The antifungal activity and phytochemical of ethanol extracts from leaves of Azadirachta indica plants in Iraq

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Abstract---The present study appearing various compounds that have been isolated from Azadirachta indica and showed natural compounds from leaves against three types of pathogenic fungi are as follows: "Alternaria alternate, Neoscytalidium dimidiatum and Sordaria fimicola". The Phytochemical of Azadirachta indica leaves were exposed to (GC-MS) analysis. The results showed highest activity against all reviewed fungal, in Alternaria alternate, Neoscytalidium dimidiatum and Sordaria fimicola. the three concentrations of extract (20, 30, 40 mg/ml) in respectively were given a results (0.00) mm in the diameter of colonies. The GC-MS analysis of Azadirachta indica leaves parts showed the presence of: 1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)- 1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)-1-ethylcyclohexane 1-Methyl-4-(1-methylethylidene)-2-1-methylvinyl)-1-vinylcyclohexane) trans-beta-caryophyllene Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylenedimer 1-R,1α,3α,4β-4-Ethenyl-a,a,4-trimethyl-3-(1-methylethenyl) cyclohexanemethanol diethyl benzene-1,2-dicarboxylate Neantine Selinenol 4(-2 a,8-Dimethyl-1,2,3,4,4a,5,6,8a-octahydro-2-naphthalenyl)-2-propanol tert-Butyl[6-bromofuro[3,2-b]pyridin-2-yl]-methylcarbamate Palmitic acid (hexadecanoic acid) Palmitic acid, ethyl ester 2-Hexadecan-1-ol, 3,7,11,15-tetramethyl-, (2E,7R,11R)- cis-9-Octadecenoic acid 9-Octadecenoic acid (Z), ethyl ester Behenic acid ethyl ester Oleic Acid GurudeebanSatyavani Acetyl-O-anisidine 1-Hydroxy-3-methylbenzene 1-(1Z-octadecenyl)-2-hexadecanoyl-sn-glycero-3-phosphocholine 2-[23]-Methyl-1-(1-methylethyl)cyclopropyl]-24-nor-5alpha-cholester Nicco Squalane
EX 24 ·S-Ethylcholest-5-en-3β-ol · (2-Dodecen-1-yl)succinic anhydride, technical

Keywords---Azadirachta indica, gas chromatography, mass spectrometry, bioactive phytochemical, antifungal activity.

Introduction

Azadirachta indica, known as Neem, belong to family - Meliaceae. It is one of two species of the genus Azadirachta. It is native to India, Pakistan, most countries in Africa and Bangladesh, growing in tropical and semi-tropical regions. It is deciduous, fast growing tree, reach to 20-40 meters in height, leaves opposite, pinnately compound, are 20-40 cm long, leaflets dark green, 3-8 cm long, flowers white, bisexual, the male flower exist on the same tree, flowers arranged in axillary panicles. Fruits glabrous. (1)

Extracts from seeds and leaves exhibits immunomodulatory, anti-inflammatory, antiulcer, antifungal, antibacterial [Rai et al.,2011, Neycee et al, 2012] antihyperglycaemic, antiviral, antioxidant, , anti malarial, anti mutagenic and anti carcinogenic properties and also as insecticides for agricultural use. so Fungicidal properties of neem extracts are significantly reduced conidal germination in several fungi. (Hanif,etal 2013, Giri etal,2019, Passosa etal,2019 Patil etal ,2013) so a studies were carried out by Abd El-Ghany et al ,( 2015) to test the antifungal activity of four plant extracts (Azadirachta indica one of them) ; The study evaluating the antifungal activity of natural compounds of plant extracts with comparing of chemical fungicide (against Fusarium oxysporum, Alternaria alternata and Aspergillus flavus). Shrivastava and Swarnkar(2014) indicates that the antifungal activity of Neem leaves against pathogenic fungi like Alternaria solani, Aspergillus flavus and Cladosporium. The biochemical components are taxonomically and chemically awfully diverse composites with incomprehensible function. They are used in agriculture, scientific research and the human therapy., (Azam etal 2013) So this study aimed to explain a synthetic drugs from herbal plant extract and Propolis and their effects on mentioned fungi.

Materials and Methods

Study area and sampling

The studied fungi were isolated from infected plants by these fungi in Kerbala fields, the fungi were identifying in the agricultural college laboratory, Kerbala university.

Microscopic assessment

The samples were examined using a method [11] " the area were cleaning with a cotton saturated swab with 70% alcohol to get rid of a bacteria and Saprophytes fungi, and then taken a scrape from the influenced parts infected by a tool Loop fertilization and then placed on a pure glass slide with a drip of 0% KOH and
then put the glass slide cover and heat the sample on a benzene flame and examined by a microscope for the occurrence of pathogenic fungi spores or hypha, Mentioned Fungi were diagnosed based according to: [13] [12] . The phenotypic characteristics of spores and fungal colonies and microscopic properties and were espoused by identifying the appearance and color of the colony from the bottom of the dish .

