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In vitro investigation of antifungal impact of various plant extracts

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Abstract

Natural compounds derived from plant extracts have a considerable role in the control of fungal serious diseases. Recent approaches investigate, antifungal protective roles from natural sources and recommend their effects relative to synthetic compounds. The motive of this research is to compare between methanol, dichloromethane and water extracts of fifteen plant extracts against various endophytic fungal isolates. Antifungal activities were measured using hole - plate diffusion method. Results showed that methanol extract exhibit the most significant activity followed by dichloromethane, while, water extract exhibited the least activity. *Artemisia herba-alba* methanol extract was highest active against *Aspergillus niger* isolated from Zee seeds. In conclusion, this study recommended the use of these plant extracts as a potent antifungal agent.

Keywords: antifungal agents, plant extracts, plant pathogenic fungi

1. Introduction

Some fungi are pathogenic to plants and leads to variety of diseases. Fungi can infect any part of the plant body. The ability to isolate and identify these phytopathogens is an essential step to investigate and treat these pathogens. During different forms of microbial infection, precise investigation and using combined drugs are recently used for a successful control of infection **[1].** Despite, the presence of natural and synthetic products to eradicate fungal threats, many dangerous fungal infections are present and threaten human, animals and plants **[2].** There is a recent trend to develop a wider variety of antifungal agents that are more effective, less toxic, and cheaper from natural sources. Plants contains different molecules which could be useful, effective and affordable cost. They have compounds, that could be useful in the eradication of many diseases **[3].** The plant extracts have been investigated and characterized for application to control microbial infections **[4].** Many plants from different groups used in traditional medicine are available in rural regions and less expensive than other forms of drugs **[5].**

During primary steps to explore antifungals, it seems reasonable to assume that, many forms with different chemical formulas were tested to have advantages rather than regular used forms of drugs. Various extracts of traditional plant were also being examined. In the new trends, testing extracts of plants using various solvents from same plant to get innovate results. Researchers looking for plants that have well developed parts that could have different groups of compounds especially secondary metabolites to be beneficial for applications **[6]**. Antifungal agents are very common between plants with well-developed structures, some research groups have been evaluated activity of these plant extracts versus pathogenic fungi. **[7].** Plants have been reported to have important origins of different beneficial compounds and a vast yield of extra-ordinary components which have been separated and used in various uses including traditional medicine which could be used by about 80% of the world's populations **[8]**.

Plant extracts exhibit antifungal roles versus large classes of fungi **[9-13]**. Several reports tested the effect of different plant extracts against the growth of fungi, e.g: *Cymbopogon proximus* against the toxigenic fungi *Aspergillus flavus* **[14]**; *Allium sativum*, and *Eugenia caryophyllus* against *Fusarium oxysporum*, and *Rhizoctonia solani* **[15]**; and *Aristea ecklonnii* against *Botrytis cinerea*, **[16].**

Plants extracts have been recognized since time immemorial. They are still used in cure, feeding and cosmetics **[17]**. Among these plant species, *Teucrium polium, Cymbopogon proximus, Artemisia herba-alba, Moringa oleifera, Punica granatum, Ecballium elaterium, Sinapis alba, Humulus lupulus, Arctium tomentosum, Salvia officinalis L., Lavendula pinnata L., Cleome droserifolia, Acacia nilotica, Cassia angustifolia* and *Solenostemna arghel* were described as broad-spectrum antimicrobial agents **[18, 19].** Therapeutic effect of these plants can generally be attributed to their volatile fractions rather than their extracts **[17].**

In spite of much of plant species have been examined for antifungal properties, large groups have not been characterized in a proper way **[20]**. The objective of the present study is to assess antifungal action of various plant extracts against various plant fungal pathogens.

2. Materials and Methods:

Preparation of samples and isolation of plant pathogenic fungi:

Plant samples from pear, wheat grains, zee seeds, apple fruits, beans, peaches, rice, lemon, were collected (in summer and autumn) between March-November, 2019.The plant samples were collected in a bags, stored in proper place and used for isolation of microorganisms within 3 days. Then, rinsing the plant with NaOCl for 2 minute, washing with sterile distilled water for several times, dried using sterile filter papers and cut into 1 cm pieces. Three pieces per

each plant tissue were placed over the surface of three isolation media: Malt extract agar, Potato dextrose agar and Tryptic Soy Agar **[21]**.

Morphological, examination and identification studies of fungal isolates:

The morphological features of all plant fungal isolates were investigated and subjected to the direct microscopic examination at **The Regional Center for Mycology and Biotechnology (RCMB)**, **Al- Azhar University** by using Atlas of clinical fungi **[22]** and *Penicillium* species were identified by using A laboratory guide to common *Penicillium* species **[23].** Using **Image analysis system** [soft imaging system GmbH software at **(RCMB)** was used for examining the morphological features of fungal isolates. The cultures were tested by using light microscopy, after 5-7 days' incubation.

Plants:

Fifteen plants from a were collected as following: *Teucrium polium, Cymbopogon proximus, Artemisia herba-alba, Moringa oleifera, Punica granatum, Ecballium elaterium, Sinapis alba, Humulus lupulus, Arctium tomentosum, Salvia officinalis L., Lavendula pinnata L., Cleome droserifolia, Acacia nilotica, Cassia angustifolia* and *Solenostemna arghel*

Preparation of plant extracts:

The plants were thoroughly washed in running water and sterile distilled H_2O, dry, then ground by blender and stored in clean container. Methanol, dichloromethane and water were used as separated extraction solvents. 500 ml of each solvent are added to 60 gm of powdery plants and mixed for 30 min by a homogenizer, then stand for 2 hours and filtered by Whatman filter paper to remove the residual materials and were used as 100% pure extracts. Mixtures were then centrifuged at 7000 rpm for 15 min to collect extracts. Solvents were left to evaporate using a rotary evaporator. Complete the total extracts of used plants to 5 ml of each solvent. **[24-26]**.

Determination of antifungal potentialities of the plants:

To perform this experiment; The diameter of inhibition zones using hole - plate diffusion method; 0.5 cm diameter holes were cut in the agar using cork borer in sabouraud dextrose agar sterile plates, which had previously been seeded with the test fungal strain by using sterile cotton swabs; the swabs were spread over the surface of the medium. The holes were filled by 200 µl of each concentrated plant extract filtrate, plates were left at 4 °C for two hours for penetration, and then incubated for and 5-7 days. After the end of the incubation period, the inhibition zones were measured **[27]**.

The inhibition zones were assessed at two points along the diameter of the plate and the mean of these two measures were calculated as the mean diameter of the colony. The inhibition zone in control sets was compared with that of various treatments **[28]**.

3. Results:

Plants & Identification of the fungal isolates:

Various plants used in this study were seen in table (1), Furthermore, different plant fungal isolates were identified at (RCMB) Al-Azhar University. The most fungal isolates have been affected with different plant extracts were *Aspergillus niger, Penicillium citrinium* (B) and *Aspergillus fumigatus* as shown in (**Fig. 1).**

Table (1) Different Plant species, their families, and their uses**.**

No.	Plants	Family	Uses
1	*Teucrium polium*	*Lamiaceae*	Severe abdominal pain, reduce heat. Treatment of Diabetes
2	*Cymbopogon proximus*	*Poaceae*	To clear the urinary tract, to treat wound compresses. Arthritis and rheumatism and Fever.
3	*Artemisia herba-alba*	*Asteraceae*	antiseptic, antispasmodic and the treatment of diabetes mellitus
4	*Moringa oleifera*	*Moringaceae*	Antimicrobial-Anti-inflammatory, Strengthens the immune system, Protects liver and kidney Rebuilds the weak bones, It treats patients with diabetes and high blood pressure patients. Milk dispenser
5	*Punica granatum*	*Lythraceae*	Anti-Inflammatory, anticancer, Anti-bacterial Treatment of osteoporosis, Diabetes , Blood Fat -Bleeding gums and Acidity of the stomach
6	*Ecballium elaterium*	*Cucurbitaceae*	Jaundice - Hepatitis, Epilepsy, Sinus Abdominal ascites - Reduce Cholesterol.
7	*Sinapis alba*	*Brassicacea*	Reduce the risk of cancer, especially stomach cancer. Reduces high blood pressure , arthritis,
8	*Humulus lupulus*	*Cannabaceae*	Calming the Nerves-Aperitif, Anti-Bloating-Anti-contractions, control digestion
9	*Arctium tomentosum*	*Asteraceae*	Cure diabetes patients, immune system stimulation, skin problems
10	*Salvia officinalis L.*	*Lamiaceae*	Sprains, swelling, ulcers and bleeding
11	*Lavendula pinnata L.*	*Lamiaceae*	Headache, general, calming, aromatherapy and anxiolytic
12	*Cleome droserifolia*	*Capparaceae*	Wounds and dermatitis

13	*Acacia nilotica*	*Mimosaceae*	Healing, urinary tract infections and conorrhea
14	*Cassia angustifolia*	*Fabaceae*	Constipation
15	*Solenostemna arghel*	*Aselepiadaceae*	Antispasmodic, digestive and menstruation

Table (2) Antifungal activities of different plant extracts against Aspergillus fumigatus, Aspergillus niger, *Penicillium aurantiogriseum* using different solvents. Data are expressed as mean diameter of inhibition zones (cm).

Fungal Isolates		***Aspergillus fumigatus***			***Aspergillus niger***			***Penicillium aurantiogriseu***		
Solvents us / **Collected Plants**		**Methanol**	**Dichloromethan**	**Water**	**Methanol**	**Dichloromethan**	**Water**	**Methanol**	**Dichloromethan**	**Water**
1	***Teucrium polium***	**1.3**	**-**	**-**	**1.0**	**-**	**-**	**1.8**	**-**	**-**
2	***Cymbopogon proximus***	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
3	***Artemisia herba-alba***	**2.5**	**-**	**1.5**	**6.0**	**3.0**	**3.5**	**4.0**	**-**	**2.5**
4	***Moringa oleifera***	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
5	***Punica granatum***	**2.0**	**4.0**	**1.5**	**2.0**	**-**	**-**	**3.5**	**1.2**	**-**
6	***Ecballium elaterium***	**-**	**2.0**	**-**	**-**	**3.8**	**-**	**-**	**2.0**	**-**
7	***Sinapis alba***	**-**	**2.0**	**2.5**	**-**	**5**	**2.5**	**-**	**3.2**	**2.5**
8	***Humulus lupulus***	**-**	**2.0**	**-**	**-**	**4.5**	**-**	**-**	**3.0**	**-**
9	***Arctium tomentosum***	**1.0**	**-**	**-**	**1.5**	**-**	**2.0**	**2.0**	**-**	**-**
10	***Salvia officinalis L.***	**1.2**	**-**	**-**	**1.0**	**1.8**	**1.0**	**0.8**	**1.2**	**-**
11	***Lavendula pinnata L.***	**1.5**	**-**	**-**	**1.0**	**1.2**	**1.0**	**1.0**	**2.0**	**-**
12	***Cleome droserifolia***	**1.0**	**1.0**	**-**	**-**	**0.8**	**-**	**1.0**	**2.2**	**0.8**
13	***Acacia nilotica***	**-**	**1.0**	**0.8**	**-**	**0.8**	**-**	**-**	**0.8**	**-**
14	***Cassia angustifolia***	**-**	**-**	**1.0**	**-**	**1.0**	**-**	**1.2**	**-**	**-**
15	***Solenostemna arghel***	**-**	**0.8**	**-**	**-**	**1.0**	**-**	**-**	**0.8**	**-**

Table (3) Antifungal activities of different plant extracts against *Aspergillus flavus, Epicoccum nigrum, Trichoderm pseudokoningii* using different solvents. Data are expressed as mean diameter of inhibition zones (cm).

Fungal Isolates		***Aspergillus flavus***			***Epicoccum nigrum***			***Trichoderma pseudokoningii***		
Solvents used / **Collected Plants**		**Methanol**	**Dichloromethane**	**Water**	**Methanol**	**Dichloromethane**	**Water**	**Methanol**	**Dichloromethane**	**Water**
1	***Teucrium polium***	**0.8**	**1.3**	**-**	**-**	**-**	**-**	**1.0**	**-**	**-**
2	***Cymbopogon proximus***	**-**	**-**	**-**	**-**	**-**	**-**	**0.8**	**-**	**-**
3	***Artemisia herba-alba***	**4.0**	**4.0**	**3.5**	**4.0**	**2.5**	**3.0**	**-**	**1.5**	**2.0**
4	***Moringa oleifera***	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
5	***Punica granatum***	**2.0**	**2.5**	**-**	**3.5**	**5.2**	**2.0**	**2.3**	**2.0**	**3.5**
6	***Ecballium elaterium***	**-**	**2.2**	**-**	**-**	**4.5**	**-**	**-**	**3.0**	**-**
7	***Sinapis alba***	**-**	**2.5**	**1.5**	**-**	**4.7**	**3.0**	**-**	**2.5**	**2.5**
8	***Humulus lupulus***	**-**	**2.5**	**-**	**-**	**5.3**	**-**	**-**	**2.5**	**-**
9	***Arctium tomentosum***	**-**	**-**	**-**	**-**	**-**	**1.5**	**-**	**2.0**	**-**
10	***Salvia officinalis L.***	**1.0**	**2.0**	**0.8**	**1.5**	**-**	**-**	**1.5**	**1.8**	**1.0**
11	***Lavendula pinnata L.***	**-**	**-**	**-**	**-**	**1.2**	**-**	**1.2**	**-**	**-**
12	***Cleome droserifolia***	**-**	**1.5**	**-**	**0.8**	**1.5**	**1.2**	**-**	**-**	**-**
13	***Acacia nilotica***	**-**	**1.5**	**-**	**-**	**-**	**-**	**-**	**2.0**	**-**
14	***Cassia angustifolia***	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1.2**	**-**
15	***Solenostemna arghel***	**-**	**1.0**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

Table (4) Antifungal activities of different plant extracts against *Alternaria alternata, Fusarium culmorum, Rhizoctonia solani* using different solvents. Data are expressed as mean diameter of inhibition zones (cm).

Fungal Isolates		*Alternaria alternata*			*Fusarium Culmorum*			*Rhizoctonia solani*		
Solvents used / Collected Plants		Methanol	Dichloromethane	Water	Methanol	Dichloromethane	Water	Methanol	Dichloromethane	Water
1	*Teucrium polium*	1.2	-	-	1.0	-	-	-	-	-
2	*Cymbopogon proximus*	1.2	-	-	-	-	-	-	-	-
3	*Artemisia herba-alba*	2.5	1.8	2.5	0.3	2.5	2.0	0.8	2.5	1.2
4	*Moringa oleifera*	-	4.3	2.0	-	-	-	-	2.5	2.0
5	*Punica granatum*	3.0	3.8	-	3.0	1.5	1.8	-	-	-
6	*Ecballium elaterium*	-	2.5	-	-	2.2	-	-	2.5	-
7	*Sinapis alba*	-	3.0	1.8	-	2.0	2.0	-	-	-
8	*Humulus lupulus*	-	3.0	-	-	2.8	-	-	-	-
9	*Arctium tomentosum*	-	-	-	1.8	2.0	1.8	-	-	-
10	*Salvia officinalis L.*	-	-	-	1.5	-	-	2.5	2.5	1.5
11	*Lavendula pinnata L.*	1.8	2.5	1.5	1.8	1.8	1.5	-	-	-
12	*Cleome droserifolia*	-	-	-	-	-	-	-	-	-
13	*Acacia nilotica*	1.5	2.5	-	2.5	2.0	1.8	-	1.8	-
14	*Cassia angustifolia*	-	-	-	1.8	-	-	2.0	-	-
15	*Solenostemna arghel*	-	-	-	-	2.5	-	-	1.8	-

Table (5) Antifungal activities of different plant extracts against *Fusarium oxsprium, Fusarim graminearum* and *Pencillium citrinium using different solvents.* Data are expressed as mean diameter of inhibition zones (cm).

Fungal Isolates		*Fusarium oxysporum*			*Fusarim graminearum*			*Pencillium citrinium*		
Solvents used / Collected Plants		Methanol	Dichloromethane	Water	Methanol	Dichloromethane	Water	Methanol	Dichloromethane	Water
1	*Teucrium polium*	-	-	-	0.8	1.0	-	-	-	-
2	*Cymbopogon proximus*	-	-	-	-	1.5	-	-	-	-
3	*Artemisia herba-alba*	2.2	3.0	3.0	2.5	2.6	2.0	2.0	2.0	2.5
4	*Moringa oleifera*	-	-	2.5	-	-	1.5	-	-	-
5	*Punica granatum*	2.3	-	1.8	3.0	-	-	2.5	2.3	-
6	*Ecballium elaterium*	-	-	-	-	-	-	-	4.8	-
7	*Sinapis alba*	-	-	2.0	-	-	2.3	-	4.5	2.5
8	*Humulus lupulus*	-		-	-	2.5	-	-	4.8	-
9	*Arctium tomentosium*	3.0	-	3.0	1.0	2.5	3.0	-	-	-
10	*Salvia officinalis*	-	-	-	-	-	-	2.5	2.5	1.8
11	*Centaurea cyanus*	-	-	-	-	-	-	-	1.8	-
12	*Cleome droserifolia*	1.5	1.8	-	1.8	1.0	-	-	-	-
13	*Acacia nilotica*	2.0	1.8	-	-	1.8	-	1.8	1.8	-
14	*Cassia angustifolia*	-	-	-	-	-	-	-	-	-
15	*Solenostemna arghel*	2.5	2.8	2.8	0.8	2.0	1.5	1.5	0.8	-

Fig. (1) Light microscopy micrograph of *Aspergillus niger* (A), *Pecillium citrinium* (B) and *Aspergillus fumigatus* (C), Magnification = 40X.

Screening test of antifungal activity:

This study was conducted to detect the antifungal activities of different botanical extracts using different solvent systems (methanol, dichloromethane and water) against various plant fungal pathogens isolated from various crops. The crude extracts had antifungal actions versus the majority of the fungal isolates, and the of inhibition potentiality differ with the fungi according to the type of plant extract.

Among the solvents used for extraction, methanol extracts revealed the most inhibitory effect against mycelial growth of the plant fungal isolates, especially, *Artemisia herba-alba* against *Aspergillus niger* isolated from the Zee seeds.

The methanol extract results were as the follows: *Artemisia herba-alba* exhibited good results against *Aspergillus niger* (6.0 cm), *Epicoccum nigrum* (4.0 cm), *Fusarim Culmorum* (3.0 cm), *Fusarim graminearum* (2.5 cm)

The dichloromethane extract results were as the follows: *Humulus lupulus* exhibited good results against *Aspergillus niger* (4.5 cm), *Epicoccum nigrum* (5.3 cm), *Fusarim Culmorum* (2.8 cm), *Fusarim graminearum* (2.5 cm).

However, water extracts had the least effect against most of the tested fungal isolates.

4. Discussion

Plants commonly yield many metabolites which have many biomedical applications. Plant molecules have been widely used in traditional medicine **[29, 30].** The last periods witnessed an emerging in the testing on plants as a repertoire of disease control **[31, 32].** More natural antimicrobial agents lead the investigators to examine the success of compounds extracted from plants **[33.**

Plant parts are subjected to infection by several micorganisms, mainly fungi named endophytes **[34, 35].** The antimicrobial action of ethanol, chloroform, and water extracts of *Centella asiatica* has been studied against different fungi such as *A. niger* and *C. albicans* with inhibitory effect against all the tested microorganisms **[36].**

The crude extracts of *Dodonaea viscosa* have promising antifungal role on fungal plant pathogens, *Aspergillus niger*, *Aspergillus flavus.* Chloroform extract had maximum inhibition activity of 50-90.91% as compared to methanol, ethyl acetate and aqueous extracts have active inhibition action against tested plant pathogens **[37],** which agrees with this study; methanol extracts recorded the most inhibitory effect against the most of tested fungal isolates.

Ali, et al., **[38]** tested nematicidal activities of *Artemisia. herba-alba* Furthermore, Ahmed-Laloui, *et al.,* **[39]** examined methanolic extract of *A. herba-alba* (AH), *A. campestris* subsp**.** *glutinosa***,** and *A. judaica subsp sahariensis* reported that artemisinin and antioxidant activities of three wild Artemisia species of Algeria.

The current study agrees with Pavel and Alcu **[40]** who reported that the rise of fungal resistance to regular treatments and the regimen cost, and its fungistatic activity. *Artemisia herba-alba* extract tested its role to be used as a topical antifungal material against fungi. Recently, El-Shatoury *et al.,* **[41]** reported that biodiversity of entophytic actinobacteria to inhabiting *Artemisia herba-alba.*

Adegoke, *et al*., **[42]** reported that the antimicrobial role of the aqueous, methanol and chloroform leaf extracts of *Cissus multistriata*. Cosoveanu and Cabrera **[43]** reported various roles characterized by fungal endophytes of *Artemisia* spp. Aziz *et al.,* **[44]** reported that *Artemisia herba-alba* could be used in preparation of nanoinsecticides versus microbial pathogens. In accordance with current results, the methanol extract of *Artemisia herba-alba* revealed promising antifungal activity compared with dichloromethane extract, with *A. niger*

5. Conclusion=

Thus, this study revealed that methanol extract of the screened plants would be beneficial in treating plant pathogens especially *Artemisia herba-alba* against *Aspergillus niger* isolated from the zee seeds. More tests are required to isolate, characterize and elucidate the structure of the bioactive agents of these plant organs for antifungal drugs formulation.

References:

[1] M. Yosri, B.H. Amin, N.N. Abed, Elithy, A.S. Kareem S.M., N.M. Sidkey. Identification of

Novel Bioactive Compound Derived from *Rheum officinalis* against *Campylobacter jejuni* NCTC11168. ScientificWorldJournal.:3591276, 2020

[2] A.Y. Maksimov, S.Y. Balandina, P.A. Topanov, I.V. Mashevskaya, S. Chaudhary Organic Antifungal Drugs and Targets of Their Action. Curr Top Med Chem.; Vol.21(8), pp.705-736, 2021.

[3] L. Luo, J. Yang, C. Wang, J. Wu, Y. Li, X. Zhang, H. Li, H. Zhang, Y. Zhou, A. Lu, S. Chen Natural products for infectious microbes and diseases: an overview of sources, compounds, and chemical diversities. Sci China Life Sci.:1–23, 2021.

[4] R.M.O.F. Sousa, A.C. Cunha, M. Fernandes-Ferreira. The potential of Apiaceae species as sources of singular phytochemicals and plant-based pesticides. Phytochemistry.;187:112714, 2021.

[5] A. Logiel, E. Jørs, P. Akugizibwe, P. Ahnfeldt-Mollerup Prevalence and socio-economic factors affecting the use of traditional medicine among adults of Katikekile Subcounty, Moroto District, Uganda. Afr Health Sci.; Vol. 21(3), pp.1410-1417, 2021

[6] D. Krüzselyi, J. Bakonyi, P.G. Ott, A. Darcsi, P. Csontos, G.E. Morlock, Á.M. Móricz Goldenrod Root Compounds Active against Crop Pathogenic Fungi. J Agric Food Chem.; Vol. 69(43), pp.12686-12694, 2021.

[7] L. Parikh, B.O. Agindotan, M.E. Burrows Antifungal Activity of Plant-Derived Essential Oils on Pathogens of Pulse Crops. Plant Dis. Vol. 105(6), pp.1692-1701, 2021.

[8] H. Cimen, M. Touray, S.H. Gulsen, O. Erincik, S.L. Wenski, H.B. Bode, D. Shapiro-Ilan, S. Hazir Antifungal activity of different *Xenorhabdus and Photorhabdus* species against various fungal phytopathogens and identification of the antifungal compounds from *X. szentirmaii*. Appl Microbiol Biotechnol.; Vol.105(13), pp.5517-5528, 2021.

[9] N. Kurita, M. Makoto, R. Kurane and Y. Takahara Antifungal activity of components of essential oils. Agric. Biol. Chem. 45: 945-952, 1981.

[10] M. Grane and S. Ahmed Handbook of Plants with Pest Control Properties, John Wiley and Sons, New York, USA. p. 431, 1988.

[11] C. L. Wilson, J. M. Solar, A. E. l. Ghaouth and M. E. Wisniewski Rapid evaluation of plant extracts and essentials oils for antifungal activity against Botrytis cinerea. Plant Dis. Vol. 81, pp. 204-210, 1997.

[12] M. M. Cowan. Plant products as antimicrobial agents. Clin. Microbiol. Rev. Vol.12., pp. 564-582, 1999

[13] A. Hernández-Ceja, P.D. Loeza-Lara, F.J. Espinosa-García, Y.M. García-Rodríguez, Medina- J.R. Medrano, G.F. Gutiérrez-Hernández, L.F. Ceja-Torres *In Vitro* Antifungal Activity of Plant Extracts on Pathogenic Fungi of Blueberry (*Vaccinium* sp.). Plants (Basel).; Vol.10(5), pp. 852, 2021.

[14] E. M. El-Assiuty, F. M. Bekheet, Z. M. Fahmy, A. M. Ismael and T. S. M. El-Alfy 2006. Potentiality of some isolated compounds from Halfa Barr (*Cymbopogon proximus* Stapf.) against the toxigenic fungi *Fusarium verticillioides* and *Aspergillus flavus*. Egypt. J. Phytopathol. Vol. 34, pp. 75-84, 2006.

[15] A. A. Aba AlKhail. Antifungal activity of some extracts against some plant pathogenic Fungi. Pak. J. Biol. Sci. Vol. (8), pp. 413-417, 2005

[16] J. Pretorius, P. Zietsman and D. Eksteen Fungi toxic properties of selected South Africa plant species against plant pathogens of economic importance in agriculture. Annals Appl. Biol. Vol. 141, pp. 117-124, 2002

[17] D.G. Nadeeshani Dilhara Gamage, R.M. Dharmadasa, D. Chandana Abeysinghe, R.G. Saman Wijesekara, G.A. Prathapasinghe, T. Someya Global Perspective of Plant-Based Cosmetic Industry and Possible Contribution of Sri Lanka to the Development of Herbal Cosmetics. Evid Based Complement Alternat Med.; 2022:9940548, 2022

[18] M.S. Mueller and E. Mechler Medicinal Plants in Tropical Countries: Traditional Use - Experience – Facts., Stutgart, Germany, 2005

[19] N.C.C. Silva and A. Fernandes-Junior. Biological properties of medicinal plants: a review of their antimicrobial activity. Journal of venomous animals and toxins including tropical diseases 16, 402-413, 2010.

[20] J. Iantas, D.C. Savi, R.D.S. Schibelbein, S.A. Noriler, B.M. Assad, G. Dilarri, H. Ferreira, J. Rohr, J.S. Thorson, K.A. Shaaban, C. Glienke Endophytes of Brazilian Medicinal Plants with Activity Against Phytopathogens. Front Microbiol.;12:714750, 2021.

[21] M. H. Naziha, M. E. Mervat and M. A. Noha. Entophytic fungi of some medicinal plants in Egypt. Egypt. Acad. J. Biolog. Sci., Vol.8(1), pp. 65-78, 2016

[22] G.S. De Hoog, J.G. Guarro and M.J. Figueras Atlas of clinical fungi 2nd edition, 2000.

[23] I. J Pitt. A laboratory guide to common *Penicillium* species. Book,1991.

[24] L.A.J. Shittul, M.A. Bankole, T. