

# Determination of Antimicrobial and Antioxidant Activities of Extracts from Selected Medicinal Plants

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## Abstract

In this work, attempt has been made to determine the antimicrobial and antioxidant activities of aqueous and organic extracts of Vernonia amygdalina (**1**), Ajuga integrifolia (**2**), Artemisia afra (**3**), and Artemisia absinthium (**4**). The plant extracts were tested against clinically important bacteria- Escherichia coli, Shigella, staphylococcus aureus, and Bacillus subtilis. The results showed that methanol extract is more effective than that of aqueous extract which is in turn more effective than that of petroleum ether. The methanol extract of **3** could inhibit all test bacteria and exhibited significant ( $P \leq 0.05$ ) antimicrobial activity when compared with other herbs, with the zone of inhibition ranging from  $18.33 \pm 0.58$  to  $26.00 \pm 1.73$  mm. On the other hand, **4** demonstrated a moderate activity against the test bacteria with the zone of inhibition ranging from  $5.57 \pm 0.58$  to  $8.00 \pm 1.67$  mm. However, that of **1** and **2** had no significant antibacterial activity against test bacteria except a moderate activity against Escherichia coli with the zone of inhibition ranging from  $3.09 \pm 1.47$ . The minimum inhibitory concentration and minimum bactericidal concentration of **3** were 0.1-2.5 and 1.2-2.5 (gm/ml) respectively against E. coli- a common enterogenic bacteria. Mechanistic insight regarding the active principle is deciphered through alkaloid free against free alkaloid. The results reinforced the fact that oxygenated metabolites are most responsible for the antimicrobials when compared with alkaloids. **1**, **2** and **3** showed significant antioxidant activity with an IC<sub>50</sub> value of 24.2, 18.9 and 20.2  $\mu\text{g/mL}$  respectively. In conclusion, **3** is relatively most effective against the test bacteria. The recorded antioxidant activities of **1**, **2** and **3** reinforce their traditional use in the treatment of liver disease.

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**Keywords:** Medicinal plant extracts; antimicrobial activity; antioxidant properties.

## 1. Introduction

In developing countries like Ethiopia medicinal plants (MPs) are commonly used for the treatment of ailments related to microbial infections. Although limitations of resources and access to modern health care services are frequently mentioned reasons, there are additional reasons for the preference of MPs to synthetic drugs. Synthetic drugs such as antibiotics may be used to treat microbial infections. Unfortunately the microbial agents particularly bacteria develop resistance to many antibiotics. Another factor that strengthens the use of plants, instead of synthetic drugs, could be attributed to the belief that they provide some benefits over modern medicine and allow users to feel that they have some control in their choice of medication [1]. In addition, the antibiotics in some cases have allergic reaction and immunity suppression.

*Vernonia amygdalina*, *Ajuga integrifolia*, *Artemisia afra*, and *Artemisia absinthium* are traditionally used in the study area for the treatment of ailments related to microbial infections. *Artemisia afra* and *Ajuga integrifolia* have also been used in the treatment of liver diseases. This is expected as some plants have already been reported to serve as a useful source of natural antioxidants to prevent not only the presumed deleterious effects of free radicals in the human body, but also the deterioration of fats and other constituents of foodstuffs [2]. MP extracts may contain many bioactive phytochemicals which present a potential source of natural antioxidants [3]. Their ability to act as potential antioxidants has been extensively investigated [4, 5]. Despite the successful story on the use of plants in the treatments of ailments caused by microbial infection and as natural antioxidants, the general acceptability has been limited by lack of scientific data to support the indigenous knowledge [6]. It is therefore appropriate to conduct scientific scrutiny on the selected herbs in order to determine their antimicrobial activities and antioxidant properties and also determine the effective doses and possible toxicities. Such measure could possibly lead to new findings to treat diseases in lieu of depending solely on drug developments which may be time-consuming aside from causing side effects to human health after prolonged treatments.

*Vernonia amygdalina*, with up to 20 cm long elliptical leaves and rough bark, is a member of the Asteraceae family that grows as a small shrub to a height of 2--5 m. It is reputed to have several health benefits. The organic fraction extracts of the plant was shown to possess antimicrobial and antiparasitic activities [7,8], antioxidant and cytoprotective [9] activities. It is also effective against amoebic dysentery [10], gastrointestinal disorders [11] and has cytotoxic effects towards human carcinoma cells of the nasopharynx [12]. Its antioxidant activity has been attributed to the presence of flavonoids [13].

