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ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF CRUDE EXTRACTS OF MEDICINAL PLANTS GROWN IN EASTERN ETHIOPIA

KESATEBRHAN HAILE ASRESSU

Department of Chemistry, College of Natural and Computational Sciences, Haramaya University, P.O.Box:138, Dire Dawa, Ethiopia

ABSTRACT

The aim of my research was to screen secondary metabolites and antimicrobial activities of methanolic extracts of the leaves *Vernonia amygdalina* and *Plantago lanceolata,* and acetone extracts of the leaves *Rosmarinus officinalis* and *Thymus schimperi* against two bacteria (*Staphylococcus aureus* and *Escherichia coli)* and two fungi *(Aspergillus niger and Fusarium oxysporum)* using paper disc diffusion method. Phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, saponins, steroids, tannins, phenolic compounds, anthraquinones, cardiac glycosides and triterpenes in the plants investigated. The plant extracts were found to be more effective on bacteria than fungi. The bioassay has indicated that the inhibition effect of the extracts was proportional to the concentration. The acetone leaf extracts of *Rosmarinus officinalis* possess higher antibacterial properties than *Vernonia amygdalina, Plantago lanceolata* and *Thymus schimperi.* Phytochemical investigation results showed that the highest antibacterial potential of *Rosmarinus officinalis* may be attributed to flavonoids, phenolic compounds and triterpenes.

KEY WORDS: Phytochemical screening, plant extracts, medicinal plants, antimicrobial activities

KESATEBRHAN HAILE ASRESSU
Department of Chemistry, College of Natural and Computational Sciences, Haramaya University, P.O.Box:138, Dire Dawa, Ethiopia.
E-mail: kesete06@gmail.com Telephone +251- 910649665

Corresponding author

INTRODUCTION

Medicinal plants, since time immemorial, have been used in virtually all cultures as a source of medicine. It has been estimated that about 80-85% of population both in developed and developing countries rely on traditional medicine for their primarily health care needs and it is assumed that a major part of traditional therapy involves the use of plant extracts or their active principles [1]. *Vernonia amygdalina* is locally known as ''grawa'' in Amharic. It is a tropical shrub in the family of *Asteraceae* with 1-3 m in height, petiole leaf of about 6 mm in diameter, and elliptic in shape. The leaves are dark green coloured with a characteristic odour, sweet taste and a bitter taste.[2] The bitter taste of *Vernonia amygdalina (V.amygdalina)* was suspected as a guide to choose for the appropriate plant, plant part and amount of intake [3]. The species is common in most West African and Central African countries. *V.amygdalina* stimulates the digestive system and helps in reduction of fever, to treat hiccups and kidney disease. In Nigeria, it is also used in making beer, as a remedy against high blood pressure and traditionally used in post-harvest protection [4]. *V.amygdalina* is widely eaten as a vegetable after crushing and washing thoroughly to remove the bitterness and has been confirmed to have antibacterial, antifungal, nematicidal, antiplasmodial and insecticidal properties[1]. *Plantago lanceolata* is one of the East Asia plantaginceae family. The genus *Plantago lanceolata* (*P.lanceolata)* comprises 265 species and has cosmopolitan distribution [5]. *Plantago* L. is an adaptable perennial weed, indigenous to Britain but now naturalized all over the world. This plant is locally termed as "gurteb" in Amharic and has got application for many skin disorders in Ethiopian traditional medicine. Medicinally, it is astringents, demulcents, emollients, expectorants, cough, gastrointestinal disorder, diarrhoea, diuretics, antibacterials and antivirals [6, 7]. *Plantago* L. is an adaptable perennial weed, indigenous to Britain but now naturalized all over the world. It is apernial herb growing on slopes and sand hills near the sea in Japan, Korea, Sakhalin, Kurils, Kamhatka, Commandor islands and in the region of Ussuri and Amer [8]. *Rosmarinus officinalis* L**.** commonly called rosemary in English and "Siga metbesha" in Amharic. Rosemary is a member of the mint family Lamiaceae, which also includes many other herbs. It is an attractive evergreen shrub with pine needle-like leaves. It is native to the Mediterranean countries [9], reaching a height of 1.5 m. The main producers are Italy, Dalmatia, Spain, Greece, Turkey, France, Portugal, Ethiopia and North Africa (Morocco, Tunisia, and Egypt) [10]. Traditionally, rosemary has been used by herbalists to improve memory, relax muscles, relieve muscle pain and spasm, stimulate hair growth, wedding ornaments, support the circulatory and nervous systems, in the prevention of cancer and its antibacterial properties [11]. *Thymus schimperi* (Lameaceae, called *Tossign* in Amharic) is widely used in Ethiopian cooking. *Thymus schimperi (T.schimperi)* is endemic to the Ethiopian highlands growing on edges of roads, in open grassland, on bare rocks and on slopes, between 2200-4000 m altitudes [12]. It is comparatively widespread in central, eastern and northern Ethiopia. *T.schimperi is* a small perennial herb, woody at the base and 5 to 40 cm high. It is used medicinally and as culinary herbs [12]. A tea made by the herb in water has been recommended as a medicinal remedy for respiratory problems, gastrointestinal disorders and liver disease [13]. The dried leaves of *T. schimperi* are also used in traditional medicine for the treatment of headache, gonorrhea, inflammation, spasm, thrombosis, urinary retention, mental illness, eye disease, toothache, stomachache, earache, liver disease, gonorrhea, leprosy, lung TB, acne and ascaris [14]. A knowledge of the chemical constituents of plants is desirable, not only for the discover of therapeutic agents, but also because such information may be of value in disclose new sources of such economic materials as tannins, oils, gums and precursors

