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ANTIBACTERIAL ACTIVITY OF EXTRACTS FROM *MYRTUS COMMUNIS* L. (ADES) AND *DODONEAE ANGUSTIFOLIA* L.F. (KITKITA) USING BIOAUTOGRAPHY METHOD

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ABSTRACT: The increasing prevalence of antibacterial drug resistant organisms in our globe and high prevalence of infectious diseases in developing countries has led to new efforts in the search of bioactive compounds from complex chemical composition of plant extracts. A bioautographic agar overlay assay using Staphylococcus aureous as the indicator organism for the detection of antimicrobial compounds from ten extracts of *Myrtus communis* L. and *Dodoneae angustifolia* L was analyzed. Hexane, dichloromethane, acetone, methanol and water solvents are used as extractant and ethyl acetate: methanol: water, chloroform: ethyl acetate: acetic acid and benzene: ethanol: ammonia solvent systems were used to separate the components from all the extract of *Myrtus communis* L. and *Dodoneae angustifolia* L. Our results indicated that the extracts of *Myrtus communis* L.f. and *Dodoneae angustifolia* L had bioactive constituents responsible for their antibacterial potentials. Water solvents extracted small number of antibacterial compounds from both plants, followed by hexane extractant; while dichloromethane, acetone and methanol extractant shared similarities in bioactive compounds on bioautograms, and extracted the highest number of antibacterial compounds with variety of polarities. Chloroform: ethyl acetate: acetic acid solvent system separated the largest number of biologically active components in all extractants. As a high number of antibacterially active compounds were found in *M. communis* and *D. angustifolia* extracts of dichloromethane, acetone and methanol, we recommend assay guided fractionation, isolation and dosage formulation of these antibacterial compounds from these plants for clinical applications.

Key words/phrases: Antibacterial, Bioactive-compound, Bioautography, *Dodonaea angustifolia*, *Myrtus communis*

INTRODUCTION

Throughout recorded history, mankind has always faced the threat of

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infectious diseases. It was the major causes of mortality prior to the modern era of antimicrobial chemotherapy, and even today, infectious diseases worldwide account for over one-third of all deaths (NIC, 2000). In developing countries, more than one-half of all deaths annually were reported to be due to infectious diseases alone and Ethiopia is not exceptional (NIC, 2000; WWI, 2005). Even though antimicrobial agents were used to manage infectious diseases for many decades, in recent years, man is facing challenges with controlling and treating of these diseases due to the fact that resistance has developed to almost every group of antibiotics (Fish and Ohlinger, 2006). It is true that because of this antibiotic resistance many people are dying from previously curable infectious diseases (Fish and Ohlinger, 2006). The challenges due to high prevalence and antimicrobial resistance of infectious diseases necessitate many researchers to continuously search for novel antimicrobial drugs for the treatment of infectious diseases *(*Atta-ur-Rahman *et al*., 2001).

Traditional medicine is an important source of alternative medicine. Natural products especially, medicinal herbs are the source of inspiration for researchers in their search of novel compounds for the development of new antibacterials because they contain complex chemical constituents of therapeutic values (Cowan, 1999; Rios and Recio, 2005). Many of commercially used modern antimicrobial drugs were initially used in crude form in traditional practices. It is estimated that today, plant materials have supplied approximately more than one quarter of prescribed drugs for the treatment of human diseases (Cowan, 1999; Rios and Recio, 2005). However, this is only a few contribution for medicine as the traditionally used medicinal plants are thought to be store houses of complex undiscovered biologically active compounds having antimicrobial potentials (Leeds *et al*., 2006; Kaufman *et al*., 1999; Singh and Barrett, 2006; Cseke *et al*., 2006).

In Ethiopia, natural products, have been the basis of treatment of human diseases since time immemorial (Dawit Abebe and Ahadu Ayehu, 1993). As elsewhere in the world; the majority of Ethiopian population are dependent on medicinal plants for the treatment of infectious diseases (Dawit Abebe and Ahadu Ayehu, 1993). The country is rich in natural products having flora that is estimated to contain around 7000 species of higher plants and more than 800 are estimated to have therapeutic values (Dawit Abebe and Ahadu Ayehu, 1993; Sebsebe Demissew, 1993). However, the country is unable to get medical and economic advantage from these natural products and has been facing infectious diseases in the presence of therapeutic natural

products; while expending a huge amount of money annually for antimicrobial agents to treat infectious diseases.

