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Research Article

# Phytochemical screening, antibacterial and radical scavenging activities of root wood and root bark extracts of *Albizia schimperiana*

**Zelalem Abdisa**[1*] **and Kalkidan Tarekegn** [2]

[1]*Department of Chemistry, College of Natural and Computational Sciences, Wollega University, Nekemte, Ethiopia.*[2]*Department of Chemistry, College of Natural and Computational Sciences, Dambi Dollo University, Dambi Dollo, Ethiopia.*

## ABSTRACT

**Objective:** Different parts of Albizia schimperiana are used for treatment of different types of diseases. In Ethiopia traditionally people use Albizia schimperiana to treat headache and other pains. In Tanzania the stem bark is used to treat warts, the leaf has shown significant antimicrobial and potential anti-helmintic activity. The main objective of the study was to identify the phytochemical constituents, and evaluate antibacterial and radical scavenging activities of root wood and root bark extracts of A. schimperiana.

**Method:** The root of A. schimperiana was collected from east wollega zone, Nekemte town, in May 2018. The collected plant root was washed with distilled water and peeled so that the root wood and root bark were dried separately under shade at room temperature and ground in to powder using analytical mill. The bioactive constituents of the root of A. schimperiana were extracted through maceration technique using n-hexane, ethyl acetate, acetone, and methanol. The antibacterial assay was determined using disc diffusion method against four bacteria strains while the antioxidant activity of the plant extracts were investigated spectrophotometrically using DPPH.

**Results:** The phytochemical screening revealed the presence of alkaloids, phenolic compounds, saponins, flavonoids, anthraquinones and terpenoids and absence of tannins and steroids in both root parts. The root extracts were active against all the tested bacterial strains. The root wood extracts of acetone and ethyl acetate demonstrated strong zone of inhibition against S. aureus (30mm) and E. coli (30 mm) at 100 mg/mL, as compared to the reference antibiotic gentamicin (20 mm). The methanol root wood extract of A. schimperiana has shown better DPPH radical scavenging activity at 1 mg/mL (89.8%) when compared to the methanol root bark extract (71.6%) at the same concentration.

**Conclusion:** It can be concluded from this study that the root extracts of A. schimperiana owned many phytochemicals that exhibited promising antibacterial and antioxidant properties. Therefore, the root of A. schimperiana can serve as a potential source for development of new drugs as well as finding natural antioxidants that could assist the body in fighting disease like cancers.

**Keywords:** Albizia schimperiana; phytochemical; Antioxidant; antibacteria.

## BACKGROUND

Plants are naturally gifted for their ability to synthesize various medicinal and bioactive compounds. People have been used plant derived drugs worldwide for the treatment of many diseases [1]. In many parts of the developing world, especially for those people living in rural area, plant-based traditional medicine represents the primary or the only veritable form of accessible primary health care. Currently, the demand for the herbal drug treatment of various ailments is increasing and plants are being explored globally for the development of newer drugs [2].

Most of the traditional medicines are formulated from leaves, bark, stem, root, seed, fruit and tubers of plants because bioactive compounds like alkaloids, terpenoids, phenolics and flavonoids are deposited in these parts of the plant. These bioactive compounds are known to possess antioxidant, antibacterial, antifungal, antidiabetic, anti-inflammatory, and radio-protective activity [3, 4]. As a result of these properties, about 50% of modern drugs in clinical use are derived from natural products [5]. Moreover, modern isolation techniques and need for more active medicines led to the development of purified drugs [6].

