

## Phytochemical Investigation and Evaluation of Minimum Antibacterial Inhibitory Concentrations of Methanolic Extracts of the Root and Leaves of *Aloe yavellana*

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**Abstract:** The aim of this study was to carry out a preliminary phytochemical investigation on roots and leaves of *A.yavellana* and also to evaluate the antibacterial activities of the crude extracts of the abovementioned parts of *A.yavellana*. Both the experiments were carried out following standard procedures reported in the literature. The phytochemical analyses of methanolic extract of *A.yavellana* root revealed the presence of alkaloids, terpenoids, anthraquinones, phenols, flavanoids, steroids, glycosides, saponins and the absence of tannins whereas the leaf extract contained alkaloids, anthraquinones, phenols, flavanoids, steroids, saponins and the absence of tannins, terpenoids and glycosides. The minimum inhibitory concentration the of methanolic extract of root and leaf of *A.yavellana* was investigated against *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* showed that the minimum inhibitory concentration of the extracts were found to be 5 mg/ml against most of the bacterial strains used in the study. The root extract was found to be better in inhibiting the growth of all the bacterial species than the leaf extract. The result of the study justifies the traditional use of the plant. Further investigation is needed to evaluate antibacterial activities of the extracts on additional bacterial species and to isolate compounds that could be used as candidates in the discovery of new antibacterial agents.

**Key words:** *Aloe yavellana* · Antibacterial activity · Phytochemical screening · Minimum Inhibitory Concentration (MIC)

### INTRODUCTION

Infectious diseases kill about 9 million people, many of them are children under the age of five and they also cause enormous burdens through life-long disability [1]. The major contributing factor in this regard is the increase of drug resistance in human pathogenic microorganisms that could be attributed to the indiscriminate use of antibiotics in the treatment of infectious diseases [2]. This led to an urgent global call for new antimicrobial drugs from different sources including natural resources (or medicinal plants).

Medicinal plants are known to be rich sources of different types of drugs that include antimicrobial agents and are also potential sources of future antimicrobial agents [3-6]. Moreover, medicinal plants or plant materials

used in traditional medicines are readily available in rural areas (especially in developing countries) at relatively cheaper prices than modern medicines and social and religious reasons [7]. This fact is further consistent with reports from the World Health Organization (WHO). The reports also indicate that about 3.5 billion people in the developing world rely on medicinal plants as components of their health care [8]. The reports also showed that 70-80% of population in developing countries including those countries of in Africa consult Traditional Medical Practitioners (TMPs) treatment of several human and livestock diseases in different parts of the world. Medicinal properties of plants (crude plant extracts) are normally dependent on the presence of variety of chemical constituents such as alkaloids, anthraquinones, cardiac glycosides, saponins,

tannins and polyphenols and also other secondary metabolites that need isolation and characterization in order to identify the bioactive constituents that are responsible for the reported antimicrobial properties of the medicinal plants [9].

Similar to other countries of developing world, over 80% of the Ethiopian population depends on traditional medicine to treat human and animal diseases [10]. Use of traditional medicine (medicinal plants) is more acceptable from spiritual and cultural perspectives [7]. Of the medicinal plants used by human being, plant species that belong to genus aloe are well known for their medicinal uses [11, 12]. The genus comprises approximately 600 species with centers of diversity in southern and eastern Africa, the Arabian Peninsula and Madagascar. Forty six (46) of the species are indigenous to Ethiopia and Eritrea [13, 14]. These species, for instance, used in the treatment of several human illnesses such as stomach ailments, gastrointestinal problems, skin diseases, constipation for radiation injury, inflammatory problems, wound healing and burns, as an antiulcer and diabetes and also used in skin care, cosmetics and in nutraceuticals [12,15]. There are also reports that show the presence of several types of biologically active compounds and with over 130 phytoconstituents isolated from the group [16]. The use of the aloe species are is also widely used in Ethiopia to treat several human diseases [11, 17, 18].