Myrtus communis L. and *Dodonaea angustifolia* L.f. are used in folk healing practices for the treatment of infectious disease (Dawit Abebe and Ahadu Ayehu, 1993). Various studies have reported the antimicrobial effects of these plants (Alem *et al*., 2008; Amabeoku *et al*., 2001; Appendino *et al*., 2006; Khurram *et al.*, 2009; Negero Gemeda *et al*., 2008; Thring *et al*., 2007). However, the complex chemical composition of the extracts of such medicinal plants made the isolation, standardization and dosage formulation of the antimicrobial compounds difficult (Belachew Desta, 1993; Aberra Geyid *et al.*, 2005). Nevertheless, the use of bioautography agar overlay bioassay allows the detection of bioactive components in crude plants by allowing the combination of bioassay *in situ* and localization of antimicrobially active compounds in a complex chemical matrix of plant extracts (Rohalison *et al*., 1991; Valgas *et al*., 2007). Thus the present study was conducted to investigate antibacterially active components from the extracts of *M. communis* and *D. angustifolia* by bioautography assay; to introduce this method for the *in vitro* search of biologically active components from complex chemical composition of the medicinal plants.

MATERIALS AND METHODS

Plant collection

Fresh leaves of *Myrtus communis* L. and *Dodonaea angustifolia* L.f. were collected from North Shewa and Bale Zone, Ethiopia on September, 2008. Authentication of the plants identity was done in the Department of Drug Research, Ethiopian Health and Nutrition Research Institute, Ethiopia. The voucher specimens were deposited in the Herbarium of Department of Drug Research.

Plant extraction

Leaves of plants were dried in the shade and grounded to a fine powder. Samples from each plant were individually extracted by weighing five aliquots of 1g of finely ground plant material and extracting with 10ml of hexane, dichloromethane, acetone, methanol and water separately in centrifuge tubes. These were vigorously shaken and then centrifuged at 3500 rpm for 10 minutes; the supernatant was decanted into labeled containers. The procedure was repeated three times (Eloff, 1999). The solvent was removed under a cold-air stream and a different yield of dried extract was obtained. The crude dry extract was weighed and re-dissolved in

acetone to yield a solution containing 20mg of crude extract per milliliter of extractants. Fresh plant extracts were prepared for each experiment.

Microorganisms and growth conditions

The test organism used in this study was *Staphylococcus aureus* (American Type Culture Collection ATCC 25923) as a representative organism for gram positive bacteria. The organism was grown in nutrient broth (Oxoid Ltd., UK) at 37^0C and maintained on nutrient agar (Oxoid Ltd., UK) slants at 4^0C.

Phytochemical analysis

Preliminary phytochemical screening was also conducted to identify various secondary metabolites in *M. communis* and *D. angustifolia* plant materials. Chemical tests for tannin, saponins, flavonoids, phenolic glycosides, chromophers, free anthra-quinones, anthraquinone glycosides, alkaloid and poly phenols were carried out on the powdered specimens, aqueous and alcoholic extract using standard procedures adopted in our phytochemicstry laboratory to identify plants secondary metabolite constituents (Asfaw Debella, 2002).

Normal phase aluminum backed silica gel G60 F254 plates (10cm X 10cm) were used to develop chromatogram in order to separate the components of plant extracts. Each chromatogram was loaded with 5µL of 20mg/ml of solutions having the spot size of 4mm and developed in duplicate (with one plate intended for bioautography and another for reference chromatogram) simultaneously in a saturated, twin trough developing tank to minimize variation in chromatographic conditions. Ethylacetate: methanol: water (8:1:1) (polar/neutral); chloroform: ethylacetate: acetic acid (5:4:1) (intermediate polar/acidic); benzene: ethanol: ammonia (9:1:0.1) (non-polar basic) solvent system were used for the separation of chemical constituents. The UV active absorbing spots were detected at 366nm wave length on the reference chromatogram, which was finally stained by vanillin-sulfuric acid (0.1g vanillin: 28ml methanol: 1ml sulfuric acid) in order to detect the separated chemical component of each extract. The sprayed TLC chromatogram was compared with the bioautogram for the identification of active bands or compounds.

Bioautography overlays

After the chromatograms were dried for the complete removal of solvents that were used in the solvent system; the chromatogram developed for bioautogram in similar condition with the reference chromatogram were

placed in plate with cover and exposed to UV light for 30min. One milliliter of (10^6 CFU/ml of *S. aureus* (ATCC 29213) overnight nutrient broth culture was used for every 10 ml of molten Mueller–Hinton agar (Oxoid Ltd., UK). Culture was added to 42^0C Mueller–Hinton agar (Oxoid Ltd., UK), mixed and poured over the chromatograms as a thin layer. After solidification of the medium, the overlayed plates were incubated overnight at 37^0C in a 100% humid environment. Subsequently after 20 h incubation, bioautogram was sprayed with an aqueous solution of 2, 3, 5-triphenyltetrazolium chloride (TTC) and incubated at 37^0C for 4 h. Plates were then examined for the presence of zone of inhibition of bacterial growth that could be seen around the active chromatogram spot representing separated compound. White areas that were produced due to the absence of reduction of methyl tetrazolium chloride to the colored formazan that happens in the presence of bacterium, indicated the presence of active antibacterial compounds that inhibited the growth of *Staphylococcus aureus* (Rohalison *et al*., 1991; Valgas *et al*., 2007).

