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Journal of Medicinal Plants Research

25 May 2018
ISSN 1996-0875
DOI: 10.5897/JMPR
www.academicjournals.org

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DOI: 10.5897/JMPR2018.6588
Article Number: DCE1CCF57203
ISSN 1996-0875

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Full Length Research Paper

In vitro antioxidant and antimicrobial activities of *Pistacia lentiscus, Phyllanthus anderssonii* and *Cinnamomum verum* crude extracts and fractions

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Received 5 April, 2018; Accepted 14 May, 2018

In this study, phytochemical screening, antioxidant and antimicrobial activities of ethanolic (80%) extracts from leaf and stem of *Pistacia lentiscus* and *Phyllanthus anderssonii* and leaf of *Cinnamomum verum* and their fractions were evaluated. Antimicrobial activity was performed by disc diffusion method. The antioxidant activity was determined by stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical method. Fractionation improved the antimicrobial activity of *P. lentiscus* and *C. verum*. The ethyl acetate fraction of *P. lentiscus* leaf showed higher activity against *Bacillus subtilis* (19 mm), *Staphylococcus aureus* (18 mm), *Escherichia coli* (20 mm), and *Pseudomonas aeruginosa* (22 mm) than that obtained from the crude ethanolic extract and the aqueous fraction displayed the highest activity against *S. aureus* (21 mm). Fractionation also improved the antimicrobial activity of the stem against *B. subtilis* (19 mm from ethyl acetate, butanol and aqueous fractions), *P. aeruginosa* (18 mm from ethyl acetate fraction) and *Candida albicans* (20 mm from ethyl acetate fraction). Although crude ethanolic leaf extract of *C. verum* did not show antifungal activity against *Aspergillus niger*, however, upon fractionation, the ethyl acetate and aqueous fractions displayed high antifungal activity (20 and 19 mm, respectively); it also improved its activity against *C. albicans* (21 mm) and *B. subtilis* (20 mm) from the ethyl acetate and aqueous fractions, respectively. Fractionation of *P. anderssonii* stem ameliorated only the antibacterial activity against *S. aureus* (20 mm) and *E. coli* (21 mm) where the aqueous fraction exhibited the highest activity. Results of antioxidant activity showed that leaf of *P. lentiscus* (95%) and stem of *P. anderssonii* (93%) displayed the highest DPPH scavenging activity. Fractionation improved mainly the antioxidant potentiality of *C. verum* leaf where the ethyl acetate (78%) showed the highest activity. The different polarities of solvents yielded different fractions with different chemical composition and thus displayed different levels of antimicrobial and antioxidant activity.

Key words: *Pistacia lentiscus, Phyllanthus anderssonii, Cinnamomum verum,* antimicrobial activity, antioxidant activity.

INTRODUCTION

Infectious diseases have long been known as a major problem in developing countries. Development of antibiotic resistant bacteria is due to inadequate antibiotic use in human and animal health and to their continued

use as growth promoters in poultry and livestock production (Elisha et al., 2017). Two-thirds of deaths from infections in 2010 were reported to be caused by around 20 species, mainly bacteria and viruses (Dye, 2014). Novel, effective and affordable drugs are needed to combat infectious diseases especially in developing countries of the world; where up to one-half of deaths are due to infectious diseases (Awouafack et al., 2013; Srivastava et al., 2013).

Oxidative stress has been considered as a major contributing factor in the development of numerous life-threatening complications. The antioxidant potential of plants has received a great consideration as they play an important therapeutic role in human disease (Kasote et al., 2015).

Sudan harbour a high diversity of medicinal plants that play important role in health care system and consequently represent an integral part of life in Sudan. People in different regions of the Sudan use medicinal plants for the treatment of various diseases due to lack of medical doctors and exorbitant prices of pharmaceutical products. Hence, evaluation of their claimed pharmacological potential efficacy and safety is warranted (Issa et al., 2018).

Pistacia lentiscus L. (family Anacardiaceae) and *Cinnamomum verum* J. Presl (family Lauraceae) are widely used spice and have many applications in perfumery, flavoring and pharmaceutical industries (Mbaveng and Kuete, 2017; Bozorgi et al., 2013). *Phyllanthus anderssonii* Müll.Arg. (family Phyllanthaceae) is used in Sudan to treat uterus infections, female infertility, and broken bones.

The aim of this study was to evaluate the *in vitro* antimicrobial and antioxidant activities of the ethanolic (80%) extracts from leaf and stem of *P. lentiscus, P. anderssonii* and leaf of *C. verum* and their derived fractions.

MATERIALS AND METHODS

Plant

Leaves and stems of *P. lentiscus* and leaves of *C. verum* were collected from the district of Shambat-Khartoum North, Sudan. Leaves and stems of *P. anderssonii* were collected from Kasala State, Sudan. Botanical identification and authentication were performed and voucher specimens (No. 2015/4PL for *P. lentiscus*, No. 2015/4PA for *P. anderssonii* and No. 2015/4CV for *C. verum*) have been deposited in the herbarium of Al-Neelain University.

Preparation of extracts and fractions

The dried, powdered materials (500 g each) were separately extracted thrice in 80% ethanol (EtOH) (v/v) at room temperature for 24 h, followed by filtration using filter paper. The three percolates were mixed, concentrated and subsequently freeze-dried. The freeze-dried sample was sequentially extracted with petroleum ether (PE), ethyl acetate (EtOAc), and n-butanol (BuOH). Extracts were concentrated under vacuum by rotary evaporator and aqueous fractions were freeze-dried.

Preliminary phytochemical screening

The extracts and fractions were subjected to phytochemical analysis using standard phytochemical methods (Trease and Evans, 2002).

Antimicrobial activity

Test strains and culture media

Standard strains of microorganism were used in this study and were obtained from Medicinal and Aromatic Institute of Research, National Research Center, Khartoum. The bacterial species used were the Gram-negative bacteria: *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) and the Gram-positive bacteria: *Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (ATCC 25923). Fungal species were *Candida albicans* (ATCC 7596) and *Aspergillus niger* (ATCC 9763). Bacteria were grown in Mueller Hinton Agar and fungi were grown in Sabouraud Dextrose Agar. The concentration of bacterial suspensions was adjusted to 10^8 cells/mL and that of fungal suspensions to 10^7 cells/ml.

Antibacterial assay

Antibacterial activity of extracts and fractions was evaluated by the disc diffusion method (Kil et al., 2009). Extracts/Fractions solutions (20 mg/ml) were prepared by diluting with 5% dimethyl sulfoxide (DMSO). The test microorganisms were seeded into respective medium by spread plate method. After solidification, filter paper discs with a diameter of 6.0 mm were impregnated with 10 µl of crude extracts/fractions followed by drying off. DMSO was used as a negative control, while gentamicin (10 µg/disc) was used as a positive control. Antibacterial discs were dispensed onto the surface of the inoculated agar plates and Petri plates were incubated for 24 h at 37°C. Experiment was done in triplicate. Diameters of clear zone of inhibition produced around the discs were measured and recorded.

Antifungal assay

Antifungal activity was also evaluated by the disc diffusion method (Mothana and Lindequist, 2005). Paper discs were impregnated with 10 µl of extracts at 20 mg/ml followed by drying off. DMSO was used as a negative control, while nystatin (10 µg/disc) was used as a positive control. Antifungal discs were dispensed onto the surface of the inoculated agar plates, after which the plates were incubated at 27°C for 48 h. Experiment was done in triplicate. After the colonies grew, the zones of inhibition around the discs were

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measured and recorded.

Antioxidant activity

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

Antioxidant activity of the extracts was estimated using DPPH *in vitro* method (Mensor et al., 2001). Test samples were dissolved separately in 5% DMSO to get test solution of 1 mg/ml. Assay was performed in 96-well, microtiter plate. 140 µL of 0.6×10^{-6} mol/l DPPH were added to
each well containing 70 µl of sample. The mixture was shaken gently and left to stand for 30 min in dark at room temperature. The absorbance was measured spectrophotometrically at 517 nm using Cecil-Elect Spectrophotometer. Blank was done in the same way using 5% DMSO and sample without DPPH and control was done in the same way but using DPPH and 5% DMSO without sample. Ascorbic acid was used as reference antioxidant compound. All analyses were done in triplicate. The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%) = $100 - [(Abs_{sample} - Abs_{blank}) \times 100] / (Abs_{control})]$

where Abs_{sample} is the absorbance of DPPH radical + sample; Abs_{blank} is the absorbance of sample+ 5% DMSO; and $Abs_{control}$ is the absorbance of DPPH radical + 5% DMSO.

RESULTS

Extraction yield and phytochemical screening

Results depicted in Table 1 show a higher extractive yield in the leaf crude extract (34.1%) of *P. lentiscus* as compared to the stem (24.5%) and in the leaf crude extract (39.1%) of *P. anderssonii* compared to the stem (13.1%). Crude leaf extract of *C. verum* revealed an extractive yield of 29.5%. Fractions extractive yields were variable and it was observed that all aqueous fractions (except that of *P. anderssonii* stem) showed high extractive yield after different solvent-solvent fractionation.

Phytochemical screening of crude ethanolic (80%) and different fractions of *P. lentiscus, P. anderssonii* and *C. verum* revealed the presence of different classes of secondary metabolites like flavonoids, sterols, triterpenes, coumarins. Tannins were only detected in crude ethanolic (80%) extracts of the three plants and in their butanol and aqueous fractions. Saponins were present in crude ethanolic (80%) extracts as well as their aqueous fractions. Alkaloid was only detected in the ethyl acetate and butanol fractions of *P. anderssonii* stem. Anthraquinone glycosides and cardiac glycosides were not detected in the investigated plants (Table 1).

Antimicrobial activity

Crude ethanolic (80%) leaf and stem extracts of *P. lentiscus, P. anderssonii* and *C. verum* and their fractions were tested for their antimicrobial activity. Results are shown in Table 2.

P. lentiscus crude extracts and fractions from stem and leaf

The crude ethanolic (80%) extract of *P. lentiscus* stem displayed higher antibacterial activity than that obtained from the crude ethanolic (80%) extract of the leaf against *E. coli* with inhibition zone of 19 mm. The latter showed the highest antifungal activity with inhibition zones of 18 and 19 mm against *A. niger* and *C. albican,* respectively. A remarkable increase in the antibacterial activity of *P. lentiscus* leaf was observed upon fractionation where the ethyl acetate fraction showed higher inhibition zones against the four tested bacteria species than those obtained from the crude ethanolic extract. Moreover, the remained aqueous fraction after fractionation possessed higher activity against *S. aureus* (21 mm) than that obtained from the crude extract and all fractions. The antifungal activity of the leaf was not altered after fractionation and the ethyl acetate fraction displayed the same inhibition zone value as the crude extract.

Fractionation of the stem ethanolic (80%) extract of *P. lentiscus* increased generally the antimicrobial activity where the highest activity was observed in the ethyl acetate fraction against *B. subtilis* (19 mm), *P. aeruginosa* (18 mm) and *C. albicans* (20 mm) and in the butanol extract against *B. subtilis* (19 mm). The aqueous extract displayed high and similar inhibition diameter against *B. subtilis* as those obtained by the ethyl acetate and butanol fractions.

P. anderssonii crude extracts and fractions from stem and leaf

Both leaf and stem ethanolic (80%) extracts of *P. anderssonii* exhibited good antimicrobial activity against all tested microorganisms where the leaf displayed an activity in the range of 20 to 22 mm and the stem in the range of 18 to 21 mm. However, fractionation reduced the antimicrobial activity of crude extracts of both organs except aqueous fraction of the stem. The observed increase in antibacterial activity of aqueous fraction was against *S. aureus* (20 mm) and *E. coli* (21 mm).

