Antifungal Activity of the Extracts of Moleata (Dandelion) Plant
(Taraxacum officinale L.)

By

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Examination Committee:

<table>
<thead>
<tr>
<th>Members of Committee</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- Prof. Awad Mohamed Abdel-Rahim</td>
<td>Chairman</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>……………………</td>
</tr>
</tbody>
</table>

Date of Examination: 2/4/2013
Dedication

To my
dear father
beloved mother
brothers and my sisters
(my grandmother)
Uncles and my aunts
Teachers
and friends
ACKNOWLEDGEMENTS

My grateful thanks to {Allah} who gave me the health and the ability to achieve this humble study, and with his will this achievement was properly completed.

Also, I would like to express my gratitude and thanks to Professor Awad M. Abdel-Rahim, the main supervisor for his good guidance, follow up, encouragement and help during this study. I wish to thanks also to Dr. Mai Abdalla Ali the co-supervisor. My special thanks to Dr. Mutaman A. Kehail and all members of the Center of Biosciences and Biotechnology for their assistance, and valuable help.

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Deepest thanks to my family, friends for encouragement and support.
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**ABSTRACT**

There is an increasing demand for biologically active substances from plant origin which is the current interest and focus of new research approach. The synthetic chemical pharmaceuticals showed various side effects on the functioning of different parts of the body, both internally and externally. However, Sudan possess an immense wealth of medicinal plants, that still unexploited. The present study investigated the antifungal activity of the most important and widely distributed; the Moleata (Dandelion) Plant (*Taraxacum officinale* L.). The PDA medium was used for the inhibition zone (cup plate) and the radial growth methods, while the PDB medium was used for the mycelial weight test. Extraction with different solvents confirmed that, hexane, ethanol and aqueous extracts were highly effective in inhibiting growth of both fungi (*Aspergillus niger* and *Penicilium italicum*). The aqueous extracts of the different parts of the plant were found to inhibit mycelial radial growth of the two fungi. Mycelial fresh and dry weights of both fungi, were also greatly reduced. The results also confirmed that the different plant parts were showing different effects, the extracts of the leaf are the more effective giving about 2.0 cm and 1.4 cm radial growth diameter of *A. niger* and 1.4 cm and 0.9 cm of *P. italicum*, at the higher concentrations (75 and 100 mg/ml), respectively. However, the extracts of both roots and flowers were less effective, compared to the leaf. From the results, it could be concluded that, the extracts of Moleata are highly effective as antifungal agents, specially the leaves of the plant. It was recommended that, further tests should be carried on other microorganisms, and the active antifungal components need also to be verified.
الخصائص المضادة للفطريات لمستخلصات نبات الموليته (الهندباء)

هند السر محمد عمر

الخلاصة

هناك زيادة في الطلب على المواد الفعالة بيولوجيا من أصل نباتي وهو الاهتمام الحالي والتركيز على نهج بحثي جديد. أظهرت الأدوية الكيميائية الاصطناعية آثارا جانبية مختلفة على أداء أجزاء مختلفة من الجسم، الداخلية وخارجيا. ومع ذلك، يمتلك السودان ثروة هائلة من النباتات الطبية، والتي لا تزال غير مستغلة.

الدراسة لمعرفة الخصائص المضادة للفطريات لنبات الموليته (الهندباء) Taraxacum officinale استخدمت في هذه الدراسة طريقة المنطقة المثبطة وطريقة النمو الميسليومي لقياس تأثير المستخلصات على نمو الفطر بواسطة الوسط الغذائي الصلب PDA في حين تم استخدام الوسط PDB لدراسة الوزن الميسلومي الرطب والجاف. أكد استخدام المذيبات المختلفة أن الهكسين، والإيثانول والمستخلص المائي كانت فعالة للغاية في تثبيط النمو على حد سواء تقريبا. المستخلصات المائية من الأجزاء المختلفة من النباتات اعطت فعالية عالية في تثبيط نمو ميسليوم الفطريين (A. niger و italicum P. italicum). الأوزان الرطبة والجافة هي أيضا تقلصت إلى حد كبير لكلا الفطريين. وأكدت النتائج أن المستخلص المائي للأوراق هو الأكثر فعالية حيث أعطى حوالي 2.0 سم و 1.4 سم طول النمو الميسليومي لللفطريين A. niger و 1.4 سم و 0.9 سم لللفطريين P. italicum في التراكيز العالية (75 و 100 مل/مل على التوالي). من جهة أخرى، كانت مستخلصات الجذور والزهر أقل فعالية مقارنة بالورقة. يستدعي من النتائج أن مستخلصات نبات الموليته لها فعالية كمواد مضادة للفطريات وخاصة أوراق النبات. وينبغي إجراء مزيد من الاختبارات اللاحقة على الكائنات الحية الدقيقة الأخرى. كما تحتاج المكونات الفعالة المضادة للفطريات إلى مزيد من الدراسات.
# List of Contents

<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dedication</td>
<td>IV</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>V</td>
</tr>
<tr>
<td>Abstract</td>
<td>VI</td>
</tr>
<tr>
<td>Arabic Abstract</td>
<td>VII</td>
</tr>
<tr>
<td>List of Contents</td>
<td>VIII</td>
</tr>
<tr>
<td>List of Tables</td>
<td>X</td>
</tr>
<tr>
<td>List of Plates</td>
<td>XI</td>
</tr>
<tr>
<td>List of Figures</td>
<td>XII</td>
</tr>
<tr>
<td><strong>CHAPTER ONE : INTRODUCTION</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>CHAPTER TWO: LITERATURE REVIEW</strong></td>
<td>4</td>
</tr>
<tr>
<td>2.1 Natural Products</td>
<td>4</td>
</tr>
<tr>
<td>2.2. Dandelion or moleata plant</td>
<td>7</td>
</tr>
<tr>
<td>2.2.1. Introduction</td>
<td>7</td>
</tr>
<tr>
<td>2.2.2. Scientific classification</td>
<td>8</td>
</tr>
<tr>
<td>2.2.3. Plant description</td>
<td>9</td>
</tr>
<tr>
<td>2.2.4. Chemical constituents</td>
<td>11</td>
</tr>
<tr>
<td>2.2.5. Beneficial uses</td>
<td>11</td>
</tr>
<tr>
<td>2.2.6. How it works in the body</td>
<td>14</td>
</tr>
<tr>
<td>2.3. Fungi Used</td>
<td>16</td>
</tr>
<tr>
<td>2.3.1 <em>Pencillium italicum</em></td>
<td>16</td>
</tr>
<tr>
<td>2.3.2 <em>Aspergillus niger</em></td>
<td>17</td>
</tr>
<tr>
<td>2.3.2.1. Description</td>
<td>17</td>
</tr>
<tr>
<td>2.3.2.2. Industrial uses</td>
<td>18</td>
</tr>
<tr>
<td><strong>CHAPTER THREE : MATERIALS AND METHODS</strong></td>
<td>20</td>
</tr>
<tr>
<td>3.1. Source of materials</td>
<td>20</td>
</tr>
<tr>
<td>3.2. Media Used</td>
<td>21</td>
</tr>
<tr>
<td>3.2.1. Potato Dextrose Agar {PDA}</td>
<td>21</td>
</tr>
<tr>
<td>Preparation of the medium</td>
<td>21</td>
</tr>
<tr>
<td>3.2.2 Potato Dextrose Broth {PDB}</td>
<td>21</td>
</tr>
<tr>
<td>Preparation of the medium</td>
<td>22</td>
</tr>
</tbody>
</table>

VIII
3.3. The inhibition zone method (cup plate) 22
3.4. The fungal radial growth method 23
3.5. The mycelial weights method 23