*Aloe yavellana* (also known as *Hargessa* by Oromiffa speaking local community) is one of the species that belong to genus *Aloe*. It is widely used for its medicinal value. Its different parts are used for the treatment of various diseases in traditional medicine/folk remedies throughout the world. In Ethiopia, the plant species is used in traditional medicine alone or in combination with other plant species such as *Acaccia-Commiphora* and also as fuel wood by Yabello community in Borena area (Southern Ethiopia). The leaves and roots of the plant are used by the community of the area for treatment of malaria and wounds [19]. The traditional medicinal use of the plant in treatment of malaria and wound suggest that it could be good/potential sources bioactive molecules that can be isolated and characterized to get their structural information. This would have much contribution in the discovery of new antimicrobial and antimalarial agents that can replace the currently existing antimalarial and antimicrobial agents that have faced resistance by the pathogens. Moreover, *Aloe yavellana*, is under the threat of extinction due to agriculture and heavy grazing by herded animals [20] and it needs conservation and a

thorough phytochemical investigation to identify secondary metabolites that are responsible for its medicinal use. In this paper, we report the Phytochemical screening of methanolic extracts of the roots and leaves of *Aloe yavellana* and also evaluation of antibacterial activities (specifically minimum inhibitory concentrations, MICs) of the extracts. Our literature survey also showed that there are no published reports, to date, on phytochemical investigation and antibacterial activity tests of any plant parts this species.

## MATERIALS AND METHODS

**Collection and Extraction of Plant Material:** The plant materials (leaves and roots) were collected from Yabello area (Oromia region) on the road to Konso and near the border of Kenya (At altitude of 1910 m, Latitude 38.04E and Longitude of 45.3N). The plant was authenticated by Professor Fikre Dessalegn, Department of Botany, Addis Ababa University and the plant specimen was deposited at the herbarium of Science Faculty, Addis Ababa University. The plant materials were dried in an open air without exposing them to direct sun light. The dried plant materials were then separately powdered to suitable size and made ready for extraction. The dried and powdered form (200 g) root and leaf (200 g) were soaked separately in 100% methanol for 72hrs. The extracts were then filtered using Whatman No.1 filter paper. The methanol filtrate was concentrated to dryness under reduced pressure at 40 °C using a rotary evaporator. The resulting crude extracts were weighted and stored in refrigerator until used for phytochemical screening and determination of minimum inhibitory concentration (MIC).

**Screening of Phytochemical Constituents:** The crude extracts were subjected to various phytochemical tests to identify the chemical constituents. The tests carried out following standard procedures reported in literature.

**Test for Alkaloids:** 0.3 g of each of the extracts were dissolved in 2 mL of dilute hydrochloric acid. The mixture was then filtered. The filtrate was treated with Dragendroff's reagent. Formation of red precipitate was inspected [21].

**Test for Glycosides:** Extracts were hydrolyzed with dil. HCl and then subjected to test for glycosides. Modified Borntrager's Test: Extracts were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted

with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides [22].

**Test for Saponins:** 0.2 g of each of the methanolic extracts were taken in small test tubes and mixed with 5 ml of distilled water. The mixture this was shaken and boiled for 15 minutes. Formation of small bubbles (Frothing) was inspected [22, 23].

**Test for Phenols:** The extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols [24].

**Test for Tannins:** Small quantity of each of the methanol extracts were mixed with water and heated on water bath. The mixture was filtered and small amount of solid  $\text{FeCl}_3$  was added to the filtrate. Dark-green solution was inspected [23, 25].

**Test for Flavonoids:** 0.3 g of each of the methanolic extracts was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid was inspected [26].

**Test for Anthraquinones:** Borntrager's test was used for this test. 0.5 g of each of the extracts was taken into dry test tubes and boiled with conc. Hydrochloric acid for few minutes and filtered. The filtrate was allowed to cool and mixed with equal volume of chloroform ( $\text{CHCl}_3$ ). Finally, 5 ml of 10% ammonia solution was add to each of the filtrates of the extracts and heated in water bath. Formation of pink, red or violet colour of the mixtures was inspected [25].

**Test for Terpenoids:** 0.2 g of each of the extracts was dissolved in a 2 ml of chloroform. 2 ml of concentrated sulphuric acid was added to the mixtures and heated for 2 minutes. Formation of a reddish-brown colored interface was inspected [27].