C. verum crude extracts and fractions from leaf

Crude ethanolic (80%) extract of *C. verum* leaf displayed good antimicrobial activity against all tested microorganisims except in the case of *A. niger* which was not susceptible to the crude extract. However, fractionation resulted in a pronounced antifungal activity where the ethyl acetate fraction displayed inhibition zone of 20 mm against *A. niger* and increased the activity

Table 1. Yield extractive and phytochemical screening of *Pistacia lentiscus, Phyllanthus anderssonii* and *Cinnamomum verum* crude extracts and fractions.

Extract/Fraction	Yield (%)*	Saponin	Coumarin	Tannin	Alkaloid	Steroid	Triterpene	Flavonoid	Cardiac glycoside	Anthraquinone
Pistacia lentiscus										
Leaf										
EtOH (80%)	34.1	+	+	+	-	+	+	+	-	-
PE	7.3	-	+	-	-	+	+	+	-	-
Chloroform	0.4	-	+	-	-	+	+	+	-	-
EtOAc	26.3	-	+	-	-	+	+	+	-	-
BuOH	10.9	-	+	+	-	+	+	+	-	-
H_2O	55.1	-	+	+	-	+	+	+	-	-
Stem										
EtOH (80%)	24.5	+	+	+	-	+	+	+	-	-
PE	8.4	-	+	-	-	+	+	+	-	-
Chloroform	0.8	-	+	-	-	+	+	+	-	-
EtOAc	21.5	-	+	-	-	+	+	+	-	-
BuOH	18.2	-	+	+	-	+	+	+	-	-
H_2O	51.1	+	+	+	-	+	+	+	-	-
Phyllanthus anderssonii										
Leaf										
EtOH (80%)	39.1	+	+	+	-	+	+	+	-	-
PE	1.9	-	+	-	-	+	+	+	-	-
Chloroform	0.8	-	+	-	-	+	+	+	-	-
EtOAc	19.5	-	+	-	-	+	+	+	-	-
BuOH	15.1	-	+	+	-	+	+	+	-	-
H_2O	62.7	+	+	+	-	+	+	+	-	-
Stem										
EtOH (80%)	13.1	+	+	+	-	+	+	+	-	-
PE	18.0	-	+	-	-	+	+	+	-	-
Chloroform	8.8	-	+	-	-	+	+	+	-	-
EtOAc	14.2	-	+	-	+	+	+	+	-	-
BuOH	43.3	-	+	+	+	+	+	+	-	-
H_2O	15.7	+	+	+		+	+	+	-	-
Cinnamomum verum										
Leaf										
EtOH (80%)	29.5	+	+	+	-	+	+	+	-	-
PE	29.680	-	+	-	-	+	+	+	-	-

Table 1. Cont'd.

Chloroform	0.467	-	+	-	-	+	+	+	-	-
EtOAc	1.169	-	+	-	-	+	+	+	-	-
BuOH	19.764	-	+	+	-	+	+	+	-	-
H_2O	44.208	+	+	+	-	+	+	+	-	-

*****Crude yield extract calculated as percentage of the weight of the raw material (500 g) and fraction yield was calculated as percentage of the weight of the crude EtOH (80%) extract.

against *C. albican* from 18 to 21 mm. Also, the aqueous fraction displayed a remarkable antifungal activity against *A. niger* (19 mm). For other microorganisms, a slight increase in the antibacterial activity against *B. subtilis, E. coli* and *P. aeruginosa* was observed in the ethyl acetate fraction.

Antioxidant activity

Crude ethanolic (80%) leaf and stem extracts of *P. lentiscus, P. anderssonii* and *C. verum* leaf and their fractions were evaluated for their *in vitro* antioxidant activity using DPPH method and results are shown in Table 3. Crude ethanolic (80%) extracts of the three investigated plants showed high DPPH scavenging capacity with ranking order of activity (in terms of % inhibition) as leaf of *P. lentiscus* (95%) > stem of *P. anderssonii* (93%) > stem of *P. lentiscus* (84%) > leaf of *P. anderssonii* (76%) > leaf of *C. verum* (8%). Fractionation of the crude leaf extract of *P. lentiscus* did not influence the scavenging capacity where butanol fraction displayed the same activity (95%) while others fractions showed minor reduction (93%) in their activity. The scavenging activity of the stem was increased upon fractionation by 7, 6, 11 and 13% in the petroleum, chloroform, ethyl acetate and butanol fractions, respectively. Fractionation of crude leaf and stem extracts of *P. anderssonii* reduced their scavenging potentiality except for the ethyl acetate fractions. The scavenging activity of the ethyl acetate fraction of the leaf increased by 25% when compared with the crude extract and that of the stem was comparable to its corresponding crude extract. The scavenging activity of the aqueous fraction of the leaf demonstrated good scavenging potential and showed 21% increase in the scavenging activity when compared with the crude leaf extract. A remarkable increase of the scavenging activity of the crude leaf extract of *C. verum* was obtained upon fractionation by 262, 475, 857 and 600% in the petroleum, chloroform, ethyl acetate and butanol fractions respectively.

DISCUSSION

Generally, the leaf gave higher yield of extract than the stem and the aqueous fractions gave the highest yield after fractionation followed by the butanol fractions. It was reported that ethanol and water extracts showed higher amount of extracted compounds in comparison with ethyl acetate extract (Tuberoso et al., 2010). Moreover, polar solvents extracted large molecules like glycosides, proteins, saponins and tannins (Tan et al., 2013).

The phytochemical analysis revealed the presence of different classes of secondary metabolites. As shown in Table 1, each extract or fraction contained at least five types of secondary metabolites and some of the extracts and fractions displayed high antimicrobial and antioxidant activities (Tables 2 and 3). It is known that secondary metabolites such as flavonoids, terpenoids, steroids, phenols, saponins, alkaloids and tannins have good antimicrobial properties (Dorman and Deans, 2000; Kuete and Efferth, 2010; Biswas et al., 2013). Flavanoids and phenols form complexes with extra cellular and soluble proteins of bacterial cell walls leading to the death of the bacteria (Cowan, 1999). Terpenoids are known to have antibacterial property by affecting the synthesis of cell membranes components, prenylation of proteins and the use of carbon source (Nayak et al., 2010). Saponins have been found to have inhibitory effects on Gram-positive organism, *S. aureus* (Biswas et al., 2013).

Crude ethanol extracts and fractions of the three plants exhibited varying degrees of antibacterial activity against the tested microorganisms. It is well known that using different solvents may extract different compounds and consequently could lead to different extract potentials (Aleksic and Knezevic, 2014). Fractionation increased mainly the antibacterial activity of *P. lentiscus* leaf; the ethyl acetate fraction showed higher activity against all tested bacteria than that obtained from the crude ethanolic extract and the aqueous fraction displayed highest activity against *S. aureus* (Table 2). Furthermore, fractionation improved mainly the antimicrobial activity of

Table 2. Antimicrobial activity of *Pistacia lentiscus, Phyllanthus anderssonii* and *Cinnamomum verum* crude extracts and fractions.

Organ	Extract/Fraction	Inhibition zone (mm)					
		Bs	***Sa***	***Ec***	***Pa***	***An***	***Ca***
	Pistacia lentiscus						
Leaf	EtOH (80%)	15 ± 0.33	15 ± 0.27	13 ± 0.31	15 ± 0.13	18 ± 0.24	19 ± 0.33
	PE	7 ± 0.07	7 ± 0.01	NA	NA	NA	NA
	Chloroform	NA	7 ± 0.07	14 ± 0.43	13 ± 0.12	10 ± 0.01	8 ± 0.11
	EtOAc	19 ± 0.42	18 ± 0.31	20 ± 0.41	22 ± 0.11	19 ± 0.42	18 ± 0.31
	BuOH	13 ± 0.11	14 ± 0.27	13 ± 0.12	9 ± 0.07	NA	NA
	H_2O	16 ± 0.41	21 ± 0.17	14 ± 0.11	17 ± 0.42	NA	15 ± 0.11
Stem	EtOH (80%)	14 ± 0.41	15 ± 0.11	19 ± 0.31	12 ± 0.21	13 ± 0.45	15 ± 0.11
	PE	NA	NA	NA	NA	NA	NA
	Chloroform	NA	14 ± 0.12	NA	13 ± 0.21	NA	12 ± 0.12
	EtOAc	19 ± 0.33	16 ± 0.42	18 ± 0.17	18 ± 0.31	15 ± 0.17	20 ± 0.42
	BuOH	19 ± 0.31	16 ± 0.41	11 ± 0.12	7 ± 0.07	NA	NA
	H_2O	19 ± 0.12	17 ± 0.44	15 ± 0.27	16 ± 0.33	NA	13 ± 0.17
	Phyllanthus anderssonii						
Leaf	EtOH (80%)	20 ± 0.24	21 ± 0.11	20 ± 0.41	22 ± 0.41	22 ± 0.27	20 ± 0.51
	PE	NA	NA	NA	7 ± 0.01	6 ± 0.01	NA
	Chloroform	11 ± 0.12	11 ± 0.12	11 ± 0.12	8 ± 0.07	NA	6 ± 0.01
	EtOAc	17 ± 0.27	19 ± 0.33	18 ± 0.17	16 ± 0.41	15 ± 0.11	17 ± 0.17
	BuOH	11 ± 0.12	14 ± 0.41	6 ± 0.01	NA	NA	NA
	H_2O	16 ± 0.11	21 ± 0.43	14 ± 0.27	15 ± 0.21	NA	15 ± 0.21
Stem	EtOH (80%)	20 ± 0.17	18 ± 0.42	19 ± 0.42	18 ± 0.33	18 ± 0.31	21 ± 0.17
	PE	NA	NA	NA	7 ± 0.01	NA	10 ± 0.10
	Chloroform	NA	8 ± 0.01	7 ± 0.07	NA	NA	NA
	EtOAc	10 ± 0.01	15 ± 0.33	10 ± 0.41	14 ± 0.11	13 ± 0.12	11 ± 0.09
	BuOH	7 ± 0.01	10 ± 0.01	NA	16 ± 0.41	NA	NA
	H_2O	19 ± 0.33	20 ± 0.44	21 ± 0.51	16 ± 0.17	16 ± 0.17	18 ± 0.42
	Cinnamomum verum						
Leaf	EtOH (80%)	17 ± 0.21	18 ± 0.42	18 ± 0.33	17 ± 0.41	NA	18 ± 0.31
	PE	NA	NA	NA	NA	NA	NA
	Chloroform	12 ± 0.07	14 ± 0.11	11 ± 0.41	NA	7 ± 0.07	NA
	EtOAc	19 ± 0.42	18 ± 0.31	19 ± 0.31	18 ± 0.31	20 ± 0.33	21 ± 0.27
	BuOH	NA	NA	NA	NA	NA	NA
	H_2O	20 ± 0.33	16 ± 0.41	18 ± 0.33	13 ± 0.27	19 ± 0.31	12 ± 0.21
	Gentamicin	16 ± 0.01	15 ± 0.01	17 ± 0.01	16 ± 0.01	-	-
	Nystatin	-	-	-	-	24 ± 0.01	20 ± 0.01

Bs, Bacillus subtilis; Sa, Staphylococcus aureus; Ec, Escherichia coli; Pa, Pseudomonas aeruginosa; An, Aspergillus niger; Ca, Candida albicans. Gentamicin and Nystatin, 10 µg/disc; NA, not active.

the stem against *B. subtilis, P. aeruginosa* and *C. albicans.* Fractionation of *P. anderssonii* stem ameliorated only the antibacterial activity against *S. aureus* and *E. coli* where the aqueous fraction exhibited the highest activity. Although crude leaf extract of *C. verum* did not show antifungal activity against *A. niger,* upon fractionation, the ethyl acetate and aqueous fractions displayed high antifungal activity; also it improved its activity against *C. albicans* (ethyl acetate fraction) and *B. subtilis* (aqueous fraction). These results suggested the existence of compounds with an antagonistic effect in the crude extract. Fractionation

Table 3. Antioxidant activity of *Pistacia lentiscus, Phyllanthus anderssonii* and *Cinnamomum verum* crude extracts and fractions.