CHAPTER FOUR: RESULTS 24

4.1 Efficiency of different extracting solvents 24
4.2. Effects of different concentrations of the extracts on mycelial radial growth 24
4.3. Effect of the extracts on mycelial weight 33

CHAPTER FIVE: DISCUSSION 41

CONCLUSIONS AND RECOMMENDATIONS 45
REFERENCES 47
## List of Tables

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Effect of the different plant parts extracts obtained by different solvents on inhibition zone of <em>A. niger</em>.</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>Effect of the different plant parts extracts obtained by different solvents on inhibition zone of <em>P. italicum</em>.</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>Effect of different concentrations of the aqueous leaf extracts on the radial growth (cm) of the <em>A. niger</em>.</td>
<td>29</td>
</tr>
<tr>
<td>4</td>
<td>Effect of different concentrations of the aqueous roots extracts on the radial growth (cm) of the <em>A. niger</em>.</td>
<td>30</td>
</tr>
<tr>
<td>5</td>
<td>Effect of different concentrations of the aqueous flowers extracts on the radial growth (cm) of the <em>A. niger</em>.</td>
<td>31</td>
</tr>
<tr>
<td>6</td>
<td>Effect of different concentrations of the aqueous leaf extracts on the radial growth (cm) of the <em>P. italicum</em>.</td>
<td>34</td>
</tr>
<tr>
<td>7</td>
<td>Effect of different concentrations of the aqueous roots extracts on the radial growth (cm) of the <em>P. italicum</em>.</td>
<td>35</td>
</tr>
<tr>
<td>8</td>
<td>Effect of different concentrations of the aqueous flowers extracts on the radial growth (cm) of the <em>P. italicum</em>.</td>
<td>36</td>
</tr>
<tr>
<td>9</td>
<td>Effect of the aqueous extracts of different parts on dry and fresh weight of <em>A. niger</em>.</td>
<td>37</td>
</tr>
<tr>
<td>10</td>
<td>Effect of the aqueous extracts of different parts on dry and fresh weight of <em>P. italicum</em>.</td>
<td>38</td>
</tr>
</tbody>
</table>
**List of Plates**

<table>
<thead>
<tr>
<th>Plate No.</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I).</td>
<td>Moleata (Dandelion) plant at the vegetative stage.</td>
<td>10</td>
</tr>
<tr>
<td>(II).</td>
<td>Moleata (Dandelion) at the flowering stage</td>
<td>10</td>
</tr>
<tr>
<td>(III).</td>
<td>Moleata (Dandelion) Flower at the seeding stage.</td>
<td>10</td>
</tr>
<tr>
<td>(IV).</td>
<td>Moleata (Dandelion) plant at the flowering stage</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>(Collected from Elselemi village).</td>
<td></td>
</tr>
<tr>
<td>(V).</td>
<td>Effect of the different plant parts of the aqueous extracts on inhibition zone (cm) of <em>A. niger</em>. (A) Leaf, (B) Root, (C) Flower.</td>
<td>27</td>
</tr>
</tbody>
</table>
### List of Figures

<table>
<thead>
<tr>
<th>Fig. No.</th>
<th>Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Effect of different plant parts extracts obtained by different solvents on inhibition zone (cm) of <em>A. niger</em> (a) and <em>P. italicum</em> (b).</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>Comparison of the effects of different concentration of the aqueous plant parts extracts on the radial growth (cm) of <em>A. niger</em> (a) and <em>P. italicum</em> (b).</td>
<td>32</td>
</tr>
<tr>
<td>3</td>
<td>Comparison of the effects of the aqueous extracts of different plant parts on fresh and dry weight of <em>A. niger</em> (a) and <em>P. italicum</em> (b).</td>
<td>39</td>
</tr>
</tbody>
</table>
CHAPTER ONE
INTRODUCTION

Throughout history, natural products from plants have played major sustaining roles in the life of humans, especially for food sources and for medicinal products. Nature has provided mankind with folk medicines for centuries and continues to be the richest source of bioactive chemicals for the development of modern drugs. Terrestrial plants in particular were used as the basis of sophisticated traditional pharmacopoeias from as early as 2600 BC, and some of the earliest documentations come from inscriptions from Mesopotamia. Records from the ancient Egyptians and Chinese showed that plants were used for the preparation of hundreds of drugs, covering a most impressive array of health problems and diseases (Osbourn and Lanzotti, 2009).

Even though medicinal and pharmaceutical sciences, through the development of technology, have created milestones, plant-based systems continue to play an essential role in the healthcare of many communities. It was estimated by the World Health Organization that, approximately 80% of the world’s inhabitants rely mainly on traditional medicines for their primary healthcare (Farnsworth, 1985), the remaining 20% of the world’s population, are mainly residing in the developed countries. Nature is equally important since approximately 25% of the prescribed drugs contain extracts or plant metabolites and an additional significant percentage of the market drugs have been developed through studies employing natural products as the lead molecules (Cragg and Newman, 2005).

Indigenous healers often claim to have learned by observation, that sick animals changed their food preference to nibble at bitter plants, they would normally reject (Huffman, 2003).

Owing to their popular use as remedies for many infectious diseases, searches for plants containing antimicrobial substances are frequent (Betoni et al, 2006). Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids
and flavonoids, which have been found in vitro to have antimicrobial properties (Lewis and Ausubel, 2006). A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases due to their availability, fewer side effects and reduced toxicity (Lee et al, 2007). There are several reports on the antimicrobial activity of different herbal extracts (Bonjar, 2004 and De Boer et al, 2005).

There is some literature dealing with the medical practices of Sudanese people. Most of the studies done by El-Ghazali (1987) were integrating ethno-botanical, agro-medical and pharmacological studies of Sudanese flora in different parts of the country. Due to the wide diversity of botanical and large number of species, Sudanese medical plants can be considered as very promising candidates as bioactive agents.

Moleata or dandelion (Taraxacum officinale) is one of the most familiar sights of spring, though not a welcome one for some gardeners. In some countries dandelions are much sought after as a salad plant and its roots are used to make soft drinks, wine and a coffee substitute. From May to July, they turn green lawns and pastures yellow with their flowers. Starting from the base of the plant, the leaves have slim stalks, but grow wider towards the tip. The fresh juice of dandelion is applied externally to fight bacteria and help heal wounds. Dandelion is also used for the treatment of the gall bladder, kidney and urinary disorders, gallstones, jaundice, cirrhosis, hypoglycemia, dyspepsia with constipation, edema associated with high blood pressure and heart weakness, chronic joint and skin complaints, gout, eczema and acne. As a tonic, dandelion strengthens the kidneys. An infusion of the root encourages the steady elimination of toxins from the body. Dandelion is a powerful diuretic but does not deplete the body of potassium. Research is revealing that the many constituents of dandelion including taraxacin, taraxacoside, insulin, phenolic acids, sesquiterpene lactones, triterpenes, coumarins, catortenoids and minerals, mainly potassium and calcium. These are very valuable in curing a number of disorders and illnesses. Dandelion is traditionally used as a tonic and blood purifier, for constipation, inflammatory skin conditions, joint pain, eczema and liver.
dysfunction, including liver conditions such as hepatitis and jaundice (Mascolo et al, 1987).

