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Antibacterial activity and phytochemical investigation of leaf and root extracts of *Aloe gilbertii Reynolds*

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The objective of the study was to test the antibacterial activities of crude extracts of roots and leaves of *Aloe gilbertii* Reynolds against clinical pathogens. The crude extracts were prepared via maceration technique employing n-hexane, acetone, chloroform, dichloromethane:methanol (50:50% V/V) and methanol solvent system. The phytochemical screening tests of the dichloromethane:methanol (50:50% V/V) root extract of *A. gilbertii* revealed the presence of alkaloids, glycosides, phenols, flavonoids, anthraquinones and terpenoids. In the same way, the phytochemical tests of dichloromethane:methanol (50:50% V/V) leaf extract of the same plant revealed the presence of alkaloids, saponins, phenols, flavonoids, anthraquinones and steroids. Antibacterial activities of both plant parts were tested against four bacterial strains namely *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae* and *Escherichia coli* using the agar well diffusion method. Root extracts were found to possess better growth inhibitory activities against all the bacterial species. The zones of inhibition were in the ranges of 8 to 23 and 8 to 18 mm, for the root and leaf extracts, respectively. The finding of the study justifies the use of the *A. gilbertii* Reynolds in traditional medicine for the treatment of various human illnesses caused by bacterial organisms; however, further investigations are needed.

Key words: Antibacterial activity, phytochemical screening, *Aloe gilbertii*, leaf extract, root extract.

INTRODUCTION

Plants have not only nutritional values but also medicinal and ritual/magical values. Literature reports revealed that medicinal plants have a long history of use in most communities in developing countries (Sofowora et al., 2013). The vast majority (70-80%) of people in the developing world consult Traditional Medical Practitioners (TMPs) for their healthcare systems (Tugume and Nyakoojo, 2019; Tezera et al., 2020). The medicinal plants contain vitamins, minerals and a variety of secondary metabolites and have been used for a long time by TMPs for the treatment of numerous human and animal diseases in various parts of the developing world (Tugume and Nyakoojo, 2019). It is believed that medicinal plants have been used and are still in use as the primary source of medicine. *Aloe gilbertii* is widely used locally by different communities in Ethiopia as a medicinal plant in the

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Figure 1. *Aloe gilbertii* species.

treatments of malaria and wound healing (Chitme et al., 2004). Aloe is a plant species with a long ethnobotanical and medicinal history around the world. The genus is comprised of approximately 420 species with centres of diversity in Southern and East Africa, the Arabian Peninsula and Madagascar. It is also reported to be an important source of biologically active compounds with well over 130 phytoconstituents isolated from the group (Wollela, 2018; Dagne et al., 2000). Aloe species are well known for their effectiveness in treating stomach ailments, gastrointestinal problems, skin diseases and constipation (Radha and Laxmipriya, 2015). They have anti-inflammatory, antiulcer and antidiabetic effects and wound healing properties (Belayneh et al., 2020; Singh et al., 2010). They are also used widely in the preparation of skincare, cosmetics products and as nutraceuticals (Upadhyay, 2018). *Aloe gilbertii* (Figure 1) is one of the *Aloes* that is endemic to Ethiopia (Sebsebe and Nordal, 2010; Wollela, 2018). Its different parts are used for the treatment of various diseases in traditional or folk remedies throughout the world (Belayneh et al., 2020). For instance, leaves and root parts have been used by local people mainly for the treatment of malaria and wounds (Fikre, 2013). Some of the pharmaceutical reports revealed that dichloromethane: methanol (50:50% V/V) root extract of *A. gilbertii* showed the presence of secondary metabolites such as flavonoids, anthraquinones, alkaloids, saponins and phenol (Mudin et al., 2018; Yadeta, 2019a). Despite its wide medicinal use and phytochemical studies, there are no as such published reports on the investigation of the antibacterial activities of root extracts of Ethiopian endemic Aloe species. This fact has initiated the present study to study the in *vitro* antibacterial activities of the root and leaf extracts of *A. gilbertii* against selected multidrug-resistant gram-positive and gram-negative bacterial pathogens.

