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The phytochemical and the antifungal activity of *Melia azedarach* Ethanol extracts from leaves of Plants in Iraq

Douaa Abd Alhameed Jawad
Department of Biology, College of Education for Pure Science, Kerbala University, Kerbala, Iraq

Neepal Imtair Al Garaawi
Department of Biology, College of Education for Pure Science, Kerbala University, Kerbala, Iraq
Corresponding author email: neepal.i@uokerbala.edu.iq

***Abstract*---**In the present study a various phytochemical compounds that have been isolated from *Melia azedarach* and showed phytoconstituents from leaves against three of pathogenic fungi are as follows: *Alternaria alternate, Neoscytalidium dimidiatum and Sordaria fimicola.* "The Phytochemical of *M. azedarach* leaves were exposed to (GC-MS) analysis. The results showed highest activity against reviewed fungal, (*Alternaria alternate, Neoscytalidium dimidiatum and Sordaria fimicola).* All 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 *Melia azedarach* leaves parts showed the presence of؛ Ethyl methyl phthalate ؛ diethyl benzene-1,2-dicarboxylate ؛ Methyl hexadecanoate Palmitic acid (hexadecanoic acid) ؛ 11-octadecenoic acid methyl ester ؛ 2-Hexadecen-1-ol,3,7,11,15-tetramethyl-,(2E,7R,11R)-؛ -9-(Z)-Octadecenoic acid؛ 1,16-Hexadecanedicarboxylic acid؛ 9(Z),12(E)-Octadecadienoic acid؛ 9,19-cyclolanost-24-en-3-ol ؛ 3-beta-5-alpha-6-beta-trihydroxycholestan ؛4-Stigmasten-3-one؛ 9,19-Cyclo-9.beta.-lanost-25-en-3.beta.-ol, 24-methyl-, (24S)- ؛ (R,R,R)-alpha-Tocopherol ؛ 4-(4-ethylcyclohexyl)-1-pentyl-cyclohexene ؛ (4,4-Dimethyl-5-.alpha.-cholestan-3-.beta.-ol ؛ 5alpha-Stigmastan-3,6-dione؛ (22E)-Stigmast-22-en-3-one؛ Cholest-8-en-3.beta.-ol؛ (2-Dodecen-1-yl)succinic anhydride, technical.

Keywords*---Melia azedarach* "gas chromatography –mass spectrometry, bioactive phytochemical, antifungal activity"

Introduction

M. azedarach has a lots of common name such as margosa, chinaberry, Barbados, Persian lilac, Indian lilac, umbrella tree, zanzalakht, zarur, bakain and think.(1)(2)(AL-Rawi,1964; Bounechada and Fenni,2007) This species originates from Southern Asia: India, Pakistan, Iran, and South of China , it has been widely cultivated in East-Southern Africa, America, Australia, middle East ,southern of Europe and Indonesia(3) (Wulandini and Widyani,2007). M. azedarach is a large ever green tree, it is a deciduous, can reach (15m)in height and (60cm) in diameter (4)(Gayatri and Sahu,2010), leaves dark green, alternately whorled bi-pinnately, compound,2-8cm long ,leaflet lanceulate with tapering tips, 2,5-8cm long and 1-3 cm wide, acute , to round base, apex acuminate,.Flowers are purplish and fragrant, bisexual or male. Fruit ellipsoid –globose drubs, 1-1.5cm in diameter, exo-carp thin and smooth, endo-carp brownish yellow(3) (Wulandini and Widyani,2007). M. azedarch have anantifungal(5)(Ashraf and Javaid,2007).

