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RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF BIOACTIVE COMPOUNDS FROM ROOT EXTRACTS OF *ALOE OTALLENSIS*

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ABSTRACT

The worldwide candidacy of plant species for treatment of various diseases and infections still stood firm today. In developing countries, such as Ethiopia, where health care coverage is less, traditional medicine has played a significant role in treating health problems in both livestock and humans. *Aloe otallensis* is one of the plant species widely used in traditional medicine in Southern parts of Ethiopia, for treatments of human illnesses with the help of traditional practitioners. Medicinal products used by the society are neither controlled nor properly regulated by quality assurance parameters and may cause serious problem when not properly utilized. This study was conducted aiming to isolate and characterize bioactive compounds from *aloe otallensis* root extracts for medicinal purposes. Extraction was carried out from root part of the plant with solvents of different polarities. IR and NMR spectroscopic techniques were used to get spectrum of the extracts. The phytochemical analysis from crude extracts confirmed the presence of alkaloids, saponins, flavonoids, phenols, steroids, glycosides, terpenoids and tannins. Two different pure compounds are isolated and the compounds are characterized using spectra of spectroscopic techniques (IR, ^{1}H-NMR, ^{13}C-NMR and DEPT-135). Results signify that the two isolated and characterized compounds are 8-acetoxy -8, 9 ,9a ,10-tetrahydrobenzo[g]isoquinoline-2(3H)-carboxylic acid and 7-acetoxy – 7, 8, 9 – dihydrobenzo[g] oxy-nitro isoquinoline -2(5aH) - carboxylic acid . These compounds are alkaloids and can be used for antimalarial and anti-pain treatment and this result supports the knowledge of the community of using the plant parts for antimalarial and anti-bacterial treatments.

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INTRODUCTION

The use of medicinal plants as a source for relief from illness can be traced back over long years. Today, plants remain the most common source of antimicrobial agents. *Aloe*, a genus belonging to family *Xanthorrhoeaceae*, comprises over 460 species of succulent flowering plants varying from small herbs to large woody trees (Wollela, 2018; Nebiyu, 2019). *Aloe otallensis* is one of the species of *Aloe* and is endogenous plant to Ethiopian (Wollela, 2018; Paulos, 2012). Different species of the genus *Aloe* contain variety classes of secondary metabolites as it is showed by their extraction using different solvents. Water extraction of *Aloe vera* has been screened for tannins, saponins, anthraquinones, flavonoids, alkaloids and phenols (Nwaoguikpe, 2010) while methanol extraction of *Aloe vera* showed positive response to presence of tannins, flavones, alkaloids and quinines (García, 2014). The phytochemical investigation from *Aloe* g *ilbertii Reynolds* revealed the presence of alkaloids, saponins, flavonoids, phenols, steroids, glycosides, terpenoids and tannins. Phytochemical investigation of the root extracts of *A. pulcherrima* has resulted in identification of anthraquinones (Abdissa *et al*., 2017; Teka, 2016). Studies have also been conducted on identification of antimalarial activities of the *Aloe debrana* species of the same genus (Gemechu, 2014; Deressa, 2010). Abeje and his co-workers carried out phytochemistry and antileishmanial activity of the leaf latex of *Aloe calidophila Reynolds* from Yabello and Mega, Southern Ethiopia, and found the presence of anthronesaloinoside, aloin, and microdontin. Research works from Oumer and co-workers (Oumera, 2014) revealed potential candidacy of antimicrobial anthrone from leaf latex of *Aloe trichosantha* in samples collected from Afar region

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of eastern Ethiopia. *In Vitro* antimicrobial and antioxidant activities of anthrone and chromone from the latex of *Aloe harlana Reynolds* showed positive response (Asamenew, 2011). The antibacterial activity potential assessment of *Aloe weloensis* showed positive response against bacteria strains (Emiru, 2019). As *Aloe otallensis* is species in the genus *Aloe*, it could be expected that it could have similar constituents obtained from other species of the same genus. Sofar only very few researches have been done on *Aloe otallensis* species in Ethiopia regarding pure compound isolation and characterization. According to study conducted in southern part of Ethiopia, therapeutic compounds are isolated and identified from *aloe otallensis* for their antimalarial activity (Paulos, 2012; Paulos, 2011) and antioxidant (Paulos, 2011). Phytochemical screening was done for *Aloe otallensis* in southern Ethiopia (Nigusse, 2016). Gamo Community of Southern part of Ethiopia extensively use *Aloe otallensis* for malarial (Paulos, 2012) and tonsillitis treatments. Irrespective of attempts made to identify and screen phytochemicals in *Aloe otallensis* species, it is obvious that more advanced research is need for further investigation of bioactive compounds in *Aloe otallensis* that are responsible for traditional medicinal treatments. This paper aims for isolation and characterization of bioactive compounds from *aloe otallensis*.

