# ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES FROM LEAVES AND ROOTS OF (*ELEPHANTOPUS SCABER* L.) EXTRACTED USING DIFFERENT SOLVENTS

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by

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Mohammed Riyadh Mohammed AL-Shaheen

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| | |
|---|---|
| DMSO | Dimethyl Sulpooxide |
| DPPH | Gallic Acid Equivalent |
| MIC | Minimum Inhibitory Concentration |
| MRSA | Methicillin Resistant *Staphylococcus aureus* |
| MTCC | Microbial Type Culture Collection Centre |
| NCCLS | National Committee for Clinical Laboratory Standard |
| UTI | Urinary Tract Infection |
| GC-MS | Gas chromatography mass spectrometry |

**Aktiviti Antimikrob dan Antioksidan dari Daun dan Akar *Elephantopus scaber* L. Ekstrak Menggunakan Pelarut yang berbeza**

## ABSTRAK

Tumbuhan perubatan digunakan secara tradisional dalam rawatan pelbagai jenis penyakit sejak zaman dahulu lagi. Kajian ini telah dijalankan untuk menilai kegiatan antimikrob dan antioksidan akar dan daun *Elephantopus scaber* dengan menggunakan pelarut yang berbeza (etil alkohol, aseton dan air). Etil alkohol, aseton dan air ekstrak akar dan daun tertakluk kepada analisis fitokimia, yang mendedahkan kehadiran alkaloid, fenol, flavonoid, saponin, steroid, tanin, kumarin, kuinon dan glikosida, sebahagian besar ianya dipilih dari akar dan daun menggunakan pelarut yang berbeza. Etil alkohol, aseton dan ekstrak air dari akar dan daun diselidik di dalam makmal untuk aktiviti antimikrob terhadap *Escherichia coli, Salmonella typhis, Klebsiella pneumonia, Streptococcus pyogenes, Staphylococcus aureus,* dan *Candida Albican* dengan kaedah penyebaran yang baik dengan menggunakan kepekatan yang berbeza (20, 40 , 60, 80, 100) mg / ml. Data bioassay diperoleh daripada ekstrak aktif dan minyak menjadi sasaran analisis varians (ANOVA). Pengubatan ini menunjukkan perbezaan yang ketara ($p \leq 0,05$) yang dipisahkan dengan menggunakan ujian yang dibuat oleh Student-Newman-Keuls (SNK). Etil alkohol dan ekstrak aseton daripada akar menunjukkan aktiviti antimikrob yang menjanjikan terhadap semua ujian mikroorganisma. Ekstrak etil alkohol daripada akar memberi aktiviti pengaruh yang ketara terhadap semua ujian organisma dibandingkan dengan parameter lain, juga mencatatkan peningkatan ekstrak etil alkohol terhadap daun berbanding ekstrak aseton daripada akar untuk kepekatan 100 dan 20 mg / ml. Ekstrak air daun memiliki aktiviti yang lemah terhadap semua ujian mikroorganisma dan tidak menghalang pertumbuhan *Klebsiella pneumonia* untuk kepekatan 100mg / ml tetapi pada kepekatan 20mg/ml tidak menghalang pertumbuhan semua ujian organisma. Kepekatan Minimum penghalang (MIC) dari akar dan daun ekstrak dengan menggunakan pelarut yang berbeza juga ditentukan terhadap ujian mikroorganisma yang berbeza. Nilai (MIC) akar dan daun ekstrak antara 100 mg / ml untuk 500 mg / ml. Etil alkohol, aseton dan ekstrak air akar dan daun dipilih untuk ujian aktiviti antimikrob. Aktiviti antimikrob telah dikaji terhadap enam mikroorganisma iaitu *Escherichia coli, typhis Salmonella, Klebsiella pneumonia, Streptococcus pyogenes, Staphylococcus aureus*, dan *Candida Albican* ianya didapati bahawa etil alkohol ekstrak akar menunjukkan pengaruh yang tinggi terhadap *Escherichia coli, Salmonella typhis, Streptococcus pyogenes, Staphylococcus aureus*, dan *Candida Albican* tetapi kurang berkesan terhadap *Klebsiella pneumonia*. Ekstrak air menunjukkan kesan lemah terhadap semua ujian organisma dan tidak memiliki kesan terhadap *Klebsiella pneumonia*. Akar dan ekstrak daun daripada *Elephantopus scaber* menggunakan pelarut yang berbeza untuk ekstrak yang telah dinilai untuk aktiviti antioksida dengan menggunakan DPPH menggunakan kaedah kepekatan yang berbeza (1, 10, 20, 30, 40, 60, 80, 100) ug / ml. Hasil daripada analisis dibandingkan dengan asid askorbik sebagai piawai. Nilai IC50 piawai (asid askorbik) adalah 20 mg / ml dan untuk etil alkohol dan aseton daripada akar nilai IC50 adalah 30 mg / ml, untuk air, nilai IC50 adalah 60 mg / ml. Ekstrak etil alkohol bagi daun memiliki nilai IC50 sebanyak 40 mg / ml dan untuk aseton dan air bagi daun bernilai IC50 adalah 60 mg / ml. Kajian ini menunjukkan ekstrak yang memberi khasiat lebih tinggi adalah daripada pelarut etil alkohol daripada akar di mana ianya kesan sebatian bioaktif. Analisis GC-MS dari sampel aktif mengesahkan adanya sebatian yang mengandungi -OOH, -OH, -N, dan -S kumpulan yang dikaitkan dengan penghalangan bakteria antibiotik konvensional. 10 komponen utama yang diperoleh dari sampel disyaki mengandungi aktiviti anti-bakteria termasuk