RESULTS

Table 1 shows the results of qualitative chemical screening that of secondary methabolites in the *M. communis* and *D. angustifolia*. As it is clearly noted *M. communis* tested positive for chromophers, free anthraquinones, flavonoids, tannins, polyphenols, and quaternary alkaloids; while *D. angustifolia* tested positive for chromophers, flavonoids, tannins, polyphenols, and quaternary alkaloids secondary metabolites.

Table 1. Chemical Screening of *Myrtus communis* and *Dodoneae angustifolia.*

Plants	S	Ph	C	F	T	Fa	An	Pp	A
M. communis	-	-	+	+	+	+	-	+	+
D. angustifolia	-	-	+	+	+	-	-	+	+

a-, test negative; +, test positive; S, saponins; Ph, phenolic glysides; C, chromophors; Fa, flavonoids; T, tannins; Fa, free anthraquinones; An, anthraquinone glycosides; Pp, poly phenols; A, alkaloids.

Thin layer chromatography finger print was used for the better screening of antibacterial activity of *M. communis* and *D. angustifolia* extracts by different extractant and solvent systems (Fig. 1). Out of the five extractants used for the extraction, the smallest numbers of compounds from the plant materials were extracted by water solvents followed by hexane solvent while the other extractants shared many similarities in number of compounds extracted from both plants. As it can be clearly seen from this figure, the highest polar and the highest non polar solvents extracted the

least number of compounds in all the solvent systems used for the separation. Out of the solvent systems used, benzene: ethanol: ammonia solvent system separated the highest number of the compounds from the plant extracts (Fig. 1).

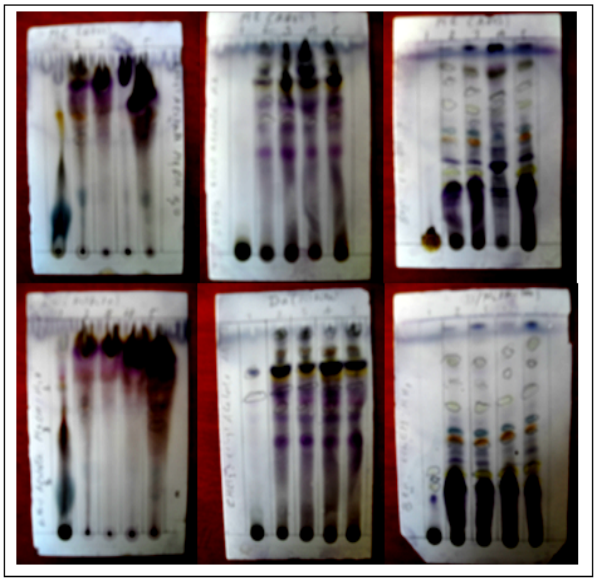

Fig. 1. Chromatogram of *Myrtus communis* (Top) and *Dodoneae angustifolia* (Bottom) developed by Ethyl acetate: Methanol: Water, Chloroform: Ethylacetate: Acetic acid and Benzene: Ethanol: ammonia in left to right order and sprayed with vanillin sulfuric acid to indicate compound extracted with aqueous, methanol, dichloromethane, hexane and acetone in left to right order in each chromatogram.

Bioautogram of *M. communis* and *D. angustifolia* showed antibacterial potentials of these plants (Fig. 2). Our bioautogram results revealed that there were differences in number of antibacterial compounds between the extractant and the solvent system used in the study. Benzene: ethanol: ammonia solvent system separated the highest number of compounds; whereas ethylacetate: methanol: water separated the smallest number. But the chloroform: ethylacetate: acetic acid separated the highest number of biologically active compounds followed by benzene: ethanol: ammonia. Water extractant showed active compounds when separated in polar solvent system and it showed activity at the baseline of chromatogram in the intermediate acidic and non-polar basic solvent system, this extractant showed the lowest number of active compounds while the methanol, acetone and dichloromethane showed the highest (Fig. 2).

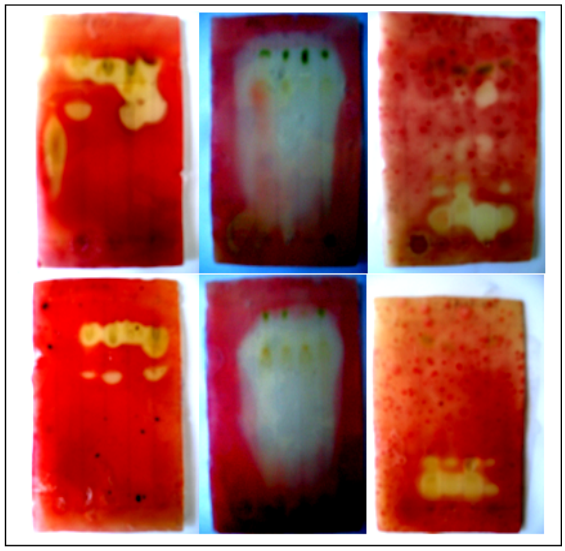

Fig. 2. Bioautogram of *Myrtus communis* (Top) and *Dodoneae angustifolia* (Bottom) separated by Ethyl acetate: Methanol: Water, Chloroform: Ethyl acetate: Acetic acid and Benzene: Ethanol: ammonia solvent system left to right order and overlay with agar containing Staphylococcus aureus (ATCC 25923).