MATERIALS AND METHODS

Plant Materials: Roosts of *Aloe otallensis* were collected in June 2019 from Gamo Zone, Southern part of Ethiopia. Authentication and Botanical identification was done using standard identification keys in Department of Biology, Wolaita Sodo University, Ethiopia.

Chemicals: N-hexane dichloromethane/methanol (1:1) and methanol were used for gradient extraction; ethyl acetate and n-hexane were used for column elution. Pre-coated Thin Layer Chromatography (Silca gel, UV254) plats were used for chromatographic analyses. The chemicals used in this study were all of analytical grades and were purchased from Ranchem co.ltd Agents in Addis Ababa, Ethiopia

Materials: Rotary evaporator (Heidolph, UK) was used for concentration of crude extracts while Grant (Gl S400) thermostatic bath shaker was used for maceration of plant materials. Oven (model: N50L, GENLAB, WIDNES, England) for heating, analytical balance (AFP-110L) for weight measuring, UV chamber (Uvitec) for visualizing molecular segregation were used. 1H-NMR, 13C-NMR and DEPT-135 spectra were recorded using Bruker (400 MHz) spectrometer while IR spectra were obtained from Perkin Elmer BX infrared spectrometer (400-4000 cm^{-1}).

Preparation of Sample: The collected plant materials (root) were chopped into small pieces and were air–dried for 30 days without exposing to sun light. It was then milled to suitable size for extraction using local available mortar. About 1000 g plant powder was prepared for further extraction procedure.

Extraction: Thousand grams of powdered *Aleo otallensis* root was macerated by using n-hexane, dichloromethane/ methanol (50%:50%) and methanol for 72 h with continuous shaking using shaker machine one after another. The resulting supernatant solution was filtered using Whatman filter paper No. 1 and the residual solvent in each gradient extract was removed using Rota Vapor (Heidolph, UK) under reduced pressure and then dried on an oven at a temperature of 40 °C to remove the solvent and kept in the refrigerator. The percent yields of the extracts were calculated using

$$\text{Percentage yield} = \frac{\text{mass of the extract}}{\text{mass of plant material used for extraction}} \text{X100}$$

Preliminary Phytochemical Screening Tests: Phytochemical examinations were carried out for all the extracts as per standard method reported in literatures (Nigusse, 2016). Based on these methods, identification tests for the presence of secondary metabolites namely steroids, terpenoids, saponins flavonoids, tannins, alkaloids, phenols and glycosides were carried out.

Isolation and Characterization of Compounds: Among the crude extracts carried out in this experiment, the crude extract of methanol showed the best TLC profile. Then, 15.4 g of methanol extract was adsorbed onto silica gel (20 g) and subjected to column chromatographic isolation. The column was then eluted using ethyl acetate and n-hexane mixture starting from 100% ethyl acetate and gradually increasing non polarity by adding n-hexane. The TLC analysis of crude extract in various combination of solvent varying polarity was used to select the solvent system for the isolation of pure compounds.

In doing so, ethyl acetate, n-hexane and combination of the two were able to show separation of pigments on TLC of the methanol crude extracts. Hence, the mixture of ethyl acetate and n-hexane has been used in different combination with slight increment in non-polarity. The collected fractions were concentrated using rotary evaporator. Fractions were tested using TLC. The spots on the TLC plates were visualized using UV light (at 254 nm and 365 nm) followed by iodine vapor. Pure isolates were produced in fractions (%:%): 95:5, 90:10 and 85:15 in combination of ethyl acetate and n-hexane, respectively. The column chromatographic separation led to isolation of two compounds. The structural elucidations of the compounds were carried out based on data obtained from spectroscopic (IR and NMR) techniques.

RESULTS AND DISCUSSION

Mass of Crude Extracts: Extractions of the plant material (roots) were carried out using different solvents of different polarities and in sequential extraction approach. It was started with n-hexane and followed by dichloromethane/methanol and methanol. As can be seen from Table 1, the amounts of extracts were 3.4 g, 7.8 g and 15.4 g for n-hexane, dichloromethane/methanol and methanol, respectively.

The resulted amount of crude extract of methanol was found to be higher than the other crude extracts. On top of that, the crude extract of methanol had an excellent TLC profile when compared to other solvent crude extracts. Due to these reasons, crude extract of methanol was the best candidate for isolation of compounds.