**Tests for Steroids:** 0.5 g of each of the extracts was mixed 2 ml of acetic anhydride. Then 2ml concentrated sulphuric acid was added. Color change from violet to blue or green was inspected [28].

**Test Organisms:** The test organisms *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*

were obtained and confirmed at Chromopark Research Laboratory, Trichy Road, Nammakal 637001, Tamil Nadu, India. They were maintained on Mueller-Hinton Agar medium. Twenty-four hour old pure cultures were prepared for use each time. All the antibacterial activity tests were carried out at Chromopark Research Laboratory, Trichy Road, Nammakal- 637001, Tamil Nadu, India.

**Determination of Minimum Inhibitory Concentration (MIC) of Extracts:** This test was carried out using procedure reported in literature [29]. Plates were prepared under aseptic conditions. A volume of 100  $\mu\text{L}$  of test material in 10% (v/v) DMSO or sterile water (usually a stock concentration of 1 mg/mL for purified compounds and 10 mg/mL for crude extracts) was pipetted into the first row of the plate. To all other wells 50  $\mu\text{L}$  of nutrient broth or normal saline was added. To each well 10  $\mu\text{L}$  of resazurin indicator solution was added. Finally, 10  $\mu\text{L}$  of bacterial suspension ( $5 \times 10^6$  cfu/mL) was added to each well to achieve a concentration of  $5 \times 10^5$  cfu/mL. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Different extract concentrations (0.25mg/ml, 0.5g/ml, 1.0 g/ml, 1.5 g/ml and 5 g/ml) of extracts were applied with the help of micropipette and incubated at 37°C for 24 h. The standard drug (Ciproflaxcin) was used as positive control in the experiment. The sensitivity of the bacterial species to the extracts was determined by measuring the diameter of the inhibitory zones (in mm).

## RESULTS AND DISCUSSION

It is known that phytochemical constituents or secondary metabolites are responsible for most of observed biological (antibacterial, antifungal, antiviral and pesticidal) activities of medicinal plants [30]. In order to find out the phytochemical constituents, phytochemical tests were carried out on the methanol extracts of the leaves and the roots of *A. yavellana* following standard procedures reported in literature (*See experimental section*). The results revealed that the root extract contained alkaloids, glycosides, saponins, phenols, flavanoids, anthraquinones, terpenoids and steroids. Tannins were not detected in the root extract (Table 1). Similar phytochemical investigation also revealed that the leaf extract contained alkaloids, saponins phenols, flavanoids, anthraquinones and steroids. Tannins, terpenoids and glycosides were not detected (Table 1). The presence these secondary metabolites substantiates the claim by traditional health care givers about the use of

Table 1: Showing phytochemical constituents of root and leaf extracts of *A. yavellana*

S.No	Test	Root extract	Leaf extract
1	Alkaloids	+	+
2	Glycosides	+	-
3	Saponins	+	+
4	Phenols	+	+
5	Tannins	-	-
6	Flavanoids	+	+
7	Anthraquinones	+	+
8	Terpenes	+	-
9	Steroids	+	+

(+): presence of constituent; (-): absence of constituent

Table 2: Minimum inhibitory concentration of the root extracts of *Aloe yavellana* (in mm)

S.No	Bacterial strains	Cip	0.25	0.5	1	1.5	5
1.	<i>E. faecalis</i>	29	-	-	-	-	11
2.	<i>S. aureus</i>	25	-	-	11	13	15
3.	<i>K. pneumoniae</i>	17	-	-	-	-	11
4.	<i>P. aeruginosa</i>	28	-	-	-	-	12

Cip: the standard drug Ciproflaxcin; -: No zone of inhibition or no growth inhibition

Table 3: Minimum inhibitory concentration of leaf extract of leaf of *Aloe yavellana* (Zone of inhibition in mm)

S.No	Isolates	Cip	0.25	0.5	1	1.5	5
1	<i>E. faecalis</i>	25	-	-	-	-	13
2	<i>S. aureus</i>	25	-	-	-	-	-
3	<i>K. pneumoniae</i>	17	-	-	-	13	17
4	<i>P. aeruginosa</i>	28	-	-	-	-	-

Cip: Ciproflaxcin; -: No zone of inhibition or no growth inhibition

this plant species in the treatment of bacterial infections. Moreover, this fact is consistent with literature reports that discuss antibacterial activities of secondary metabolites such as alkaloids [31] and polyphenols [32].