Plant name	Organ	DPPH Scavengin activity (%)					
		Extracts/Fractions					
		EtOH (80%)	PE	$CHCl_3$	EtOAc	BuOH	H_2O
Pistacia lentiscus	Leaf	95	93	93	93	95	80
	Stem	84	90	89	93	95	87
Phyllanthus anderssonii	Leaf	76	50	33	95	61	92
	Stem	93	82	40	94	53	32
Cinnamomum verum	Leaf	8	29	47	78	56	27
Vitamin C (Standard)		98					

reduced the antimicrobial activity of both the leaf and stem of *P. anderssonii*; this could be probably due to synergistic activities of compounds present together in the crude extract but separated from each other upon fractionation. Petroleum ether and chloroform fractions of the investigated plants had no or poor activity against all tested microorganisms suggesting that the observed antimicrobial activity of the studied plants could mainly be to the presence of compounds of hydrophilic nature.

Results of antioxidant activity showed that leaf of *P. lentiscus* and stem of *P. anderssonii* displayed the highest DPPH scavenging activity. Fractionation improved mainly the antioxidant potentiality of *C. verum* leaf where the ethyl acetate showed the highest activity. Also, fractionation improved the DPPH scavenging activity of *P. anderssonii* leaf and slightly improved that of *P. lentiscus* stem. However, fractionation (except aqueous fraction) slightly alter the DPPH scavenging activity of *P. lentiscus* leaf and generally all fractions showed high DPPH scavenging activity indicated that the activity might be attributed to the presence of several antioxidant agents with polar and non-polar nature.

Previous studies on *P. lentiscus* were mainly reported for essential oil and gum where they were found to possess remarkable antimicrobial (Hayder et al., 2005; Koutsoudaki et al., 2005; Miyamoto et al., 2014) and antioxidant (Benhammou et al., 2008; Chryssavgi et al., 2008; Bampouli et al.,2014) activities. Essential oil and oleoresins (acetone extract) from *C. verum* leaf displayed high antioxidant, antibacterial and fungal activity (Singh et al., 2007). Moreover, it is worth mentioning that this is the first phytochemical study and biological activity evaluation of *Phyllanthus anderssonii.*

Conclusion

Data obtained in the present study showed that crude extracts of *P. lentiscus, P. anderssonii* and *C. verum* demonstrate good antimicrobial and DPPH scavenging potential. Fractionation with solvents of different polarities yielded different fractions with different chemical composition and thus displayed different levels of antimicrobial and antioxidant activity. This study provides scientific insight to further determine the antimicrobial and antioxidant principles in the three studied plants.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Prof. Maha Kordofani (Botany Department, Faculty of Science, University of Khartoum) for the identification of the plants.

REFERENCES

Aleksic V, Knezevic P (2014). Antimicrobial and antioxidative activity of extracts and essential oils of *Myrtus communis* L. Microbiological Research, 169(4):240-254.

Awouafack MD, McGaw LJ, Gottfried S, Mbouangouere R, Tane P, Spiteller M, Eloff JN (2013). Antimicrobial activity and cytotoxicity of the ethanol extract, fractions and eight compounds isolated from *Eriosema robustum* (Fabaceae). BMC Complementary and Alternative Medicine, 13(1):289.

Bampouli A, Kyriakopoulou K, Papaefstathiou G, Louli V, Krokida M, Magoulas K (2014). Comparison of different extraction methods of *Pistacia lentiscus* var. *chia* leaves: yield, antioxidant activity and essential oil chemical composition. Journal of Applied Research on Medicinal and Aromatic Plants, 1(3):81-91.

Benhammou N, Atik Bekkara F, Panovska TK (2008). Antioxidant and antimicrobial activities of the Pistacia lentiscus and atlantica extracts. African Journal of Pharmacy and Pharmacology, 2(2):22-28.

Biswas B, Rogers K, McLaughlin F, Daniels D, Yadav A (2013). Antimicrobial Activities of Leaf Extracts of Guava (*Psidium guajava* L.) on Two Gram-Negative and Gram-Positive Bacteria. International Journal of Microbiology, 2013:1-7.

Bozorgi M, Memariani Z, Mobli M, Surmaghi MHS, Shams-Ardekani MR, Rahimi R (2013). Five *Pistacia* species (*P. vera, P. atlantica, P. terebinthus, P. khinjuk* and *P. lentiscus*): A Review of their traditional uses, phytochemistry, and pharmacology. The Scientific World Journal, 2013:1-33.

Chryssavgi G, Papageorgiou V, Mallouchos A, Theodosis K, Michael K, (2008). Essential oil composition of *Pistacia lentiscus* L. and *Myrtus communis* L.: evaluation of antioxidant capacity of methanolic extracts. Food Chemistry, 107:1120-1130.

Cowan MM (1999). Plant products as antimicrobial agents. Clinical

Microbiology Review, 2(4):564-582.
Dorman HJ, Deans SG (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. Journal of Applied Microbiology, 88(2):308-316.
Dye C (2014). After 2015: Infectious diseases in a new era of health and development. Philosophical Transactions of The Royal Society B, 369(1645):20130426.
Elisha IL, Botha FS, McGaw LJ, Eloff JN (2017). The antibacterial activity of extracts of nine plant species with good activity against *Escherichia coli* against five other bacteria and cytotoxicity of extracts. BMC Complementary and Alternative Medicine, 17(1):133.
Hayder N, Ammar RB, Abdelwahed A, Kilani S, Mahmoud A, Chibani JB, Mariotte AM, Ghedira K, Dijoux-Franca MG, Chekir-Ghedira L (2005). Antibacterial and antimutagenic activity of extracts and essential oil from (Tunisian) *Pistacia lentiscus*. Toxicological and Environmental Chemistry, 87(4):567-573.
Issa TO, Mohamed YS, Yagi S, Ahmed RH, Najeeb TM, Makhawi AM, Khider TO (2018). Ethnobotanical investigation on medicinal plants in Algoz area (South Kordofan), Sudan. Journal of Ethnobiology and Ethnomedicine, 14(1):31.
Kasote DM, Katyare SS, Hegde MV, Ba H (2015). Significance of Antioxidant Potential of Plants and its Relevance to Therapeutic Applications. International Journal of Biological Sciences, 11(8):982-991.
Kil HY, Seong ES, Ghimire BK, Chung IM, Kwon SS, Goh EJ, Heo K, Kim MJ, Lim JD, Lee D, Yu CY (2009). Antioxidant and antimicrobial activities of crude sorghum extract. Food Chemistry, 115(4):1234-1239.
Koutsoudaki C, Krsek M, Rodger A (2005). Chemical composition and antibacterial activity of the essential oil and the gum of *Pistacia lentiscus* Var. *chia.* Journal of Agricultural and Food Chemistry, 53(20):7681-7685.
Kuete V, Efferth T (2010). Cameroonian medicinal plants: pharmacology and derived natural products. Frontiers in pharmacology, 25(1):123.
Mbaveng AT, Kuete V (2017). Cinnamon Species. In Medicinal Spices and Vegetables from Africa. pp. 385-395.
Mensor LI, Menezes FS, Leitao GG, Reis AS, Santos DOS, Leitao SG (2001). Screening of Brazilian plants extracts for an antioxidant activity by the use of DPPH free radical method. Phytotherapy Research, 15(2):127-130.
Miyamoto T, Okimoto T, Kuwano M (2014). Chemical composition of the essential oil of mastic gum and their antibacterial activity against drug-resistant Helicobacter pylori. Natural Products and Bioprospecting, 4(4):227-231.
Mothana RA, Lindequist U (2005). Antimicrobial activity of some medicinal plants of the island Soqotra. Journal of Ethnopharmacology, 4:96(1-2):177-181.
Nayak BS, Ramdath DD, Marshall, JR, Isitor GN, Eversley M, Xue S, Shi J (2010). Wound-healing activity of the skin of the common grape (*Vitis Vinifera*) variant, Cabernet Sauvignon. Phytotherapy research, 24(8):1151-1157.
Singh G, Maurya S, deLampasona MP, Catalan CAN (2007). A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food and Chemical Toxicology, 45(9):1650-1661.
Srivastava J, Chandra H, Nautiyal AR, Kalra SJS (2013). Antimicrobial resistance (AMR) and plant-derived antimicrobials (PDAms) as an alternative drug line to control infections. Biotechnology, 4(5):451-460.
Tan MC, Tan CP, Ho CW (2013). Effects of extraction solvent system, time and temperature on total phenolic content of henna (*Lawsonia inermis*) stems. International Food Research Journal, 20(6):3117-3123.
Trease GE, Evans WC (2002). Textbook of Pharmacognosy. 15th Ed. Saunders Publishers, London.
Tuberoso CIG, Rosa A, Bifulco E, Melis MP, Atzeri A, Pirisi FM, Dessì MA (2010). Chemical composition and antioxidant activities of *Myrtus communis* L. berries extracts. Food Chemistry, 123(4):1242-1251.

Vol. 12(16), pp. 194-202, 25 May, 2018
DOI: 10.5897/JMPR2017.6445
Article Number: D9C96A857205
ISSN: 1996-0875

http://www.academicjournals.org/JMPR

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Journal of Medicinal Plants Research

Full Length Research Paper

Indigenous knowledge on medicinal plants used in and around Robe Town, Bale Zone, Oromia Region, Southeast Ethiopia

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Received 6 July, 2017; Accepted 14 November, 2017

A study on medicinally important plants in and around Robe town, Southwest Ethiopia was been carried out to document the medicinal plants and the associated knowledge in the area. Thirty informants with age range between 18 and 70 years took part in this study. Semi-structured interview was used to collect the data from the informants. The collected data was then analyzed using micro-soft excel spread sheet 2007 and summarized by descriptive statistical methods. Fifty five medicinally important plants were documented from the study area. The medicinal plants comprised of 33 families and 49 genera. Fabaceae was 30.4% followed by Solanaceae (21.7%) dominatedthe family distribution. Herbs are the most harvested (45.4%) plant habits followed by shrubs (30.9%) and trees (21.8%). Leaves are the most (52.7%) important plant parts used for medicinal value. Oral (47.3%) is the most common administration method used by the local people of the area. Crushing dominates (60%) the preparation method of the medicine. The practice on the use of traditional medicine is commonin the study area. Conservation practices and awareness on the use of the medicinally important plants is suggested.

Key words: Indigenous knowledge, medicinal plants, semi-structured interview, Robe Town, Ethiopia.

INTRODUCTION

About 80% of the populations in developing countries rely on plants for their health care system (Hostettmann and Marston, 2002). The firm dependence on plant products for the treatment of human and livestock diseases might be have resulted from the lack of facilities and inadequate access to modern medicine (Tolossa et al., 2013). Furthermore, affordability and efficacy on treatment are contributing to the preference of medicinal plants than modern medicine (Yirga, 2010a). Like in other developing countries, majority of the indigenous people in Ethiopia are also depending on traditional medicine (Kassaye et al., 2006).

