vernonia amygdalina* or *Justica schimperiana* were determined by the gravimetric method. Five (5) gram of the powdered sample was placed into a conical flask and 50 mL of water and 2 mL HCl solution was

added. The solution was allowed to boil for 30 minutes. The boiled mixture cooled before it was filtrated through what-man filter paper. 10 mL of ethyl acetate which contained flavonoid was recovered, while the aqueous layer was discarded. A pre weighed what man filter paper was used to filter the second (ethyl- acetate layer), the residue was then placed in an oven to dry at 60°C. It was cooled in a

desiccators' and weighed. The quantity of flavonoid was determined using the relation shown in Eq. 2 (Okwulehie et al., 2015).

$$\text{Flavonoids (\%)} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100 \quad (2)$$

Where, W_1 = weight of empty filter paper, W_2 = weight of paper + flavonoids extracts

Determination of total saponin

The saponin content of the sample was determined by double extraction gravimetric method. 5 grams of the powdered samples were mixed with 50 mL of 20% aqueous ethanol solution in a flask. The mixtures were heated with periodic agitation in water bath for 90 minutes at 55°C. They were then filtered through what man filter paper. The residue were extracted with 50 mL of 20% ethanol and both extract were poured together and the combined extract were reduced to about 40 mL at 90°C and transferred to a separating funnel where 40 mL of diethyl ether was added and shaken vigorously. Separation was by partition during which the ether layer was discarded and the aqueous layer reserved. Re-extraction by partitioning was done repeatedly until the aqueous layer become clear in color. The saponins were extracted, with 60 mL of normal butanol. The combined extracts were washed with 5% aqueous sodium chloride (NaCl) solution and evaporated to dryness in a pre-weighed evaporation dish. They were dried at 60°C in the oven and reweighed after cooling in desiccators. The process was repeated three more times to get an average. Saponin contents were determined by difference and calculated as shown in Eq. 3 (Okwulehie et al., 2015).

$$\text{Saponin (\%)} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100 \quad (3)$$

Where, W_1 = weight of evaporating dish, W_2 = weight of dish + sample

Determination of steroid

The steroid content of the samples leave of *Cordia africana*, *Croton macroactachyus*, *Vernonia amygdalina* or *Justica schimperiana* were determined as follow, Five (5) grams of the powdered sample were hydrolyzed by boiling in 50 mL of hydrochloric acid solution for about 30 minutes. It was filtered using whatman filter paper. The filtrates were transferred into separating funnels. Equal volume of ethyl acetate were added to them, mixed well and allowed to separate into two layers. The ethyl acetate layer (extract) was used while the aqueous was discarded. The extracts were dried at 100°C for 5 minutes in a steam bath. They were then heated with concentrated amyl alcohol to extract the steroids. The mixture becomes turbid and a reweighed what man filter paper was used to filter the mixtures properly. The dry extract were then cooled in desiccators and reweighed.

The process was repeated three times and an average was obtained. The concentration of steroid was determined as expressed in Eq. 4 (Okwulehie et al., 2015).

$$\text{Steroid (\%)} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100 \quad (4)$$

Where, W_1 = weight of evaporating dish, W_2 = weight of dish + sample

DPPH free radical scavenging activity

The free radical scavenging activity of the leave extracts were measured using 1,1-diphenyl-2-picryl-hydrazyl (DPPH). 1 mL of 0.1mM DPPH was added to 1 mL of leave extract of *Cordia africana*, *Croton macroactachyus*, *Vernonia amygdalina* or *Justica schimperiana* with concentrations of (30, 60, 120, 240, 500, and 1000) µg/mL. The mixtures were left to stand for 30 min in the dark and the absorbances were recorded at 517 nm using UV-VIS spectrometer. An equal amount of DPPH and Methanol served as negative control. The experiment was done in triplicate. Ascorbic acid was used as standard control. The percentage scavenging was calculated using the Eq. 5 (Oktay et al., 2003).

$$\text{DPPH activity (\%)} = \left[\frac{AC - AS}{AC} \right] \times 100 \quad (5)$$

Where, AC absorbance of the control and AS absorbance of sample

Peroxide value determination using IDF modified

The test is based on the Co-oxidation of Fe(II) to Fe(III) by hydroperoxides from sample and the formation of the reddish Fe(III)-thiocyanate complex which is read at 500 nm to a spectrophotometer (Scheme 1).