for the synthesis of complex chemical substances. Such phytochemical screening of various plants is reported by many workers [15, 16, 17]. At present, the demand for these medicinal plants is increasing due to their use in traditional medicine, pharmaceutical industries, cosmetic fields and agribusiness, and for the quality of their essential oil [18]. However, there was no previous research work on phytochemical screening and antimicrobial activities of solvent extracts of these medicinal plants grown in Eastern Ethiopia. Therefore, in this study, preliminary phytochemical analysis and bioassay test were carried out in 4 plants of Eastern region of Ethiopia.

MATERIALS AND METHODS

Collection of Plant Materials and Extraction Procedure

Fresh leaves of *V.amygdalina* and *P.lanceolata* were collected from the area where they grow in Haramaya University in March, 2012. The fresh leaves of *R.officinalis* and *T. schimperi* were purchased from the local market of Harar town in February, 2012. The plant materials (leaves) were identified and authenticated at the Herbarium of Plant Sciences Department, Haramaya University. The leaves were sort to remove the dead ones, washed with distilled water without squeezing to remove debris and dust particles and then air-dried at room temperature (16 °C) for one week. The air-dried leaves were chopped into small pieces and finally milled into a uniform powder with the aid of mortal and weighed [1, 19, 20]. A 100 g of dry powder leaves of *V. amygdalina* and *P.lanceolata* were soaked in 500 mL of pure methanol (99.8%, HPLC grade, Northampton, U.K)) at room temperature for 48 hrs up on shaking and stirring three times per 24 hrs. Similarly, the acetone extracts were prepared by soaking 100 g of fine powder of *R.officinalis* and *T.schimperi* leaves in 500 mL of pure acetone (99.7%, ACS grade, Northampton, U.K) separately at room temperature for 48 hrs up on shaking and stirring three times per 24 hrs. The extracts were filtered after 48 hrs through a Whatmann filter paper No.1 [21]. They were concentrated using a rotary evaporator under reduced pressure and at a maximum temperature of 35 °C. The yields were calculated and preserved at 5 °C refrigerator in airtight bottles for phytochemical and antimicrobial screening.

Screening of Antimicrobial Activity

Methanol Crude Extracts (MCE) and Acetone Crude Extracts (ACE) of the plant leaves were evaluated in vitro for antimicrobial activity by using the paper disc diffusion method against gram positive bacterium *(Staphylococcus aureus* (*S.aureus*)) and gram negative bacterium (*Escherichia coli* (*E.coli*)) and two fungi, *Aspergillus niger (A. niger)* and *Fusarium oxysporum (F. oxysporum)* [22]. The bacterial cultures were inoculated into the Muller Hinton Agar (MHA) (and incubated at 37 ^{o}C). Fungal cultures were inoculated into Potato Dextrose Agar (PDA) and incubated at 27 ^{o}C. The bacteria were obtained from plant pathology laboratory of the school of plant sciences, Haramaya University. *A.niger* and *F.oxysporum* were obtained from infected ground nut and infected maize, respectively. Chloroamphenicol (CAL) was used as standard drug against bacteria where as bavistin was used against fungi. Approximately, 10 mL of sterile MHA (for bacteria) and PDA (for fungi) were poured into sterile culture plates and allowed to set wells of about 6 mm in diameter which were punched on the plates. Standard solution of 20 and 40 mg/mL concentration of the extracts were prepared and 10 and 20 µL solution from each concentration were loaded to the discs in three replications. The plates were incubated at 37 ^{o}C (for bacteria) and 27 ^{o}C (for fungi). The antifungal and antibacterial activity of the plant extracts were evaluated by measuring the zone of inhibition against the test organism after 24 hrs [23].