MATERIALS AND METHODS

Plant material collection and authentication

The plant materials (root and leaf parts) were collected from July 2015 to September 2016 from Alamura Hill, on the road to Dilla and Alaba Mountain slopes of Southern Nation Nationalities People Region (SNNPR), Southern Ethiopia. The plant material was authenticated by Professor Fikre Dessalegn, Department of Botany, Addis Ababa University and the plant specimen was deposited at the Herbarium Faculty of Science, Addis Ababa University. The plant materials were dried, powdered and made ready for extraction.

Preparation of plant extract

Powdered plant materials (500 g root and 500 g leaf) were sequentially extracted with n-hexane, acetone, chloroform (2 l each) for 24 h and dichloromethane: methanol (50:50% V/V) and methanol (2 l each) for 72 h, respectively by maceration. The extracts were filtered and concentrated under reduced pressure using a rotary evaporator at a temperature of 40°C. The resulting crude extracts of n-hexane, acetone, chloroform and methanol (100%) were discarded as the percent yields were too small to be used for the evaluation of antibacterial activities. The dichloromethane: methanol (50:50% V/V) was weighed and stored in a refrigerator until used for antimicrobial activity and phytochemical screening tests.

Phytochemical screening tests

Phytochemical screening was carried out on the crude extract of dichloromethane: methanol (50:50% V/V) roots and leaves to identify secondary metabolites. The screening was done following standard procedures reported in the literature. Test for alkaloids (Dragendroff's test): About 0.3 g of each of the crude extracts was mixed with concentrated hydrochloric acid (2 ml). The mixture was then filtered and mixed with a small amount of amyl alcohol at room temperature. Few drops of Dragendroff's reagent (Solution of potassium bismuth iodide) were added to the acid layer and a reddish-brown precipitate was observed (Ganjewala and Dipita, 2009). Test for tannins (Gelatin Test): Small amount of the extract was mixed with water and heated in a water bath. Then, a gelatin solution (0.5 ml) that contains sodium

chloride was added to the above mixture. The formation of a white precipitate indicates the presence of tannins (Saklani et al., 2012). Test for phenols: The extract (0.5 g) was dissolved in distilled water (5 ml). Then few drops of neutral 5% ferric chloride solution were added to the mixture. The formation of a dark green color was used as an indicator for the presence of phenolic compounds (Rohit, 2015).

Test for anthraquinones: About 0.5 g of the methanol extract was boiled with concentrated hydrochloric acid for a few minutes in a water bath and filtered. The filtrate was allowed to cool and an equal volume of chloroform was added to it. A Few drops of ammonia were added to the mixture and heated in a water bath. The formation of rose-pink color was inspected for the presence of arthroquinones (Evans, 2002).

Test for saponins (Froth Test): About 0.1 g of the crude extract was dissolved in water (20 ml) shaken in a graduated cylinder for 15 min. The formation of a 1 cm layer of foam indicates the presence of saponins (Roopashree et al., 2008).

Test for terpenes: About 0.25 g of extract was mixed with chloroform (2 ml) and concentrated sulfuric acid (30 ml) was added carefully to form a layer. The reddish-brown coloration of the interface was inspected for the presence of terpenes (Alamzeb et al., 2013).

Test for flavonoids (Alkaline Reagent Test): Few drops of sodium hydroxide solution were added to the extract and the formation of intense yellow color, which becomes colorless on the addition of dilute acid, indicates the presence of flavonoids (Saklani et al., 2012).

Tests for steroids (Liebermann-Burchard test): Small amount (0.1 g) of each extract was shaken with chloroform in a test tube; a few drops of acetic anhydride were added to the test tube and boiled in a water bath and rapidly cooled in iced water. Concentrated sulfuric acid (2 ml) was added to the above mixture. Formation of a brown ring at the junction of two layers and turning the upper layer to green shows the presence of steroids (Joshi et al., 2013).

Bacterial activity tests

Test organisms: The selected bacterial strains two gram-positive (*Staphylococcus aureus* (ATCC 25923), *Enterococcus faecali* (ATCC29212) and two gram negative bacteria, *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae,* (ATCC700603) 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, Tamil Nadu, India. The bacterial strains were reactivated by sub culturing on a nutrient broth at 37°C and maintained on a nutrient agar slant at 4°C for further activity.

