

In Vitro Antimicrobial Efficacy of Fractions from Onion (*Allium Cepa*) Leaves Extract from Wukro, Ethiopia

Teklit Gebregiorgis Amabye

Department of Chemistry, College of Natural and Computational Science, Mekelle University, Mekelle, Ethiopia

Email address:

teklitgeb@gmail.com

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Abstract: To examine the *in vitro* anti bacterial activities of the ethanol extract of fresh leaves of *Allium cepa* (onion) and to determine and quantify the phenol compounds of the investigated plantparts. This study was drifting out at the Mekelle university department chemistry and Adigrat pharmaceutical industry, from March 2015 to April 2015. Clinical strains of *Streptococcus pneumoniae* and ethanol extracts of the plant species was used for the antimicrobial study. Thirty grams of the sample was ground, filtrated, and each filtrate mixed with 100 ml ethanol and placed in a shaker for 48 hours. The ethanol was evaporated from the sample, weighed, and subjected to an antibacterial activity test using the agar diffusion method. The high-performance liquid chromatography was used to identify and quantify the phenols extracts of investigated samples. Ethanol extract of the investigated plant parts showed antibacterial activities against different pathogenic bacteria. Leaf extracts of *Allium cepa* showed the highest antibacterial activity and contains more phenols. The ethanol extract of the tested plants could be considered as an alternative source of new antibacterial drugs.

Keywords: HPLC, Antimicrobial Empire, *Streptococcus Pneumoniae*, Medicinal Plants

1. Introduction

Development of antibiotic resistance among common respiratory pathogens is a major cause of concern worldwide. *Streptococcus pneumoniae* (*S. pneumoniae*) is the most common respiratory pathogens. (*S. pneumoniae*) remains a common pathogen and leading cause of morbidity and mortality. [1] Transmission of *S. pneumoniae* occurs as the result of direct person-to-person contact via respiratory droplets and by autoinoculation in persons carrying the bacteria in their upper respiratory tract. [2] Many human diseases are known to have been treated with herbal medicine throughout the history of human beings. The increasing evolution of multi drug resistant bacteria and the recent appearance of strains with reduced susceptibility to antibiotics lead to the emergence of untreatable bacterial diseases. [3,4] In addition, the revival of interest in plant derived drugs is mainly due to the widespread belief that „natural medicines“ are safe and more dependable than the costly, synthetic drugs, many of which are toxic and possess adverse effects. Thus, there is a growing interest to explore the alternative drugs from different plant species that have antimicrobial properties and can be used as antibiotic resources. [5, 6]. In this respect, *Allium*

cepais applying significant change remedy against various microbial diseases. At the present time, the *Allium* family has over 500 members, each differing in appearance, color and taste, but close in biochemical, photochemical and nutraceutical content. [7] Recent studies revealed many bioactive compounds from this medicinal species. Hence, the present study was undertaken to evaluate antimicrobial activity of pure fractions from this plants against human pathogenic organism *S. pneumoniae*.

2. Materials and Methods

2.1. Collection of Plant Species

An *allium cepa* (onion) fresh leaf was collected from various area of Wukro district and analysis was carried out at Mekelle university department of chemistry and Adigrat pharmaceutical industry Tigray Adigrat Ethiopia. The plant materials were free from disease, and washed thoroughly 2-3 times with water and once with sterile distilled water.

2.2. Extraction of Plant Material

Thirty grams of fresh leaves of *Allium* was weighed out and

crushed directly by grinder for 15 minutes, and the solution samples were filtered through 2-layered muslin cloth. The filtrates were mixed with 100 ml ethanol, placed in a shaker for 48 hours, and then filtered through Whatman No. 1 filter paper. The ethanol in each filtrate was evaporated completely, and each extract was weighed and subjected to an antibacterial activity test.

2.3. Collection of Organism

Human pathogenic microbes *S. pneumoniae* were clinical isolates of patients isolated from clinical patients at dental clinics in and around Mekelle and Adigrat hospitals.

2.4. Analysis of Antimicrobial Activity

The antimicrobial activity was determined by the agar well diffusion method against different strains of bacteria 100 µl of standardized inoculum Nephelometer Standards of each test bacterium was spread onto sterile Muller-Hinton Agar (Hi-Media). A 9 mm diameter well was cut from the agar using a sterile cork-borer; subsequently each well was filled with 0.1 ml of the ethanol plant extraction. Sterile dimethyl sulfoxide (DMSO) served as negative and gentamicin (10 µg/disc) as positive control to determine the sensitivity of each bacterial species tested. The plates were kept at room temperature for one hour to allow proper diffusion of the extract into agar and then incubated at 37°C for 48 hours. Nine clinical strains of bacteria and two ethanol extracts of the plant species was used for the antimicrobial study. The test was performed in triplicate, and the minimal inhibitory concentration (MIC) using micro titer plates and the McFarland standard of a suspension formed from a liquid culture means or eugenic medium in which the biological sample is inoculated, and in which the turbidity is continuously measured directly from the suspension of the sample analyzed during the growth step of the bacteria, until a determined threshold of turbidity is reached expressed

according to the McFarland standard. and diameter of the cleared zones was determined. All tests were lugged out from March 2015 to April 2015 at the Mekelle university Department of chemistry and Adigrat pharmaceutical industry tigray Adigart Ethiopia.