Aqueous, methanol, dichloromethane, hexane and acetone extract of both plants all showed zone of inhibition. Methanol, acetone and dichloromethane extract showed similarity in the three solvent systems. Hexane extractant shared few bioactive compounds with dichloromethane, acetone and methanol extracts. All the extracts of *M. communins* and methanol, dichloromethane and acetone extracts of *D. angustifolia* all showed two active zones in ethylacetate: methanol: water (polar/neutral) solvent system (Table 2a and 3a). Methanol, dichloromethane and acetone extract of *M. communins* and *D. angustifolia* all showed seven different clear spots/zone indicating antibacterial compounds when separated by chloroform: ethylacetate: acetic acid (intermediate polar/acidic) solvent system (Table 2b and 3b). Moreover hexane extracts of *D. angustifolia* similar number of bioactive spots in this solvent system. In non polar basic (benzene: ethanol: ammonia) solvent system the methanol extracts of both plants showed three bioactive compounds, dichloromethane extract of *M. communis* and hexane extracts of *D. angustifolia* indicated highest number of bioactive compound while the aqueous extracts of both plants showed no bioactive compounds (Table 2c and 3c).

Table 2a. Bioautography results from *Myrtus communis* aqueous, methanol, dichloromethane, hexane and acetone extracts developed by Ethyl acetate: Methanol: Water.

Solvent System	Extraction solvent	Rf value	Inhibition size of zone (w x h) mm	Band color under UV 366nm	Visible Band color	Band color after V-S sprayed
Ethyl acetate: Methanol: Water (8:1:1)	Aqueous	0.9125	-	Fluoresce white	NS	Purple
		0.675	2 x 1	Green	Yellow	Orange
		0.4375	10 x 29	Green	NS	Brown P
	Methanol	0.8125	11 x 8	Green	NS	Violet
		0.6875	11 x 7	Green	Yellow	Orange
	Dichloromethane	0.8	11 x 8	Green	NS	Violet
	Hexane	0.825	9 x8	Green	NS	Light Violet
	Acetone	0.825	12 x 15	Green	Green Y	Violet
		0.7625	12 x 10	Green	Yellow	Orange-V
		0.65	15 x 11	Green	Yellow	Orange V

[a]-, no inhibition; h, height; NS, not seen; P, purple; V, violet; V-S, vanillin sulfuric acid; w, width; Y, yellow.

Table 2b. Bioautography results from *Myrtus communis* aqueous, methanol, dichloromethane, hexane and acetone extracts developed by Chloroform: Ethylacetate: Acetic acid.

Solvent System	Extraction solvent	Rf value	Inhibition size of zone (w x h) mm	Band color under UV 366nm	Visible Band color	Band color after V-S sprayed
Chloroform: Ethyl acetate: Acetic acid (5:4:1)	Aqueous	-	-	-	-	-
	Methanol	0.95	6 X 5	Red	Green	Green
		0.8625	11 X 4	Violet	NS	Yellow
		0.8	11x 3	Green	NS	Yellow
		0.7875	6 X 2	Green	Orange G	Violet
		0.7625	4 X 3	Green	NS	Yellow
		0.6875	3 X 3	Light green	NS	Violet
	Dichloromethane	0.925	6 X 6	Red	Green	Green
		0.8625	10 X 4	Violet	NS	Yellow
		0.8	12 x 5	Green	NS	Yellow
		0.79375	8 X 3	Green	Orange-G	Violet
		0.7	4 X 4	Light green	NS	Violet
	Hexane	0.98	7 X 8	Red	Green	Green
		0.8375	10 x 4	NS	NS	Violet
		0.8	7 x 8	Green	NS	Yellow
		0.775	5 x 3	Green	Orange-G	Light gray
		0.75	4 x 4	NS	NS	Yellow
		0.66875	3 x 2	NS	NS	Violet
	Acetone	0.98	8 X 7	Red	Green	Green
		0.875	11 X 5	Violet	NS	Yellow
		0.8375	10 X 5	NS	NS	Violet
		0.825	12 X 5	Green	NS	Yellow
		0.8	7 x 4	Green	Orange-G	Violet
		0.7625	5 x 3	NS	NS	Yellow
		0.69375	3 x 2	Light green	NS	Violet

[a]-, no inhibition; G, green; h, height; NS, not seen; V-S, vanillin sulfuric acid; w, width

Table 2c. Bioautography results from *Myrtus communis* aqueous, methanol, dichloromethane, hexane and acetone extracts developed by Benzene: Ethanol: Ammonia.