*Ajuga integrifolia*, Lamiaceae, is locally known as 'Annamura' (Wolayta), has a very bitter taste, leaves moderately to densely hairy, grayish green, simple, and flowers pale blue, pale violet, light blue or white [14]. In traditional health system of Ethiopia, the aqueous and sometimes alcohol infusion of the fresh or dried leaves of the plant is used for the treatment of various diseases including diabetes, malaria, pain and fevers, toothache, skin disease, hypertension, stomachache, pneumonia, liver problem, swelling of legs, retained placenta [15], and Epilepsy [16] and others [17,20].

A. afras one of the important and most widely used herbs in the traditional medicine. In recent years, it has gained significant attention from the scientific community. Studies have been conducted either to verify or substantiate the traditional use of this herb. Despite a series of work going around over a span of a century on A. afra, scientific publications based on laboratory work were very minimal and generally confined in determining the constituents of A. afra essential oil and assigning chemical structure to it. Some scientific studies made on essential oil from the plant extracts from 1993 onwards were in the direction of finding out the activity namely antifungal [21], antibacterial [22], antioxidant [23], toxicity [24], anti-cancer [25], antituberculous [26], antimalarial [27] antitrypanosomal [28], protective myocardial [29], protective intestinal epithelial Caco-2 cells [30], anti-ulcerative [31] etc.

*Artemisia absinthium* L. (wormwood), native to temperate regions of Europe, Asia and northern Africa, is a herbaceous, perennial plant with fibrous roots. It is grown as an ornamental plant and is used as an ingredient in the spirit absinthe as well as some other alcoholic drinks. The volatile oil distilled from dried leaves and flowers is used in fragrance compounding and in some external analgesics [3–4]. The composition of the essential oil from *A. absinthium* has been the object of several studies [3–12], especially for its contents of such compounds as thujone isomers and chamazulene with pharmacodynamic properties.

Most of the studies on the above plants to date, however, focus on specific constituents such as essential oils of the plant extracts. Targeting specific compound might be relevant for the development of scientific drugs. Scientific developments on crude extracts are even more appropriate considering the cost and accessibility. In addition to this, the antimicrobial studies [32, 33] to date on the above plants target a different bacteria.

In this work, attempt has been made to determine and compare the antimicrobial and antioxidant activities and also determine their efficacy and dosage regimes of the aqueous and methanol extracts of *Vernonia amygdalina*, *Ajuga integrifolia*, *Artemisia afra*, and *Artemisia absinthium* that are tested against clinically important bacteria- *Escherichia coli*, *Shigella*, *Staphylococcus aureus*, and *Bacillus subtilis*. A comparison of their activities with that of a well-known antimicrobial drug should give a better indication as to whether the plants have any value as potential candidate for control of the disease.

## 2. Materials and Methods Structure

### 2.1. Collection of plant materials

Fresh parts of four medicinal plants, *Vernonia amygdalina* (Leaves), *Ajuga integrifolia* (areal parts), *Artemisia afra* (Leaves), and *Artemisia absinthium* (Leaves) were collected from Sodo Zuriya districts of Wolayta Zone (SNNPR). The plant materials were taxonomically identified and authenticated by the Department Biology, Addis Ababa University. The plants were selected based on information collected from informants through structured interview. The level of homogeneity among information provided by different informants was calculated by the Informants' Consensus Factor, FIC [34] using the following formula:  $FIC = \frac{Nur - Nt}{(Nur - 1)}$  Where, Nur = number of use reports from informants for a particular plant-use category; Nt = number of taxa or species that are used for that plant use category for all informants. FIC Values range between 0 and 1, where

'1' indicates the highest level of informant consent. The fidelity level (FL), the percentage of informants claiming the use of a certain plant species for the same major purpose, was calculated for the most frequently reported diseases or ailments as:  $FL (\%) = (N_p / N) \times 100$  Where,  $N_p$  = number of informants that claim a use of a plant species to treat a particular disease;  $N$  = number of informants that use the plants as a medicine to treat any given disease [35].

## **2.2. Preparation of plant extracts**

Prior to extraction, the fresh samples were dried under shade and ground to fine powders using a blender. 300 gm of the dried, ground samples were then soaked in methanol (1.5 L) for 3 days at room temperature. The solvent-containing extracts were then decanted and filtered. The extractions of the ground samples were further repeated (2x) with methanol (1.5 L each time). The filtrate from each extraction was combined and the excess solvent was evaporated under reduced pressure using a rotary evaporator to give crude methanol extracts. Serial dilutions of the concentrated extract gave 0.01, 0.1, 1 and 10 mg/L that are used to study the in vitro antibacterial activity by agar well diffusion method. Similar procedure was applied for aqueous extract too.

## **2.3. Qualitative phytochemical analysis**

The extract was tested for the presence of bioactive compounds by using standard methods [36-38].

Test for flavonoids (Shinoda test): 10 mg of crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet color appeared after few minutes which indicated the presence of flavonoids.