Scheme 1: The oxidation reaction mechanism

An amount of 10 mg sample (w) will be dissolved in 9.8 ml mixture of chloroform-methanol, 7:3 (v/v); 50 µl of ammonium thiocyanate will be added followed by 50 µl of Fe(II) solution. After 10 minutes the absorbance will be measured at 500 nm against a blank using a spectrophotometer (UV-VIS 1700 Shimadzu). The blank contains all the reagents except the fat. PV is expressed as mEqO₂/Kg fat using the formula (Eq. 6). This test was done twice, after 15 days of storage (treatment 1) and after 30 days of storage (treatment 2).

$$\text{PV} = \frac{\text{Abs}}{55.84 \times w} \times \frac{1}{b} [\text{mEqO}_2/\text{Kg fat}] \quad (6)$$

Where, w- weight of sample, Abs- absorbance, 55.84-atomic weight of Fe³⁺, b-the slope of the Fe (III) calibration curve.

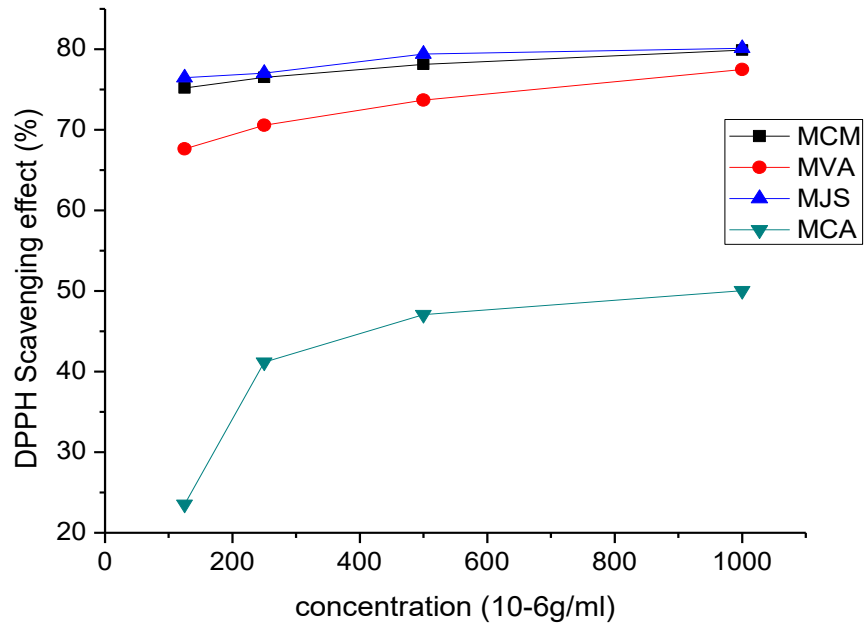


Fig. 3: DPPH scavenging activities in different concentration of four different plants leave extracts

