ABSTRACT
The human nature has never been free of health related problems neither has found single ever curing drug. Plants have been used until today as immediate remedy for various alignments. Essential oils are complex mixture of component unlike chemicals which are based on single products. Thus, a plant with essential oil is believed to have medicinal value. In this study the anthelmintic and antioxidant activity of eucalyptus essential oil was investigated. Eucalyptus leafs were brought from Adigrat and surrounding areas and washed before they shade dried for ten days. Then they were extracted with distilled water in hydro distillation with Clevenger apparatus. The crude essential oil of the plant was screened for phytochemicals employing various standard methods. Biological activity of essential oil was also tested with focus to its anthelmintic and antioxidant activity against Pheretima posthuma and DPPH (2, 2-diphenyl-1-picrylhydrazyl) respectively. In both assays significant inhibitions was recorded and even in the anthelmintic activity the oil extracts recorded relatively higher efficacy than the standard drug used in almost all of the concentrations used. Even if the oils are not rich in the polar phytochemicals but their activity was significant against the worms and radicals at all concentration. It was found out that the oil inhibited the radicals to about 68% and it kill the earth worms at 37±4min at the highest concentration (100g/ml v/v).

KEYWORDS: Eucalyptus globules, Pheretima posthuma, 2, 2-diphenyl-1-picrylhydrazyl, Hydro distillation, Invitro biological activity, UV spectroscopy.
1. INTRODUCTION

Diseases caused by different organisms have been fought all living creature till today. Thus, to look for plant derived long lasting drug has been a concern of all times for all scientists in the field. [1, 2]

Helminthes infections, commonly called helminthiasis are among the most important animal diseases inflicting heavy production losses causing more morbidity and greater economic and social deprivation among humans and animals than any single group of parasites. The disease is highly prevalent particularly in third world countries due to poor management helminthiasis practices. They pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia. [1, 3, 4, 5]

Free radical mediated disorders on the other hand are the cause for many diseases. Considerable evidence have accumulated to implicate cellular damage arising from reactive oxygen species (ROS), which lead to arthritis, hemorrhagic shock, atherosclerosis, diabetes, hepatic injury, aging, tumor promotion, neurodegenerative disorders (e.g. Alzheimer disease, Parkinson disease, multiple sclerosis, Down’s syndrome), inflammation, viral infections, autoimmune pathologies, and digestive system disorders such as gastrointestinal inflammation and ulcer. A number of medicinal plants have been used to treat parasitic infections and free radical mediated disorders. [2, 6, 7]

Essential oil also known as ethereal oil is a concentrated, hydrophobic liquid that contains hundreds of aromatic compounds, organic constituents, including hormones, vitamins and other natural elements. These compounds are extracted from leaves, stems, flowers, bark, roots or other elements of a plant. Essential oil contains highly volatile components. [8, 9]

Essential oils are used to treat many ailments in different ways. They have a profound effect on the central nervous system, relieving depression and anxiety, reducing stress and relaxing. Many essential oils are used in perfumery. It takes many pounds of flowers to construct ounce of essential oil. [8, 9, 10, 11]

*Eucalyptus* oil has numerous traditional uses. *Eucalyptus* oil for example has been used as a traditional non-ingestive treatment for coughs and colds, a topically applied medication for relief of muscular pain and as a solvent/sealer in root canal dentistry. It has uses as a fragrance in soaps, detergents and perfumes and as a flavoring in food. Household uses
include spot and stain remover and wool wash component. It has also been used as a flotation agent in the mining industry. [12]

Oils of many plants especially essential oils have Antibacterial, fungicidal, anthelmintic, antioxidant, insecticidal and cytotoxic potency. [11, 12, 13] Essential oil, could act as a chemical defense against plant pathogenic diseases. [9]

The essential oil 1, 8-Cineole (often just "cineole") is the pharmaceutically active component of Eucalyptus oil. Alcohols, Aldehydes, Terpenes, Ketones are extremely useful due to their antiviral, antibacterial, antiseptic, anti-fungal, anti-inflammatory, disinfectant and sedative properties. They are present in geraniol, geranium, lemon and Eucalyptus oils. [8, 11, 14, 15]

In Ethiopia there are many plants with wide traditional use mainly to treat different diseases. However, almost all of them are not studied involving scientific methods. [16] Peoples also use these plants as last alternative for already known illness. This shows scientific information regarding the medicinal plant should be incorporated as there is less know how in the people and less documented information. Eucalyptus plant is one amongst the victims. It is distributed in all most every part of the country than any plant species. However, with respect to its potential use there is insignificant information about it. Therefore, the aim of this study is to assess the antioxidant and anthelmintic activity of essential oil from Eucalyptus globulus leaves.