asid Methanehydrazonic, N- [3- (methylthio) -1, -2,4-thiadiazol-5-il] -, ethylester (8,87%); asid 2-Butenoic , 3-metil, metilester (0,60).

**Antimicrobial and Antioxidant Activities from Leaves and Roots of *Elephantopus scaber* L. Extracted using Different Solvents**

## ABSTRACT

Medicinal plants are used traditionally in treatment of various kinds of diseases since time immemorial. The present study was carried out to evaluate the antimicrobial and antioxidant activities of roots and leaves of *Elephantopus scaber* by using different solvent (ethyl alcohol ,acetone and water). The ethyl alcohol, acetone and water extracts of the roots and leaves were subjected to phytochemical analyses, which revealed the presence of alkaloids, phenols, flavonoids, saponins, steroids, tannins, coumarins, quinones and glycosides in most of the selected of roots and leaves useing different solvents. Ethyl alcohol, acetone and water extracts of roots and leaves were investigated for in vitro antimicrobial activity against *Escherichia coli, Salmonella typhis, Klebsiella pneumonia, Streptococcus pyogenes, Staphylococcus aureus,* and *Candida Albican* by well diffusion method using different concentrations (20, 40, 60, 80, 100)mg/ml. Bioassay data obtained from the active extracts and oils were subjected to statistical analysis of variance (ANOVA). Treatment means showing significant difference ($p \leq 0.05$) were separated using Student-Newman-Keuls test (SNK). Ethyl alcohol and acetone extract of roots showed promising antimicrobial activity against all test organisms. Ethyl alcohol extract of root gave a significant effect activity against all test organisms compared with the other sample. Ethyl alcohol extract of leaves was also higher compared with the acetone extract of roots for a concentrations of 100 and 20mg/ml. The water extract of leaves has a weak activity against all test organisms and did not inhibit the growth of *Klebsiella pneumonia* for a concentrations of 100mg/ml but at a concentrations of 20mg/ml did not inhibit the growth of all test organisms. The Minimum inhibitory concentration (MIC) of the roots and leaves extracts by using different solvent were also determined against different test organisms. The MIC value of roots and leaves extract ranged from 100 mg/ml to 500 mg/ml. The ethyl alcohol, acetone and water extract of roots and leaves were selected for possible antimicrobial activity. The antimicrobial activity was studied against six microorganisms namely *Escherichia coli, Salmonella typhis, Klebsiella pneumonia, Streptococcus pyogenes, Staphylococcus aureus,* and *Candida Albican.* It was found that ethyl alcohol extract of roots showed height influence against *Escherichia coli, Salmonella typhis, Streptococcus pyogenes, Staphylococcus aureus,* and *Candida Albican* but less effective against *Klebsiella pneumonia*. The water extract showed a weak effect against all tests organisms and did not has any effect against *Klebsiella pneumonia.* The roots and leaves extract from *Elephantopus scaber* using different solvents to extract were evaluated for antioxidant activity by using DPPH method using a different concentrations (1, 10, 20, 30, 40, 60, 80, 100) µg/ml. The results from the analysis were compared with ascorbic acid as standard. The IC50 value of standard (ascorbic acid) was 20 mg/ml and for ethyl alcohol and acetone of roots the IC50 value was 30 mg/ml, for water the IC50 value was 60 mg/ml. The ethyl alcohol extract of the leaves had the IC50 value of 40 mg/ml and for acetone and water of leaves the IC50 value was 60 mg/ml. This study showed that the extract which gave higher efficacy is ethyl alcohol for the roots where it the bioactive compounds was detecte in this extract by (GC-MS). GC-MS analysis of the active samples confirmed the presence of compounds containing -OOH, -OH, -N, and –S groups which were associated with bacterial inhibition in conventional antibiotics. The 10 major constituents obtained from samples suspected to contain antibacterial activity include Methanehydrazonic acid, N-[3-(methylthio)-1,-2,4-thiadiazol-5-yl]-,ethylester (8.87%); 2-Butenoic acid, 3-methyl-, methylester (0.60%).

# CHAPTER 1

# INTRODUCTION

Throughout the world plants are used to treat various diseases. They provide natural products that are used against diseases. Plant-derived materials or products with therapeutic properties are known as herbal medicines; they may contain processed or raw ingredients from one or more plants that are beneficial for human health (De et al., 2010). Medicinal plants are important with respect to new drug and pharmacological research development. They are widely used and accepted as home remedies and raw materials for the pharmaceutical industry. Indigenous knowledge of plants and animals that are used to maintain health is known as Ethnopharmacology. Nowadays, people are exposed to many types of pollution such as air, water and soil pollution. Such pollutions effect on people to high levels of free radicals, that are capable of attacking the healthy cells of the body (Evans, 2009).



Bioactive compounds from diverse sources have been isolated and characterized around the world. Systematic screening of plant materials represent an important effort to find new bioactive compounds with the needed therapeutic potential to fight against pathogenic microorganisms for example, Salmonella typhae, Klebsella pneumonia, Streptococus aureus. The clarification of the chemical structures of some of these compounds had led to the synthesis and production of more potent and safer drugs. However, within the last few decades, microbial resistance has emerged for most of the available agents, thus, necessitating the search for newer drugs (Dhanasekaran et al., 2015). The increasing reliance on drugs from natural sources has led to the extraction and development of several drugs and chemotherapeutic agents from traditional herbs which are present in abundance in the tropics (Falodun et al., 2006). In fact, the use of

medicinal plants to treat diseases of varying etiology is part of the Asian tradition, although, it has been used for thousands of years. None of these bioactive plant compounds have been exploited for clinical uses as antibiotics. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antioxidant and antimicrobial traits which are chiefly synthesized during secondary metabolism of the plant. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Prusti et al., 2008).

Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that can damage cells. In fact antioxidants can terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves (Ahmad, 2012). Natural antioxidants can be obtained from the extraction of fruits or vegetables and herbs. For those who need the antioxidant in their bodies, they can get it through the fresh fruit when they eat. But in the food and pharmaceutical industries, the extract of antioxidants from fruits and vegetables is needed for their manufacturing process. The natural antioxidants can be extracted through the fruits, vegetables, and herbs. Extraction is used to separate substances and the process of extracting the antioxidant can be done by different solvents extraction. For example, it can be done by using solvent which is a desired substance dissolves in the extraction and the undesired substance does not dissolve (Sehwag and Das, 2013).

Synthetic antimicrobial agents are ineffective against multi-drug resistant bacteria and it caused the global trouble for the lost of budget use for the treatment

infectious diseases. So, there is an urgent need to develop alternative antimicrobial drugs for the treatment of infectious diseases. One approach is used of medicinal plants screen for their possible antimicrobial properties which is novel, inexpensive and effective against pathogenic microorganism (Liu, 2013). Herbal medicine is most popular for treatment of disease because it is easily available, cheap and less side effects as compared to antibiotic. Since ages, plants have been used for curing and restoring health as they contain components of therapeutic value. Medicinal plants used in folklore remedies have drawn interest of researchers in discovering solutions to multiple drug resistance against commercial antimicrobial drugs (Snyder et al., 2013). Many naturally occurring compounds found in plants have shown to possess antifungal, antibacterial and antiprotozoan activities and serve as a source of antimicrobial agents that could be used either systemically or locally (Croteau et al., 2000). The antimicrobial activity of medicinal plant extracts is very well documented . Also the recently reported concept of drug synergism between known antimicrobial agents and bioactive plant extract can also be implemented as a new form of treatment of infectious diseases (Guaadaoui et al., 2014). Combinations of agents with different lethal mechanisms may have synergistic or additive antimicrobial activities. Thus combination therapy also helps patients with severe infections due to drug resistant pathogens (Snyder et al., 2013).



The genus *Elephantopus scaber* was established in Asteraceae family by Linnaeus in 1753. It is a genus of about 32 species centered in the Neotropics (extending from southern Mexico through Central America and Northern South America to Southern Brazil), Europe, Asia (India, Nepal, Pakistan, Sri Lanka, China, Taiwan, Hong Kong, Japan, Malaysia, Indonesia, Vietnam, Philippines, Thailand and Myanmar), Australia and Africa. The lectotype species of Elephantopus genus, i.e., Elephantopus scaber

Linn. family Asteraceae, is a common wild weed that forms undergrowth in shady places. It is a rather coarse, rigid, erect, hairy herb, 30–60 cm high. The *E. scaber* plant is primarily used as a diuretic, febrifuge and to relieve anuria and blennorrhagia. Other applications include employment as an antibiotic, anti-swelling agent, anti-inflammatory bechic and emollient (Roy et al., 2015). It is believe to be a good remedy for leucorrhea, anemia and beneficial during parturition, In Malaysia, tutup bumi is taken internally as a diuretic, febrifuge and applied externally as a poultice for abdominal pains and other complaint. A decoction made from the leaves or roots is used as a tonic for eliminating roundworms and treating coughs and venereal diseases (Ndam et al., 2014).

The aim of this study was to evaluate leaves and roots of *Elephantopus scaber* extracts as potential sources of natural antioxidants and antimicrobials. In this study, the effects of extraction solvents (ethyl alcohol, aqueous and acetone) on the extract yield, antioxidant capacity and antibacterial activity. Information on the bioactive compounds content, antioxidant capacity and antibacterial activity of these leaves and roots extracts could be helpful in the selection of suitable solvents for extracting bioactive compounds from other similar plant materials.



## 1.1 PROBLEM STATEMENTS

Nowadays, the production of antimicrobial drugs in pharmaceutical industries has increased due to the increase in untreatable diseases. This disease cause by microbes which have become resistant towards commonly used antimicrobial drugs. Thus, researches try to find other source of compounds which can be turned into antimicrobial and antioxidant drugs. Recently, attempts made to use herbal plants as one of the alternative drugs in the treatment of diseases have increased due to the impact it gives

on the human health and disease prevention. Till now, there is less than thirty researches published on this plant species; *E. scaber* and mostly do not focus on the antimicrobial and antioxidant activity from different parts of this plant, the research on this plant only using the whole plant extract, therefore, in this study, the leaves and root extract of this plant was investigated for its antimicrobial and antioxidant activity by different solvent, were selected protic solvent (ethyl alcohol) and aprotic solvent proton (acetone) and solvent inorganic (aqueous), ethyl alcohol has not been studied for this plant while the acetone and aqueous has been studied for whole plant only.

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## 1.2 RESEARCH OBJECTIVES

1- To evaluate the bioactive compounds of roots and leaves from *Elephanthopus scaber L.* using different solvents .

2- To assess the antimicrobial activities of roots and leaves from *Elephanthopus scaber L.* extracts against five different types of bacteria and one fungus.

3- To determine the antioxidant activity of roots and leaves from *Elephantopus scaber L.* extracts.

4- To identify the bioactive compounds from the extract which has the highest effect against microorganism using GC-MS.



## 1.3 SCOPE OF STUDY

The expectation of this research will cover the extract from the roots and leaves of *Elephanthopus scaber* using different type of solvent (water , ethyl alcohol and acetone) and also determine the effect of the extracts on different types of bacteria and fungi. The bacteria used in this study are *Escherichia coli* and *Streptococcus pyogenes, Salmonella typhimurium, Staphylococcus aureus, Klebsiella pneumoniae* and Fungus *Condida*

*Albican. Elephanthopus scaber* extracts will also be investigated on the effect of free radical. The extract that gave the highest effect from the leaves and roots extract will be further tested for bioactive compounds using Gas Chromatography Mass Spectrometry (GC-MS).