2.5. Quantization of Phenols Using HPLC

A HPLC system (Shimadzu) consisting of a solvent delivery module (LC- 10AD) with a double plunger reciprocating pump, UV-VIS detector (SPA-10A), column oven (CTO-10A) and 20-µl injection loop were used. The column used was an Apex octadecyl 104 C18 (25x0.4 cm ID) with 5-µm packing (Jones Chromatography Limited, Colorado, USA). [9]. Phenol compounds present in the samples was identified by comparing retention time (RT) of the standards and by the co-injection. Contents of phenol compounds were calculated by Comparing peak areas of reference compound with those in the samples run under similar elution conditions.[10, 11]

Statistical analysis: The result of the antimicrobial activity was expressed as the means obtained from 3 independent analyses. Analysis of variance was used to compare between data. All analyses were performed at $p < 0.05$ using Minitab version 2000 13.1 (Minitab, State College, PA, USA).[11]

3. Results

The results of the antimicrobial activity of the ethanol extract of fresh leaves of *A. cepa* by agar well diffusion method revealed that all extracts showed inhibitory activity against *S. pneumoniae*. As shown in Table 1. Among the plants from various geographical origin tested, the ethanol leaf extracts of *Allium cepa* showed the highest antibacterial activity with a zone of inhibition ranging from 11.87- 19.23 mm at 20mg/ml concentration and MIC value at 10mg/ml.

Table 1. Antibacterial activity and MIC values of ethanol extract of fresh leaves of *Allium cepa*. Values are expressed as mean of the two replicates.

Human Pathogenic Bacteria	<i>Allium cepa</i> (MIC)		DMSO	Gentamicin 95%CI 10µg/disc (95%cl)
	20mg/ml	10mg/ml		
<i>Streptococcus Pneumoniae</i>	11.87(9.43-14.87)	18.62(16.63-23.68)	NIL	35.63(35.52-36.89)

Nil-no inhibition zone, DMSO-dimethyl sulfoxide, MIC-minimum inhibitory concentration, CI- confidence interval at $p < 0.05$

Table 2. Constitutions of five phenols in the ethanol extract of fresh leaves of *Allium cepa*

Analyte	<i>Allium cepa</i>	
	RT(min)	mg/100g
Catechin	ND	ND
Cinnamic acid	ND	ND
Ferulic acid	13.83	0.028
P-coumaric acid	14.45	0.011
Sinapic acid	ND	ND

RT-retention time of the peak, Tr-Concentration < 0.001 mg/100mg, ND-not detected, min- minutes, CI-confidence interval at $p < 0.05$

13.33 mm at 25mg/ml concentration and MIC value of 12.5 mg/ml. The HPLC scrutiny of the ethanol extract of fresh

leave of *A. cepa* fresh grand the description of five phenolic compounds: catechin, p-coumaric acid, ferulic acid, cinnamic acid and sinapic acid. As presented in Table 2, it perhaps certain that the type and amount of phenolic compounds detected varied in terms of the tested plants.

4. Discussion

Medicinal plants play a crucial role in the search for alternative antimicrobial components. According to the World Health Organization, it is estimated that around 80% of the earth's population use some form of herbal medicine in their health care, whereas natural products are preferable option than synthetic ones[12]. The literature indicates that medicinal

plants have secondary compounds that are of great importance in human life in terms of acting as antioxidants, being anti-inflammatory, and involved in the modulation of detoxification enzymes, the stimulation of the immune system, the modulation of steroid metabolism and antimicrobial effects [13]. The results obtained in the present study indicate that the ethanol extract of fresh leaves of *A. cepa* have antimicrobial activities against the test organism used. The results also show that plants assayed here possess different levels of antimicrobial activities, that ethanol extracts of fresh leaves of *Allium cepa* exhibited the highest activity. The findings support the idea that many plants are used in the treatment of various diseases whose symptoms might involve microbial infection leading to the discovery of novel bioactive compounds [14-16].

In this study, the antimicrobial activity of ethanol extracts of investigated plant could be explained by phenolic compounds present including catechin, *p*-coumaric acid, ferulic acid, cinnamic acid, and sinapic acid (13). The contents of the main phenols in *A. cepa*. Many bioactive components that are naturally occurring in most plant materials have been reported to account for the exertion of antimicrobial activity (14). The results highlight the strong positive relationship between antimicrobial activity and the phenol content in all the plant extracts examined. The fact that the ethanol extract of the plants studied were active against both clinical and laboratory isolates, is an indication that it could be probably used as an important source of supportive therapy or in combination with antibiotic drugs. Nevertheless, it remains to investigate potential toxic effects of ethanol extracts of *A. cepa* (14-16) what could be a limitation of its use.

5. Conclusion

This study has shown that ethanol extracts from the fresh leaves of *Allium cepa* possess antimicrobial properties and could serve as potential source of antibacterial compounds that could be used in the formulation of new antimicrobial drugs. The bioactive phenolic substances obtained from this plant can therefore be a promising source for the treatment of various bacterial infections, especially *S. pneumoniae*. Isolation, characterization, and purification of these phytoconstituents and the determination of its respective antibacterial activities, together with toxicological analysis should be the future direction for researchers.

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