This study has been conducted to investigate the antifungal activity of the extracts from dandelion (Taraxacum officinale). The extracts from three different parts of the plant were examined for activity against two fungi (Aspergillus niger and Penicillium italicum) using different concentrations.
CHAPTER TWO

LITERATURE REVIEW

2.1 Natural Products

Natural products have, until recently, been the primary source of commercial medicines and drug leads. A recent survey revealed that 61% of the 877 drugs introduced worldwide can be traced to or were inspired by natural products. However, beginning in the 1990s, natural product drug discovery was virtually eliminated in most big pharmaceutical companies. This was primarily due to the promise of the then-emerging field of combinatorial chemistry (Cseke et al, 2004), whereby huge libraries of man-made small molecules could be rapidly synthesized and evaluated as drug candidates. From 1981 to 2002, no combinatorial compounds became approved as drugs, although several are currently in late-stage clinical trials. At the same time, the number of new drugs entering the market has dropped by half, a figure of which the large pharmaceutical corporations are painfully aware. The haystack is larger, but the needle within it is more elusive. This has led only recently to a new found respect for the privileged structures inherent within natural products (DeSimone et al, 2004). Of the roughly 350,000 species of plants believed to exist, one-third of those have yet to be discovered. Of the quarter million that have been reported, only a fraction of them have been chemically investigated. Many countries have become aware of the value of the biodiversity within their borders and have developed systems for exploration as well as preservation. At the same time, habitat loss is the greatest immediate threat to biodiversity (Frankel et al, 1995). Plants produce an amazing array of organic chemicals with an enormous diversity of structural types. Many of these phytochemicals are essential for plant growth and development and are widely used by humans and other animals as food sources. They include a wide variety of polysaccharides such as cellulose, starch, and fructans; the polyphenol, lignin; fatty acids and lipids; proteins such as enzymes and structural
components in cell membranes; and nucleic acids such as DNA and RNA. Many more have undoubtedly evolved in response to ecological pressures of competition, including plant-to-plant competition for light and space, herbivory from marauding insects and other fauna, as well as bacterial and fungal infections (e.g., phytoalexins). These biologically active compounds are not only necessary for the well-being, survival, and evolution of the plants that produce them, but also for humans, who have exploited them for industrial (e.g., ethanol from corn and sugarcane as an alternative energy source and, currently, pharmaceutical biotechnology and nanotechnology), 5-methylcytosine vicine convicine theobromine zeatin construction (e.g., houses, bridges, barrels, baseball bats, fences, insulation), fuel (e.g., crop residues, wood chips, and sawdust), agricultural (e.g., fruits, vegetables, herbs and spices, wine and beer products, animal feeds, forest and horticultural products, and landscape plants), medical/pharmaceutical, recreational, and even spiritual/religious purposes (Leland et al, 2006). There is an increasing demand for biologically active substances from plant origin which is the current interest and focus of new research approach. The synthetic chemical pharmaceuticals showed various side effects on the functioning of different parts of the body, both internally and externally. Plant products have been shown to have no side effect, good therapeutic potential, due to the presence of active pharmacologically important substances, such as terpenes, alkaloids, flavonoids and glycosides (Yusuf et al, 2002 and Farrukh and Ahamed, 2003). Screening is a tool in discovering new biologically active molecules which have been found to be most productive in the area of antibiotics. In view of this, many plants have been screened for antimicrobial activity in India and abroad (Grierson and Afolayan, 1999 and Karuppusamy et al, 2001). Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a believe in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of
microorganisms (Harbottle \textit{et al}, 2006). Thus, in light of the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy (Coates \textit{et al}, 2002). For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against the multi-drug resistant microbe strains (Braga \textit{et al}, 2005). For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief. It is estimated that local communities have used about 10\% of all flowering plants on Earth to treat various infections, although only 1\% have gained recognition by modern scientists (Kafaru, 1994). Cytotoxic compounds have been isolated from the species of \textit{Vismia} (Hussein \textit{et al}, 2003). Antibacterial activity of the essential oil as well as eugenol purified from \textit{Ocimum gratissimum} to treat pneumonia, diarrhea and conjunctivitis has also been reported earlier (Nakamura \textit{et al}, 1999). According to the WHO, medicinal plants would be the best source for obtaining variety of drugs (Santos \textit{et al}, 1995).

Mainstream medicine is increasingly receptive to the use of antimicrobial and other drugs derived from microorganisms or their synthesized derivatives, which become ineffective and new, particularly viral diseases remain intractable to this type of drugs. Another driving factor for the renewed interest in plant antimicrobials in the past 20 years has been the rapid rate of plant species extinction (Lewis and Elvin, 1995). There is a feeling among natural-products chemists and microbologists that the multitude of potentially useful phytochemical structures could be at risk of being lost irretrievably (Borris, 1996). There is a scientific discipline known as ethanobotany (or ethnopharmacology), whose goal is to utilize the impressive array of knowledge assembled by indigenous people about the plant and animal products they have used to maintain health (Georges and Pandelai, 1949). Lastly, the human
immunodeficiency virus (HIV) has spurred intensive investigation into the plant derivatives which may be effective, especially for use in underdeveloped nations with little access to expensive Western medicines (De Clercq, 1995).

The use of plant extracts, as well as other alternative forms of medical treatments, is enjoying great popularity in the late 1990s. Earlier in this decade, approximately one-third of people surveyed in the United States used at least one “unconventional” therapy during the previous year (Eisenberg et al, 1993). It was reported that in 1996, sales of botanical medicines increased 37% over 1995 (Klink, 1997). Many plants have been found to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections (Brantner and Grein, 1994).

2.2. Dandelion or Moleata Plant:

2.2.1. Introduction:

Dandelion is a perennial herb thought to be introduced to Sudan from Europe and Asia. It is now naturalized throughout the Northern Hemisphere. No one is sure exactly how the dandelion has spread so widely, and there is some debate on the origin of the plant. Dandelion is found growing in pastures, lawns, waste ground, sand, rocks, even cracks in concrete.

The genus name of the dandelion, *Taraxacum*, comes from the Greek word taraxos, which means disorder, and akos, which means remedy. The species name, *officinale*, means that it is used medicinally. The common name may come from the Greek word leontodon, which means lion’s tooth. Other sources claim that the word dandelion comes from the old French word Dent-de-lion or from the Latin dens leonis, both also meaning lion’s tooth or teeth. The common dandelion is a member of the Composite family, with relatives including *Echinacea* (purple coneflower), chicory and other daisy-like flowers. Medicinal species, *Taraxacum officinalis* aka *Leontodon taraxacum* (roots are *Radix taraxaci*; leaves are *Herba taraxaci*).
Europeans have developed over 100 specialized varieties for salads, cooking, wine, and as a coffee substitute. The common names used include, blowball, canker wart, fairy clock, lion’s tooth, piss-in-bed, white endive, wild endive; Lowenzahwnurzel (Germany); pissenlit (France); pu gong ying (China), as was reported by Peirce and Huang (1999).

2.2.2. Scientific Classification:

Kingdom Plantae – Plants

Subkingdom Tracheobionta – Vascular plants

Superdivision Spermatophyta – Seed plants

Division Magnoliophyta – Flowering plants

Class Magnoliopsida – Dicotyledons

Subclass Asteridae

Order Asterales

Family Asteraceae – Aster family

Genus Taraxacum – dandelion

Species Taraxacum officinale – common dandelion

2.2.3 Plant Description:

The common dandelion is a perennial, herbaceous plant with long, lance-shaped leaves that are deeply toothed (plate I), as was reported by Steven and Ehrlich (2011). A new dandelion can also grow if some of the root, called a taproot because there is one long thick root that holds the plant in place and seeks out water, is left even if the plant and flower are removed. The leaves are 3 to 12 inches long and grow from a basal rosette. The leaves are green and jagged, they will appear before the
stem and the flower. The leaves are the first thing to look for when staking out your dandelion to observe. The stem of the plant is hollow, and when broken a sticky white bitter sap will ooze out. Please avoid breaking the flower stem so that can track when seeds are released, later. The flower of the Dandelion is shaped like a circle and is bright yellow. It is symmetrical - meaning if were to fold it in half it would look the same on both sides (plate II). When finished flowering, the dandelion flower turns to seed. The whole head of the flower will close up and the green bracts underneath the flower will close around the seeds. The yellow petals will disappear and the seeds mature. Then this new seed head will open up. These big puffy white seed heads, when blown by the wind, can start 200 or more new plants (plate III). Remember this the next time make a wish and blow the seeds away (Asad, 1995).