The phytochemical compounds of M. azedarach revealed the presence of alkaloids, resins, tannins, gum, benzoic acid, cinnamic acid, , phenol, coumarin, teranor-triterpenoid , triterpens and glycoside,(6) (Ahmed et al., 2008) . Extracts from different parts of Melia azedarach L. were studied as potential antifungal agents for selected phytopathogenic fungi. Aspergillus flavus,Diaporthe phaseolorum var. meridionales, Fusarium oxysporum, Fusarium solani, Fusarium verticillioides, and Sclerotinia sclerotiorum.(7)(Carpinella et al 2003). Jabeen etal (2011)(8) reported that The antifungal activity of Melia azedarach L. leaves was investigated against Ascochyta rabiei (Pass). Neycee etal (2012)(9) showed that the Antifungal effects of methanol extract of chinaberry (M. azedarach) against strains of Trichoderma spp Sclerotium spp spp Fusarium oxysporum and Rhizoctonia solani. Asman etal (2021)(10) reviewed the Antifungal activity of extracts of Melia azedarach against Lasiodiplodia pseudotheobromae.

"The biochemical componentes are taxonomically and chemically awfully diverse composites with incomperhensible function. They are used in agriculture, scientific research and the human therapy,.(11]So ,this study aimed to explain a synthetic drugs from herbal plant extract and Propolis and their effects on mentioned fungi".

Materials and Methods

1. 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.

2. Microscopic assessment

"The samples were examined using a method [12] " 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 amicroscope for the occurrence of dermatophytes spores or hypha, Mentioned Fungi were diagnosed based according to: [14] [13] ,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".

3. Plant Extract perperation

Wahid & Jafar method (15) was followed in the extraction process.

4. Cultivated Method of alcoholic extract of *M. azedarach* plant on pathogenic fungi growth

"El-Kady *etal (16)* Method were chased, "The alcoholic extract of *Melia azedarach* 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 expermented 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 [17] ":

Inhibition

$$\textbf{ratio} = \frac{Average\ diameter\ of\ fungus\ in\ control\ dish(1) - Average\ diameter\ of\ fungus\ in\ tretment\ dish}{Average\ diameter\ of\ fungus\ in\ control\ dish(1)} \times \mathbf{100}$$

5-*Collection and preparation of plant materials*

"Melia azedarach 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.

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

Phytochemical identification of *Melia azedarach***.** 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 45minutes". [18, 19]

Results and Discussion

1- Antifungal activity

In the current study, three types of fungi were selected to test the efficacy of the ethanol extract of *Melia azedarach* leaves on the growth and development of three types of plantpathogenic fungi are as follows: *Alternaria alternate, Neoscytalidium dimidiatum and Sordaria fimicola.*

The ethanolic extract of *M. azedarach* leaves showed "a high antifungal activity against three types of plantpathogenic fungi studied.
The results showed that all studied fungal, at 3 concentrations of extract (20,30,40 mg/ml)respectively were give 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 [8] who confirmed that ethanol leaves M. azedarach extract works to inhibit the growth of fungal pathogens. [6] found that ethanolic of *M. azedarach* leaves extract inhibits plant pathogenic fungi because the leaves contain some secondary metabolites that have antimicrobial properties.

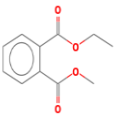
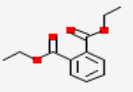
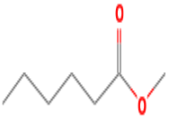
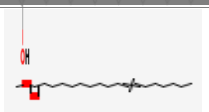
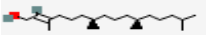
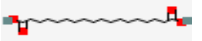
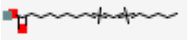
Table (1) Antifungal activity of ethanol extracts from *M. azedarach*

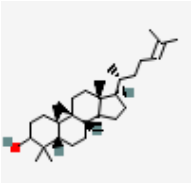
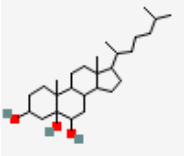
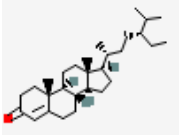
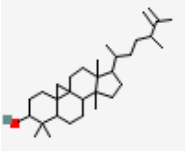
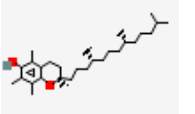
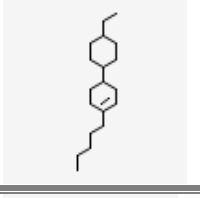
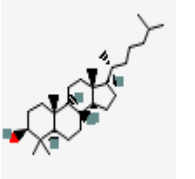
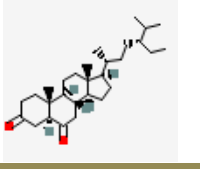
Fungal type	Mean of Inhibition zone (mm)				
	Comparison 1 with distilled water (0.00 mg /ml)	Comparison 2 with Clotrimazole 2mg/ml)(	**Concentration (20mg/ml)**	**Concentration (30mg/ml)**	**Concentration (40mg/ml)**
A. alternate	80.00	0.00	0.00	0.00	0.00
N. dimidiatum	80.00	0.00	0.00	0.00	0.00
S. fimicola	80.00	0.00	0.00	0.00	0.00