Solvent System	Extraction solvent	Rf value	Inhibition size of zone (w x h) mm	Band color under UV 366nm	Visible Band color	Band color after V-S sprayed
Benzene: Ethanol: Ammonia (9:1:0.1)	Aqueous	-	-	NS	NS	NS
	Methanol	0.3	6 X 3	Light red	Orange	Yellow
		0.2875	10 X 5	Dark Green	Orange-Y	Violet-Y
		0.225	12 X 8	Dark Green	NS	Purple
	Dichloromethane	0.85	5 x 4	Red	Green	Yellow
		0.6625	5 x 4	Green Br	NS	Yellow
		0.35625	7 x 6	Light red	Orange	Yellow
		0.3	12 x 7	Dark Green	Orange	Violet-Y
	Hexane	0.83125	9 x 10	Red	Green	Yellow
		0.8375	6 x 7	Light red	Light green	NS
		0.3875	6 x 5	White-Fl	Orange-Y	Violet
		0.2875	10 x 10	Red	Ns	violet
		0.24375	8 x 6	NS	NS	Violet
	Acetone	0.3625	5 x 4	Light red	Orange-Y	Yellow
		0.2875	6 x 9	Dark Green	Orange-Y	Violet-Y
		0.1825	5 x 2	Dark Green	NS	Purple

[a]-, no inhibition; Br, brown; Fl, fluoresce; h, height; NS, not seen; V-S, vanillin sulfuric acid; w, width; Y, yellow

Table 3a. Bioautography results from *Dodoneae angustifolia* aqueous, methanol, dichloromethane, hexane and acetone extracts developed by Ethyl acetate: Methanol: Water.

Solvent System	Extraction solvent	Rf value	Inhibition size of zone (w x h)	Band color under UV 366nm	Visible Band color	Band color after V-S sprayed
Ethyl acetate: Methanol: Water (8:1:1)	Aqueous	0.5125	2 x 5	Green	NS	Purple
	Methanol	0.85	9 x 8	Green	Yellow	Violet
		0.6875	5 x 3	Light Green	NS	Light violet
	Dichloromethane	085	9 x 8	Green	NS	Violet
		0.625	7 x 4	Light Green	NS	Light violet
	Hexane	0.8	8 x 7	Green	NS	Violet
	Acetone	0.7625	10 x 12	Green	Yellow	Violet
		0.625	9 x 5	Light Green	Yellow	Light Violet

[a]-, no inhibition; h, height; NS, not seen; V-S, vanillin sulfuric acid; w, width

Table 3b. Bioautography results from *Dodoneae angustifolia* aqueous, methanol, dichloromethane, hexane and acetone extracts developed by Chloroform: Ethylacetate: Acetic acid..

Solvent System	Extraction solvent	Rf value	Inhibition size of zone (w x h) mm	Band color under UV 366nm	Visible Band color	Band color after V-S sprayed
Chloroform: Ethyl acetate: Acetic acid (5:4:1)	Aqueous	0.75	-	Green	NS	Violet
	Methanol	0.825	5 x 3	Violet	NS	Orange-purple
		0.775	9 x 4	NS	NS	Yellow
		0.75	9 x 2	Green	Orange	Violet
		0.7625	14 x 5	NS	NS	Yellow
		0.6625	7 x 3	Green	NS	Violet
		0.55	6 x 6	NS	NS	Violet
		0.4375	4 x 3	Light green	NS	violet
	Dichloromethane	0.825	10 x 3	Violet	NS	Orange-purple
		0.79375	10 x 4	NS	NS	Yellow
		0.7625	10 x 2	Green	Orange	Violet
		0.71875	14 x 7	NS	NS	Yellow
		0.6875	8 x 3	Green	NS	Violet
		0.5375	5 x 2	NS	NS	Violet
		0.4375	4 x 3	Light green	NS	Violet
	Hexane	0.8375	10 x 2	Violet	NS	Orange-purple
		0.7875	9 x 3	NS	NS	Yellow
		0.7625	10 x 3	Green	Orange	Violet
		0.725	11 x 7	NS	NS	Yellow
		0.6875	8 x 2	Green	NS	Violet
		0.5375	5 x 4	Light green	NS	Violet
		0.4375	3 x 4	Light green	NS	violet
	Acetone	0.825	7 x 2	Violet	NS	Orange-purple
		0.8	9 x 3	NS	NS	Yellow
		0.775	10 x 4	Green	Orange	Violet
		0.75	14 x 5	NS	NS	Yellow
		0.6875	6 x 4	Green	NS	Violet
		0.5	4 x 5	NS	NS	Violet
		0.45	4 x 3	Light green	NS	violet

[a]-, no inhibition; h, height; NS, not seen; V-S, vanillin sulfuric acid; w, width

Table 3c. Bioautography results from *Dodoneae angustifolia* aqueous, methanol, dichloromethane, hexane and acetone extracts developed by Benzene: Ethanol: Ammonia.