Ethiopia is endowed with a diversified topography and climate favorable for diversified plant taxa. The country has an estimated 6000 species of vascular plants of which 10% are endemic (Yasin et al., 2015). Apart from the plant diversity, Ethiopia is also home for many languages, cultures and beliefs which in turn have

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contributed to the diversity of traditional knowledge and practices of the people (Limenih et al., 2015). Due to this, in Ethiopia there is a long history of using plants to treat different human and livestock ailments (Mesfin et al., 2014). Moreover, most of the Ethiopian population still depends on traditional medicine (Giday et al., 2009). This is because the growth and development of modern health care in Ethiopia has been very stunted and to date, its coverage is less than 50% of the population (Yirga, 2010b).

When compared to the role the practice play in the health system, the indigenous knowledge system in Ethiopia is not fully documented (Enyew et al., 2014). Due to deforestation and land degradation the medicinal plants together with the associated knowledge are at the risk of lost (D' avigdor et al., 2014).Furthermore, the local communities could face cultural changes due to the development activities in the areas where the communities live and this will lead to knowledge lose (Belayneh et al., 2012). The oral knowledge transfer between generations is also making the knowledge more fragile and the probability to be lost in the transfer process is high. Although the country has a written language, the knowledge on traditional medicine is not well documented and is transferred through words of mouth.

Although different studies have been made in different parts of the country, most of them are more general and do not focus on a specific Ethnic group or agro-ecological zone of the country (Yineger et al., 2008). This shows that documentation and conservation of medicinal plants and the knowledge of the society in specific agro ecological zones and specific ethnic group of the country is very important. Robe is one of the fast growing towns of Bale zone located in one of the biodiversity rich areas of Oromia region. In every corner of the town, it is very common to come across traditional healers selling their traditional medicines prepared from natural resources. On the other hand, the town is growing faster and the native people are facing cultural changes due to immigrants from different areas. Thus, unless the traditional knowledge is documented soon, the risk of traditional knowledge lose is expected. However, no related study has been conducted in the town so far. Therefore, this study was initiated to document the medicinal plants and the knowledge associated with medicinal plants in and around Robe town.

MATERIALS AND METHODS

Study area

The study was conducted in Robe town located at 430 km from Addis Ababa, the capital city of the country, Oromia region, Southeastern Ethiopia. The town is also situated in 7° 8' 0" North and 40° 0' 0" East position and serving as a capital city of the zone (Duressa et al. 2014).. The estimated population of the town is 73859 of them 37668 are males and 37191 are females. The town has minimum and maximum temperature ranges between 9.42 and 21.16°C, respectively and minimum and maximum annual rainfall are 535 and 1018 mm, respectively (Figure 1) (Chala, 2012).

Method

Descriptive survey approach was employed in this study to record the medicinal plants in the area. The medicinal plants data were collected from the indigenous people in and around Robe town. Purposive sampling method was used to select the traditional healers. Recommendations from the local elders were considered to select the healers. Accordingly, 30 informants (25 males and 5 females) were systematically selected. The age ranges of the informants taking part in the study were between 18 and 70 years. The lower age limit was considered to be 18 years since informants below 18 years are not believed to have such indigenous knowledge. According to Martin (1995), semi-structured interview was used to collect the data from the informants. The informants were convinced upon the purpose of the research and each informant was requested for permission before the interview.

The interview prepared in English was translated into Afaan Oromo and Amharic, the local languages of the informants. After researchers obtain an oral consent from each informant, information concerning the traditional healers was collected. Medicinal plants information such as the plant local name, treated disease, and the use plant parts, preparation and route of administration were recorded from the informants. At the end of each interview, the researchers together with the traditional healers and local assistant carried out a field observation to observe and collect the reported medicinal plants. The collected specimens during field observation were pressed, dried and identified using different volume Flora books of Ethiopia and Eritrea. The collected data was analyzed by the descriptive statistical methods such as table, chart and percentages.

RESULTS

Fifty five medicinally important plants were documented from the study area (Table 3). The medicinal plants comprised of 33 families and 49 genera (Table 1). Fabaceae (30.4%) followed by Solanaceae (21.7%) and Asteraceae (17.4%) are dominant families in the area. Majority of the plants (85.45%) are used for the treatment of human ailments and the rest (14.54%) are used for both human and livestock. All of the medicinal plants are collected from the wild.

Plan habit

Herbs are the most (25. 45.4%) used plants in the area for medicinal purpose followed by shrubs (17. 30.9%), trees (12. 21.8%) and climbers (1.1.8%) (Figure 2).

Form of plant

Most of the medicinal plants in the area (31. 56.4%) are used in fresh form and both fresh (14. 25.4%) and dry (10. 18.2%) occupy the rest of the plant form (Figure 3).

Concerning the purpose of the medicinal plants, majority

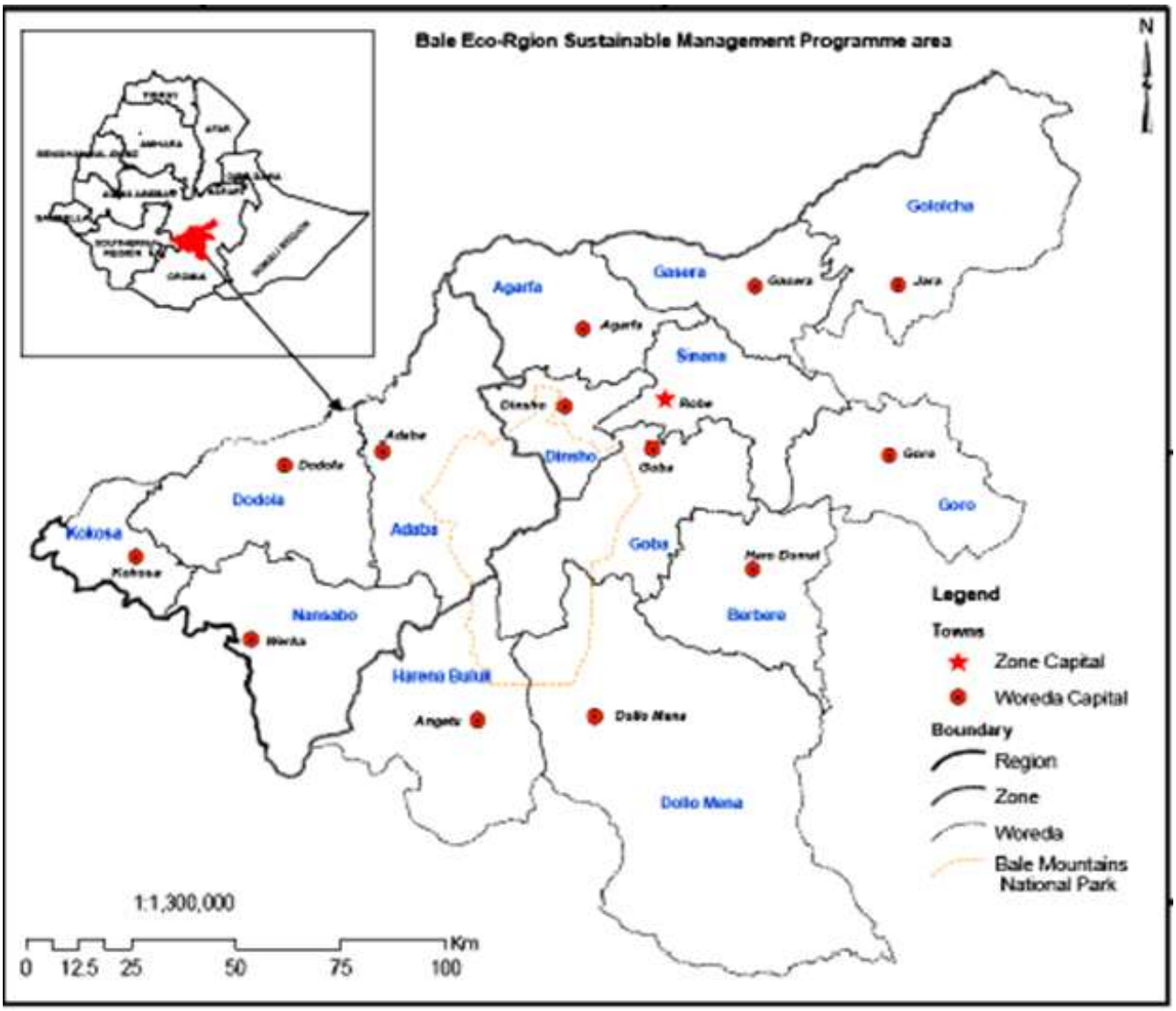

Figure 1. Map of Bale Zone.
Source: Duressa et al. (2014).

of them (47. 85.4%) are used for the treatment of the human disease, while the others (8. 14.5%) are used for both human and livestock diseases (Figure 4).

Plant parts used

Leaf is the most harvested plant part (29. 52.7%) in the study area for medicinal value followed by seed (9. 16.4%), root (8.14.5%),stem (4. 7.3%), root and leaf (2. 3.6%), fruit (2. 3.6%) and bulb (1, 1.8%) (Figure 5).

Administration method

The administration method in the area is dominated by oral method (26.47.3%) followed by dermal (19.34.5%). Oral and dermal (6.10.9%), nasal (3. 5.4%), and oral and nasal (1.1.8%) also occupied the rest administration method (Figure 6).

Preparations methods of medicinal plants

Of the preparation methods recorded in the study area, crushing accounts for the largest (33.60%) preparation. Chewing (8.14.5%), boiling (7.12.7%), squeezing (5.9.1%), smoking (1.1.8%) and pasting (1.1.8%) are also the other common methods of preparations (Table 2).

DISCUSSION

Medicinal plant diversity

The result from the study area revealed that, the area is

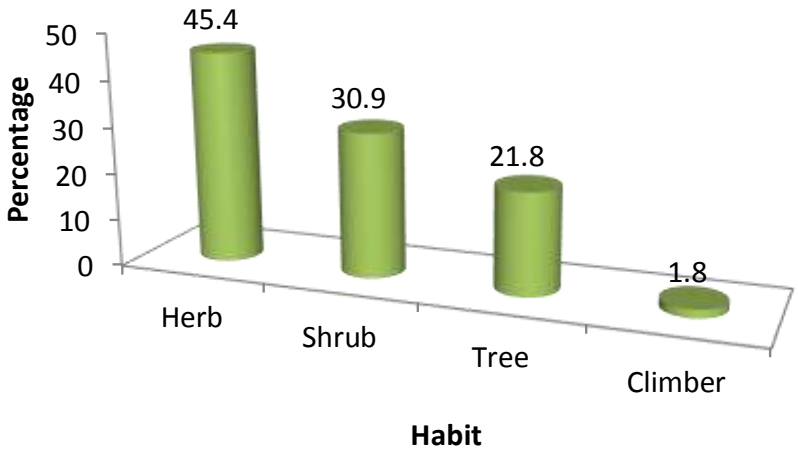

Figure 2. Growth forms (habits) of the medicinal plants.