In vitro antibacterial activity

Agar well diffusion assay

The antibacterial activity was carried out using the agar diffusion method (Ba-Hamdan et al., 2014). The 20 ml of Muller Hinton agar media was placed in the Petri dishes (100 mm diameter) and then the medium surface was impregnated with the 24 h grown selected bacterial strains (1.5×10^6 cells per ml). Different concentrations (5-12.5 mg) of the dichloromethane: methanol (50:50% V/V) root and leaf parts of *A. gilbertii* extracts were dispensed in separate well with the help of a micropipette incubated at 37 °C for 24 h. The dissolution of the organic extracts was facilitated with the addition of 10% (v/v) dimethyl sulfoxide (DMSO) which did not affect the growth of microorganisms (as shown by our control experiments). The formation of an inhibition zone was measured using the ruler that measured in millimeters. Lack of bacterial growth represented the antibacterial effect in the medium. The 10 µg of standard antibiotic (Ampicillin) was used as a reference drug.

Determination of minimum inhibition concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of root and leaf parts of *Aloe gilbertii* extracts showed significant antibacterial activity against gram-positive bacterial strains *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecali* (ATCC29212) and two negative bacteria, *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae,* (ATCC700603) were determined using Mueller–Hinton broth microdilution method of the Clinical and Laboratory Standards Institute, M07-A8 AS. The original stock solutions of selected plant extracts were prepared with at 50 mg extract/ml 10% DMSO solution. The MIC of tested plant extract was determined as the lowest concentration inhibiting the visual growth of the tested bacterial cultures. The initial test concentration was serially diluted in a 96 well plate to obtain final concentrations of 10, 9, 8, 7, 6 and 5mg/ml with the DMSO solution and inoculated with 5 µl of suspension containing 10^8 CFU ml^{-1} of selected bacterial strains. The 96 well plates were incubated for 24 h at 37 °C for bacterial growth. The culture intensity of each well was read at 600 nm and compared with the untreated control. The experiments were conducted in triplicates

RESULTS AND DISCUSSION

Phytochemical screening tests of *A. gilbertii* extracts

The results from the phytochemical screening of the dichloromethane: methanol (50:50% V/V) A*. gilbertii* roots extract revealed the presence of secondary metabolites such as alkaloids, glycosides, phenols, flavonoids, anthraquinones and terpenoids whereas alkaloids, saponins, phenols, flavonoids, anthraquinones and steroids were detected the leaf extract (Table 1). The present finding is consistent with previous reports by (Mudin et al. 2018) and Yadeta (2019b). In this study, glycosides and terpenes were found in the root extracts whereas these two classes of compounds were not detected from the leaves of *A. gilbertii*. Moreover, saponins and steroids were detected in leaf extracts but not in the root extract (Table 1). The presence of these secondary metabolites could be responsible for the wide medicinal uses of *A. gilbertii*.

Antibacterial activities of the crude extracts

It is known that phytochemical constituents or secondary metabolites are responsible for most of the biological activities such as antibacterial activities of medicinal plants (Anushia et al., 2009). 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* activity tests against four bacterial strains namely *S. aureus, E. faecalis, K. pneumoniae* and *E. coli,* using four different concentrations (5, 7.5, 10 and 12.5 mg/ml). The results indicated that the gram negative

Table 1. Secondary metabolites identified in the *A. gilbertii* roots and leaves crude extracts.

Phytoconstituents	Root extract	Leaf extract
Alkaloids	+	+
Glycosides	+	-
Saponins	-	+
Phenols	+	+
Tannins	+	+
Flavanoids	+	+
Anthraquinones	+	+
Terpenes	+	-
Steroids	-	+

Present (+); Absent (-).

Table 2. The zones of inhibitions of the root and leaf extracts of *A. gilbertii* at different concentrations.

Bacterial strain	Conc. of extracts (mg/ml)/zone of inhibition (in mm)								Zone of inhibition of Ampicillin (10 µg/ml)
	5		7.5		10		12.5		
	R.E	L.E	R.E	L.E	R.E	L.E	R.E	L.E	
E. coli	12	10	16	12	18	15	21	18	-
K. pneumoniae	-	-	8	-	11	8	14	11	-
E. faecalis	13	8	16	12	18	15	23	17	-
S. aureus	-	9	-	12	-	15	8	17	8

No zone of inhibition or no growth inhibition; R.E: root extract; L.E: leaf extract.