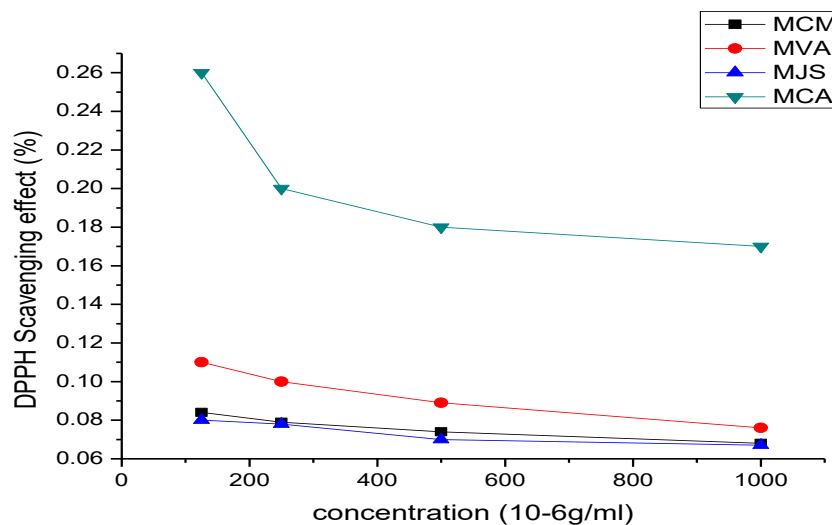


Fig. 4: Absorbance of different concentrations of leaves of four medicinal plants

Calibration

For calibration curve, a standard solution of Fe(III) 10 $\mu\text{g/ml}$ was prepared. The absorbance of Fe(III) standards [5-40 $\mu\text{g Fe(III)}$] was plotted vs. their concentrations (Shantha et al., 1994).

Statistical analysis

All measurements were carried out in triplicate ($n=3$), and values expressed are the mean of three repetition \pm standard deviation (SD). The graphs were displayed using origin 8 software and difference between mean were determined by the least significant difference test, and significance was defined as a confidence limit of $P < 0.05$.