2. Methodology

2.1 Chemicals and Solvent

Sodium chloride (NaCl), Potassium iodide (KI), Ferric chloride (FeCl₃), Potassium Mercuric Iodide(K₂HgI₄), Sodium hydroxide (NaOH ), Hydrochloric acid (HCl), Mercuric II Nitrate(Hg(NO₃)₂), Ninhydrin, Hydrogen per oxide(H₂O₂), sulphric acid (H₂SO₄), picric acid(C₆H₃N₃O₇ ), Tween 80, DPPH (2,2- diphenyl-1-picrylhydrazyl) assay, Ammonia solution, Ammonium hydroxide , Ethanol, Benzene, Dichloromethane, Dimethylsulfoxide (DMSO).
2.2 Instruments and Equipments
Clevenger apparatus, round bottom flask, rotatory evaporator, fridge, condenser, heating mentel, electrode beam balance, Petridish, thermometer, distiller, pestle and mortal, UV-visible spectroscopy.

2.3 Plant Material Collection
Eucalyptus plant fresh leafs were collected from Adigrat and surround areas in the month of August to September 2013 and identified by botanists in the University.

2.4 Extraction of Essential Oil
Before Extraction, the collected samples was washed with tap water, to eliminate soil and other surface contaminants and shade dried at room temperature for 8-10 days. The dried sample was crashed to increase surface area using the pestle and mortal. The essential oil was then extracted from samples of Eucalyptus using hydro distillation in Clevenger type apparatus. First 180g of the sample was measured using digital beam balance and put in to round bottom flask using spatula. Then ¾ of flask was filled with distilled water, the Clevenger and condenser was fixed and the set up was placed on heating mantle. Then the flask was covered by aluminum foil (to have uniform heating) and was allowed to heat for 4 hours. While it was heating the oil and water evaporated as stem. The essential oil was obtained by removing the water until it riches the layer of the oil. The obtained essential oil was purified and stored under refrigerator for further use. [17]

2.5 Phytochemical Screening
The crude oil extract of Eucalyptus was tested for the presence of different phytochemicals such as Phenol, alkaloids, quinones, flavonoids, tannins, saponins, amino acids and terpenoids. The test was carried out by the standard phytochemical tests described elsewhere. [1, 18, 19]

2.6 In vitro Antioxidant Activity
2.6.1 DPPH (2,2- diphenyl-1-picrylhydrazyl) assay
A 0.3 mM solution of DPPH radical solution in ethanol 90% was prepared and then 1 ml of this solution was mixed with 2 ml of different concentrations of each extract (sample). After 30 min incubation in dark and at room temperature, absorbance (A) was measured at 518 nm in UV spectrophotometer. The percentage of the radical scavenging activity (RSA) was calculated by the following equation:
Ethanol 90% (1 ml) plus each sample solution (2 ml) was used as a blank. DPPH solution (1 ml) plus ethanol 90% (2ml) was used as a negative control. Ascorbic acid (at the concentrations of 100, 50, 25, 10, 5, 2.5 mg/ml) was used as a positive control. \[20, 21\]

2.7 Anthelmintic Activity

The assay was performed on adult earth worm _Pheretima pasthuma_ due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. The worms were washed with normal saline to remove all the extraneous matter. Standard drug (Piperazine citrate, 1%) and sample extracts at different concentrations (100, 50, 25, 10, 2.5 mg/ml v/v) were prepared in 5% DMSO in normal saline (0.85%) with Tween 80 (0.5% v/v for easy diffusion) and poured in to respective labeled petri plates (20 ml). Six worms of equal size (or nearly equal) were introduced into each of the plates. Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors. Death was also confirmed by dipping the worms in slightly warm water. The mortality of parasite was assumed to have occurred when all signs of movement had ceased. \[2\]

2.8 Statistical Analysis

Experiments were carried out in triplicate with their mean values and standard deviations by formula.