Test for glycosides (Liebermann's test): 10 mg of crude was mixed with each of 2ml of chloroform and 2ml of acetic acid. Then the mixture was cooled in ice. Carefully concentrated  $H_2SO_4$  was added. Color change from violet to blue green indicates the presence of glycoside.

Test for alkaloids: 1 g of crude extract was dissolved in dilute 1% HCl and filtered then the filtrate was treated Mayer's reagent; formation of yellow colored precipitate indicate the presence of alkaloids.

Test for phenols: 1 g of crude extract was treated with 3-4 drops of 2%  $FeCl_3$  solution. Formation of violet color indicated the presence of phenols.

Test for terpenoids (Salkowski test)

To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated  $H_2SO_4$  (3 ml) was carefully added to form a layer. A reddish brown coloration of the interface indicates the presence of terpenoids.

Test for saponins: 0.5 g of crude extract was mixed with 2 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for Steroid: Crude extract was mixed with 2 ml of chloroform and concentrated H<sub>2</sub>SO<sub>4</sub> was added sidewise. A red color produced in the lower chloroform layer indicates the presence of steroid.

#### **2.4. Test microorganisms and microbial culture**

Five bacterial strains were used in this study: Escherichia coli, Shigella, staphylococcus aureus, and Bacillus subtilis. The test microorganisms were obtained from the Microbiology Laboratory, Dilla University. Bacterial strains were cultivated at 37°C and maintained on nutrient agar slant at 4°C.

#### **2.5. Antimicrobial activity assay**

Antimicrobial activity was determined against four bacterial pathogens by the agar well diffusion assay. The crude methanol and aqueous extracts were dissolved in Dimethyl Sulfoxide (DMSO) with the exception of the water fraction and then antimicrobial effect methanol was tested using different concentrations. Petri dishes (measuring 90 mm each side) containing 20 mL of Mueller Hinton agar (OXOID). Appropriate concentration of the extracts in DMSO and water were applied onto the wells. The plates were incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the wells. Standard antibiotic ampicillin (1mg/ml) served as the positive antibacterial controls for the test bacteria. Negative controls were done using the nutrient media loaded with DMSO and water. After that, the diameter of inhibition zone was measured in millimeters. All tests were repeated three times to minimize test error. An inhibition zone of 16 mm or greater (including diameter of the wells) was considered as high antibacterial activity.

#### **2.6. Antioxidant properties**

Spectrophotometric determination of antioxidant values of Vernonia amygdalina, A. integrifolia and A. afra was carried using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent according to a standard method [39, 40]. Briefly, 50 mL of various concentrations of the extracts were added to 5 mL of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm using a Jasco 7800 spectrophotometer. The change in absorbance was then related to concentration by the well-known Beer's law. Tests were carried out in triplicate.

### **3. Results**

#### **3.1. Qualitative phytochemical analysis**

Antimicrobial activities of plants are linked to class of secondary metabolites the elaborate. The presence of oxygenated compounds such as terpenoids, phenolic biosynthetic origin or hydrolytic enzymes (glucanases and chitinases) and proteins are primary indications as to whether the plant could possess antimicrobial activity [41]. Therefore, phytochemical screening was carried out to verify whether the test plants contain the above class of compounds. The phytochemical characteristics of the four medicinal plants tested were summarized in the Table 1. The results revealed the presence of medicinally important class of compounds in the four plants studied. From the Table, it can be seen that, phenols/tannins, flavonoids, alkaloids, glycosides and saponins were present

in all the plants.

**Table 1:** Phytochemical constituents of the five medicinal plants studied

	Phenols/ Flavonoids	Saponins	Glycosides	Steroids	Terpenoids	Alkaloids
Ajuga integrifolia	+	+	+	+	+	+
Artemisia afra	+	+	+	+	+	+
Artemisia absinthium	+	+	+	+	+	+

+ = positive result (present)

### 3.2. Determination of antimicrobial activity

Antimicrobial activities of the methanol, aqueous and petroleum ether extracts of the four plants were characterized by recording diameter (mm) of growth inhibition zone by agar well diffusion assay. The susceptibility of the test microorganism is related to the inhibition zone. Microorganisms are termed susceptible to the plant extract when the zone inhibition is equal to or more than 7 mm ( $\geq 7$ ) in diameter, or resistant with a zone of inhibition less than 7 mm ( $< 7$ ) therapies [42]. Our results are summarized in Table 2 and 3. All the water extract of the selected plants showed no inhibition against all the bacteria tested in this study. As indicated in Table 2, the crude extracts of *Artemisia afra* exhibited the highest antibacterial activities against all bacteria. The presence of such class of compounds as terpenoids and flavonoids might contribute to the highest result of antibacterial activities. The results show the wide variation in the antibacterial properties of methanol, aqueous and petroleum extracts. The methanol extract is generally exhibited the highest antibacterial activity while the aqueous extract displayed the least. The fractions of alkaloid have insignificant effect on all the test bacteria reinforcing the fact that antimicrobial activities of plants are usually attributed to the presence of oxygenated compounds such as terpenoids, phenolic compounds and flavonoids. Table 3 shows the antimicrobial activities of *Verinonia amygdaline*. As indicated in the Table, the plant didn't exhibit significant effect against the test bacteria. An attempt to investigate synergistic effect, if any, of the plant on the activity of standard drug also led to a negative conclusion.