Plate (I). Moleata (Dandelion) plant at the vegetative stage.
Plate (II). Moleata (Dandelion) at the flowering stage.

Plate (III). Moleata (Dandelion) Flower at the seeding stage.

2.2.4. Chemical Constituents:

Dandelion’s active ingredients are found in both the roots and leaves. The leaves contain bitter sesquiterpene lactones such as taraxinic acid and triterpenoids such as cycloartenol. The roots contain these compounds as well as phenolic acids and insulin (Bradley, 1992). Potassium is present in the leaves at 297 mg per 100 grams of leaves. The leaves also contain substantial amounts of vitamin A (14,000 units per 100 grams of leaves, compared with 11,000 units per 100 grams of carrots), as was reported by Newall et al. (1996). The sesquiterpene lactones found in both leaves and root have demonstrated diuretic effects (Racz-Kotilla et al., 1974). They also stimulate bile flow from the liver. Different compounds may be present in different products depending on extraction methods. For example, the alcoholic extracts stimulate bile excretion.

2.2.5. Beneficial Uses:
Dandelion has long been used medicinally as a diuretic. Arab physicians of the 10th century relied on dandelion as a liver tonic, laxative and diuretic. In the Middle Ages, European physicians continued to use the leaves and roots of the yellow-flowering plant to treat diseases of the yellow bile (liver and gall bladder) and as a diuretic (Lewis, 1977). The folk medicines of China, India and Russia have recognized dandelion’s effects as a liver tonic. Traditional Chinese medicine combines dandelion with other herbs to treat hepatitis, to enhance the immune response to upper respiratory tract infections, bronchitis and pneumonia, and as a topical compress to treat mastitis (Hansel et al., 1980; Huang, 1999).

Moleata or dandelion (Taraxacum officinalis) is one of the medicinal plants which was included in the British Pharmaceutical Codex and United States Dispensatory from 1831 through 1926 and in the National Formulary until 1965. The use of the dried roots of dandelion is listed in the United States Pharmacopoeia. Traditionally, in Europe and America, dandelion was used as a favorite spring health tonic and is cultivated in India as a liver remedy. In Germany, there is an over-the-counter preparation containing this common weed that has been found effective against gallstones. It is also listed in over-the-counter treatments in Canada, France and the United Kingdom (Peirce and Huang, 1999).

As recently as 1957, more than 20 tons of dandelion leaves were imported into the US annually for medicinal purposes (Duke, 1985). Dandelion was included as a diuretic in the US pharmacopeia from 1831 to 1963. Based on its diuretic effects, dandelion is often included in herbal weight loss and premenstrual syndrome remedies. Some herbalists also recommend it as a way to help prevent atherosclerosis (Ottariano, 1999). Others suggest it may be a useful spring and fall tonic for patients with chronic osteoarthritis and those with a tendency to form gallstones (Weiss, 1988). Dandelion leaf extract was found to stop the proliferation of breast cancer, only a crude extract of dandelion was found to have this effect. An extract of
Dandelion in a water solution did not stop the spread of the breast cancer cells (Sophia et al, 2008).

Dandelion (*Taraxacum officinale*) is one of the best known European medicinal plants, rich in triterpenoids, which has been used for the treatment of various inflammatory diseases such as rheumatoid arthritis and also for many infectious disorders. The anti-inflammatory activity of dandelion extracts has been recently confirmed in animal studies (Mascolo et al, 1987) and aqueous extracts seem to have anti-tumour activity (Newall et al, 1996). Based on pharmacological studies, Dandelion (*Taraxacum officinalis*) is one of the components of phytomedicines used in therapy for hepatitis and the drug also has diuretic and choleretic actions (Bissett, 1994 and Bradley, 1992).

Dandelion extract was found to prevent the spread of ovarian cancer cells, the researchers found these effects with an extract of dandelion in ethanol alcohol. Dandelion extract was also found to reduce pro-inflammatory cytokines, demonstrating one manner by which dandelion could affect cancer cells. Pro-inflammatory cytokines are proteins that regulate inflammation in the body, they have been associated with various types of cancer. Therefore, reducing these pro-inflammatory cytokines can play an important role in the fight against cancer (Choi and Kim, 2009). Dandelion herb may help people with chemo-resistant melanoma. Specifically, dandelion herb caused apoptosis, or the death of melanoma cells. Yet, dandelion did not lead to the apoptosis of healthy cells. The researchers mentioned that this is significant because while many herbal preparations can kill cells, they do not target specific cells. However, dandelion kills cancerous cells while leaving healthy cells intact (Chatterjee et al., 2011). The German Commission approves it for use as a diuretic and to treat dyspepsia, liver and gallbladder complaints and appetite loss (Blumenthal and Fleming, 1998).

In addition to its medicinal uses, dandelion serves as a salad green in the gourmet’s garden. The leaves are an excellent source of vitamin A. The ground roots are sometimes used as a substitute for chicory roots or coffee beans. The flowers are
sometimes fermented into wine. The root is the part most often used medicinally (Duke, 1985).

Provision of dosage information does not constitute a recommendation or endorsement, but rather indicates the range of doses commonly used in herbal practice. Doses are given for single herb use and must be adjusted when using herbs in combinations. Doses may also vary according to the type and severity of the condition treated and individual patient conditions. There is disagreement on the optimal form and dose of dandelion. Reputable physicians and herbalists recommend a range of doses (Ottariano, 1999.; Bradley, 1992 and Newall et al, 1996).

2.2.6. How it Works in the Body:

The essential mineral potassium is found in very high amounts in the leaves of the dandelion herb, this mineral balances important biochemical functions in the body and the leaves themselves contain other chemicals that function as powerful diuretic agents, the potassium acts as a balancing agent of these diuretics. When compared to conventional diuretics, which always require a supplement of the potassium mineral to balance the total requirements of the body for minerals, the difference between dandelion and these conventional medications becomes apparent. The dandelion plant is used as an herbal remedy for alleviating painful urinary ailments in the Chinese system of medicine. Dandelion roots are used for other forms of herbal remedies and their essential function in the body is different, mostly they are used in the treatment of the liver and are used to bring about improvements in its overall functioning, and also they find use as a mild laxative. Heat disorders are treated in the Chinese system using the herbal remedies sourced from the dandelion, heat disorders especially those affecting the liver, the symptoms of which can include redness, swelling, and the development of painful eyes are all treated using dandelion, the remedies made from the dandelion are also used in the treatment of damp or heat jaundice in different patients. The gallbladder is treated using a tonic made from both the leaves and the
roots of the dandelion, this herb is very useful for such conditions. The dandelion is used to holistically cleans the body and as detoxification agent, it is believed that the herb produces beneficial effects by removing the chemical pollutants in the body, thus cleansing it of harmful and toxic substances accumulated over time. Firm and hard abscesses are also treated using the dandelion remedies in the Chinese system, this is especially so, if such abscesses involve tissues in the breast and in the digestive system of the person. Topical as well as internal herbal remedies can be derived from the dandelion to treat a variety of internal and external disorders. Lactation is promoted in nursing women, through the use of specific herbal dandelion remedies during the period of breastfeeding. In the Chinese system, the dandelion is credited with having bitter, sweet and cold properties. Multi-ingredient preparations containing dandelion included: agnuchol, agrimonas, aristochool N, berberis complex, bio-garten tee, chol-grandelat, cholsonal, cholosom, fluid loss, galleb forte, gallexier, hepafungin, helalixier, herbal diuretic complex, phytomed hepato, stomach mixture, uva ursi complex, uvacin, waterlex (Kathi and Kemper, 1999).
2.3. Fungi Used