bacterial strains such as *K. pneumonia* at 5 mg/ml, gram positive *S. aureus* at 5, 7.5 and 10 mg/ml concentrations were found not to be sensitive to the root extract of *A. gilbertii* whereas gram positive *E. faecalis* and gram-negative *E. coli* were found to be sensitive to the extracts at 5 mg/ml concentration. At 5, 7.5, 10 and 12.5 mg/ml concentrations, the observed inhibition zones for E. *faecalis* and *E. coli* were 13, 16, 18, 23 and 12, 16, 18, 21 mm, respectively (Table 2). The observed inhibition zone for the root extract at a concentration of 12.5 mg/ml was found to be the same (8 mm) as that of the reference antibiotic drug (Ampicillin -10 µg/ml) against *S. aureus* (Table 2). The observed inhibitions zones were also found relatively to be significant at higher concentrations than that of the reference drug for most of the bacterial strains (*E.coli, K. pneumoniae and E. faecalis*). The data also showed that the zones of inhibition increase with the increasing concentrations of the extracts (Table 2). A similar trend was observed for the leaf extracts on three bacterial strains (*E. coli, E. faecalis* and *S. aureus*). On the other hand, no growth of *K. pneumoniae* was observed at low concentrations (5 and 7.5 mg/ml) (Table 2). Despite their comparable inhibition zones (at 12.5 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 one bacterial species namely, *S. aureus*. The difference in inhibition activities of root and leaf extracts could be attributed to the absence of some phytochemicals (such as, terpenes and glycosides) in the leaf extract (Table 1). This is consistent with the literature reports that discuss antibacterial activities of terpenes (Yoshihiro et al., 2008; Naoko et al., 2008), alkaloids (Singh and Verma, 2011) and polyphenols (Dua et al., 2013) as well as glycosides (Nazemiyeh et al., 2008; Sameerah et al., 2013). The fact that the root extract is active against both gram-negative (*K. pneumoniae* and *E. coli*) and gram-positive (*E. faecalis* and *S. aureus*) bacteria suggests the possibility of developing new broad-spectrum agents that could be used for the treatment of bacterial infections that are resistant to currently existing drugs.

The minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the root and leaf extracts of *A. gilbertii* were assessed by 96 well plate broth micro dilution methods with a concentration range from 5mg /ml to 10 mg /ml. Only the tested bacteria, which were highly susceptible to the selected plant extracts, were taken for determining the MIC. The maximal zones of inhibition and MIC values for tested bacterial strains, which were sensitive to the *A. gilbertii* root and leaf extracts, were in the range of 8-23 mm and MIC values of 1 - 8.5 mg/ml. As shown in Table 3, among the tested plant

Table 3. Minimum inhibitory concentration (MIC) of extracts on selected bacterial strains.

Plant extract	*S. aureus* (ATCC25923) (mg/ml)	*E. feacalis (*ATCC 29212) (mg/ml)	*E. coli* (ATCC25922) (mg/ml)	*K. Pneumonia* (ATCC 700603) (mg/ml)
Leaf	1	1	1	6
Root	8.5	1	1	3.5

extracts, a root extract of *A. gilbertii* showed strong antibacterial activity against *E. faecalis* and *E. coli* with a significant zone of inhibition were in the range of 13-23 mm and 12-21 mm respectively with MIC values of 1 mg /ml.

Conclusion

This work is one of the few attempts to analyze the phytochemical constituents and *in vitro* antibacterial activity of polar extracts of the roots and leaf of *A. gilbertii* indigenous to Ethiopian flora. Phytochemical screening tests of the crude dichloromethane:methanol (50:50% V/V) root extract of *A. gilbertii* revealed the presence of alkaloids, terpenoids, anthraquinones, phenols, flavonoids, steroids, glycosides, saponins whereas alkaloids, anthraquinones, phenols, flavonoids, steroids and saponins were detected in the leaf extracts. In agreement with the previous study, the wide traditional use of the plant may be attributed to its rich anthraquinones and phenolic compound constituents. Moreover, the *in vitro* antibacterial activity of root and leaf extracts of *A. gilbertii* showed significant antibacterial activity. Thus, further work is recommended on this endemic plant to validate its use in traditional use and to identify more bioactive secondary metabolites and compounds in support of its traditional use.

CONFLICT OF INTEREST

The authors have not declared any conflict of interest.

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