### 3.3. Antioxidant activity

The antioxidant activity was studied based on DPPH radical scavenging activity which is reported in % as:

$$(\%) = \frac{(A_0 - A_1)}{A_0} \times 100$$

Where  $A_0$  was the absorbance of DPPH radical + methanol;  $A_1$  was the absorbance of DPPH radical + sample extract or standard. The 50% inhibitory concentration value (IC50) was calculated as the effective concentration of the extract that is required to scavenge 50% of the DPPH free radicals. Accordingly, IC50 values of 24.2, 18.9 and 20.2  $\mu\text{g/mL}$  were recorded for *Vernonia amygdalina* (Leaves), *Ajuga integrifolia* (areal parts), and

Artemisia afra (Leaves) respectively. The strongest effect was measured for Vernonia amygdalina with an IC50 of 24.2 µg/mL. A. afra took 20.2 µg/mL to produce a 50% inhibition.

**Table 2:** In vitro antibacterial bioassay of Artemisia afra (Growth inhibition zone diameter (mm))

Types of extract and conc.	Growth inhibition zone diameter (mm)			
	E.coli 25922	Bacillus subtilis	Shigella 5223	Staphylococcus aureus 25923
Aqueous extract	-	-	-	-
Methanol extract 1 g/ml	29	23	20	27
0.75g/ml	23	20	19	22
0.5 g/ml	20	18	17	20
0.25 g/ml	16	16	16	19
0.1 g/ml	19	14	23	17
Petroleum ether	-	20	26	21
Alkaloid free( F1)	24	22	25	24
Free alkaloid (F2)	16	19	15	-
Positive control (1mg/ml)		30	34	24
DMSO	-	-	-	-

**Table 3:** In vitro antibacterial bioassay of methanol extract of Vernonia amygdalina

Types of extract and concentration	Growth inhibition zone diameter (mm)				
	Staphylococcus aureus 25923	Streptococcus	Bacillus subtilis	Shigella 5223	E.coli 25922
1 g/ml	-	-	-	-	-
Vernonia 1mg/ml + control	26	28	34	22	16
0.25mg/ml					
Positive control (1mg/ml)		30	34	24	28
Negative control	-	-	-	-	-

#### 4. Conclusion

Among the plants, Vernonia amygdalina, Ajuga integrifolia, Artemisia afra, and Artemisia absinthium, investigated in this study for the antibacterial activity, Artemisia afra exhibited significant inhibition zone against

all the test bacteria *Escherichia coli*, *Shigella*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Streptococcus*. *Escherichia coli* is the most susceptible to the crude extract confirming the potential use of the plant in stomach complaints related to the bacteria. However, the lack of antibacterial activity of the aqueous extract justifies the claim that water extract is not as such effective. Our study points out that the use of ethanol as in “katikala” or “tella” as a solvent could be appropriate to enjoy health benefits of *A. afra*. Although the phytochemical screening of the methanol extracts revealed the presence of several classes of compounds, the presence of terpenoids and flavonoids strengthens the scientific validity of the plant as an antimicrobial agent as justified, in this study, by the result of free alkaloid vs. alkaloid free fractions. Similarly, the significant antioxidant properties of *Vernonia amygdalina*, *Ajuga integrifolia*, and *Artemisia africana* can be attributed to the presence of phenolic compounds and flavonoids in the plants. Since the determination of antimicrobial activities, in this study, is based on recording zone of inhibition that could not indicate the effectiveness of the antibacterial activity, though useful as screening tools in antibacterial assays, other potentially useful techniques such as the micro dilution method should be considered in further study to strengthen the findings. We also recommend that the practices of traditional healers and the use of plant extracts for treatment of bacterial infections should be supported by scientific procedures to enhance the efficacy and avoid resistance. Appropriate solvents should be used. Finally, it is declared that the study is limited to certain zones of SNNPR (Ethiopia) and that the medicinal plants included in this study are by no means the only ones in the study area. More plants with potential antibacterial activities could be identified through a more thorough study.

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