Fungi are ancient group of organisms. Many fungi are harmful because they decay, rot and spoil many different materials as they obtain their food and cause serious diseases in animals and plants. Beside that, fungi have beneficial effects as they have been used in the production of bread, beer, wine, steroids and many other industrial products (George, 2006). The majority are multicellular, but a few like yeasts, are single-celled (Alexopolus and Mims, 1979). Although fungi are nonmotile, they successfully survive and disperse because of their ability to form spores. Some spores are produced sexually and others are produced asexually. Spores may be produced internally or externally. When released, the spores can be transported by wind or water. Because of their small size, spores can remain in the atmosphere for a long time and travel thousands of kilometers. (Eldon et al, 2005).

2.3.1. *Pencillium italicum*

The fungus *Pencillium italicum* causes a disease called green mold. The green mold is most prevalent in cold stored fruits where it is able to develop slowly at cold temperature. Spores of *P. italicm* are airborne and large numbers are produced on the surface of the infected fruit. These spores will contaminate the packing house, equipment, water used in drenchers and soak tanks, storage room, transit containers and even the retail market area. The fungus survive in the field on soil debris and produces spores that infect fruits in the tree and on the ground. At cooler fall and winter temperature that favor fungal development, large numbers of spores are produced and carried by wind current to the surface of fruit in the tree canopy. The spores germinate and infect fruits when nutrients and moisture are released at injuries that are formed during harvesting and handling and even injuries that involve only a few oil glands are sufficient to induce infection. The fungus can also invade fruit through certain physiologically induced injuries, such as injuries associated with
chilling and stem-end rind breakdown. Condition known as spoilage occur when masses of spores are produced on fruits and contaminate surfaces of healthy fruit in the cartons. Fruits containing soil particles must be cleaned before retail sale. The infection and sporulation cycle can be repeated many times in a packing house and in a storage room during an extended storage. The prolific spore production ability of the fungus enables it to eventually develop strains with resistance to chemical fungicide treatments. Initial symptoms of the blue mold are similar to those of green mold and sour rot. The small decayed area appear as a soft watery spot that is more firm than comparable stages of sour rot. When lesions enlarge to 1-2 inches in diameter, white mycelium is formed in the center and green spores are soon produced. The sporulation area is surrounded by a narrow band of white mycelium, which is encompassed by a definite band of water-soaked rind. Occasionally, P. italicum will sporulate in the flesh of the fruit. The infected fruit can be covered entirely by a mass of green spores, which are easily dispersed by any physical movement or by air current (Brown, 1994).

2.3.2. Aspergillus niger

2.3.2.1. Description

Aspergillus niger is a fungus and one of the most common species of the genus Aspergillus. It causes a disease called black mould on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments, where its black colonies can be confused with those of Stachybotrys (species of which have also been called black mold (Samson et al., 2001). The fungus is included in the subgenus Circumdati, section Nigri. The section Nigri includes 15 related black-spored species that may be confused with A. niger, including A. tubingensis, A. foetidus, A. carbonarius, and A. awamori (Klich, 2002 and Samson et al., 2004). A number of morphologically similar species were recently described by Samson et al.,
(2004). *A. niger* is a haploid filamentous fungi and is a very essential microorganism in the field of biology. In addition to producing extracellular enzymes and citric acid, *A. niger* is used for waste management and biotransformations. The fungus is most commonly found in mesophilic environments such as decaying vegetation or soil and plants (Schuster *et al.*, 2002). The fungus was isolated from the plant *Welwitschia mirabilis* in Namibia and Angola, a plant estimated to be about 3000 years old. It is also easily isolated from common things such as dust, paint, and soil. Commonly in labs, *A. niger* is isolated via chemostat cultures which can test positively or negatively for the fungus (Van de vandervoort *et al.*, 2004). *A. niger* is not only a xerophilic fungus, but a mold that doesn’t require free water for growth, or grow in humid environments), it is also a thermotolerant organism (capable of growing at high temperatures). Because of this property, the filamentous fungus exhibits a high tolerance to freezing temperatures (Schuster *et al.*, 2002). Although The fungus is relatively harmless compared to other filamentous fungi, there have been some medical cases that have been accounted for, such as lung infections or ear infections in patients that have a weakened immune system, or a immune system that has been impaired by a disease or medical treatment. In the case of ear infections, the fungus invades the outer ear canal which can cause damage to the skin it came in contact with (Schuster *et al.*, 2002, May and Adams, 1997).

### 2.3.2.2. Industrial Uses

The fungus *A. niger* is cultured for the industrial production of many substances. Various strains of *A. niger* are used in the industrial preparation of citric acid and gluconic acid which have been assessed as acceptable for daily intake by the World Health Organisation. Fermentation by the fungus is generally recognized as safe by the United States Food and Drug Administration under the Federal Food, Drug, and Cosmetic Act (IGN, 2008). Many useful enzymes are produced using industrial fermentation of *A. niger*. For example, glucoamylase is used in the production of high fructose corn syrup, pectinases are used in cider and wine
clarification and α-galactosidase. An enzyme that breaks down certain complex sugars.
CHAPTER THREE

MATERIALS AND METHODS

3.1. Source of Materials

Samples of different parts of Moleata or Dandelion plant (*Taraxacum officinale*), were collected in July 2012 from the Elselemi village near Wad Medani city, Gezira state (plate IV). The dried parts were blended into a powder using a blender. Five different concentrations (0, 25, 50, 75 and 100%) were prepared for each part separately, Potato Dextrose Agar (PDA) or Potato Dextrose Broth (PDB) media were used. The cultures of both *Aspergillus niger* and *Penicillium italicum* were obtained from the Center of Biosciences and Biotechnology, Faculty of Engineering and Technology, University of Gezira. The media used in this study were prepared locally, using chemicals from Oxoid Corporation.

Plate (IV). Moleata (Dandelion) plant at the flowering stage

(Collected from Elselemi village).
3.2. Media Used

3.2.1. Potato Dextrose Agar {PDA}

This medium was used for isolation and maintenance of fungi, and for other experiments whenever needed. The medium consists of the following materials:

- Potato (peeled and diced) : 200 g
- D-Glucose : 20 g
- Agar : 20 g
- Distilled water : 1000 ml

**Preparation of the medium**

The potato samples 200g were peeled, diced and boiled for 1 hour in 800 ml distilled water, then the extract was filtered and made up to 1 liter and then 20 g agar were added. The medium was then dispensed in 100 ml samples in conical flasks covered with cotton plugs and aluminum foil before being sterilized in the autoclave at 121°C (15lb/ in²) for 15 minutes. However glucose was sterilized separately by filtration and then added to the medium under aseptic conditions. The medium was then stored at 4°C in a refrigerator. When needed, it was melted in a water bath and poured into sterilized Petri dishes.