Solvent System	Extraction solvent	Rf value	Inhibition size of zone (w x h) mm	Band color under UV 366nm	Visible Band color	Band color after V-S sprayed
Benzene: Ethanol: Ammonia (9:1:0.1)	Aqueous	0.31875	-	Green	NS	Yellow
	Methanol	0.325	8 x 6	Dark Green	Yellow	Yellow
		0.25	12 x 9	Dark Green	Yellow	Yellow-Violet
		0.1	6 x 6	Green	NS	Violet
	Dichloromethane	0.3125	6 x 3	Dark Green	Yellow	Yellow
		0.25	10 x 10	Dark Green	Yellow	Yellow-violet
		0.18125	5 x 3	Light green	NS	Violet
	Hexane	0.2375	8 x 6	NS	NS	Violet
		0.18425	10 x 10	NS	NS	Violet
		0.125	4 x 2	NS	NS	Purple
	Acetone	0.35625	5 x 3	Dark G	Green	Yellow
		0.1875	6 x 6	Light green	Green	NS

[a]-, no inhibition; G, green; h, height; NS, not seen; V-S, vanillin sulfuric acid; w, width

Table 2 and 3 shows the solvent systems used for the development of chromatogram had significant effect on the separation of bioactive components from plant extracts. Chloroform: ethylacetate: acetic acid (intermediate polar/acidic) solvent system separated the highest number of active compounds from both plants with a wide range of retention factor values while the Ethyl acetate: Methanol: Water (polar/neutral) solvent system showed the least number of active compounds followed by Benzene: Ethanol: Ammonia (non-polar basic) solvent system (Table 2a-3c).

The number of inhibition zone (bioactive compounds) extracted from *M. communis* were higher than that of *D. angustifolia* by accounting 51 inhibition to 47 inhibition, respectively. Aqueous extract only showed inhibition zone in the chromatogram separated by Ethyl acetate: Methanol: Water (8:1:1) for both plants (Table 4). Methanol, dichloromethane, acetone solvent extracted the same number of active compounds (7) in both plants. Chloroform: Ethyl acetate: Acetic acid (5:4:1) solvent system separated the larger number of inhibition zone from *D. angustifolia* extracts than *M. communis* extracts by accounting 28 to 27, respectively. As both plants showed many compounds that were activity against the bioautogram of *S. aureus* in almost all extractants; it was necessary to conduct phytochemical screening of the plants.

Table 4. Number of spots that have antibacterial activity on the bioautograms of *Myrtus communis* and *Dodoneae angustifolia* extracts.

Plants	Solvent system	Type of extract						
		Aqueous	Methanol	Dichloromethane	Hexane	Acetone	Subtotal	total
M. communis	E/Me/W	2	2	1	1	3	9	
	C/E/Aa	0	7	7	6	7	27	51
	B/Et/A	0	3	4	5	3	15	
D. angustifolia	E/Me/W	1	2	2	1	2	8	
	C/E/Aa	0	7	7	7	7	28	47
	B/Et/A	0	3	3	3	2	11	
Total		3	24	24	23	24	98	98

[a]A, ammonia; Aa, acetic acid; B, Benzene; C, Chloroform; E, Ethyl acetate; Et, Ethanol; Me, Methanol; W, Water

DISCUSSION

Natural products have been used for purpose of medicine throughout the world for hundreds if not thousands of years. Natural products especially medicinal herbs have a reputation not only in traditional healings; but also in modern medicine as they provided large inputs for the discovery of new drugs (WHO, 2001). Nowadays evaluating herbal medicine as the inspiration for the discovery of novel antibacterial drugs to combat the

problems aroused by infectious diseases and antimicrobial drug resistance are conducted here and there throughout the world by various researchers. Plants are reservoirs of complex chemical constituents that can serve as a store house for antimicrobially active compounds. They contain various medically important secondary metabolites (Sarker *et al*., 2006). The beneficial effects of *M. communis* and *D. angustifolia* have been documented by researchers from the Ethiopian community; thus it has been an advantage to develop drug (Dawit Abebe and Ahadu Ayehu, 1993; Negero Gemeda *et al*., 2008).

Our qualitative chemical screening confirmed the presence of medically important secondary metabolites. In our study, the isolation and characterization chemical constituents of the plant were not performed. However, our results of the preliminary phytochemical screenings conducted in our laboratory showed that the leaf of *M. communis* and *D. angustifolia* contained flavinoids, tannins, polyphenols and quaternary alkaloids for which antimicrobial activity were reported by various studies (Cowan, 1999; Cseke *et al*., 2006). The active compounds shown in the results of bioautography may be due to the presence of these compounds or their derivatives.

Our chromatogram results corroborate that the highest polar and non-polar solvents used for extraction showed the least number of compounds when separated by all the solvent systems used for the separation. This could be due to the solvent affinity of secondary metabolites confirmed present in the plants (Eloff, 1999; Sarker *et al.*, 2006). Polyphenolic compounds such as flavonols, tannins and other phenolic compounds are generally soluble in polar solvents such as methanol, aqueous, acetone and ethanol (Eloff, 1999; Sarker *et al.*, 2006). Our study that used chloroform, dichloromethane and hexane for the extraction of antibacterial compounds that have affinity to the non-polar solvent were in agreement with the recommendation of Ncube and his collaborators (Ncube *et al.*, 2008). Moreover, our finding that indicated antibacterial compounds in the plant materials have differences in their affinity for the polarity and pH of solvent system used for the separation on chromatogram were replicated in the finding of Scott (2003).

