How to Cite:
Jawad, D. A. A., & AlGaraawi, N. I. (2022). The antifungal activity and phytochemical of ethanol extracts from leaves of Azadirachta indica plants in Iraq. *International Journal of Health Sciences*, *6*(S8), 3909–3917. https://doi.org/10.53730/ijhs.v6nS8.13014

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

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

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

***Abstract*---**The present study appearing various compounds that have been isolated from Azadirachta indica and showed natural compounds from leaves against three typs of pathogenic fungi are as follows:" Alternaria alternate, Neoscytalidium dimidiatum and Sordaria fimicola" . The Phytochemical of 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)- ؛(R)-(+)-Limonene 1؛-methyl-3-(1-methylethyl)-cyclohexane 1 ؛-Methyl-4-(1-methylethylidene)-2-1-methylvinyl)-1-vinylcyclohexane) ؛ trans-beta-caryophyllene ؛ Bicyclo [2.2.1]heptane, 2,2-dimethyl-3-methylene-,dimer 1 ؛R,1α,3α,4β-4-Ethenyl-α,α,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 ؛-Hexadecen-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-cholane ؛ Nikko 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 etal.,2011, Neycee etal, 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 conidial 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 componentes are taxonomically and chemically awfully diverse composites with incomperhensible 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 amicroscope 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 perperation

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 *etal (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 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 [16] ":

Inhibition ratio

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

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 45minutes". [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) regirested 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*

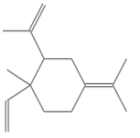
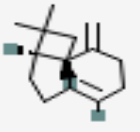
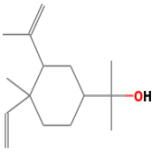
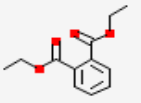
Fungal type	Mean of Inhibition zone (mm)				
	Comparison 1 with distilled water (0.00 mg /ml)	Comparison 2 with Clotrimazole (2mg/ml)	Concentration (20mg/ml)	Concentration (30mg/ml)	Concentration (40mg/ml)
A. alternate	80.00	0.00	0.00	0.00	0.00
N. dimidiatum	80.00	0.00	0.00	0.00	0.00
S. fimicola	80.00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

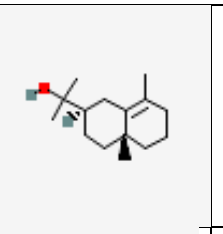
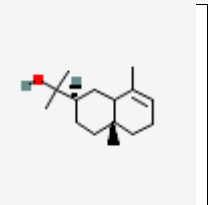
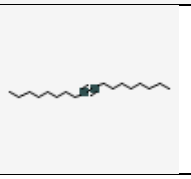
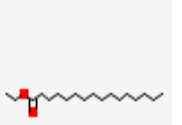
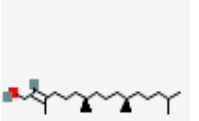
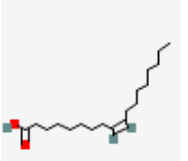
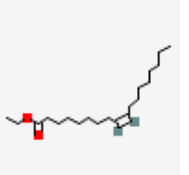
Assessment of Biochemical compounds of *A. indica* leaves

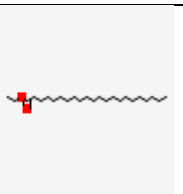
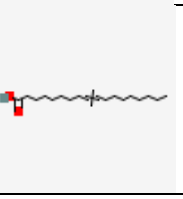
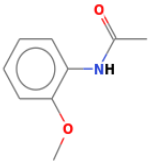
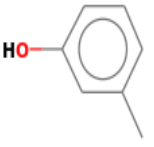
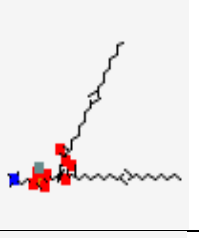
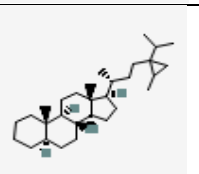
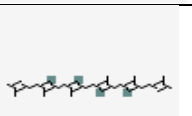
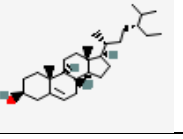
"The GC-MS analysis of ethanol extract of *A.indica* leaves are appeared the presence of 25 components performed in Table 2. the separated compounds has different biological activities, as . anxiolytic antimicrobial, anti-inflammatory spasmolytic, , antiproliferative, , antialgal effects and antioxidant".