Albizia schimperiana Oliv. is one of traditionally used medici-

**Correspondence to:** Zelalem Abdisa, 1Department of Chemistry, College of Natural and Computational Sciences, Wollega University, Nekemte, Ethiopia, Tel: 0938046937; E-Mail: zelalem2ab@yahoo.com

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nal plant that belongs to the family Fabaceae (Leguminosae) and subfamily Mimosoideae and consists of approximately 150 species and most of the species are deciduous woody trees and shrubs [7, 8]. Albizia schimperiana Oliv is known by the local name 'Hambabeesa' in Afaan Oromo and 'Sesa' in Amharic). It is an evergreen tree that grows at an altitude of 1600 m-2600 m. Different parts of this plant are used to treat various types of diseases. The stem bark is used in Tanzania to treat warts. The leaf of Albizia schimperiana has shown significant antimicrobial activity on different bacterial species and potential anti-helmintic activity [9]. In Ethiopia traditionally people use Albizia schimperiana to treat headache and other pains. Leaf extract and some pure compounds isolated from stem bark have shown antibacterial activities, antimalaria, antileishmanial, antimicrobial and cytotoxic activities [9-11].

In this study phytochemical screening, antibacterial and radical scavenging activities of the root wood and root bark extracts of Albizia schimperiana was presented.

Figure 1: Aerial part of Albizia schimperiana plant (Photo taken by Kalkidan T. from Wollega University compound in May, 2018).

**Figure 1:** Aerial part of Albizia schimperiana plant (Photo taken by Kalkidan T. from Wollega University compound in May, 2018).

## MATERIALS AND METHODS

## COLLECTION OF PLANT MATERIAL

The root of A. schimperiana was collected from east wollega zone, Nekemte town, in May 2018 and the plant was identified by Dr Tena Regasa, a botanist from Wollega University, Biology department. The collected plant root was washed with distilled water to remove dirt or unwanted materials. The cleaned root part was peeled so that the root wood and root bark were dried separately (Figure 2) under shade at room temperature and ground in to powder using analytical mill. The powdered root parts were subjected to solvent extraction using maceration technique.

**Figure 2:** Photo of samples root bark and root wood of A. schimperianaCHEMICALS, REAGENTS AND INSTRUMENTS

Extraction solvents used during this research work were of analytical reagent grade and include n-hexane (99%), ethyl acetate (99.5%), acetone (99 %) and methanol (99.8%) (Loba Chemie: laboratory reagents and fine chemicals); Ferric chloride (99%), H2SO4 (98%), Lead acetate (99%), benzene, Mayer's reagent, Wagner's reagent, NaOH, NH3 and Acetic anhydride were utilized for phytochemicals screening. DMSO, nutrient agar, Barium chloride and Gentamicin were used for antibacterial test while ascorbic acid and DPPH were used for antioxidant activity test. Solvents were removed using rotary evaporator. Absorbance measurement was taken with UV spectrophotometer.

## EXTRACTION

Dried and powdered root wood (500g) and root bark (500g) of A. schimperiana were soaked separately in n-hexane (1500mL each) for 48 hrs with random shaking, filtered first using cotton plug and then with Whatmann  1 filter paper. The marc was dried and soaked using ethyl acetate (1500mL) for 48 hrs with random shaking, and filtered. The same procedure was repeated for acetone and methanol with equal volume and soaking time as before, filtered, solvents were removed by rotary evaporator under reduced pressure and fractions were collected separately. The dried extracts were weighed and the percentage yield was calculated.

## PHYTOCHEMICAL SCREENING

The preliminary qualitative phytochemical screening of the root wood and root bark of Albizia schimperiana were performed to identify the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, saponins, steroids and anthraquinones present in the solvent extracts. The following chemical tests were carried out on the extracts by following standard procedures reported in [12, 13].

## TEST FOR ALKALOIDS

Mayer's reagent: To a 2 mL sample extract, 1 mL of Mayer's reagent was added along with the sides of the test tube. Formation of white or creamy precipitate is used as positive test.

Wagner's test: To a 1 mL of the extract, 1 mL of Wagner's reagent was added along with the sides of the test tube. Formation of reddish brown precipitate indicates the presence of alkaloids.

## TEST FOR PHENOLIC COMPOUNDS

Ferric chloride test: To 2 mL of extracts 2 mL 2% ferric chloride solution was added. A dark green colour indicates the presence of phenolic compound.

Lead acetate test: To 2 mL extracts 3 mL of 10% lead acetate solution was added. A bulky white precipitate indicates the presence of phenolic compounds.

### Test for Saponins

5 mL of the extract was diluted with distilled water to 20 mL. The suspension was shaken for 15 minutes. A two centimeter layer of foam or froth which is stable for 10 minutes indicates the presence of saponins.

### Test for Tannins

The extracts 2 mL was treated with 1 mL FeCl3. Formation of green colour indicates the presence of condensed tannin.

## TEST FOR FLAVONOIDS

Alkaline reagent test: 2 mL of extract was treated with 10% NaOH solution, formation of intense yellow colour indicates presence of flavonoid.

## TEST FOR STEROIDS

1mL extract was dissolved in 10 mL of chloroform & equal volume of concentrated H2SO4 acid was added from the side of test tube. If the upper layer turns red and H2SO4 layer showed yellow with green fluorescence, it is used as indicator for the presence of steroid.

## TEST FOR ANTHRAQUINONES

5 mL of extract was hydrolyzed with dilute H2SO4, and then add 1mL of benzene and 1mL of NH3, formation of Rose Pink coloration suggest anthraquinone.

## TEST FOR TERPENOIDS

2 mL of extract was added to 2 mL of acetic anhydride and concentrated H2SO4. Blue or green ring was form to confirm presence of terpenoids.

## ANTIBACTERIAL TEST

Source of Test Organisms: One Gram positive bacterium (Staphylococcus aureus) and three Gram negative bacterial strains (Escherichia coli, Salmonella typhimurium and Pseudomonas aeruginosa) were obtained from Wollega University, Microbiology laboratory.

Antibacterial activity: Dry extracts were dissolved in 10% DMSO to prepare solutions of 100 mg/mL. The growth inhibition of bacteria was determined by using disc diffusion method. The bacterial inoculum was prepared from overnight broth culture in physiological saline (0.8 % of NaCl). Standardized inoculum was transferred and spread evenly on a MHA plate. Sterile discs were placed on inoculated agars, by test bacteria, filled with 50 µL diluted active extract. DMSO and Gentamicin were used as negative and positive controls, respectively. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the discs. Then, Petri dishes were incubated at 37°C during 24 hrs. After incubation, inhibition zone diameters were measured and documented [14].

Free Radical Scavenging Activity: The free radical scavenging activity of the extracts and ascorbic acid as positive control was measured in terms of hydrogen donating ability using the stable radical DPPH [15] with slight modifications. 2mL of each extract and control at various concentrations (1, 0.5, 0.25, 0.125, and 0.0625 mg/mL) was added to 2mL of freshly prepared DPPH solution (0.004%) in methanol. The reaction was allowed for 30 min in dark and absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The degree of decolorization of DPPH from purple to yellow indicated the scavenging efficiency of the extract. The percentage inhibition of DPPH free radical scavenging activity was calculated using the following equation:

DPPH radical scavenging activity (%) = [(A control –A test sample) /A control] x 100

Where; A control - is the absorbance of DPPH radical + methanol

A test sample - is the absorbance of DPPH radical + sample extract.

## RESULTS AND DISCUSSION

### Yield Of Dried Extracts

The extraction yield is a measure of the solvent efficiency to extract specific components from the original plant powder. The mass of crude extract as well as the percentage yield of the dry sample from the four solvents is recorded in Table 1. The percent yield was calculated using the formula:

**Table 1:** Weight of crude extract and corresponding yield in percentage of root wood and root bark of A. Schimperiana.

| Solvent/ Plant part | Root wood | | Root bark | |
|---|---|---|---|---|
| | Mass of extract (g) | Yield in % | Mass of extract (g) | Yield in % |
| n-Hexane | 0.13 | 0.026 | 0.74 | 0.14 |
| Ethyl acetate | 1.24 | 0.25 | 3.39 | 0.67 |
| Acetone | 22.74 | 4.56 | 18.13 | 3.65 |
| Methanol | 27.43 | 5.76 | 62.96 | 13.17 |

Plant crude extraction yield (%) = (wt of dried extract/wt of plant powder extracted) x 100

Among the solvents used methanol extracted the highest mass of secondary metabolities in both the root wood and root bark of the plant. This observation is common in most of the extraction of bioactive compounds and associated with the ability of methanol to dissolve polar compounds and to some extent non-polar groups. Methanol having a polarity index of 5.1 next to water is chosen as an extraction solvent to extract bioactive compounds [16, 17]. More phytochemicals were extracted from root bark compared to the root wood of A. schimperiana. This indicates that the root bark of the plant contained more phytochemicals that are extractable with organic solvents used than the root wood.

## PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical screening of crude extracts of root wood and root bark of A. schimperiana indicated the presence of various secondary metabolites and the result of phytochemical test has been summarized in Table 2. Alkaloids, phenolic compounds, flavonoids, saponins, terpenoids and anthraquinones were found in both the root wood and root bark solvent extracts. Methanol extract showed positive test for most of the secondary metabolities screened as compared to the remaining solvents utilized in this experiment. Earlier phytochemical screening reports showed the presence of alkaloids, saponins, tannins, flavonoids, and steroids in stem bark as well as leaf extracts [9, 18, 19], phenolic compounds and terpenoids in the stem bark [19], anthraquinones in the leaf extract [9, 18] of A. schimperiana. However, in this study tannins and steroids were not observed in both the root wood and root bark extracts of the plant. The phytochemicals like phenolic, alkaloids, flavonoids and terpenoides are known to possess a variety of biological activities including antibacterial, antifungal, antioxidant, antiviral, anti-inflammatory, antitumor, anticancer, enzyme inhibition, oestrogenic, angiostatic and antidiabetic [20]. The presence of these secondary metabolities supports the traditional medicinal use of different parts of A. schimperiana to treat various diseases caused by microorganisms.

**Table 2:** Phytochemical screening of extracts achieved from root wood and root bark.

| Phytochemicals | Root wood extracts | | | | Root Bark extracts | | | |
|---|---|---|---|---|---|---|---|---|
| | Hexane | Ethyl acetate | Acetone | Methanol | Hexane | Ethyl acetate | acetone | Methanol |
| Alkaloids | - | - | - | + | - | - | + | + |
| Phenolic compounds | - | - | - | + | - | - | + | + |
| Saponins | - | - | - | + | - | - | - | + |
| Tannins | - | - | - | - | - | - | - | - |
| Flavonoids | - | + | + | + | - | + | + | + |
| Steroid | - | - | - | - | - | - | - | - |
| Anthraquinones | + | - | - | - | + | + | - | - |
| terpenoids | + | + | + | - | + | + | + | + |

## ANTIBACTERIAL ACTIVITY TEST

Worldwide, infectious diseases are the leading cause of morbidity and mortality especially in the developing countries. Among infectious diseases known in the developing world, wound infections are the most common because of poor hygienic conditions that cause infection by many classes of bacteria [21].

In this study, the antibacterial activity of root wood and root bark of A. schimperiana crude extracts were evaluated against E. coli, P. aeruginosa, S. typhimurium (Gram-negative), and S. aureus (Gram-positive). Both root parts of the plant extracts exhibited promising antibacterial activity against these pathogenic microorganisms as compared to the reference standard drug tested, and the results were summarized in Table 3. The zone of growth inhibition observed ranges from 13mm to 30 mm. The highest zone of inhibition (30 mm) was measured for the root wood against methicillin-resistant S. aureus and E. coli followed by the root bark (28 mm) against S. typhimurium, E. coli and P. aeruginosa. However, the n-hexane extract from both root parts didn't show any inhibition zone against the four human pathogenic bacteria tested. The result showed that the root extract of A. schimperiana demonstrated its broad spectrum nature.