**Plant Extract preparation**

Wahid & Jafar method (14) was followed in the extraction process,

**Cultivated Method of alcoholic extract of Azadirachta indica plant on pathogenic fungi growth**

"El-Kady et al (15) Method were chased, "The alcoholic extract of Azadirachta indica was merged with (PDA) cultivated media with three concentrations (20, 30, 40) mg/ml (three replicates for each concentration) . After a solidifying a medium, a hole was made at a center of each dish by a cork borer piercing (5 mm) in a diameter with A control treatment. The dishes were inoculated with experimented fungus inoculum and grown on the PDA medium for 10 days each by fixing a disk with a diameter of 5 mm each in the center of the dish. Astudied dishes were incubated at 25 ° C and for 10 days, the diameter of the growing colony was measured . Results were recorded", and the inhibition ratio was calculated by using the following [16] :

\[
\text{Inhibition ratio} = \frac{\text{Average diameter of fungus in control dish} - \text{Average diameter of fungus in treatment dish}}{\text{Average diameter of fungus in control dish}} \times 100
\]

**Collection and preparation of plant materials**

" Azadirachta indica leaves were located from various spots in Iraq. Then leaves were washed and dried at room temperature. 40g of plants powdered had taken in 200 ml ethanol and then filtered.

**Constituents Identification of Extract by Gas chromatography – mass spectrum (GC/MS)**

Phytochemical identification of Azadirachta indica. were carried out by GC-MS analysis in 'a (QP 2015 Plus SHIMADZU) instrument under computer designed control at 60 eV. About 1μL of them ethanol extract was injected into the GC-MS column using a micro syringe and the scanning was done for 45 minutes". [17, 18]
**Results and Discussion**

**Antifungal activity**

In the current study, three types of fungi were selected to test the efficacy of the ethanol extract of *Azadirachta indica* leaves on the growth and development of three types of pathogenic fungi are as follows: *Alternaria alternate*, *Neoscytalidium dimidiatum* and *Sordaria fimicola*. were ethanolic extract of *Azadirachta indica* leaves were showed "a high antifungal activity against three types of pathogenic fungi studied. The results publicized that all studied fungal, at 3 concentrations of extract (20, 30, 40 mg/ml) were showed a results (0.00 mm ) in the diameter of colonies in *Alternaria alternate*, *Neoscytalidium dimidiatum* and *Sordaria fimicola*, the results are obtained in Table (1). the results of the current study are in agreement with the findings of Abd El-Ghany et al., (2015) (Shrivastava and Swarnkar , 2020) who confirmed that ethanol leaves *A.indica* extract works to inhibit the growth of *Alternaria* and some pathogenic fungi . while (Govindachari et al, 1998). And (Singh & Sastry, 1997) registred some secondary metabolites that have Antifungal properties. (Natarajan, Venugopal & Menon, 2003; Mahmoud et al, 2011; Amadioha & Obi, 1998)found that ethanolic extract of *Azadirachta indica* leaf inhibits pathogenic fungi.

Table (1) Antifungal activity of ethanol extracts from *A.indica*

<table>
<thead>
<tr>
<th>Fungal type</th>
<th>Mean of Inhibition zone (mm)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Comparison 1 with distilled water (0.00 mg/ml)</td>
</tr>
<tr>
<td><em>A. alternate</em></td>
<td>80.00</td>
</tr>
<tr>
<td><em>N. dimidiatum</em></td>
<td>80.00</td>
</tr>
<tr>
<td><em>S. fimicola</em></td>
<td>80.00</td>
</tr>
</tbody>
</table>

**Assessment of Biochemical compounds of *A. indica* leaves**

"The GC-MS analysis of ethanol extract of *A.indica* leaves are appeared the presence of 25 components performed in Table 2. the separated compounds has different biological activities, as . anxiolytic antimicrobial, anti-inflammatory spasmolytic, , antiproliferative, , antialgal effects and antioxidant".

Table(2) Major phytochemical composites in ethanolic extract of *A. indica* leaves

<table>
<thead>
<tr>
<th>No.</th>
<th>Chemical name</th>
<th>Retention time</th>
<th>%</th>
<th>Chemical structure</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
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<tbody>
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<td></td>
<td>Formula</td>
<td>Mr</td>
<td>1H</td>
<td>13C</td>
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<tr>
<td>1</td>
<td>1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)-</td>
<td>136.23</td>
<td>3.85</td>
<td>C_{10}H_{16}</td>
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<td>2</td>
<td>(R)-(+-)Limonene</td>
<td>136.23</td>
<td>15.63</td>
<td>C_{10}H_{16}</td>
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<td>1-methyl-3-(1-methylethyl)-cyclohexane</td>
<td>140.27</td>
<td>2.49</td>
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<td>4</td>
<td>1-Methyl-4-(1-methylethylidene)-2-(1-methylvinyl)-1-vinylcyclohexane</td>
<td>204.35</td>
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<td>trans-beta-caryophyllene</td>
<td>204.35</td>
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<td>6</td>
<td>Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, dimer</td>
<td>272.5</td>
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<td>1R,1α,3α,4β-4-Ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)cyclohexanemethanol</td>
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<td>diethyl benzene-1,2-dicarboxylate Neantine</td>
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<td>9</td>
<td>Selinenol</td>
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<td>2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,8a-octahydro-2-naphthalenyl)-2-propanol</td>
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<td>579</td>
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<td>tert-Butyl[6-bromofuro[3,2-b]pyridin-2-yl]-methylcarbamate</td>
<td>377</td>
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<td>Palmitic acid (hexadecanoic acid)</td>
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<td>15</td>
<td>cis-9-Octadecenoic acid</td>
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<td>484</td>
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<td>C_{29}H_{50}O</td>
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References