3.2.2. Potato Dextrose Broth {PDB}

This medium was used for mycelial weight experiments, for growth and maintenance of fungi, and for other experiments, whenever needed. The medium consists of the following:

XXXII
Potato (peeled and diced) 200 g
D-Glucose 20 g
Distilled water 1000 ml

**Preparation of the Medium**

The potato samples 200g were peeled, diced and boiled for 1 hour in 800 ml distilled water, then the extract was filtered and made up to 1 liter. The medium was then dispensed in 50 ml samples in conical flasks covered with cotton plugs and aluminum foil before being sterilized in the autoclave at 121° C (15lb/ in²) for 15 minutes. However glucose was sterilized separately by filtration and then added to the medium under aseptic conditions. The medium was used as a liquid culture for fungal experiments.

**3.3. The Inhibition Zone Method (cup plate)**

This method was used for measuring the inhibition zone made by each extract concentration against the growth of both *A. niger* or *P. italicum* on PDA medium. The medium was prepared, sterilized by autoclaving and distributed into sterile petri-dishes. After solidification, about 0.1 ml spore suspension of each fungus was poured onto the surface of the medium and spreaded, using a sterile glass rod. A sterile Whatman glass filter paper disc (No. 5) was saturated with each of the different concentration of the extracts, allowed to dry and transferred on the center of the surface of the incubated medium in the Petri-dishes. Inoculated dishes were then incubated at room temperature for 8 days and the inhibition zones were measured as was described by Barry et al. (1970), Cruickshank et al. (1975) and Abdel-Rahim et al. (2012). Three replicates were made for each treatment.
3.4. The Fungal Radial Growth Method

The medium used for fungal radial growth was the PDA as was mentioned in section 3.2.1. The five concentrations under test were prepared by serial dilution of the extract with the medium. The medium was then sterilized and poured in petri-dishes and left to solidify at room temperature (28°C – 30°C). Each solidified petri-dish was inoculated by a fungal growth disc cut by a sterile cork-borer (2.0 mm diameter) from the edge of an actively growing culture of A. niger or P. italicum grown on PDA. The inoculated Petri-dishes were then incubated at room temperature for 8 days. All treatments were done in triplicates. The diameter of growth was measured, every 48 hours by taking the average of two crossed dimensions for each disc in a Petri-dish. The radial growth was calculated as a percentage from the diameter of the Petri-dish.

3.5. The Mycelial Weights Method

The potato dextrose broth (PDB), described above was prepared and dispensed in 100 ml conical flasks (50 ml in each flask), then five concentrations (0.0, 25, 50, 75 and 100 mg/ml) were made by serial dilution of the extract with the medium, in flasks. All solutions were sterilized in an autoclave at 121°C (15 lb/in²) for 15 minutes and then cooled to room temperature. Each flask was inoculated by three discs made by a sterile cork-borer (2.0 mm diameter) from the edge of an actively growing culture of A. niger or P. italicum grown on a solidified PDA medium. Inoculated flasks were incubated at room temperature (28-30°C) for 8 days. After incubation, the cultures were filtered through whatmans (No.1) filter papers and the mycelial mats were collected, weighed (fresh weight), and dried at 80°C for 24 hrs, before being reweighed (dry weight). All treatments were done in triplicates.
CHAPTER FOUR

RESULTS

4.1. Efficiency of Different Extracting Solvents:

The present study investigated the antifungal activity of the extracts of Moleata or Dandelion plant, using two fungi (A. niger and P. italicum). Different extracting solvents were used including; ethanol, hexane, petroleum ether, as well as water (aqueous extract). The cup plate inhibition zone method was used for the comparison between the different solvents. Results on Tables (1 and 2) showed effects of the extracts obtained by the different solvents on the inhibition zone of growth of both fungi (A. niger and P. italicum), respectively. The results indicated that hexane and ethanol were better than the others. However, water extracts were also effective compared to petroleum ether. The results also confirmed that the different plant parts were showing antifungal effects (Tables 1 and 2, plate V and Fig. 1).

4.2. Effect of Different Concentrations of the Extracts on Mycelial Radial Growth:

The effects of the different concentrations of different aqueous plant parts (leaves, roots and flowers) of Taraxacum officinale on radial growth of both fungi (A. niger and P. italicum) were investigated in the present study. The results of the extracts of the different parts on A. niger are shown in Tables 3, 4 and 5; and Fig. (2). The finding showed that the leaf extracts were highly effective giving radial growth of about (2.0 cm) and (1.4 cm) in diameter at the higher concentrations (75 and 100 mg/ml), respectively. The effect was statistically significant compared to the control and the lower
**Table (1):** Effect of the different plant parts extracts obtained by different solvents on inhibition zone (cm) of *A. niger* (8 days).

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Plant parts</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Roots</td>
</tr>
<tr>
<td>Ethanol</td>
<td>5.7</td>
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<td>Hexane</td>
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<td>4.0</td>
</tr>
<tr>
<td>Mean</td>
<td>5.1</td>
<td>4.4</td>
</tr>
</tbody>
</table>

**Rows:** $F = 18.91$  \  $F_{crit} = 4.76$  \  $P. value = 0.0018$

**Columns:** $F = 1.31$  \  $F_{crit} = 5.14$  \  $P. value = 5.14$

$R^2: 0.99$

$SE: 0.15$
Table (2): Effect of different plant parts extracts obtained by different solvents on inhibition zone (cm) of *P. italicum* (8 days).

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Plant parts</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Roots</td>
</tr>
<tr>
<td>Ethanol</td>
<td>6.3</td>
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<td>5.8</td>
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<tr>
<td>Petroleum ether</td>
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<tr>
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<td>3.7</td>
</tr>
<tr>
<td>Mean</td>
<td>5.2</td>
<td>4.72</td>
</tr>
</tbody>
</table>

Rows: \( F = 16.91 \) \hspace{1cm} F crit = 4.76  
Columns: \( F = 0.52 \) \hspace{1cm} F crit = 5.14  
\( R^2: 0.99 \) \hspace{1cm} SE: 0.15
Plate (V): Effect of the different plant parts of the aqueous extracts on inhibition zone (cm) of *A. niger*. (A) Leaf, (B) Root, (C) Flower.
**Fig. (1)**: Effect of different plant parts extracts obtained by different solvents on inhibition zone (cm) of *A. niger* (a) and *P. italicum* (b).

**Table (3)**: Effect of different concentration of the aqueous leaf extracts on the radial growth (cm) of the *A. niger*.
<table>
<thead>
<tr>
<th>Incubation period (days)</th>
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<th>4</th>
<th>6</th>
<th>8</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrations %</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.7</td>
<td>1.6</td>
<td>2.5</td>
<td>3.7</td>
<td>2.13</td>
</tr>
<tr>
<td>25</td>
<td>0.7</td>
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<td>2.3</td>
<td>3.4</td>
<td>1.93</td>
</tr>
<tr>
<td>50</td>
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<td>2.0</td>
<td>2.5</td>
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</tr>
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<td>1.4</td>
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<td>1.08</td>
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</tbody>
</table>

Rows: $F = 13.95$  \quad F_{crit} = 3.26

Columns: $F = 50.54$  \quad F_{crit} = 3.49

$R^2 : 0.97$

$SE : 0.75$

**Table (4)**: Effect of different concentration of the aqueous roots extracts on the radial growth (cm) of the *A. niger*.
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<th>Incubation period (days)</th>
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<th>4</th>
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</tr>
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<td>3.7</td>
<td>4.7</td>
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<td>2.5</td>
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<tr>
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<td>2.08</td>
</tr>
<tr>
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<td>1.6</td>
<td>2.3</td>
<td>2.4</td>
<td>1.78</td>
</tr>
<tr>
<td>Mean</td>
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<td>3.06</td>
<td>3.74</td>
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</tbody>
</table>