3. RESULTS AND DISCUSSION

3.1 Yield of Essential Oil from Eucalyptus Leaves

From 180g leave 3ml of essential oil and total of 27 ml essential oil was obtained.

3.2 Phytochemical Results

The presence of phytochemicals is directly related to medicinal activity of the plant, in our concern the inhibition data recorded in anthelmintic and antioxidant activity could be due to the finding of the bioactive ingredients listed in the table below.

Tannins in their mechanism of anthelmintic action are known to interfere with energy generation by uncoupling oxidative phosphorylation or they may interfere with glycoprotein of cell surface. Tannins can also react with nematode's cuticle and toughens the skin. Alkaloids act on central nervous system and caused paralysis of the worms. Flavonoids have
a number of hydroxyl groups connected with the aromatic which enhance toxicity to the worms and antioxidative effectives. [1, 2, 3, 5]

Table 1: Results of Phytochemical Screening of Eucalyptus essential oils

<table>
<thead>
<tr>
<th>No</th>
<th>Phytochemical</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Quinines</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Amino Acids</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Triterpenes</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Phenolic compounds</td>
<td>-</td>
</tr>
</tbody>
</table>

(++) Indicates significant presence, (+) Indicates presence (-) Indicates absence.

3.3 Anthelmintic Activity

When extracts were added to Petri dish containing the worms they die at different time as shown in Table 2. The oils record dose dependent inhibition that earth warm treated with highest concentration die at 37±4 min and the earth worms exposed to lowest concentration were eliminated at 85±8 min. From the observation oil extracted of eucalyptus was found to show a potent anthelmintic activity when compared to standard drug at all concentrations.

Table 2. Anthelmintic Activity of Eucalyptus Essential Oils

<table>
<thead>
<tr>
<th>S/N</th>
<th>Concentration(mg/ml% v/v)</th>
<th>Time taken for Paralysis (min)</th>
<th>Time taken for Death (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Essential oil</td>
<td>Positive control</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>26±4</td>
<td>32±4</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>38±6</td>
<td>41±2</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>44±4</td>
<td>46±5</td>
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<tr>
<td>4</td>
<td>10</td>
<td>48±3</td>
<td>51±4</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>65±5</td>
<td>64±5</td>
</tr>
<tr>
<td>6</td>
<td>2.5</td>
<td>79±3</td>
<td>88±5</td>
</tr>
</tbody>
</table>

3.4 In Vitro Antioxidant Activity Results

Eucalyptus grown here in Adigrat has showed to have antioxidant potency. However, compared to their anthelmintic activity they were less potent. The presence of some of the secondary metabolites responsible for antioxidant activity could be a clue to the recorded percentage inhibition. On the other hand, the less abundance in most of them and the absence of phenolics can be the reason to the decrements in activity. [22]
4. CONCLUSION AND RECOMMENDATION

4.1 Conclusion

It is concluded that the Euclayptus oil extract showed more potent anthelmintic activity. Their antioxidant effectiveness is also dose dependent but less compared to the standard drug. Recently plant antioxidants are required and preferred than their counter synthetic ones attributed different factors (side effect, adaptation, cost). Plants such as eucalyptus leaves can be an immediate solution. There are very less invitro antioxidant test reports about eucalyptus plants and in some of the findings the oil has either no inhibition or it is insignificant. The findings here could be due to geographical variation. The findings of this study are significant inputs.

4.2 Recommendations

In vitro studies are always inputs to in vivo and further investigations. Besides, in vitro data are full of circumstances from the controlled parameters until the artificial environment of the organisms. Therefore, further studies in elucidation, including in vivo as well, are required to identify the actual chemical constituents that are present in the crude extracts of this plant which are responsible for anthelmintic activity and antioxidant activity to establish the effectiveness and pharmacological use of Eucalyptus as an anthelmintic and antioxidant drug. A search for plant derived drug should not be on various plants. A given plant can also show different potency at different places (attributed to geographical variation of both the plant and the organisms) at different situations (extraction solvent, method of the assay).
ACKNOWLEDGMENT
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REFERENCES