RESULTS AND DISCUSSION

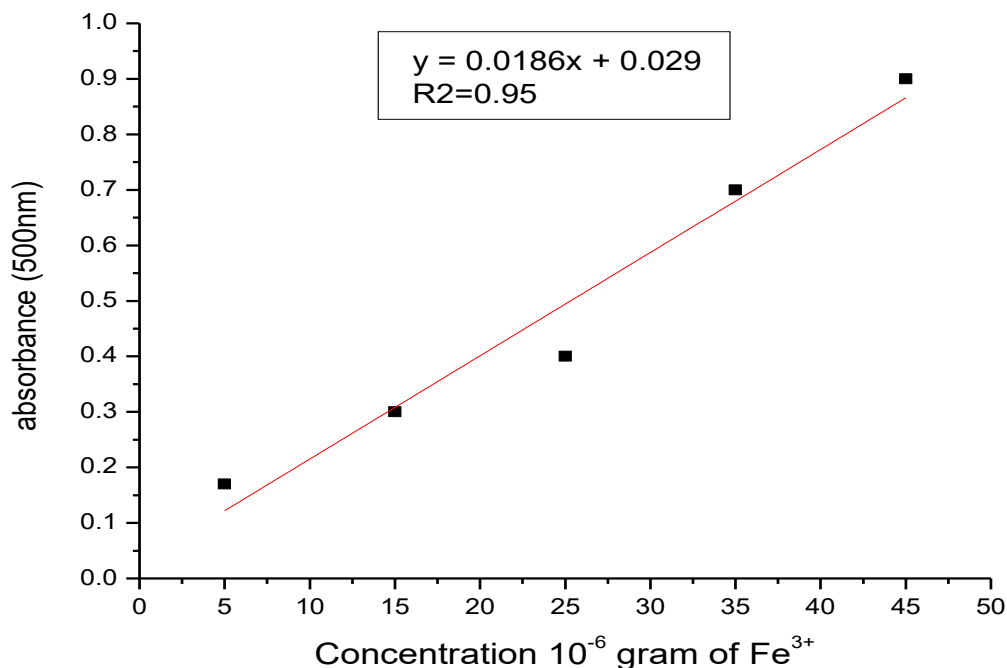


Fig. 5: Shows the calibration plot with a slope of 0.0186 and $R^2 = 0.95$

The amounts of crude extract from 20 grams of leave of *Cordia africana*, *Croton macroactachyus*, *Vernonia amygdalina* or *Justica schimperiana* is shown in figure 1. Methanol extracts showed highest amount of crude extracts (20.5%) for *Justica schimperiana* leave compared to the amount obtained from other plants such as *Croton macroactachyus* leave (8.75%), *Cordia Africanaleave* (6.4%), *Vernonia amygdalina* leave (12.9%). Variations in the yield of extracts from different plant materials might be attributed to the environmental variations, age of the plant, extraction process, nature of the extraction solvent (Hilaire et al., 2013).

Qualitative analysis

For testing the presence of some selected phytochemicals in plants leave extracts, different phytochemical tests were carried out. The results are shown in Table 1.

Quantitative analysis

The results obtained from the quantitative analysis of leave extracts of all the selected four medicinal plants showed the presence of phytochemicals from highest to least extent. The highest amounts of alkaloids were reported in plant *Cordia Africana* ($5.4 \pm 0.12\%$) and least amount of $2.4 \pm 0.11\%$ were observed in the leave extract of *Croton macrostachyus*. The highest amounts of flavonoids are reported in *Justicla Schimperiana T. leave* extract with $4.08 \pm 0.21\%$. The least

values of flavonoids are observed in *Vernonia amygdalina* ($1.24 \pm 0.34\%$). The flavonoids concentrations of the leave extract of all the selected are ($4.08 \pm 0.21\%$, $1.74 \pm 0.21\%$, $1.4 \pm 0.21\%$, $1.24 \pm 0.34\%$) for *Justicla Schimperiana*, *Croton macrostachyus*, *Cordia Africana* and *Vernonia amygdalina* respectively. Steroids showed the highest amount in three of the plants leave extract such as *Croton macrostachyus*, *Vernonia amygdalina*, *Justicla Schimperiana* but saponnins showed the highest amount in *Cordia Africana* leave extracts. There is no significant difference ($P \leq 0.05$), between the amount of flavonoids, saponnins and alkaloids in *Justicla Schimperiana* but in *Cordia Africana* leave extracts there is significant difference between the amount of flavonoids, alkanoids, saponnins and steroids (Fig. 2)

DPPH free radical scavenging activity

The free radical scavenging activities of leave of *Cordia africana*, *Croton macroactachyus*, *Vernonia amygdalina* or *Justica schimperiana* were studied by its ability to reduce the DPPH. At highest concentration the *Justica schimperiana* leave extracts showed highest value ($80.12 \pm 0.03\%$) and *Cordia africana* showed the lowest value ($50.03 \pm 0.04\%$). In MVA extract DPPH scavenging activity ranged from ($67.64 \pm 0.03\%$) to ($77.48 \pm 0.04\%$) in case of MJS extract, it ranged from ($76.47 \pm 0.32\%$) to ($80.12 \pm 0.12\%$). With all four medicinal plant leave extracts, as concentration increases free radical scavenging activity or percentage inhibitions also increase and the absorbance showed

Table 1: Phytochemical composition of the plant extracts

Phytochemicals	<i>C. Africana</i>	<i>C. macrostachyus</i>	<i>V. amygdalina</i>	<i>J. Schimperiana</i>
polyphenol	+	+	+	+
tannins	+	+	+	+
glycoside	-	+	-	-
Flavonoids	-	+	+	+
Terpenoids	-	-	-	+
Alkaloids	+	+	-	-
Saponnins	+	-	-	-
Steriods	+	+	+	+
Anthraquinons	+	+	+	+
Phlobatannins	-	-	-	-
Carotenoids	-	+	-	+
Quinones	+	-	-	-
Xanthoproteins	-	+	+	+
Carbohydrate	+	+	-	-
Coumarins	-	+	+	+
proteins	+	-	+	+
Emodins	-	-	-	+
Anthocyanins	-	-	+	+