Rows: $F = 43.50$  $F_{crit} = 3.26$

Columns: $F = 47.88$  $F_{crit} = 3.50$

$R^2: 0.87$

$SE: 0.63$

**Table (5):** Effect of different concentrations of the aqueous flowers extracts on the radial growth (cm) of the *A. niger*.
<table>
<thead>
<tr>
<th>Incubation period (days)</th>
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<th>6</th>
<th>8</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
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<td>2.8</td>
<td>4.4</td>
<td>5.2</td>
<td>3.53</td>
</tr>
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<td>2.95</td>
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<tr>
<td>75</td>
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<td>2.5</td>
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<td>1.65</td>
</tr>
<tr>
<td>Mean</td>
<td>0.86</td>
<td>1.98</td>
<td>3.22</td>
<td>3.74</td>
<td></td>
</tr>
</tbody>
</table>

Rows : \( F = 30.92 \) \( F \text{ crit} = 3.26 \)

Columns : \( F = 108.24 \) \( F \text{ crit} = 3.49 \)

\[ R^2 : \] 0.65

\[ \text{SE} : \] 0.23
Fig. (2) : Effects of different concentrations of the aqueous plant parts extracts on the radial growth (cm) of *A. niger* (a) and *P. italicum* (b).
concentrations (Table,3). It was followed by the extracts of the roots which gave about (2.8 cm) and (2.4 cm) radial growth at the higher concentration (Table,4). The flowers extracts was the least effective giving (2.8 cm). Fig. (2) Compared the effects of the different plant parts concentrations on A. niger at the 8th day, only. From the results it is clear that the leaf was the highly effective in reducing the radial growth of the fungus.

On the other hand, the extracts of the different parts of the plant was also effective in reducing radial growth of the fungus P. italicum as shown in Tables (6, 7, and 8). and Fig. (2). The results indicated that the leaf extracts were highly effective, giving about (0.9 cm) reduction in radial growth at the highest concentrations (Table, 6). It was followed by the extracts of the roots which gave of about (1.5 cm) radial growth at the highest concentration (Table,7). This was followed by the flowers (Table, 8). Results in Fig. (2) was compared the effects of the different plant parts concentrations on P. italicum at the 8th day, only. From the results it is clear that the leaf was the highly effective in reducing the radial growth of the fungus.

4.3 . Effect of the Extracts on Mycelial Weight:

The different concentrations of the Dandelion plant parts extracts, were also investigated against mycelial weight of the two fungi (A. niger and P. italicum). Data in Table (9) showed the effects the aqueous extracts of the different parts on fresh and dry weight of A. niger, and Table (10) showed the effects of the aqueous extracts of the different parts on fresh and dry weight of P. italicum. Data on Fig. (3), compared the effects of the aqueous extracts on the fresh and dry weight of both fungi.
Table (6) : Effect of different concentrations of the aqueous leaf extracts on the radial growth (cm) of the *P. italicum*.

<table>
<thead>
<tr>
<th>Incubation period (days)</th>
<th>Concentrations %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>0.0</td>
<td>0.4</td>
</tr>
<tr>
<td>25</td>
<td>0.3</td>
</tr>
<tr>
<td>50</td>
<td>0.0</td>
</tr>
<tr>
<td>75</td>
<td>0.0</td>
</tr>
<tr>
<td>100</td>
<td>0.0</td>
</tr>
<tr>
<td>Mean</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Rows: \[ F = 12.2 \quad F \text{ crit } = 3.26 \]

Columns: \[ F = 40.6 \quad F \text{ crit } = 3.5 \]

\[ R^2 : 0.92 \]

\[ \text{SE} : 1.13 \]
Table (7) : Effect of different concentrations of the aqueous roots extracts on the radial growth (cm) of the *P. italicum*.

<table>
<thead>
<tr>
<th>Incubation period (days)</th>
<th>Concentrations %</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.7</td>
<td>1.4</td>
<td>2.8</td>
<td>3.0</td>
<td>1.98</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>0.4</td>
<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
<td>1.23</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0.0</td>
<td>0.8</td>
<td>1.2</td>
<td>1.8</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>0.0</td>
<td>0.8</td>
<td>1.0</td>
<td>1.7</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
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<td>0.0</td>
<td>0.3</td>
<td>0.8</td>
<td>1.5</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.22</td>
<td>0.86</td>
<td>1.46</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rows : \( F = 17.30 \) \( F \) crit = 3.26

Columns : \( F = 48.47 \) \( F \) crit = 3.5

\( R^2 \) : 0.74

\( SE \) : 2.31
Table (8) : Effect of different concentration of the aqueous flowers extracts on the radial growth (cm) of the *P. italicum*.

<table>
<thead>
<tr>
<th>Incubation period (days)</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.8</td>
<td>2.7</td>
<td>3.9</td>
<td>4.4</td>
<td>2.95</td>
</tr>
<tr>
<td>25</td>
<td>0.7</td>
<td>2.6</td>
<td>3.8</td>
<td>4.2</td>
<td>2.83</td>
</tr>
<tr>
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<td>2.6</td>
<td>3.2</td>
<td>4.0</td>
<td>2.58</td>
</tr>
<tr>
<td>75</td>
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<td>2.1</td>
<td>2.7</td>
<td>3.2</td>
<td>2.1</td>
</tr>
<tr>
<td>100</td>
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<td>0.5</td>
<td>1.3</td>
<td>1.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Mean</td>
<td>0.48</td>
<td>2.1</td>
<td>2.98</td>
<td>3.52</td>
<td></td>
</tr>
</tbody>
</table>

Rows : \( F = 19.24 \) \( F \text{ crit} = 3.26 \)

Columns : \( F = 61.37 \) \( F \text{ crit} = 3.49 \)

\( R^2 \) : 0.76

\( SE \) : 2.13

XLVII
**Table (9)**: Effect of the aqueous extracts of different parts on dry and fresh weight of *A. niger*.

<table>
<thead>
<tr>
<th>Concentration %</th>
<th>Plant parts</th>
<th></th>
<th></th>
<th></th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Leaf</td>
<td>Roots</td>
<td>Flowers</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>6.33</td>
<td>0.72</td>
<td>5.95</td>
<td>0.98</td>
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<tr>
<td>25</td>
<td></td>
<td>5.91</td>
<td>0.63</td>
<td>5.43</td>
<td>0.91</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td>5.14</td>
<td>0.54</td>
<td>4.61</td>
<td>0.83</td>
</tr>
<tr>
<td>75</td>
<td></td>
<td>4.70</td>
<td>0.41</td>
<td>3.92</td>
<td>0.75</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>4.20</td>
<td>0.28</td>
<td>3.34</td>
<td>0.66</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>4.20</td>
<td>0.52</td>
<td>4.65</td>
<td>0.83</td>
</tr>
</tbody>
</table>

Rows: $F = 9.02$  $F_{crit} = 2.87$

Columns: $F = 147.47$  $F_{crit} = 2.71$

$R^2 : 0.98$

$SE : 0.65$
**Table (10)**: Effect of the aqueous extracts of different parts on dry and fresh weight of *P. italicum.*