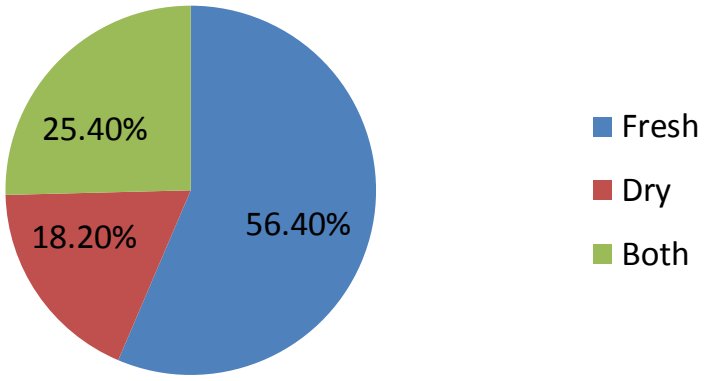

Figure 3. The plant form used in the in the area.

rich in traditional medicine. The family distribution in the study area shows that Fabaceae followed by Solanaceae dominated. The same results have also been documented in the studies carried out previously (Wondimu et al., 2007; Asefa and Abebe, 2014) in other areas in Ethiopia. Majority of the medicinal plants in the study area are used in fresh form. This may be due to the comfortable temperature and rainfall distribution which leads to the availability of fresh plants. On the other hand, traditional healers believe that fresh plants have more curing ability than the dry plants. Most of the medicinal plants recorded in the study area are used for the treatment of human ailments. This shows that the local people give more priority for themselves than animals. All of the medicinal plants documented for medicinal value are collected from the wild. This is may be due to the secrecy nature of traditional knowledge. Of the total medicinal plants documented in this study, 11 were mentioned by Bekele and Reddy (2015), 8 mentioned by Tolesa (2007), 7 mentioned by Getaneh and Girma (2014), 7 mentioned by (Enyew et al., 2014), 1 mentioned by Maryo et al. (2015) and 1 mentioned by Assefa and Abebe (2014). Data on the knowledge transfer process shows poor relationship among the generations and the knowledge on the traditional medicine is in a verge of disappearing in the near future. Most of the young respondents interviewed in the study know very few or even no one. However, the reverse is true on the senior respondents. Even the way they explain the specific medicine is totally different. This may beeither the elders are not properly transferring their knowledge or the young generations are not willing to receive the knowledge.

Plant habit and part used

The plant habit of the medicinal plants in the study area is dominated by herbs and this result is in agreement with those documented elsewhere in Ethiopia (Yineger et al., 2008; Teklay et al., 2013; Agisho et al., 2014; Getaneh and Girma, 2014). However, this finding is in contrary to the study conducted previously (Belayneh et al., 2012;

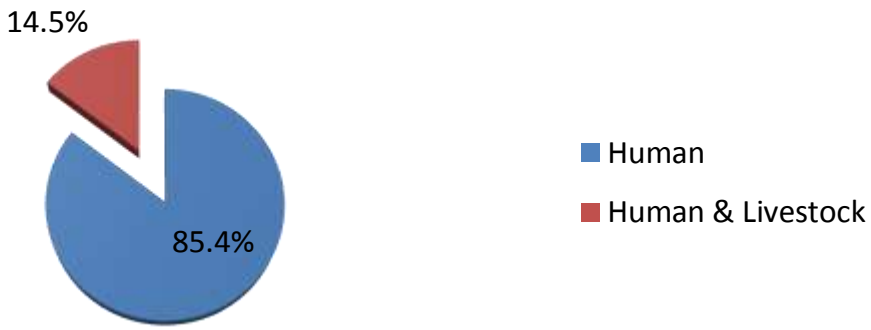

Figure 4. The purpose of the medicinal plants in the area.

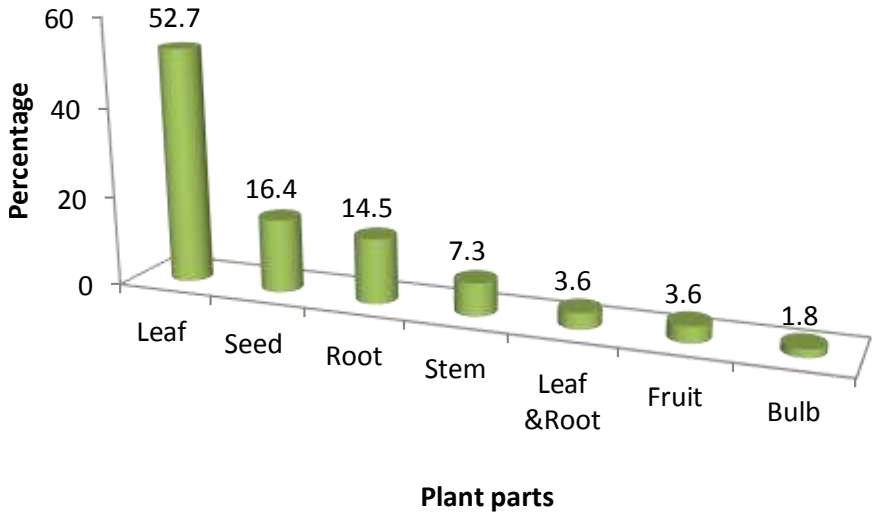

Figure 5. Plant parts used in the area.

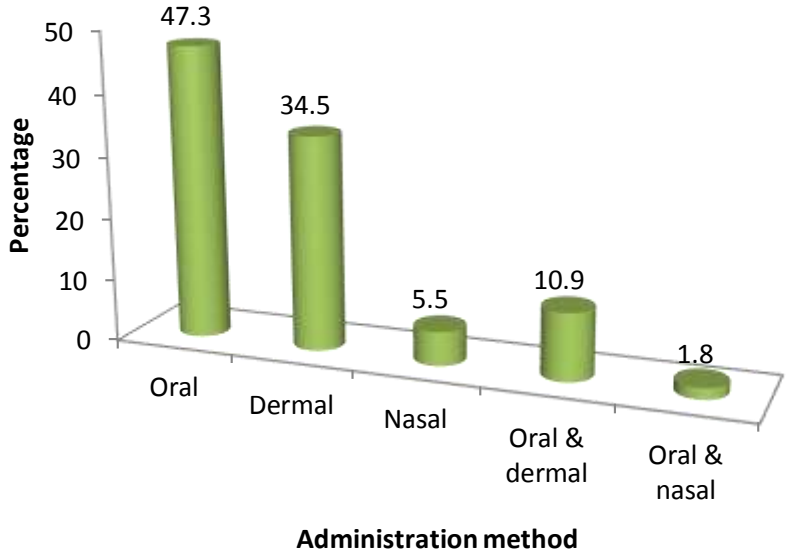

Figure 6. Administration method of the medicinal plants in the area.

Table 1. Taxonomic diversity of the medicinal plants in the study area.

Family	Number of genera	Percentage	Number of species	Percentage of species
Fabaceae	6	12.2	7	12.7
Solanaceae	3	6.1	5	9.1
Asteraceae	3	6.1	4	7.3
Oleaceae	2	4.1	2	3.6
Euphorbiaceae	2	4.1	2	3.6
Rutaceae	2	4.1	2	3.6
Cucurbitaceae	2	4.1	2	3.6
Rosaceae	2	4.1	2	3.6
Other 15 families	27	55.1	29	52.7
Total	49	100	55	100

Table 2. The preparation methods of the traditional medicine in the study area.

Method of preparation	Number of preparations	Percentage
Crushing	33	60
Chewing	8	14.5
Boiling	7	12.7
Squeezing	5	9.1
Smoking	1	1.8
Pasting	1	1.8

Bekele and Reddy, 2015) in which shrub is the most commonly used plant habit for medicinal value followed by herbs. Leaf is the most harvested plant part in the study area. This result is in line with the work of other studies (Wondimu et al., 2007; Yirga, 2010c; Mesfin et al., 2013; Regassa, 2013) where as another study by Asefa and Abebe (2014) in Benna Tsemay district of southern region and a study by Limenih et al. (2015) Dega Damot district of Amhara region of the Ethiopia documented that, root is the dominant plant part collected for medicinal purpose which is in disagreement with this study finding. Unlike roots using leaves for medicinal purpose has a very less effect on the survival of the mother plant.

Preparation and administration methods

The majority of the medicines (60%) in the area are prepared through crushing and this report is in line with the study carried out in Fiche town of Oromia region by Enyew et al. (2014). In the preparations of the medicines, different additives like honey, milk, water, coffee and tea will be used to reverse adverse effect of the traditional medicines such as vomiting, itching and diarrhea. The same result was also documented elsewhere in Ethiopia (Yirga, 2010a, b, c). Most of the prepared medicines are administered orally in the area followed by dermal. This may be due to the fact that the local people perceive that the medicines taken through mouth are more effective than the other body parts. The study agrees with work of Limenh et al. (2015). Concerning the dosage of the medicine, the local people use tea cup as a measuring instrument and they simply order the same for most of the patients but pregnant and children. For the children the traditional healers order half of the cup and for the pregnant women they prohibited some of the medicines.

Conclusions

Although the modern medicine is growing from time to time, traditional medicine is still playing a great role in the treatment of different ailments. In the study area, 55 medicinally important plants were recorded indicating that the area is rich in traditional medicine. As the study result indicates, most of the medicinal plants documented are used for the treatment of human ailments. This may be due to the fact that human diseases are more common in the area than livestock diseases. Leaf is the most commonly harvested plant part and this is an opportunity for the plants as leaf has less effect on the mother plants survival compared with root. Majority of the medicinal plants administered through mouth due to the believe medicine swallowed via mouth can easily cure the disease.

Table 3. Medicinal plants used for the treatment of different diseases in and around Robe town.

Scientific name	Local name (Or/Am)	Habit	Dt	Pu	Mp	Am
TavernieraabyssinicaA.Rich.	Dingetegna	S	Abdominal pain (megagna)	R	Crushed root will be drunk	Oral
Commiphoramyrrha	Qumbi	T	Evil eye,poisonous of snake	S	For evil eye smoking the dried part and for snake bite chewing and spraying in the affected area	Oral and dermal
Nigella sativa	Tikurazmud	H	Headache, sudden disease	S	Crushed seed drunk or applied	Oral and dermal
Solanum anguivi Lam.	Yeayitembuay	S	Nose bleeding	L	Squeezed leaf inserted in to the nose	Nasal
Rumexnepalensis	Shabbe	H	Dandruff	L	Crushed and mixed with vaseline and apply	Dermal
Olea europaea	Weyira	T	Wound	L	Oil from leaf pasted on the wound area	Dermal
Garcinia livingstoneiT. Anders	Abuqurxo	S	Teeth ache	S	Chewing	Oral
Embelia schimperiVatke	Hanquu	T	Ascaris	F	Chewing and drinking	Oral
Artemisia abyssinica Sch. Bip. ex A. Rich	Chiqugn	H	Evil eye	L	The crushed leaf to be smell as well as put around	Nasal
Senna petersiana (Bolle) Lock	Udussaliim	S	Magic, animal diarrhea, rabis disease	R	Chewing for human and crushing for animals via mouth	Oral
Calpurnia aurea (Ait.) Benth.	Ceekataa	S	Dandruff, ascaris	L	Dried and crushed mixing with Vaseline and pasted	Dermal
AnthemistigreensisJ.Gay ex A.Rich.	Barfeelii or sifaayii	S	For emergency headache	R	Dried and crushed by mixing with coffee then drink	Oral
Embeliaschimperi Vatke	Enqoqo	S	Koso	F	Crushed and mixed with banana and drunk	Oral
ClausenaanisataWilld. Hook.f. ex Benth.	Ulmayii	S	Snake bite,rabis	L	Crushed leaf will be filtered and used	Oral and dermal
Cocciniaabyssinica (Lam) Cong.	Ancootee	H	TB	R	Crushed and eat as a food	Oral
Cordia africana	Waddessa	T	Spider poison	L	The leaf is crushed and rubbed on the affected area	Dermal
Cucurbita pepo L.	Buqee /dabaaqula	H	Tape worm	Sd	The seed coat will be removed and boiled. Finally eaten with salt	Oral
Lens culinaris	Misir	H	Spider poison	Sd	The healer chew the seed and vomit it on the affected area	Dermal
Allium cepa	Qullubbiiadii	H	Cough,malaria, headache	B	The bulb of garlic is crushed and mixed with butter and eat	Oral and dermal
Nigella sativum L	Absuudaquraach	H	Abdominal pain,cough	Sd	For cough, the seed either chew or boil with milk and drink. For abdominal pain mix withmilk and drink	Oral
Sesamumorientale L.	Saliixa	H	Abdominal pain	Sd	The crushed seed boiled and mixed with water and sugar then drink	Oral
CommicarpussinuatusMeikle	Kantoma	S	Wound, tumor disease	L	The fresh leaf boiled and then rubbed	Dermal

Table 3. Cont'd.

