

*Full Length Research Paper*

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

**Emtinan A. Alhadi<sup>1</sup>, Omer A. A. Hamdi<sup>1</sup>, Saad M. H. Ayoub<sup>2</sup> and Sakina Yagi<sup>3\*</sup>**

<sup>1</sup>Department of Chemistry, Faculty of Science and Technology, University of Alneelain, P. O. Box11121, Khartoum, Sudan.

<sup>2</sup>Department of Pharmacognosy, Faculty of Pharmacy, University of Medical Sciences and Technology, P. O. Box12810, Khartoum, Sudan.

<sup>3</sup>Department of Botany, Faculty of Science, University of Khartoum, P. O. Box 321, Khartoum, Sudan.

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  $\mu$ l of crude extracts/fractions followed by drying off. DMSO was used as a negative control, while gentamicin (10  $\mu$ g/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  $\mu$ l of extracts at 20 mg/ml followed by drying off. DMSO was used as a negative control, while nystatin (10  $\mu$ g/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

\*Corresponding author. E-mail: sakinayagi@gmail.com.

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  $\mu$ L of  $0.6 \times 10^{-6}$  mol/l DPPH were added to each well containing 70  $\mu$ l 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:

$$\text{DPPH radical scavenging activity (\%)} = 100 - \left[ \frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}) \times 100}{(\text{Abs}_{\text{control}})} \right]$$

where  $\text{Abs}_{\text{sample}}$  is the absorbance of DPPH radical + sample;  $\text{Abs}_{\text{blank}}$  is the absorbance of sample+ 5% DMSO; and  $\text{Abs}_{\text{control}}$  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 microorganisms 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 andersonii* and *Cinnamomum verum* crude extracts and fractions.

Extract/Fraction	Yield (%)*	Saponin	Coumarin	Tannin	Alkaloid	Steroid	Triterpene	Flavonoid	Cardiac glycoside	Anthraquinone
<b><i>Pistacia lentiscus</i></b>										
Leaf										
EtOH (80%)	34.1	+	+	+	-	+	+	+	-	-
PE	7.3	-	+	-	-	+	+	+	-	-
Chloroform	0.4	-	+	-	-	+	+	+	-	-
EtOAc	26.3	-	+	-	-	+	+	+	-	-
BuOH	10.9	-	+	+	-	+	+	+	-	-
H <sub>2</sub> O	55.1	-	+	+	-	+	+	+	-	-
Stem										
EtOH (80%)	24.5	+	+	+	-	+	+	+	-	-
PE	8.4	-	+	-	-	+	+	+	-	-
Chloroform	0.8	-	+	-	-	+	+	+	-	-
EtOAc	21.5	-	+	-	-	+	+	+	-	-
BuOH	18.2	-	+	+	-	+	+	+	-	-
H <sub>2</sub> O	51.1	+	+	+	-	+	+	+	-	-
<b><i>Phyllanthus andersonii</i></b>										
Leaf										
EtOH (80%)	39.1	+	+	+	-	+	+	+	-	-
PE	1.9	-	+	-	-	+	+	+	-	-
Chloroform	0.8	-	+	-	-	+	+	+	-	-
EtOAc	19.5	-	+	-	-	+	+	+	-	-
BuOH	15.1	-	+	+	-	+	+	+	-	-
H <sub>2</sub> O	62.7	+	+	+	-	+	+	+	-	-
Stem										
EtOH (80%)	13.1	+	+	+	-	+	+	+	-	-
PE	18.0	-	+	-	-	+	+	+	-	-
Chloroform	8.8	-	+	-	-	+	+	+	-	-
EtOAc	14.2	-	+	-	+	+	+	+	-	-
BuOH	43.3	-	+	+	+	+	+	+	-	-
H <sub>2</sub> O	15.7	+	+	+		+	+	+	-	-
<b><i>Cinnamomum verum</i></b>										
Leaf										
EtOH (80%)	29.5	+	+	+	-	+	+	+	-	-
PE	29.680	-	+	-	-	+	+	+	-	-

Table 1. Cont'd.

Chloroform	0.467	-	+	-	-	+	+	+	-	-
EtOAc	1.169	-	+	-	-	+	+	+	-	-
BuOH	19.764	-	+	+	-	+	+	+	-	-
H <sub>2</sub> O	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). Flavonoids 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
<b><i>Pistacia lentiscus</i></b>							
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 <sub>2</sub> O	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
	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 <sub>2</sub> O	19 ± 0.12	17 ± 0.44	15 ± 0.27	16 ± 0.33	NA	13 ± 0.17
<b><i>Phyllanthus anderssonii</i></b>							
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 <sub>2</sub> O	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
	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 <sub>2</sub> O	19 ± 0.33	20 ± 0.44	21 ± 0.51	16 ± 0.17	16 ± 0.17	18 ± 0.42
<b><i>Cinnamomum verum</i></b>							
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 <sub>2</sub> O	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 <sub>3</sub>	EtOAc	BuOH	H <sub>2</sub> O
<i>Pistacia lentiscus</i>	Leaf	95	93	93	93	95	80
	Stem	84	90	89	93	95	87
<i>Phyllanthus anderssonii</i>	Leaf	76	50	33	95	61	92
	Stem	93	82	40	94	53	32
<i>Cinnamomum verum</i>	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, Spittler 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, Pappageorgiou 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 Soqatra. *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*. 15<sup>th</sup> Ed. Saunders Publishers, London.
- Tuberoso CIG, Rosa A, Bifulco E, Melis MP, Atzeri A, Pirisi FM, Dessi MA (2010). Chemical composition and antioxidant activities of *Myrtus communis* L. berries extracts. *Food Chemistry*, 123(4):1242-1251.