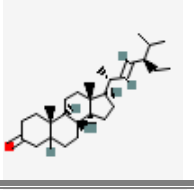
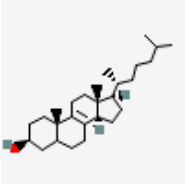
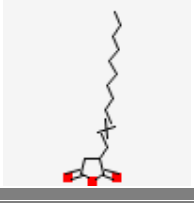
2- Assessment of Biochemical compounds of *M. azedarach* leaves

"The GC-MS analysis of ethanol extract of *M. azedarach* leaves are appeared the presence of 20 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 *M. azedarach* leaves

No.	Chemical name	Retention time	Exact mass	Chemical structure	Molecular formula	Molecular weight
1	Ethyl methyl phthalate	11.883	1.36		$C_{11}H_{12}O_4$	208.2
2	diethyl benzene-1,2-dicarboxylate	12.898	44.71		$C_6H_4(COOC_2H_5)_2$	222.2
3	Methyl hexadecanoate	17.040	0.68		$C_7H_{14}O_2$	130.1
4	Palmitic acid (hexadecanoic acid)	17.558	3.75		$C_{16}H_{32}O_2$	256.4
5	11-octadecenoic acid methyl ester	19.112	1.90		$C_{19}H_{36}O_2$	296.5
6	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, (2E,7R,11R)-	19.274	9.62		C20H40O	296.5
7	(Z)-Octadec-9-enoic acid	19.651	12.88		C18H34O2	282.5
8	1,16-Hexadecanedicarboxylic acid	19.910	2.71		$C_{18}H_{34}O_4$	314.5
9	9(Z),12(E)-Octadecadienoic acid	20.158	1.29		C18H32O2	280.4

10	9,19-cyclolanost-24-en-3-ol	21.820	0.90		C30H50O	426.7
11	3-beta-5-alpha-6-beta-trihydroxycholestan	22.036	0.92		C27H48O3	420.7
12	4-Stigmasten-3-one	22.834	1.51		C29H48O	412.7
13	9,19-Cyclo-9.beta.-lanost-25-en-3.beta.-ol, 24-methyl-, (24S)-	23.632	6.57		C31H52O	440.7
14	(R,R,R)-alpha-Tocopherol	24.215	0.69		$C_{29}H_{50}O_2$	430.7
15	4-(4-ethylcyclohexyl)-1-pentyl-cyclohexene	25.434	0.81		C19H34	262.5
16	4,4-Dimethyl-5-.alpha.-cholestan-3-.beta.-ol	25.984	2.55		C29H52O	416.7
17	5alpha-Stigmastan-3,6-dione	26.329	0.78		C29H48O2	428.7

18	(22E)-Stigmast-22-en-3-one	27.106	1.87		C29H48O	412.7
19	Cholest-8-en-3.beta.-ol	29.944	3.58		C27H46O	386.7
20	(2-Dodecen-1-yl)succinic anhydride, technical	30.321	0.92		C16H26O3	266.3