decrease in value (Fig. 3-4). DPPH scavenge activities takes place by donating hydrogen to it, and they produce relatively stable antioxidant radicals with low standard reduction potential (Choe et al., 2005). The higher stability of antioxidant radicals than that of DPPH radicals is due to resonance delocalization throughout the phenolic ring structure (Choe et al., 2006). The percentage inhibition of DPPH free radical was higher when higher concentration of extract was used. This may be due to the presence of high amount of free radical scavengers at high concentration and polar compounds are more responsible for this activity (Tailor et al., 2014). Flavonoid, phenols and other phytochemical listed in Table 1, might be accountable for this DPPH scavenging activities or antioxidant activity (Sofna et al., 2014; Jakubowski et al., 1997).

Peroxide value

Peroxides are the main initial products of oil oxidation and can be determined using the peroxide value (Asnaashari et al., 2015). An increase in PV values of sun flower oils with and without added extracts was observed during the 30-day storage. The presence of unstable compounds that are susceptible to oxidation might be the cause of increase in the PV values. Peroxide value of sun flower oil (control) was 40.98 ± 0.11 meqO₂/kg for the first treatment and increased to 96.18 ± 0.11 meqO₂/kg for the second treatment. The lowest peroxide value was recorded in samples contain sun flower oil plus MJS (8.04 ± 0.11 meqO₂/kg) for the first treatment. This showed

that the sample had the highest antioxidant activities compare to others. The peroxide value of all tested samples showed increment from treatment 1 to treatment 2. This indicated that the power of extracts to retard oxidation decrease as days of storage increase. Peroxide value increments observed in this study might be due to the increase of primary products (peroxides) followed by an increase in secondary products (aldehydes and ketones) (Basturk et al., 2017). Thus it might be possible to treat the diseases with regular use of these plants along with a healthy lifestyle and also, previous findings are in line with present investigation (Abbas et al., 2018; Akyuz et al., 2019; Aleksic Sabo and Knezevic, 2019; Chabán et al., 2019; Elghandour et al., 2018; Khan et al., 2019; Kuruppu et al., 2019; Marabini et al., 2019; Martins et al., 2015; Pallavali et al., 2019; Ponzilacqua et al., 2019; Rocha-Miranda and Venâncio, 2019; Vujanović et al., 2019).

CONCLUSIONS

The qualitative and quantitative secondary metabolites test reveals that leave of *Cordia africana*, *Croton macroactachyus*, *Vernonia amygdalina* or *Justica schimperiana* have different kinds of bioactive compounds. In comparison with their amount, there is no significant difference between the amount of flavonoids, saponins and alkaloids in MJS but in MCA leave extracts there is significant difference between the amount of flavonoids, alkanoids, saponnins and steroids. DPPH assay and peroxide value change indicated that leave of *Cordia africana*, *Croton macroactachyus*, *Vernonia*

Table 2: Peroxide values of sun flower oil with different treatments of extracts

Medicinal plants	Peroxide value (meq O ₂ /Kg)	
	Treatment 1	Treatment 2
Sun flower oil + MCA	35 ±0.34 ^b	86 ±0.04 ^b
Sun flower oil +MVA	24 ±0.53 ^c	55.26 ±0.23 ^c
Sun flower oil + MJS	8.04±0.11 ^d	16.24±0.15 ^e
Sun flower oil + MCM	8.09± 0.12 ^d	18.19±1.03 ^d
Sun flower oil (Control)	40.98 ±0.11 ^a	96.18 ±0.11 ^a

Values are mean ± SD of triplicate analysis. Different superscript letters within columns showed significant difference ($P \leq 0.05$)

amygdalina or *Justica schimperiana* had good antioxidant activity but leave of *Cordia Africana* showed the lowest activities.

ACKNOWLEDGEMENT

Debre Tabor University is acknowledged for financial support and providing necessary facilities to carry out this research.

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