Preliminary Phytochemical Screening of Crude Solvent Extracts

Standard screening test of the extracts was carried out for various plant constituents. The MCE and ACE was screened for the presence

or absence of secondary metabolites such as triterpenes, alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins, anthraquinones and cardiac glycosides using standard procedures [21, 23].

RESULTS AND DISCUSSION

Percent Yield of Plant Extracts

The yields of MCE of *V.amygdalina* and *P.lanceolata* leaves were 9.35% and 14.1% (w/w), respectively. In the preparation of ACE from the dried leave of *R.officinalis* and *T. schimperi* , a yield of 2.63% and 5.44% (w/w) was obtained, respectively.

Phytochemical Screening

Isolation of pure, pharmacologically active constituents from plants remains to be a long and tedious process. Several methods could be used for the identification, characterization and screening of the active components of plant extracts. One such technique is chemical screening that will allow localization and targeted isolation of constituents with potential activities. This procedure enables recognition of known metabolites in extracts at the earliest stages of separation and is thus economically very important. Phytochemical screening was done using colour forming and precipitating chemical reagents on the dried leaves of *V.amygdalina*, *P.lanceolata, R.officinalis* and *T.schimperi* to generate preliminary data on the constituents of the plant extracts. The results obtained from the tests are summarized in Table 1. The results of this study indicated the presences of flavonoids, saponin, tannins, alkaloids, steroids, triterpenes, phenolic compounds, anthraquinones and glycosides in *V.amygdalina* leaves. This implied that the plant grown in Ethiopia retained the constituents which were found when it was grown in its natural habitat. According to the previous study, phytochemical screening of the methanolic and aqueous extracts of *V.amygdalina* revealed the presence of antimicrobial compounds such as alkaloids, flavonoids, saponins, steroids, cardiac glycosides, tannins, phlobatannins, phenols and terpenoids [21, 24]. *P.lanceolata* was observed to contain flavonoids, saponin, anthraquinones, tannin, steroids, phenolic compounds and glycosides. Table 1 also depicted that flavonoids, triterpenes and phenolic compounds were present in the ACE of *R. officinalis.* This study was supported by previous research works. Hydro-alcoholic extracts of *R.officinalis* leaf has phytochemical constituents like flavonoids, phenolic acids (caffeic, chorogenic and rosmarinic) and essencial oils (camphor and cineole) and diterpenes (carnosol) [25]. Saponins, tannin and alkaloids were absent in both ACE of *R. officinalis* and *T.schimperi* leaves. Phytochemical screening of diethyl ether extracts of *P.lanceolata* leaves revealed the presence of flavonoids, coumarins, terpenes and the absence of steroids and alkaloids [26].

Table 1
Phytochemical constituents of MCE and ACE of leaves

Plant Extracts	Components screened								
	alkaloid	Saponin	Tannins	anthraquinone	Flavonoid	Triterpen	Phenolic cpds	Steroids	glycoside
MCE of *V. amygdalina*	+	+	+	+	+	+	+	+	+
MCE of *P.lanceolata*	-	+	+	+	+	-	+	+	+
ACE of *R. officinalis*	-	-	-	-	+	+	+	-	-
ACE of *T. schimperi*	-	-	-	+	-	-	+	+	+

+ = indicates presence of each component and – indicates absence of component

Antimicrobial Studies

The secondary plant metabolites (phytochemicals) with antibacterial potency have been actively investigated as alternatives to and/or in combination with antibiotics in the therapy of bacterial infections [27, 28]. In this study, the plant extracts were found to be more effective on bacteria than fungi. Table 2 depicted that the growth of the two bacteria was highly inhibited by ACE of *R. officinalis* than the other three plant leaves. The bioassay has indicated that the inhibition effect of the extracts was proportional to the concentration and higher concentration has stronger effect.