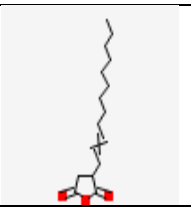
Table(2) Major phytochemical composites in ethanolic extract of *A. indica* leaves

No.	Chemical name	Retention time	%	Chemical structure	Molecular formula	Molecular weight

1	1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)-	4.396	3.85		$C_{10}H_{16}$	136.23
2	(R)-(+)-Limonene	4.688	15.63		$C_{10}H_{16}$	136.23
3	1-methyl-3-(1-methylethyl)-cyclohexane	5.486	2.49		$C_{10}H_{20}$	140.27
4	1-Methyl-4-(1-methylethylidene)-2-(1-methylvinyl)-1-vinylcyclohexane	9.219	1.00		$C_{15}H_{24}$	204.35
5	trans-beta-caryophyllene	10.535	1.03		$C_{15}H_{24}$	204.35
6	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, dimer	11.635	1.20		$C_{20}H_{32}$	272.5
7	1R,1α,3α,4β-4-Ethenyl-α,α,4-trimethyl-3-(1-methylethenyl)cyclohexanemethanol	12.326	3.31		$C_{15}H_{26}O$	222.36
8	diethyl benzene-1,2-dicarboxylate Neantine	12.801	12.87		$C_{12}H_{14}O_4$	222.24

9	Selinenol	13.480	1.46		$C_{15}H_{26}O$	222.37
10	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,8a-octahydro-2-naphthalenyl)-2-propanol	13.815	5.12		$C_{15}H_{26}O$	222.37
11	tert-Butyl(6-bromofuro[3,2-b]pyridin-2-yl)-methylcarbamate	15.973	1.42		$C_{18}H_{34}$	250.5
12	Palmitic acid (hexadecanoic acid)	17.548	4.15		$C_{16}H_{32}O_2$	256.42
13	Palmitic acid, ethyl ester	17.861	0.95		$C_{18}H_{36}O_2$	284.47
14	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, (2E,7R,11R)-	19.241	2.72		$C_{20}H_{40}O$	296.5
15	cis-9-Octadecenoic acid	19.630	11.98		$C_{18}H_{34}O_2$	282.5
16	9-Octadecenoic acid (Z)-, ethyl ester	19.889	5.81		$C_{20}H_{38}O_2$	310.5

17	Behenic acid ethyl ester	20.159	1.97		$C_{24}H_{48}O_2$	368.6
18	Oleic Acid_GurudeebanSatyavani	22.769	1.10		$C_{18}H_{34}O_2$	282.4
19	Acetyl-O-anisidine	23.201	4.70		$C_9H_{11}NO_2$	165.18
20	1-Hydroxy-3-methylbenzene	25.175	1.31		C_7H_8O	108.13
21	1-(1Z-octadecenyl)-2-hexadecanoyl-sn-glycero-3-phosphocholine	25.596	3.36		$C_{42}H_{84}NO_7P$	746.1
22	23-[2-Methyl-1-(1-methylethyl)cyclopropyl]-24-nor-5alpha-cholane	26.189	1.80		$C_{30}H_{52}$	412.7
23	Nikko Squalane EX	26.437	1.40		$C_{30}H_{50}$	410.7
24	24S-Ethylcholest-5-en-3β-ol	27.829	6.87		$C_{29}H_{50}O$	414.71

25	(2-Dodecen-1-yl)succinic anhydride, technical	29.350	2.50		$C_{16}H_{26}O_3$	266.38