In order to evaluate their antibacterial activities and their potential as sources of new antibacterial agents, both the root and leaf extracts were subjected to *in vitro* tests (or minimum inhibitory concentration test). Minimum inhibitory concentration (MIC) is the lowest (minimum) concentration that inhibits growth of bacteria. As discussed above, the antibacterial activities of the crude extracts were carried out against four bacterial strains namely *S. aureus*, *K. pneumoniae*, *E. faecalis* and *P. aeruginosa*. Four different concentrations (0.5, 1.0, 1.5 and 5 mg/ml) of each of the extracts were used. The results indicated that all the bacterial strains were found not to be sensitive to low growth was observed at concentrations of 1 and 1.5 mg/ml against *E. faecalis*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa*. *S. aureus* was found to be sensitive to the extract at and above 1

mg/ml concentration (1, 1.5 and 5 mg/ml). At these concentrations, the observed inhibition zones were 11, 13 and 15 mm, respectively, against *S. aureus* (Table 2). These data also showed that with increasing the concentrations, zone of inhibition against this bacterial strain also increased. For the rest of the bacterial strains namely *E. faecalis*, *K. pneumoniae* and *P. aeruginosa*, the MICs values were found to be 11, 11 and 12 mm, respectively. The results also revealed that the observed zones of inhibitions are comparable to each other but lower than that of the reference drug (Ciproflaxcin, Table 2).

With regard to antibacterial activity of the crude extract of leaves of *Aloe yavellana*, the observed data revealed that there is no inhibition of growth of *S. aureus* and *P. aeruginosa* in all of the concentrations of the extract used in the test (Table 3). Among the five concentrations of the crude extract of the leaves of *Aloe yavellana*, only 1.5 and 5 g/ml of were observed to inhibit growth of *K. pneumoniae*. The inhibition zones were 13 and 17 mm, respectively (Table 3). Another bacterial strain that was inhibited by a crude extract of concentration of 5 mg/ml was *E. faecalis* (13 mm). Moreover, the observed inhibition of the leaf extract at concentration of 5mg/ml was found to be the same with that of the reference drug (Ciproflaxcin) against *K. pneumoniae*.

The results also revealed that growth inhibition of the bacterial could effectively occur at concentration of = 5mg/ml of the crude extracts. Despite their comparable inhibition zones (at 5 mg/ml), the root extract was found to be effective against the four bacterial strains whereas the leaf extracts were more effective than root extract only against two bacterial species (namely, *K. pneumoniae* and *E. faecalis*). The difference in inhibition activities of root and leaf extracts could be attributed to the absence some phytochemicals (e.g., terpenes and glycosides) in the leaf extract (Table 1). This is consistent with the literature reports that discuss antibacterial activities of terpenes [33, 34] and glycosides [35, 36]. The fact that the root extract is observed to be active against both gram-negative (*K. pneumoniae* and *P. aeruginosa*) and gram-positive (*E. faecalis* and *S. aureus*) bacteria suggests the possibility of developing new broad spectrum agents that could be used for treatment of bacterial infections that are resistant to currently existing drugs. Further investigation is recommended on additional bacterial species to evaluate antibacterial activities of the extracts and isolation of compounds from the extracts to be tested for their antibacterial activities.

## CONCLUSION

Searching for new antimicrobial agents from plants and detect its ability to treat diseases caused by resistant microorganisms is needed. *Aloe yavellana* is one of the most common endemic medicinal plants used in traditional medicine in Ethiopia. The result of the phyto chemical analysis of the methanol extracts of the root and leaves of the plant indicated the presence of important secondary metabolites. Its traditional use as medicinal plant could be attributed to these compounds. Moreover, the test on minimum inhibitory concentrations of the extracts on gram-negative and gram negative bacterial species showed promising results. However, further investigation is needed to evaluate on antibacterial activities of the extracts on additional bacterial species and to isolate bioactive compounds that could be used as candidates in the discovery of novel antibacterial agents.

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