References

1. Al-Rawi, A. and Farty, J. L. J.1964. Medical plants in Iraq. 2nd Ed. Al-Eaqaza poplishers. Ministry of water and agriculture.100pp.
2. Bounechada, M. and Fenni, M. 2007. Laboratory evaluation of Melia azedarach L. extracts on Ocneridia bolivar (Orthoptera, Pamphaginae) adults. African Corp. Science Conference Proceeding. 8:1009-1011.
3. Wulandini, R. and Widyani. N. 2007. Melia azedarach L. Forest and landscape Denmark.117:1-2.
4. Gayatri, N. and Sahu, R. K.2010. In vitro antioxidative activity of azadirchta indica and Melia azedarach leaves by DPPH scarenging assay. J. Amr. Sci. 6 (6): 123-127.
5. Ashraf, H. and Javaid, A.2007. Evaluation of antifungal activity of Meliaceae family against Macrophomina phaseolina. Mycophath. 5(2):81-84.
6. Ahmed, M. F.; Ahmed, M. A.; Thayyil, H.; Zameeruddin, K. and Ibrahim, M . 2008 .Antioxidative Activity of Melia azedarach Linn. leaf extract. IJPT Archive of SID. 7(1):31-34.
7. Carpinella, M.C., Giorda L.M., Carlos G Ferrayoli C.G., and Palacios,S.M. Antifungal effects of different organic extracts from Melia azedarach L. on phytopathogenic fungi and their isolated active components . J Agric Food Chem. 2003;51(9):2506-11
8. Jabeen,K., Javaid,A., Ahmad,E. Athar ,M. Antifungal compounds from Melia azedarach leaves for management of Ascochyta rabiei, the cause of chickpea blight. Nat Prod Res .2011.;25(3):264-76.
9. Neycee, M.A., Nematzadeh,GH.A. Dehestani, Alavi, A.M. Assessment of antifungal effects of shoot extracts in chinaberry (Melia azedarach) against 5 phytopathogenic fungi. Intl J Agri Crop Sci. 2012. Vol., 4 (8), 474-477,
10. Asman,A., Cahyani, A. B., Nufus ,A. H., Rosmana,A. , Fakhruddin A.and NatsirN.U. Antifungal activity of extracts of Melia azedarach and Ageratum

conyzoides against Lasiodiplodia pseudotheobromae through in vitro test. IOP Conf. Series: Earth and Environmental Science 886 (2021) 012007.
11. Adem., R.S.,.Ayangbenro ,A.S and Gopane,R.O. (2020) Phytochemical screening and antimicrobial activity of Olea europaea subsp. africana against pathogenic microorganisms, Scientific African 10 (2020) e00548.
12. Szepietowski, J. C.; Schwart, R. A. (2005).Tineabarbae. Umdnj-New Jersew medical school.
13. Ayman Y El-Khateeb, Elsherbiny A Elsherbiny, Louis K Tadros, Safaa M Ali and Hassan B Hamed.(2013) . Phytochemical Analysis and Antifungal Activity of Fruit Leaves Extracts on the Mycelial Growth of Fungal Plant Pathogens. J Plant Pathol Microb. Vol 4 , Isse 9 • 1000199
14. Champion, R.; Burton, J. ; Burns, D. and Breathnach, S.(1998). Text book of dermatology. 6th. ed. Blackwell Science Ltd. P. 1277-1376.
15. Wahid, A.Z and Jafar,F.N .(2005).Test of Life effectiveness ofCarthamustinctorius Extract toward germ and fungi .AlBasrah research journal.Volume: 31 Issue: 3BPages: 39-47.
16. El-Kady, I. A.; Mohamed, S. S. and Mostafa, E. M.(1993). Antibacterial and antidermatophyte activities of some essential oils from spices. Qatar Univesity. Sci. J. 13 (1): 63-69..
17. Gahukar R.T. (2012). Evaluation of plant-derived products against pests and diseases of medicinal plants: A review., Crop Protection,Vol 42, PP: 202-20
18. Abu-Serag N.A, Al-Gara،awi N. I and, A M Ali.Analysis of bioactive phytochemical compound of (*Cyperus aucheri* Jaub.) By using gas chromatography –mass spectrometry. IOP Conf. Series: Earth and Environmental Science (2019). 388(1):012063
19. SA Allaith, DF Alfekaik, MA Alssirag. (2019). Identification of Pistacia vera and Prunus amygdalus Batsch seed oils using GC-MS as useful methodology for chemical classification., IOP Conference Series: Earth and Environmental Science 388 (1), 012061.