References

1. Abd El-Ghany T. M. Roushdy M. M. and M. A. Al Abboud. Efficacy of Certain Plant Extracts as Safe Fungicides Against Phytopathogenic and Mycotoxigenic Fungi . Agricultural and Biological Sciences Journal Vol. 1, No. 3, 2015, pp. 71-75.
2. 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
3. Amadioha A. C. and Obi, V. I. (1998).Fungitoxic activity of extracts from Azadirachta indica and XylopiaaethiopicaonColletotrichumlindemuthianum in cowpea. Journal of Herbs, Spices and Medicinal Plants. 6(2), 33–40.
4. 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
5. Azam M. M., Mamun-Or-Rashid A. N. M, Towfique N. M.,and Sen M. K.. S. Pharmacological potentials of *Melia azedarach* L. American Journal of BioScience. Vol. 1, No. 2, 2013, pp. 44-49.
6. Champion, R. ; Burton, J. ; Burns, D. and Breathnach, S.(1998). Text book of dermatology. 6th. ed. Blackwell Science Ltd. P. 1277-1376.
7. 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..
8. 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
9. Giri ,R.P. Gangawane ,A.K. and Giri ,S.G .Neem the Wonder Herb A Short Review. International Journal of Trend in Scientific Research and Development.Vol. 3 , Issue: 3 . 2019.p.962-969.
10. Govindachari, T. R. Suresh, G. Gopalakrishnan,G. Banumathy, B.and Masilamani,S. (1998). Identification of antifungal compounds from the seed oil of Azadirachtaindica. Phytoparasitica.26(2),109–116.
11. Hanif S, Naz S and Iqbal S. 2013. Antifungal activity of *Azadirachta indica* against Alternaria solani. Journal of Life Sciences and Technologies, 1(1): 89-93.
12. Mahmoud, D.A. Hassanein, N.M. Yousuf, K.A. and Abouzeid, M.A. (2011). Antifungal activity of different neem leaf extracts and the nimonol against some important human pathogens. Braz. J. Microbiol. 42(3).
13. Natarajan,V. Venugopal,P.V. and Menon,T. (2003). Effect of Azadirachtaindica (Neem) on the growth pattern of dermatophytes. Indian Journal of Medical Microbiology. 21(2), 98–101.

14. Neycee MA, Nematzadeh GHA, Dehestani A and Alavi M. Evaluation of antibacterial effects of chinaberry (Melia azedarach) against gram-positive and gram-negative bacteria. Int. J. Agri. Crop Sci. 2012; 4(11): 709-712.
15. Passosa, M.S. Juniorb,A.R.C. Boenoa,S.I. Virgensa,L.L.G.D. Calixtoc,S.D. Venturac,T.L.B. Lassounskaiac, E. Braz-Filhoa,R. Vieiraa, I.J.C. (2019) . Terpenoids isolatedfrom Azadirachta indica roots and biological activities. Brazilian J. Of Pharmacognosy,29,p 40–45.
16. Patil,P.R. Patil,S.P. Mane, A.and Verma, S. (2013). Antidiabetic activity of alcoholic extract of Neem (Azadirachtaindica) root bark. National Journal of Physiology, Pharmacy and Pharmacology. 3 (2), 142–146.
17. Rai N, Grover A and Bhandari BS. Antimicrobial Activity of Medicinal plants-Azadirachta indica A. Juss, Allium cepa L. and Aloe vera L. Int. J. PharmTech Res. 2011; 3: 1059-1065.
18. 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.
19. Singh,N. and Sastry,M.S. (1997). Antimicrobial activity of Neem oil. Indian Journal of Pharmacology. 13, 102–106.
20. Srivastava,S.K. Akhilesh Kumar,B.A. and Pandey,A. Phytochemicals of AzadirachtaIndica Source of Active Medicinal Constituent Used for Cure of Various Diseases: A Review. Journal of Scientific Research, Vol. 64, Issue 1, 2020.
21. Szepietowski, J. C.; Schwart, R. A. (2005).Tineabarbae. Umdnj-New Jersew medical school.
22. Townsend C C and Guest E. (1985).Flora of Iraq ,Monocotyledones , excluding Gramineae .Vol.8.ministry of Agriculture & Agrarian Reform Republic of Iraq.p310 – 355
23. 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.