**Table 3:** Antibacterial efficacy of extracts against pathogens at 100 mg/mL concentration.

| Solvent/ Root part | Root bark | | | | Root wood | | | |
|---|---|---|---|---|---|---|---|---|
| | *S. aureus* | *S. typhi* | *E. coli* | *P. aerug* | *S. aureus* | *S. typhi* | *E. coli* | *P. aerug* |
| n-Hexane | - | - | - | - | - | - | - | - |
| Ethyl acetate | 10 | 20 | 28 | 20 | 18 | 15 | 30 | 20 |
| Acetone | 18 | 25 | 25 | 28 | 30 | 15 | 20 | 13 |
| Methanol | 15 | 28 | 22 | 15 | 18 | 25 | 15 | 25 |
| Gentamicin | 20 | 25 | 20 | 20 | 20 | 25 | 20 | 20 |

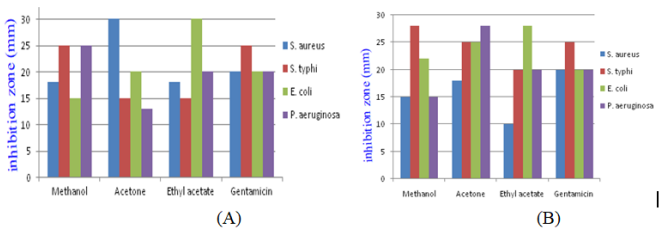


**Figure 3:** Antibacterial activity of A) root wood and B) root bark extracts of Albizia schimperiana at 100 mg/ml

Medicinal plants represent a rich source of antimicrobial agents [22]. A. schimperiana was reported to contain macrocyclic spermine alkaloids called Budmunchiamines [23] and these budmunchiamines are known to possess antibacterial activity [24]. Bacha and coworkers also reported moderate activity against some bacterial strains and fungi for methanol root extract of Albizia schimperiana [25].

## DPPH RADICAL SCAVENGING ACTIVITY

DPPH is stable free radical at room temperature and accepts an electron/hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radical is determined by the decrease in its absorbance at 517 nm, induced by antioxidants and visually noticeable by the color change from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidative activity [26].

In this study, the methanol extracts of root wood and root bark and its serially diluted solutions were tested for their antioxidant activity using DPPH radical scavenging assay (Table 4). Generally, scavenging activity of each extract was increased in a concentration dependent manner. The root wood extract showed better antioxidant activity with percentage inhibition of (89.8 %) at 1 mg/mL as compared to that of root bark (71.6 %) which is promising compared to the scavenging ability of ascorbic acid (96.4%)

at the same concentration. However, at lower concentration the root wood showed very weak activity.

**Figure 4:** Line graph plot of % DPPH radical scavenging activity versus concentration of standard and methanol extracts of root wood and root bark of A. schimperiana.

Antioxidants are substances which can either directly scavenge Reactive Oxygen Species (ROS) or prevent the generation of ROS [27]. By doing this, antioxidants reduce oxidative stress which is an imbalanced state where excessive quantities of reactive oxygen species overshadow the endogenous antioxidative capability of the cells which stimulates the oxidation of macromolecules such as proteins, enzymes, lipids and DNA [28]. Natural products of plant origin have been proposed as a potential source of natural antioxidants with strong activity. Plant phenolics are a major group of compounds acting as primary antioxidants or free radical scavengers [29]. Typical phenolics that possess antioxidant activity are known to be mainly phenolic acid and flavonoids. Thus the antioxidant potential of the root of A. schimperina can be possibly attributed to its phenolic compounds [30]. Literature report indicated that the leaf of Albizia amara possesses strong antioxidant and free radical scavenging properties. The ethanolic extract of bark increases the activity of super oxide dismutase and catalase which indicate it as good antioxidant [31, 32].

Though the DPPH radical scavenging abilities of the extracts were less than that of ascorbic acid at 1mg/mL, the study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors, acting possibly as natural antioxidants.

## CONCLUSION

The focus of this study was to identify the phytochemical constituents of the root of Albizia schimperiana as well as evaluation of its antibacterial and antioxidant activities. The root of the plant is rich in secondary metabolities since it contains alkaloids, phenolic compounds, saponins, flavonoids, anthraquinones and terpenoids among others. The strong growth inhibition zone observed against known human pathogenic bacteria may be due to the presence of these bioactive natural products. The findings of this study might add value to the uses of A. schimperiana and investigating this plant for antibacterial and antioxidant properties as well as determining the presence of any major compounds that might be responsible for the biological activities might aid in validating its traditional uses.

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