<table>
<thead>
<tr>
<th>Concentration %</th>
<th>Plant parts</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Leaf</td>
<td>Roots</td>
<td>Flowers</td>
<td>Mean</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fresh</td>
<td>Dry</td>
<td>Fresh</td>
<td>Dry</td>
<td>Fresh</td>
</tr>
<tr>
<td></td>
<td></td>
<td>wt.</td>
<td>wt.</td>
<td>wt.</td>
<td>wt.</td>
<td>wt.</td>
</tr>
<tr>
<td>0</td>
<td></td>
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<td>5.73</td>
<td>0.95</td>
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</tr>
<tr>
<td>25</td>
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<td>4.35</td>
<td>0.39</td>
<td>5.31</td>
<td>0.82</td>
<td>5.31</td>
</tr>
<tr>
<td>50</td>
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<td>3.71</td>
<td>0.34</td>
<td>4.34</td>
<td>0.78</td>
<td>4.82</td>
</tr>
<tr>
<td>75</td>
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<td>0.63</td>
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<tr>
<td>100</td>
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<td>0.54</td>
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<tr>
<td>Mean</td>
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<td>3.79</td>
<td>0.32</td>
<td>4.29</td>
<td>0.74</td>
<td>4.62</td>
</tr>
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</table>

Rows : \( F = 7.86 \quad F \text{ crit} = 2.87 \)

Columns : \( F = 74.70 \quad F \text{ crit} = 2.71 \)

\( R^2 : 0.98 \)

\( \text{SE} : 0.43 \)
Fig. (3): Comparison of the effects of the aqueous extracts of different plant parts on fresh and dry weight of *A. niger* (a) and *P. italicum* (b).
From the results it is clear that the extracts reduced both weight of mycelia of both fungi. However, *P. italicum* was found more sensitive to the extracts compared to *A. niger*.
CHAPTER FIVE

DISCUSSION

There has been a remarkable resurgence of interest in natural product research over the last decade or so. With the outstanding developments in the areas of separation science, spectroscopic techniques, and micro plate-based ultrasensitive in vitro assays, natural product research is enjoying renewed attention for providing novel and interesting chemical scaffolds. The use of natural products, especially plants, for healing is as ancient and universal as medicine itself (Sarker et al., 2006). Nature has been a source of therapeutic agents for thousands of years, and an impressive number of modern drugs have been derived from natural sources, many based on their use in traditional medicine. Over the last century, a number of top selling drugs have been developed from natural products (vincristine from *Vinca rosea*, morphine from *Papaver somniferum*, Taxol from *T. brevifolia*, etc.). In recent years, a significant revival of interest in natural products as a potential source for new medicines has been observed among academia as well as pharmaceutical companies. Several modern drugs (~40% of the modern drugs in use) have been developed from natural products. More precisely, 39% of the 520 new approved drugs between 1983 and 1994 were natural products or their derivatives, and 60–80% of antibacterial and anticancer drugs were from natural origins. In 2000, approximately 60% of all drugs in clinical trials for the multiplicity of cancers had natural origins (Cragg et al., 1997). Plants contain significant levels of natural genetic and phenotypic variation between individuals within a species for traits ranging from development to metabolism to pathogen resistance. This intra-specific variation is a foundation for research by evolutionary and ecological biologists interested in understanding plant fitness as well as by plant biologists focused on increasing the fitness or yield of agricultural plants. An important component of intra-specific variation for both research groups is the secondary metabolite complement present within a plant.
Variation in these compounds controls important ecological and agronomic traits such as resistance to insect herbivores and benefit to human health (Osbourn and Lanzotti, 2009).

Moleata or Dandelion (*Taraxacum officinale*) is one of the highly valuable ethnomedicinal plants. The major modern and historical uses for Dandelion are as a diuretic and liver tonic. Both uses are supported by animal data. Although there is long historical tradition to support these uses, no randomized, controlled trials in humans have evaluated Dandelion’s effect as a diuretic, cholagogue, appetite stimulant, hepatitis remedy or weight loss agent. A side from gardener’s laments and allergic reactions, Dandelion is very safe. It is widely consumed as a salad green, the roots are roasted and used as a coffee substitute (Kathi and Kemper 1999).

Different dandelion (*Taraxacum officinale*) plant parts extracts, were investigated against two fungi (*A. niger* and *P. italicum*). The results of the study indicated that the different extracts parts were highly effective in reducing both mycelial radial growth and mycelial fresh and dry weights of both fungi. The reductions by the higher concentration of different parts were statistically significant compared to the control.

Clinical microbiologists have two reasons to be interested in the antimicrobial plant extracts. First, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans. It is reported that, on average, two or three antibiotics derived from microorganisms are launched each year (Clark, 1996). New sources, especially plant sources, are also being investigated. Second, the public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. In addition, many people are interested in having more autonomy over their medical care (Alper, 1998). Extract of many plant species were reported to have antifungal activities (Bullerman, 1974, Abdl-Rahim *et al.*, 1997, Al-Jali *et al.*, 1997). Almahi (2011) tested the extracts of Garad (*Acacia nilotica*) against two fungi
(A. niger and P. italicum). Her results showed that the extracts of the different tree parts had different effects with the extracts of the tree bark and pods as the best effective. Abdel-Rahim et al, (2012) reported that the fruit peel and flower extracts of Romman (Punica grantum L.) were effectively inhibited mycelial growth and dry weight of both A. niger and P. italicum. The effect was only significant at the higher concentrations. The results were also obtained by different researchers (Abdel-Rahim et al., 1997, Al-Jali et al., 1997, Abdel-Rahim et al., 2002 and Sulieman et al., 2009).

Finding healing powers in plants is an ancient idea. People on all continents have long applied poultices and imbibed infusions of hundreds, if not thousands, of indigenous plants, dating back to prehistory. There is evidence that Neanderthals living 60,000 years used plants such as hollyhock (Stockwell, 1988). These plants are still widely used in ethnomedicine around the world. Currently, of the one-quarter to one-half of all pharmaceuticals dispensed in the United States having higher-plant origins, very few are intended for use as antimicrobials. Since the advent of antibiotics in the 1950s, the use of plant derivatives as antimicrobials has been virtually nonexistent.

Antimicrobial activity of plant extract are well documented (Alicia, 1981). Vlietinck et al. (1995) and Odebiyi and Sofowora, (1979) and screened about 100 medicinal plant used by traditional healers to treat infection in Rwanda, Nigeria and Sudan for their antibacterial, antifungal and antiviral properties. In sudan many studies were carried out for testing the antimicrobial activity of some medicinal plants. Ahmed (2004) tested the extracts of 10 plants against Gram positive and Gram negative bacteria as well as Candida albicans. He found a marked effect against the Gram positive Staph. Aureus followed by the Gram negative E. coli and Candida albicans. Roodt (1992) reported antibacterial activity of Kigelia africana bark extract (aqueous, methanol and chloroform) against Escherichia coli, Enterobacter aerogens, Klebsiella pneumoniae, Salmonella typhi, Proteus vulgaris, Pseudomonas
aeruginosa, Staphylococcus aureus and Bacillus cereus. Also he showed that the methanolic bark extracts were highly effective against Salmonella typhi and Proteus vulgari.
CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

From the present study it could be concluded that:

1. The aqueous Dandelion extracts have antimicrobial activity against the tested fungi (*A. niger* and *P. italicum*). The effects included, mycelial radial growth (inhibition zone and mycelial weight).

2. The study indicated that all parts extracts were effective but the leaf gave high reduction in radial growth and mycelial dry weight compared to the other plant parts.

3. The study also revealed that the highest inhibitory effect of the Dandelion extracts were found against *P. italicum* in comparison with *A. niger*.

4. The inhibitory effect against the tested fungi was more effective when using the higher concentration of the extract (the effects was decreasing with increasing dilution).

RECOMMENDATIONS

1. The study recommended attention to benefit from the researches made in the field of medicinal plants and the application and use of their findings.

2. It could be recommended that Dandelion extracts can be used as antifungal agents.
3. It is suggested that Dandelion extracts which traditionally used for curing many known diseases would be useful for treating skin disease.

4. Dandelion extracts can be carried on other microorganisms.
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LXIV


LXX