Haginiaabyssinica	Heexoo	T	Ascaris,hookworm,tape worm	Sd	Dried and crushed seed filtered and mixed with water and drink	
Lepidiumsativum L.	Fexo (ፌሙ)	H	Wound	Sd	Crushed seed mixed with water and apply in the wounded area	Dermal
PhytolaccadodecandraL' Her	Andoodee	S	Gonorrhea, liver disease	L	Crushed leaf filtered and eat	Oral
Rutachalepensis L.	Tenadam	H	Stomach ache	L& R	Crush and eat	Oral
Moringastenopetala	Shiferaw	T	Blood pressure	L	Crush and mixed with '***beso***'thendrink	Oral
Nicotianatabacum L.	Tambo	H	Snake bite	L	Crush and drink	Oral
Argemunmexicana	Medafe	H	Wound	S	The milk from the stem applied on the affected part	Dermal
Kalanchoepetitiana A. Rich.	Anchrura	H	Tonsile,wound	L& R	The leaf boiled and apply	Oral &dermal
Solanum incanum	Hiddii	S	Bleeding of the gun and nose	Sd	Crushed and filtered seed is tight in the bleeding area	Dermal
Croton macrostachyus Del.	Bakkanniisaa	T	Ring worm	L	Leaf is crushed and drink	Oral
Ocimumgratissimum L.	Damakassee	S	Fibril illness or mich	L	Crushed leaf is filtered and used	Oral and nasal
IndigoferaspicataForrsk.	Qorichhadhaa	H	Diarrhea, sudden disease	R	Chewing	Oral
CucumisprophetarumL.	Yemidrembuay	H	Hemorrhoids	R	The boiled root apply on the affected area	Dermal
Erythrinabrucei Schwinef.	Waleenaa	H	Skin disease	L	Crushed leaf applied on the affected skin	Dermal
VerbascumsinaiticumBenth.	YaheyaJoro	T	Snake bite	L	chewing	Oral
Senna septemtrionalis (Viv) Irwin &Barneby	Samamakii	S	Stomach cleaning	L	The dry leaf is boiled with water and then before breakfast and after coffee	Oral
Jasminum grandiflorum L.	Tembelel	S	Bleeding via nose	L	The leaf crushed and put on the nose	Dermal
Aloe pubescens	Hammarreesaa	H	Blood clot	R	Crushed root is applied on the bleeding area	Dermal
Eucalyptus globules Labill	Barzafiiadi	T	Cough,mich	L	The leaf is boiled and the smoke	Dermal
Linumusitatissimum	Telba	H	Stomach ache	Sd	Crushed seed will be for drink	Oral
ZehneriascabraSond.	Haregresa	Cl	Chirt(fungus)	L	Squeezed leaf rub on the affected area	Dermal
GaliumaparinoidesForssk	Ashkt	H	Quaqucha (fungus)	L	Squeezed and apply on the affected area	Dermal
Cymbopogon martini (Roxb.) Wats.	Tej-sar	H	Abdominal pain	L	The leaf will be squeezed and drunk	Oral
Datura stramonium	Astenagir	H	Dandruff	L	The crushed leaf pasted on the affected area (head)	Dermal
Zingiberofficinale L.	Jingible	H	Abdominal pain, sudden disease	R	The root will be chew up or mixed with tea and drink	Oral
Vernoniaadoensis	Fereszeng	H	Mich	L	Squeezed leaf mixed with coffee and drink and apply	Oral and dermal
Vernoniaamygdalina	Grawa	T	Worms,vomiting	L	Crushed leaf drunk	Oral
Solanum americanum Miller	Awt (አዋጥ)	H	Almaze, alargic	L	Crushed leaf pasted on affected area	Dermal

Table 3. Cont'd.

Euphorbia candelabrum Kotschy	Adami	T	Cough	S	Smoke	Nasal
Lobelia rhyncopetalum	Gibra	T	STD	L	Crushed leaf for drink	Oral
Chatha edulis (Vahl) Forssk. exEndl.	Chat	S	STD	L	The crushed leaf boiled and drink	Oral
LippiaadoensisHochst. ex Walp	Koseret/ kusaye	S	Forgetting disease	L	The crushed leaf added with tea and drink	Oral
Rhamnusprinoides L' Herit	Gesho	S	Skin disease	L	The leaf mixed with lemon, salt, and *aliumcepa* crushed then apply	Dermal

T=tree; S=shrub; H=herb; Dt=disease treated; PU=parts used; R=root; S=stem; Sd=seed; F=fruit; B=Bulb; L=leaf; MP=method of preparation; AM=administration method.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The researchers are truly thankful for the traditional healers of the study area for their hospitality and dedicated contribution in sharing their accumulated indigenous knowledge to our inquiries on the information about medicinal plants.

REFERENCES

Agisho H, Osie M, Lambore T (2014). Traditional medicinal plantsutilization, management and threats in Hadiya Zone, Ethiopia. J. Med. Plants Stud. 2(2):94-108.

Assefa A, Abebe T (2014). Ethnobotanical Study of Wild Medicinal Trees and Shrubs in Benna Tsemay District, Southern Ethiopia. J. Sci. Dev. 2(1):17-33.

Belayneh A, Asfaw Z, Demissew S, Bussa N (2012). Medicinal plants potential and use by pastoral and agro-pastoral communities in Erer Valley of BabileWereda, Eastern Ethiopia. J. Ethnobiol. Ethnomed. 8:42.

Bekele and Reddy R (2015).Ethnobotanical Study of Medicinal Plants Used to Treat Human Ailments by Guji Oromo Tribes in Abaya District, Borana, Oromia, Ethiopia.UniversalJ. Plan Sci. 3(1):1-8.

Chala B (2012). A retrospective analysis of the results of a five-year (2005–2009) parasitological examination for common intestinal parasites from bale-Robe Health Center, Robe Town, Southeastern Ethiopia. ISRN Parasitology 2012:1-7.

D'Avigdor E, Wohlmuth H, Asfaw Z, Awas T (2014). The current status of knowledge of herbal medicine and medicinal plants in Fiche, Ethiopia. J. Ethnobiol. Ethnomed. 10(1):2-38.

Duressa T, Mohammed A, FeyissaG, TufaL, SirajK (2014). Comparative Analysis of Iodine Concentration in Water, Soil, Cereals and Table Salt of Horaboka, Mio and Besaso Towns of Bale Robe, South East Ethiopia. J. Environ. Pollution Hum. Health 2(1):27-33.

Enyew A, Asfaw Z, Kelbessa E, Nagappan R (2014). Ethnobotanical Study of Traditional Medicinal Plants in and Around Fiche District, Central Ethiopia. Curr. Res. J. Biol. Sci. 6(4):154-167.

Getaneh S, Girma Z (2014). Ethnobotanical study of medicinal plants in Debre Libanoswereda of Ethiopia. Afr. Plant Sci. 8(7):366-379.

Giday M, Asfaw Z, Woldu Z, Teklehaymanot T (2009). Medicinal plant knowledge of the Bench ethnic group of Ethiopia: an ethnobotanical investigation. J. Ethnobiol. Ethnomed. 5(1):34.

Hostettmann K, Marston A (2002). Twenty years of research into medicinal plants: Results and perspectives. Phytochem. Rev. 1:275-285.

Kassaye K, Amberbir A, Getachew B, Mussema Y (2006). A historical overview of traditional medicine practices and policy in Ethiopia. Ethiop. J. Health Dev. 20(2):128-132.

Limenih Y, Umer S, Wolde-Mariam M (2015). Ethnobotanical study on traditional medicinal plants in Dega damot woreda, Amhara region, North Ethiopia. Int. J. Res. Pharm. Chem. 5:258-273.

Mesfin F, Seta T, Assefa A (2014). An Ethnobotanical Study of Medicinal Plants in AmaroWoreda, Ethiopia. Ethnobot. Res. Appl. 12:341-354.

Mesfin K, Tekle G, Tesfay T (2013). ethnobotanical study of traditional medicinal plants used by indigenous people of Gemad District, Northern Ethiopia. J. Med. Plants Stud. 1(4):32-37.

Maryo M, Nemomissa S, Bekele T (2015). An ethnobotanical study of medicinal plants of the Kembatta ethnic group in Enset-based agricultural landscape of KembattaTembaro (KT) Zone, Southern Ethiopia. Asian J. Plant Sci. Res. 5(7):42-61.

Martin GJ (1995). Ethnobotany: Method manual. World wide fund for nature.

Regassa R (2013). Assessment of indigenous knowledge of medicinal plant practice and mode of service delivery in Hawassa city, southern Ethiopia. J. Med. Plants Res. 7(9):517-535.

Teklay A, Abera B, Giday M (2013). An ethnobotanical study of medicinal plants used in Kilte Awulaelo District, Tigray Region of Ethiopia. J. Ethnobiol. Ethnomed. 9:2-23.

Tolossa K, Debela E, Athanasiadou S, Tolera A, Ganga G, Houdijk J (2013). Ethno medicinal study of plants used for treatment of human and livestock ailments by traditional healers in South Omo, Southern Ethiopia. J. Ethnobiol. Ethnomed. 9(1):32.

Tolesa E (2007). Use and conservation of traditional medicinal plants by indigenous people in Gimbiworeda,western Wellega, Ethiopia.M.Sc thesis Addis Ababa University.

Wondimu T, Asfaw Z, Kelbessa E (2007). Ethnobotanical study of medicinal plants around Dheera town. J. Ethnopharmacol. 112(1):152-161.

Yineger H, Kelbessa E, Bekele T, Lulekal E (2008). Plants used in traditional management of human ailments at Bale Mountains National Park, Southeastern Ethiopia. J. Med. Plants Res. 2(6):132-153.

Yirga G (2010a). Assessment of traditional medicinal plants in Endrta District, South-eastern Tigray, Northern Ethiopia. Afr. J. Plant Sci. 4(7):255-260.

Yirga G (2010b). Ethnobotanical Study of Medicinal Plants in and Around Alamata, Southern Tigray, Northern Ethiopia.Curr. Res. J. Biol. Sci., 2(5):338-344.

Yirga G (2010c). Use of traditional medicinal plants by indigenous people in Mekele town, capital city of Tigray regional state of Ethiopia. J. Med. Plant. Res. 4(17):1-5.

Vol. 12(16), pp. 203-208, 25 May, 2018
DOI: 10.5897/JMPR2018.6579
Article Number: 7AE7E1257207
ISSN 1996-0875

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Journal of Medicinal Plants Research

Full Length Research Paper

Phytochemical screening and peroxide value determination of methanolic extract of four traditional medicinal plants from Debre Tabor Town, Ethiopia

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Received 8 March, 2018; Accepted 6 April, 2018

Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing of different disease. Terpenoids, flavonoids, alkaloids, tannins are some of bioactive compounds present in different parts of medicinal plants. Qualitative identification of bioactive compounds and antioxidant activity determination of crude extracts of the leaves of four different medicinal plants (*Cordia africana*Lam, *Croton macrostachyus* Hochst, *Vernonia amygdalina* Del. and *Justicia schimperiana* T.Anders.) based on peroxide value (POV) method were carried out in this study. For the phytochemical screening test, the results confirmed the presence of polyphenols, tannins, alkaloids, steroids and anthraquinons in all plants leave extracts, however flavonoids, glycosides and phlobatannins were absent. In case of peroxide value (POV) determination, the lowest value were recorded in crude extracts of *Cordia africana* Lam leaves (24 meq/kg) and the highest value were observed in *Justica Schimperiana* leaves extracts (101 meq/kg) in the 1st treatment. In the 4th treatment, *Schimperiana* T. Anders had lowest antioxidant activities [highest POV (290 meq/kg)] and *C. Africana* had the highest antioxidant activities [lowest POV (88meq/kg)]. The study also showed that temperature variations had its own influence on peroxide value. As the temperature of the system increased, the peroxide content of sample treated with leave extracts of plants also increased, however the degree of increment vary within different temperature range (30 to 50°C, 50 to 75°C and 75 to 100°C). In all temperatures, the peroxide production of niger seed oil containing plants leave extracts were less than oil free from extracts. The data generally indicates that the POV of plants leave extract showed antioxidant activity due to the presence of some Phytochemical (plyphenols, tannins, glycosides) present in plants leave extracts.

Key words: Medicinal plants, phytochemical, antioxidant, peroxide value (POV).

INTRODUCTION

Plant species still serve as a rich source of many novel biologically active compounds. The interest in phytomedicine and many medicinal plant species are being screened for biological activities (Mohammad, 2015). The World Health Organization (WHO) reported that 80% of the emerging world's population relies on traditional medicine for therapy. During the past decades, the developed world has also witnessed an ascending trend in the utilization of complementary and alternative medicine (CAM), particularly herbal remedies (Manish et al., 2015).Traditional medicinal plants are widely used in different part of the world for curing diseases. For instance, in China, about 30 to 50% of the total medicinal consumptions was obtained from traditional herbal

preparations. In Africa, Ghana, Mali, Nigeria and Zambia, the first line of treatment for 60% of children in Ethiopia up to 80% of the population uses traditional medicine due to the cultural acceptability of healers and local pharmacopeias, the relatively low cost of traditional medicine and difficult access to modern health facilities (Ahmed et al., 2016).

Traditional medicinal plants are rich in bioactive compounds. Medicinal and aromatic plants contain biologically active chemical substances such as saponnines, tannins, essential oils, flavonoids, alkaloids and other chemical compounds, which have curative properties. These chemical organic compounds of different compositions are found as secondary metabolites in one or more of these plants (Ammar et al., 2017). The presence of bioactive compounds in their leaves, root, stem, bark make traditional plants to have antioxidant properties and free radical scavenging activities (Mamta et al., 2013). The antioxidant properties of the plant were measured using different assays; among the methods used was the determination of peroxide value of the plant extract (Zaid et al., 2013). The peroxide value (POV) is the number that expresses in mill equivalents of active oxygen the quantity of peroxide contained in 1000 g of the substance; it provides information regarding the antioxidant activity of substances by measuring the oxidative stability of oil (Wahid et al., 2015; Andina et al., 2017).

Cordia Africana Lam, *Croton macrostachyus* Hochst, *Vernonia amygdalina* Del and *Justicia schimperiana* T. Anders are some of the traditional plants found in Ethiopia and outside the country. These different vegetation types are found in the various agro ecological zones of Ethiopia (Mirutse et al., 2006). The wood lands, Montane vegetation including grasslands and forests and the evergreen scrubs and rocky areas contain those medicinal plants with higher concentrations in the wood lands (Asefa et al., 2014). The research made so far on these plants in Ethiopian has been mostly of producing inventories and checklists; scientifically the antioxidant activities have not been investigated. The objectives of this paper were to investigate the antioxidant activates of the plants leave extract based on the peroxide value (POV) methods and qualitative phytochemical determination of some bioactive compounds found in plant leave extract in the study area.

MATERIALS AND METHODS

Collection of plant materials

The medicinal plants leaves of *C. africana* Lam, *C. macrostachyus* Hochst, *V. amygdalina* Del and *J. schimperiana* T. Anders were identified by botanist Mr. Haileab Zegeye around Debre Tabor town and the leave of these plants were collected, washed properly with tap water and dried at room temperature (23°C) without sun light. The dried plants leaves were powdered by coffee grinder (instruments used to grind the dried leaves) and these powdered leaves were stored in a clean polyethylene bags until extraction was carried out in Debre Tabor University chemistry laboratory.

Extraction procedure

Two hundred milliter methanol were added to 20 g powdered leaves of *C. Africana* Lam, *C. macrostachyus* Hochst, *V. amygdalina* Del and *J. schimperiana* T. Anders in a separate conical flask and shaken with electrical shaker for 48 h. Each solution was filtered by using Whatman number 1 filter paper in a separate conical flask. After separation, each extracts labeled as MCA, MCM, MAA and MJS to represent methanol extract of *C. africana* Lam, *C. macrostachyus* Hochst, *V. amygdalina* Del and *J. schimperiana* T. Anders respectively. Each extracts were kept in cool and shaded place until further experiments were takes place.

Phytochemical screening procedures

Test for phlobatannins

Extract of each plant leave powder were boiled separately with 1% aqueous hydrochloric acid and then deposition of a red precipitate in flask confirms the presence of phlobatannins (Sriyeta et al., 2017).

Test for carotenoids

One gram of each extract sample was mix with 10 ml of chloroform in a separate test tube with vigorously shaking. After the mixture, the extract was filtered with filter paper and 85% sulphuric acid was added. Blue color at the interface was present which indicates the presence of carotenoids (Sriyeta et al., 2017).

Test for quinones

To one millilitre of the extract, 1 ml of concentrated sulphuric acid was added. Formation of red color shows the presence of quinines (Sriyeta et al., 2017).

Test for xanthoproteins

One millilitre each of the various extracts was treated separately with few drops of conc. HNO_3 and NH_3 solution. Formation of reddish orange precipitate indicates the presence of xanthoproteins (Sriyeta et al., 2017).

Test for polyphenols and tannins

Crude extract was mixed with 2 ml of 2% solution of $FeCl_3$. A blue-

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green or blue-black coloration indicated the presence of polyphenols and tannins (Singh et al., 2015; Devi, 2015).

Tests for polyphenols

One millilitre of each crude extract of the sample was mixed with 2 ml of 2% solutions of ferric chloride. The black color indicates the presences polyphenols (Singh et al., 2015).

Tests for tannins

Two millilitres of each crude extracts of the sample was taken in a separate test tube; each sample of solution was stirred with 2 ml of distilled water. Three drops of ferric chloride solutions were added to each sample of solution. The formation of green precipitate was an indication for the presence of tannins (Singh et al., 2015; Devi, 2015).

Tests for glycosides

Two millilitres of each organic extract was dissolved in 2 ml of chloroform and 2 ml of concentrated sulphuric acid was added carefully and shaken gently for each sample of solutions. The reddish brown color indicates the presence of Steroidal ring (that is a glycone portion of glycoside) (Sriyeta et al., 2017).

Test for flavonoids

Three drops of 1% of NH_3 or NaOH solution was added to each of methanol extracts of the plant leaves sample in a test tube. A yellow coloration was observed to confirm the presence of flavonoid compounds (Yusuf et al., 2013).

Tests for saponnins

Two millilitres of each methanol crude extracts was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam indicates the presence of saponnins (Sriyeta et al., 2017).

Tests for steroids

Two millilitres of each methanol crude extracts was mixed with 2 ml of chloroform and 2 ml of sulphuric acid to each sample of solution side wisely. The development of greenish coloration indicates the presence of steroids.

Tests for terpenoides

Two millilitres of each methanol organic extract was dissolved in 2 ml of chloroform in a separate test tube. Each sample solution was dried by hot plate. 2 ml of concentrated sulphuric acid was added to each sample of solution. Each sample of solution was heated for 2 min. Grayish color formation indicates the presence of terpenoides.

Tests for anthaquinone

Two millilitres of each methanol organic extract was mixed with 2 ml of benzene. 3 ml of 1% NH_3 solvent was added to the sample of plant extract. The violet color shows the presence of anthraquinone (Sriyeta et al., 2017).

Tests for alkaloids

Two ml of each of methanol organic extract was stirred with 2 ml of 1% of HCl on four drops of Wagner's reagent. The turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids (Singh et al., 2015).

POV procedure

For peroxide value determination, Niger seed oil (NSO) was used because it is a valued source of edible oil in Ethiopia and contains linolic acid (important chemical for antioxidant research) in it (Gashaw and Getachew, 2014; Getachew, 2014). Six different samples *C. Africana* Lam leave extract and NSO, *C. macrostachyus* Hochst leave extract and NSO, *Vernonia amygdalina* Del leave extract and NSO, *J. schimperiana* T. Anders leave extract and NSO, ascorbic acid (AA) and NSO and Niger seed oil only (control) were prepared for determination of peroxide value. Six of them were placed at room temperature (23°C) for three week using four different treatments (treatment 1, treatment 2, treatment 3 and treatment 4) with five days interval to test their POV. For thermal effect test, some portion were taken from six samples that were placed at room temperature and kept at 30, 50, 75 and 100°C. From each sample, 5 g were taken and added to different 250 ml conical flask. 30 ml of a mixture of glacial acetic acid and chloroform (3:2) were added to each sample. The mixtures were shaken to dissolve, and 0.5 ml of saturated potassium iodide solution were also added to each flask which were placed at room temperature (23°C) and at 30, 50, 75, 100^{o}C and then the mixture were shaken for 1 min. Finally, 30 ml of water were added to each sample and titrated with 0.01 N sodium thiosulfate solutions. After the yellow color disappears, 5 ml of starch solutions were added to each sample to indicate the end of titration. The titrant was added slowly with continuous shaking, until the blue color was discharged. A blank determination was performed under the same condition.

Statistical analysis

All measurements were carried out in triplicate (n=3), and values expressed are the mean of three measurements. Results were analyzed statically by using Excel 2007 and 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 $P < 0.05$. The peroxide value (meq/kg oil) plants leave extracts against the niger seed oil was calculated with equation (1) (Adejumo et al., 2013).

$$\text{POV (meq/kg)} = \frac{(S-B)(C)(1000)}{m} \quad (1)$$

Where, S = volume of titrant ($Na_2S_2O_3$) needed for sample, B = volume of titrant ($Na_2S_2O_3$) needed for blank, C =Concentration of titrant ($Na_2S_2O_3$), and m = mass of plant leave extract.

RESULTS AND DISCUSSION

Preliminary phytochemical test

Preliminary qualitative phytochemical analysis of methanolic extract of the plants leaves showed the presence of different groups of secondary metabolites. In all tested plants leaves extracts, polyphenol, tannins, steroids, anthraquinons were present; however

Table 1. Results of phytochemical test of four different plants leave extracts (methanolic).

Phytochemicals	Types of medicinal plants			
	***Cordia africana*Lam**	***Croton macrostachyus* Hochst**	***Vernonia amygdalina* Del.**	***Justicia schimperiana* T. Anders**
Polyphenol	+	++	++	++
Tannins	++	++	++	++
Glycosids	-	++	-	-
Flavonoids	-	-	-	-
Terpenoids	-	-	-	++
Alkaloids	+	+	-	-
Saponnins	++	-	-	-
Steroids	++	++	+	+
Anthraquinons	++	+	+	++
Phlobatannins	-	-	-	-
Carotenoids	-	+	-	+
Quinones	++	-	-	_
Xanthoproteins	-	++	+	++

Key ++ = strong, + = medium, – = absent.

flavonoids and phlobatannins were absent. Quinones were detected only in *C. Africana* Lam leave extracts and glycosides were present only in *C. macrostachyus* Hochst leave extracts. Compared to all other leave extracts, the leaves of *V. amygdalina* Del. contained few secondary metabolites as showed in Table 1 above.