Table 2
Antimicrobial Activities of plant extracts through paper disc method

Compounds	Average inhibition (I) (mm) of Microorganism															
	Gram (+)Bacteria				Gram(-)Bacteria				Fungi							
	S.aureus				*E.coli*				*A.niger*				*F.oxysporum*			
	20 mg/mL		40 mg/mL		20 mg/mL		40 mg/mL		20 mg/mL		40 mg/mL		20 mg/mL		40 mg/mL	
	10 µL	20 µL	10 µL	20 µL	10 µL	20 µL	10 µL	20 µL	10 µL	20 µL	10 µL	20 µL	10 µL	20 µL	10 µL	20 µL
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MCE of *V. amygdalina*	8.8	11.3	12.5	14.7	7.3	7.3	9.5	11.8	7.0	7.7	9.2	10.8	-	-	-	-
MCE of *P.lanceolata*	7.7	11.2	13.6	15.8	7.0	7.7	9.4	10.6	-	-	-	-	-	-	-	-
ACE of *R. officinalis*	23.0	23.3	20.0	20.1	18.0	19.3	20.0	21.3	-	-	8.0	8.3	-	-	11.3	12.7
ACE of *T. schimperi*	11	14	12	16.3	10.7	11.3	11.3	15.0	10.7	10.7	14	19.7	11	24.4	12.7	18
CAL	23.7		28.7		25.0		28.7		-	-	-	-	-	-	-	-
Bavaisin	-	-	-	-	-	-	-	-	19.3		20.6		21.0		22.0	

Susceptibility (inhibition zone ≥ 7 mm) and (-) absence of susceptibility, DMSO = Dimethyl Sulfoxide

MCE of *V.amygdalina* and *P.lanceolata* showed moderate inhibition zone at both 20 and 40 mg/mL concentrations against S.*aureus* and *E.coli*. MCE of *V.amygdalina* has no inhibition effect against *F.oxysporum* at 10 and 20 µL concentrations (Table 2). It was observed that the fungi were not inhibited by the MCE of *P.lanceolata* leaves at all concentrations. Preliminary qualitative phytochemical screening showed the antibacterial activities of the MCE of *V.amygdalina* could be due to alkaloids, triterpenes, flavonoids, saponin, tannin, steroids, phenolic compounds, anthraquinones and glycosides. Table 2 also revealed that the antimicrobial activities of MCE and ACE of medicinal plants were less than that of Bavistin and Chloroamphenicol commercial drugs at all concentrations. DMSO was used as control for all experiments and showed no antimicrobial activities. On the basis of experimental data 20 µl concentration of MCE of *V.amygdalina* and *P.lanceolata* may be considered as good bactericidal drug. This study was in agreement with previous research works. MCE possess good antibacterial activities against *S.aureus* (22.5 mm) [21]. Qualitative phytochemical screening results depicted that the antimicrobial activities of *P.lanceolata* could be because of flavonoids, glycosides, anthraquinones, polyphenols, saponins, tannins and steroids. ACE of *R.officinalis* showed very high antibacterial inhibition activities against *S.aureus* and *E.coli* and moderate antifungal inhibition against *A.niger and F. oxysporum* in both 10 and 20 µL concentrations. Phytochemical screening results showed that

the antimicrobial activities of the leaves of *R.officinalis* could be because of the presence of flavonoids, phenolic compounds and triterpenes. The growth of *S.aureus* and *E.coli* was more inhibited by the ACE of *R.officinalis* than *A.niger and F. oxysporum.* ACE of *T.schimperi* showed moderate antifungal activities against *A.niger* and *F.oxysporum* and antibacterial inhibition against *S.aureus* and *E.coli* at all concentrations). The inhibition zone of ACE of *T.schimperi* against *F.oxysporum* (24.4 mm) was more than Bavistin (21 mm) at 20 µL concentrations. The phytochemical screening results showed that the antimicrobial activities of *T.schimperi* could be attributed because of the presence of cardiac glycoside, anthraquinones, phenolic compounds and steroids.

CONCLUSION

This study suggested that *V.amygdalina*, *P.lanceolata*, *R. officinalis* and *T. schimperi* possess antibacterial activities and they can be used to control variety of pathogens that cause diseases in plants and humans. Phytochemical analyses of *V.amygdalina* and *P.lanceolata* revealed the presence of flavonoids, saponin, tannins, steroids, phenolic compounds, anthraquinones and glycosides in the methanol leaf extracts and these compounds contribute for their antimicrobial activities. The acetone leaf extracts of *R.officinalis* possess higher antibacterial properties than *V.amygdalina, P.lanceolata* and *T.schimperi.* Phytochemical investigation results showed that the pronounced antibacterial activities of *R.officinalis* could be because of the presence of flavonoids, phenolic compounds and triterpens. However, further work is necessary to elucidate the structures of the biologically active components that are responsible for the antimicrobial activity of these plants.

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