Our results from the bioautograms confirmed the antibacterial potentials of *M. communis* and *D. angustifolia* extracts. Moreover the numbers of spots that had antibacterial compounds differed in extractants and solvent systems used in the study. The intermediate polar acidic solvents separated the highest numbers of antibacterial compounds. Our result that indicated the

number of antibacterial compounds separated by acidic intermediate polar solvent system are higher than the other solvent system were in line with the finding of Eloff (2001) where he reported that the number of antibacterial active compounds from *Sclerocarya birrea* Hochst. subsp. caffra (Sond.) Kokwaro) bark and leaves were higher in this solvent system.

Our result confirmed that different extractants have potentials of extracting biologically active principles. All solvents used for extraction of *M. communins* and methanol, dichloromethane and acetone extracts of *D. angustifolia* all showed lowest number of active zones when developed in polar/neutral solvent system. In other solvent system (intermediate polar/acidic); all extractant except the aqueous extract of *D. angustifolia* and methanol, dichloromethane and acetone extract of *M. communins* all showed similar antibacterial compounds. Our finding that indicated differences in extraction potentials of different solvents used for the extractions of *D. angustifolia* and *M. communins* was in agreement with the previous reports (Scott, 2003).

Our study suggested that solvent systems used for the development of chromatogram have significant effects on the number of bioactive components separated from the plant extracts. Intermediate polar/acidic solvent system separated the highest number of active compounds in both plants with a wide range of retention factor values while the polar/neutral solvent system showed the least number of active compounds followed by non-polar/basic solvent system. Our results replicate reports from the short communication by Eloff (2001).

Our bioautography results confirmed that both *M. communis* and *D. angustifolia* have bioactive compounds that have antibacterial potentials. Both plants showed many compounds that were active against *S. aureus* in almost all extractants. Our study result that showed extracts of *M. communis* and *D. angustifolia* have antimicrobial potentials was in agreement with the findings of the previous researches (Alem *et al*., 2008; Negero Gemeda *et al*., 2008; Khurram *et al*., 2009). The number of inhibition zone (bioactive compounds) extracted from *M. communis* are higher than that of *D. angustifolia* by accounting for 51 inhibition to 47 inhibition, respectively. This could be due to differences in the major and minor constituents present in the plants that have reported antibacterial activity (Cowan, 1999; Rios and Recio, 2005; Rotstein *et al*., 1974; Teffo *et al*., 2010).

In conclusion, the data of our study showed that *M. communis* and *D. angustifolia* had activity against the tested organism, which agrees with the

reputation of these plants in the Ethiopian traditional medicine for the treatment of infectious diseases. As the numbers of biologically active compounds seen in bioautogram of both plants were large enough; we recommend that isolation and characterization of the active components have to be done with the help of bioautography activity guided fractionation, isolation and formulation. As far as we know, bioautography method for the evaluation of antimicrobial effects is the first work in Ethiopia and we highly recommend the use of this method for indication, identification and isolation of bioactive components of medicinal plants.

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REFERENCES

Aberra Geyid, Dawit Abebe, Asfaw Debella, Zewdneh Makonnen, Frehiwot Aberra, Frehiwot Teka, Tesfaye Kebede, Kelbessa Urga, Kidist Yersaw, Teklele Biza, Bisrat Haile-Mariam and Mulugeta Guta (2005). Screening of some medicinal plants of Ethiopia for their anti-microbial properties and chemical profiles. *J. Ethnopharmacol.* **97**: 421-427.

Amabeoku, G.J., Eagles, P., Scott, G., Mayeng, I. and Springfield, E. (2001). Analgesic and antipyretic effects of *Dodonaea angustifolia* and *Salvia africana-lutea*. *J. Ethnopharmacol.* **75**:117-124.

Appendino, G., Maxia, L., Bettoni, P., Locatelli, M., Valdivia, C., Ballero, M., Stavri, M., Gibbons, S. and Sterner, O. (2006). Antibacterial galloylated alkylphloroglucinol glucosides from myrtle (*Myrtus communis*). *J. Nat. Prod.* **69**: 251-4.

Asfaw Debella (2002). **Manual for Phytochemical Screening of Medicinal Plants.** Department of Drug Research, Ethiopian Health and Nutrition Research Institute, Ethiopia.

Atta-ur-Rahman, Choudhary, M.I. and Thomson, W.J. (2001). **Bioassay techniques for drug development.** Overseas Publishers Association, Singapore.

Belachew Desta (1993). Ethiopian traditional herbal drugs. Part II. Antimicrobial activity of 63 Medicinal Plants. *J. Ethnopharmacol.* **39**: 129-139.

Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* **12**: 564-582.

Cseke, L.J., Kirakosyan, A., Kaufman, P.B., Warber, S.L., Duke, J.A. and Brielmann, H.L. (2006). **Natural products from plants.** 2nd ed., CRC Press, USA.

Dawit Abebe and Ahadu Ayehu (1993). **Medicinal and Enigmatic Practices of Northern Ethiopia.** Berhanena Selam Printing Enterprise, Addis Ababa.

Eloff, J.N. (1999). Which extractant should be used for the screening and isolation of antimicrobial component from plants? *J. Ethnopharmacol.* **60**: 1-8.

Eloff, J.N. (2001). Antibacterial activity of Marula (*Sclerocarya birrea* (A. rich.) Hochst. subsp. caffra (Sond.) Kokwaro) (Anacardiaceae) bark and leaves. *J. Ethnopharmacol.* **76**: 305-308.

Fish, D.N. and Ohlinger, M.J. (2006). Antimicrobial resistance: Factors and outcomes. *Crit. Care Clin.* **22**: 291-311.

Genetu Alem, Yared Mekonnen, Moges Tiruneh, and Andargachew Mulu (2008). *In vitro* antibacterial activity of crude preparation of myrtle (*Myrtus communis*) on common human pathogens. *Ethiop. Med. J.* **46**: 63-69.

Kaufman, P.B., Cseke, L.J., Warber, S., Duke, J.A. and Brielmann, H.L. (1999**)**. **Natural products from plants.** CRC Press LLC., New York.

Khurram, M., Khan, M.A., Hameed, A., Abbas, N., Qayum, A. and Inayat, H. (2009). Antibacterial activities of *Dodonaea viscosa* using contact bioautography technique. *Molecules* **14**: 1332-41.

Leeds, J.A., Schmitt, E.K. and Krastel, P. (2006). Recent developments in antibacterial drug discovery: Microbe-derived natural products-from collection to the clinic. *Expert. Opin. Investig. Drugs*. **15**: 211-26.

National Intelligence Council (NIC) (2000). The global infectious disease threat and its implications for the United States. National Intelligence Council, USA.

Ncube, N.S., Afolayan, A.J. and Okoh, A.I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. *Afr. J. Biotechnol.* **7**(12): 1797-1806.

Negero Gemeda, Kelbessa Urga, Ashenif Tadele, Hirut Lemma, Daniel Melaku and Kissi Mudie (2008). Antimicrobial activity of topical formulation containing *Eugenia caryophyllata* L. (Krunfud) and *Myritus communis* L. (Ades) essential oils on selected skin disease causing microorganisms. *Ethiop. J. Health Sci.* **18**: 101-107.

Rios, J.L. and Recio, M.C. (2005). Medicinal plants and antimicrobial activity. *J. Ethnopharmacol.* **100**: 80-84.

Rohalison, L., Hamburger, M., Hosttetman, K., Monod, M. and Frank, E. (1991). A bioautography agar overlay method for the detection of antifungal compound from higher plants. *J. Phytochem. Anal.* **2**: 199-203.

Rotstein, A., Lifshitz, A. and Kashman, Y. (1974). Isolation and antibacterial activity of acylphloroglucinols from *Myrtus communis*. *Antimicrob. Agents* **6**: 539-42.

Sarker, S.D., Latif, Z. and Gray, A.I. (2006). **Natural products isolation.** 2nd ed. Humana Press, New Jersey.

Scott, R.P.W. (2003). **Principle and practice of chromatography.** 1st ed. Library4Science Publisher, UK.

Sebsebe Demissew (1993). A description of some essential oil bearing plants in Ethiopia and their indigenous uses. *J. Essent. Oil Res.* **5**: 465-478.

Singh, S.B. and Barrett, J.F. (2006) Empirical antibacterial drug discovery--foundation in natural products. *Biochem. Pharmacol.* **71**: 1006-1015.

Teffo, L., Aderogba, M. and Eloff, J. (2010). Antibacterial and antioxidant activities of four kaempferol methyl ethers isolated from *Dodonaea viscosa* Jacq. var. *angustifolia* leaf extracts. *S. Afr. J. Bot.* **76**: 25-29.

Thring, T.S.A., Springfield, E.P. and Weitz, F.M. (2007). Antimicrobial activities of four plant species from the Southern Overberg Region of South Africa. *Afr. J. Biotechnol.* **6**(15): 1779-1784.

Valgas, C., Souza, S.M., Smania, E.F.A. and Smania, A. (2007). Screening methods to determine antibacterial activity of natural products. *Braz. J. Microbiol.* **38**: 369-380.

World Health Organization (WHO) (2001). **General guidelines for methodologies on research and evaluation of traditional medicine.** WHO, Geneva, Switzerland.

World Watch Institute (WWI) (2005). State of the World 2005 Trends and Facts-Containing Infectious Disease. World Watch Institute; Vision for a Sustainable World. Retrieved from: http://www.worldwatch.org/node/68