Peroxide value (POV) determination

Peroxide value is widely used to measure the primary lipid oxidation formed in fats and oils during oxidation (Wsowicz et al., 2014). The peroxide value of blank Niger seed oil sample was 105 meq/kg in the first treatment. It was increased to 295 meq/kg at the end of the treatment. These changes significantly indicate the noticeable phenomenon of lipid oxidation. Peroxide values of Niger seed oil containing MJS were found to be 101 meq/kg in the first treatment; it was 292 meq/kg at the completion of treatment. Investigations in the case of methanol extract of *C. Africana* Lam containing Niger seed oil samples expressed the peroxide value which increased from 24 meq/kg (first treatment) to 88 meq/kg (last treatment). POV of Niger seed oil containing *V. amygdalina* Del. changed from 72 to 152 meq/kg and the peroxide value of Niger seed oil that contain *C. macrostachyus* Hochst varied from 89 to 212 meq/kg.

There was increase in peroxide values (POV) of Niger seed oil containing MCA, MCM, MVA and MJS during all treatment. The increment between each treatment was highest in MJS and lowest in MCA (Figure 1). This shows that there was a variation in increasing of primary products (peroxides) and secondary products (aldehydes and ketones) in each plant leave extracts (Monika et al., 2014).

According to the graph and analyzed data, the POV of control was significantly higher, than the POV of almost all other treatments. Treatment containing MJS was significantly higher in POV than the treatment containing MCA, MCM, MAA and AA. Treatment containing AA maintained a significantly lower POV than almost all other in the first and second treatments. Treatment containing *C. Africana* Lam maintained the lowest POV for third and fourth treatment (Figure 1).

The above observations show that oxidation of Niger seed oil was highly hindered by the treatment containing AA and MCA, and oxidation of niger seed oil least hindered by the treatment containing MJS. According to these POV value, AA and MCA had the highest antioxidant activity and the least antioxidant activities were recorded in MJS (highest peroxide value).

The POV variations were statistically significant between AA, MCA, MJS, MVA and MCM in each treatment; however there were no significant variations between control and MJS and AA and MCA. The POV variation between niger seed oil containing AA and MCA and niger seed oil containing MJS extracts and control were very small. These showed that the antioxidant activities of AA and *C. Africana* Lam had almost identical (the highest value) and MJS had almost the same antioxidant power with control (lowest value).

The study also showed that temperature variations had its own influence on peroxide value. As the temperature of the system increased, the peroxide content of sample treated with contain MJS, MCA, MVA, MCM, AA and control also increased, however the degree of increment varied within different temperature range (30 to 50°C, 50 to 75°C and 75 to 100°C). The peroxide value difference between 50 to 75°C was the highest and the lowest peroxide value difference was recorded between 75 to

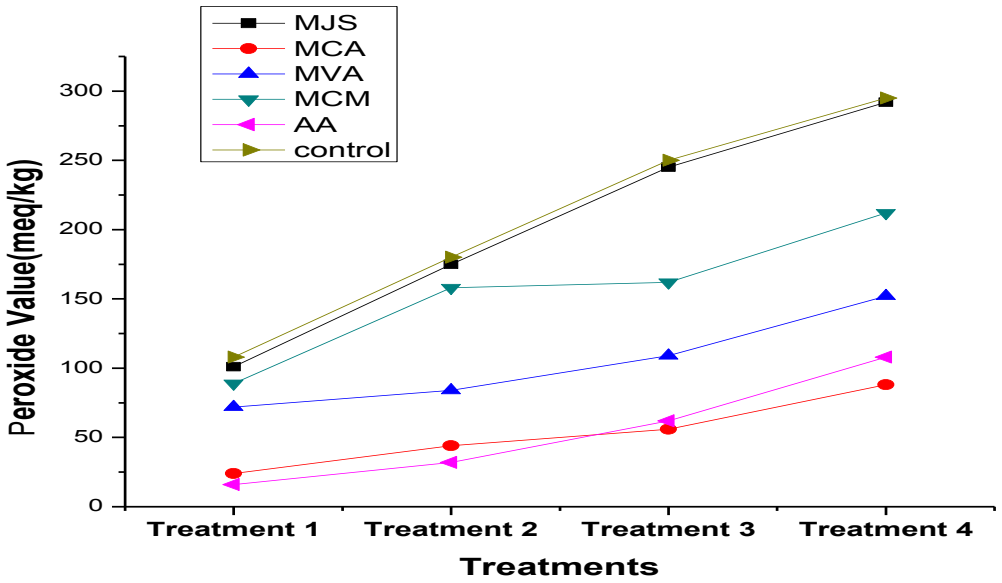

Figure 1. Changes in peroxide value (meq/kg) during storage at room temperature (23°C) for three week.

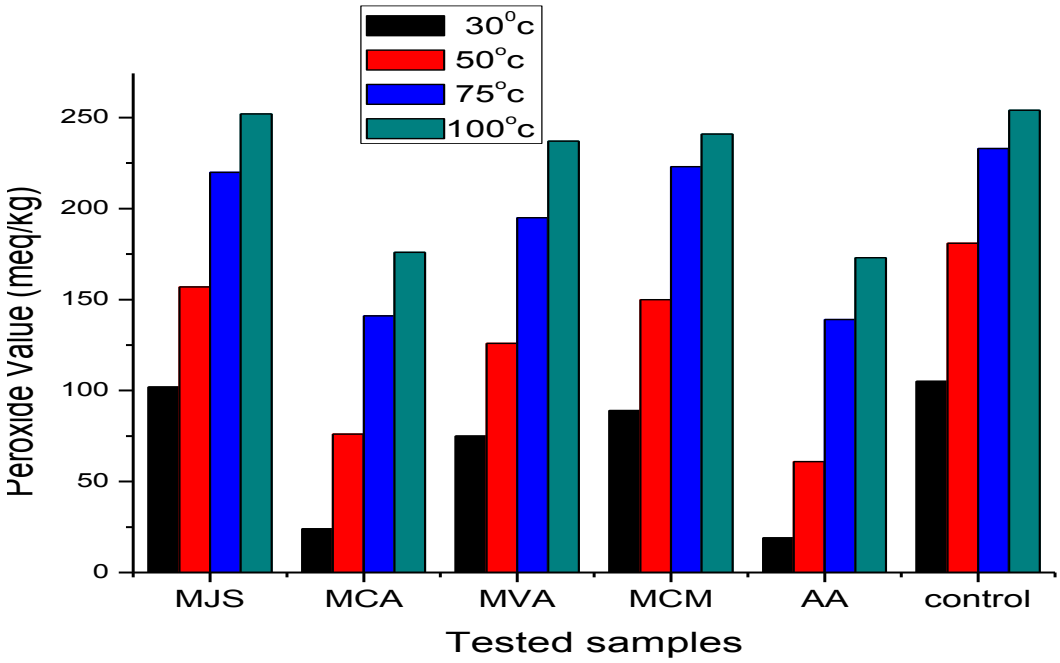

Figure 2. POV Comparison between different Levels of heat on methanolic extracts of leave of four traditional medicinal plans.

100°C temperature ranges (Figure 2). This showed that the increment of temperature above 75°C had its own negative impact on antioxidant activities of the plants leave extracts. Treatment containing *C. africana* Lam and ascorbic acid had the lowest POV than other treatments at all tested temperature. The lowest peroxide values were recorded at 30°C and the highest peroxide value were obtained at 100°C in all treatment. At all temperatures tested, the peroxide production of Niger seed oil containing MJS, MCA, MVA, MCM and AA was

less than oil free from extracts. The present study also shows that increasing temperature accelerates the oxidation rate of Niger seed oil and decreases the antioxidant properties of the extracts.

Conclusion

Phytochemicals present in leave extracts of *C. africana*, *V. amygdalina*, *J. schimperiana*, and *C. maroactachyus* indicates their potential as a source of antioxidant. The distribution and content of phytochemicals differ in medicinal plans. According to the POV value, plants leave extract showed antioxidant activity due to the presence of some phytochemicals, however the antioxidant activities varies from plant to plant and the power of antioxidant influenced by thermal variation. As thermal temperature increase, the antioxidant power of the plants leave extract decrease and this might be because the heat applied would decompose some volatile and less stable metabolites that are used to reduce oxidation. Furthermore, isolation purification and characterization of the phytochemicals present in plants will make interesting studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors are grateful to Debre Tabor University for the support of this research.

REFERENCES

Adejumo B, Inaede G, Adamu T (2013). Effect of moisture content on the yield and characteristics of oil from *moringaoleifera* seeds. Academic Research International, 4(3):160-170.

Ahmed YM, Muhammedawel K, Ashebir D (2016). Knowledge, Attitude and Practice of Community on Traditional Medicine in Jara Town, Bale Zone South East Ethiopia. Science Journal of Public Health, 4(3):241-246.

Ammar A, Naoufal L, Azam B, Dennis G, David A (2017). Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Plants 6(42):1-23.

Andina L, Riyanto S, Rohman A (2017). Determination of peroxide value of red fruit oil by FTIR spectroscopy and multivariate calibration. International Food Research Journal, 24(6):2312-2316.

Asefa A, Worku G (2014). Woody Plant inventory and diversity in traditional agro forestry of selected peasant association of South Gonder Zone, North west Ethiopia. Journal of Environment and Earth Science, 4(15):8-16.

Gashaw A, Getachew T (2014). Cholesterol Content and Free Fatty Acids in Edible Oils and Health Effects: A review. International Journal for Research in Applied Science and Engineering Technology, 2(12):120-125.

Mamta S, Jyoti S, Rajeev N, Dharmendra S , Abhishek G (2013). Phytochemistry of Medicinal Plants. Journal of Pharmacognosy and Phytochemistry, 1(6):168-182.

Manish G, Thein W, Rahul S, Amaluddin b, Jegathambigai R, Ishab K (2015). Marketing trends & future prospects of herbal medicine in the treatment of various disease. World Journal of Pharmaceutical Research, 4(9):132-155.

Mohammad A (2015). Chemistry and antioxidant activity of plants containing some Phenolic compounds. Chemistry International, 1(1):35-52.

Monika S, Victor F, Nurul A, Francisco S, Maria P (2014). The Effect of *Perillafrutescens* Extract on the oxidative stability of model food emulsions. Antioxidants, 3:38-54.

Singh C, Devi M, Thokchom D, Sengupta M (2015). Phytochemical screening, estimation of total phenols, total flavonoids and determination of antioxidant activity in the methanol extract of *Dendrobiumdenudans* D. Don stems. Journal of Pharmacognosy and Phytochemistry, 4(4):6-11.

Sriyeta C, Souvagyalaxmi S, Anjana B, Sangita D (2017). Studies on antimicrobial activity, phytochemical screening tests, biochemical evaluation of clitoreaternatealinn. Plant extracts. International Journal of Research Granthaalayah, 5(10):197-208.

Yusuf AZ, Zakir A, Shemau Z, Abdullahi M, Halima SA (2013).Phytochemical analysis of the methanol leaves extract of *Paullinia pinnata* linn. Journal of Pharmacognosy and Phytotherapy, 6(2):10-16.

Zaid O, Houshia OJ, Abu-Eid M, Zaid M (2013). Palestinian Nabali-Baladi Olive Oil Quality: Premium Ultra Fine Extra Virgin Olive Oil Classification. United States of America Research Journal, 1(2):29-34.

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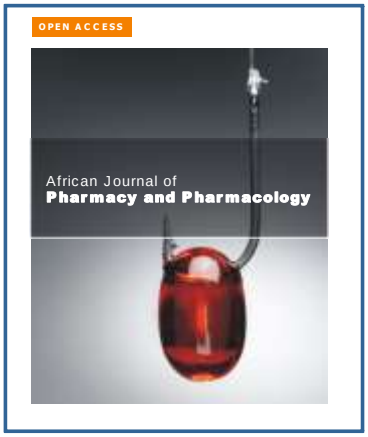

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