Phytochemical Screening: The phytochemical tests were conducted for alkaloids, flavonoids, phenols, glycosides, saponins, steroids, terpenoids and tannins.

Table 1: The mass of extracted matter in each gradient of *Aoeotallensis* crude root extracts

Solvent system used for extraction	Mass of crude extract(in gram)	Yield (%)
n-hexane	3.4	0.34
Dichloromethane/Methanol (1:1)	7.8	0.78
Methanol	15.4	1.54

Table 2. Phytochemical analysis of *Aoeotallensis* crude root extracts

Bioactive component	Reagent used	n-hexane extract		Dichloromethane/methanol extract		Methanol extract	
		Result	Observed color	Result	Observed color	Result	Observed color
Alkaloids	Dragendroff's reagent	++	Red color	++	Red color	++	Red color
Saponins	Distilled water	++	strong foam formation	++	Strong-foam formation	++	Strong foam formation
Flavonoids	Alkaline reagent	++	Yellow Color	+-	Weak-yellow color	+++	Intense yellow color
Phenols	Ferric chloride	+++	Bluish black color	+--	Fade-green color	+++	Bluish black color
Steroids	Liebermann Buchardrxn	+++	Blue-green color	+++	Blue-green color	+++	Blue-green color
Glycosides	Keller-Killani test	++	Reddish brown color	+++	Deep reddish brown color	+++	Deep reddish brown color
Terpenoids	Salkowski test	+++	Reddish brown color	+++	Reddish brown color	+++	Reddish brown color
Tannins	Ferric chloride	---	Colorless	++	Brownish green	++	Brownish green

Key: Medium (+–), Strong (++), Very Strong (+++), Absent (–)

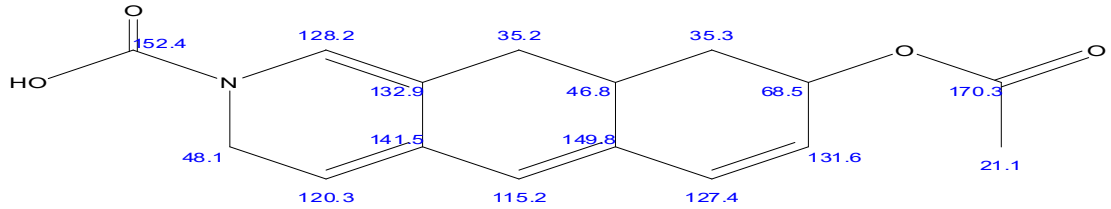

Figure 1: 8-acetoxy-8, 9,9a, 10-tetrahydrobenzo[g]isoquinoline-2(3H)-carboxylic acid

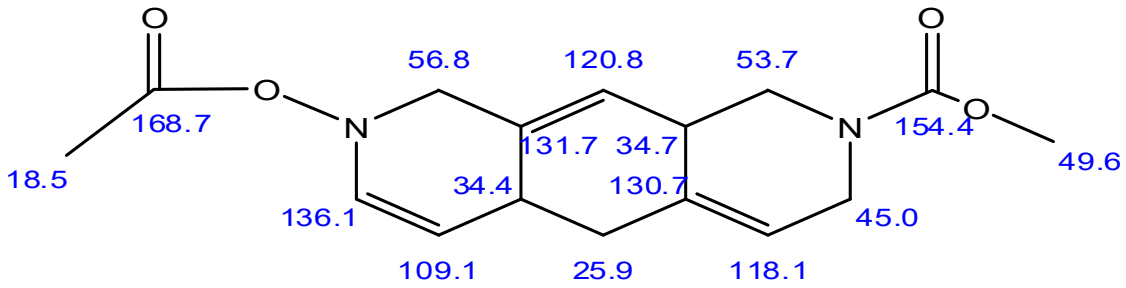

Figure 2: 7-acetoxy – 7, 8, 9 – dihydrobenzo[g] oxy-nitro isoquinoline -2(5aH) - carboxylic acid

It can be seen from Table2 that all the three crude extracts have showed positive results for secondary metabolites except n-hexane crude extract that did not show tannins for ferric chloride test.

Structural Elucidation

Structural ElucidationCompound A1: Compound A1 was obtained as a light yellowish powder appearance compound (38 mg). Analysis of its IR spectrum (Appendix A (Fig A1)) reveals stretching band at 3480 cm^{-1} that indicates the compound has alcohol (OH) functional group. The strong band at 2937 cm^{-1} represents C-H stretch of alkenes whereas the bands at 2862.9 cm^{-1} indicates the C-H stretching of methyl groups.

The observed data suggests that compound A1 could be an alcohol possessing (C=C) carbon-carbon double bond in its chain, The presence of strong band around 1700-1800 cm^{-1} also shows that the compound has carbonyl functional group. Weak bands observed in the range of 2000 and 1650 cm^{-1} indicate that the compound has aromatic functional group. ^{1}H-NMR spectrum (Appendix A (Fig A2)) peaks at 6.6-7.4 indicate the presence of aromatic protons, the signals from 3.5-4.5 indicate the presence of oxygen bearing group, the signals around 9.8 indicate the presence of carboxylic proton. Combining both IR and NMR spectra, compound A1 can be elucidated in Figure 1. ^{13}C-NMR (Appendix A (Fig A3)) and DEPT-135 spectrum (Appendix A (Fig A4)) infer the compound A1 to be alkaloid based on the NMR spectrum with 16 carbons. Investigation of ^{13}C NMR spectrum reveals

the characteristic signals for ester (δ C 168.28) and vinyl carbons (quaternary C at δ C 15).

Compound A2: Compound A2 was obtained as light greenish crystalline solid compound. The IR (KBr) spectrum (Appendix B (Fig B1)) of compound A2 has a strong band around 3400 cm^{-1} that indicates the presence of hydroxyl functional group. The strong band at 2950 cm^{-1} represents C-H stretch of alkenes whereas the band at 2870 cm^{-1} indicates C-H stretching of methyl groups. The band 16500 cm^{-1} indicates the presence of C-O bond. The observed data suggests that compound A2 could be an alcohol and C=C double bond bearing compound. ^{1}H-NMR spectrum (Appendix B (Fig B2)) shows proton peaks at 6.6-7.4 that indicates the presence of aromatic protons. Signals from 3.5-4.5indicate the presence of methoxy group, around 9.8 indicate the presence of carboxylic proton. ^{13}C-NMR (Appendix B (Fig B3) and DEPT-135 spectrum (Appendix B (Fig B4)) infer the compound A2 to be alkaloid. Investigation of ^{13}C NMR spectrum reveals the characteristic signals for ester (δ C 168.28) and vinyl carbons (quaternary C at δ C 15). The structural elucidation ofthe compound A2 is displayed in Figure 2.

The phytochemical investigation of this work has supported the presence of all the seven bioactive compounds under investigation. Previous works have identified anthraquinones, phenolics, flavonoids, saponins and alkaloids (Nebiyu, 2019). The 80% methanol extract from exudates of *Aloe otallensis* done by Nugusse *et al*. identified the presence of saponins, alkaloids and phenols while failed to detect the presence of tannins, flavonoids and anthraquinones (Nigusse, 2016). The methanol-soluble ofthe *Aloe otallensis* leaf exudate done by Paulos and co-workers elucidated a malaria preventive compound (Paulos, 2011). Well-known alkaloids contain quinine, morphine strychnine, ephedrine and nicotine (Science Direct). In this work, methanol solvent was selected depending on the yield obtained from extraction and its best TLC profile. Two pure compounds are extracted and isolated in this work. The fully characterized structure of both the compounds are alkaloids. Of these compounds quinine is known to possess antimicrobial activities while morphine is powerful of relief of pain. Results presented in this study are encouraging and verify antimalarial and anti-pain potential of the species under investigation. The findings support the traditional use of *A. otallensis* for the treatment of malaria and anti-pain.

Conclusion

The present study aimed at investigation of phytochemicals and isolation and characterization of bioactive compounds from Ethiopia indigenous *Aloe otallensis* species for antimalarial and anti-pain activities. The phytochemical investigation indicated the presence of all the seven secondary metabolites considered in this work. Two pure compounds, namely: A1 (8-acetoxy-8, 9,9a, 10-tetrahydrobenzo[g] isoquinoline-2 (3H)-carboxylic acid) and A2 (7-acetoxy – 7, 8, 9 – dihydrobenzo[g] oxy-nitro isoquinoline -2(5aH) - carboxylic acid) are extracted. From this study, it can be concluded that the root extracts of *Aloe otallensis* possesses potential bioactive compounds that can be used as antimalarial and anti-pain activities. However, further studies directed towards the *in vito* antibacterial activity investigation and side effects is advisable.

Supplementary Materials: The IR and NMR spectra of Compounds A1and A2are included in the Supplementary Materials.

Conflicts of Interest: The authors declare no conflict of interest.

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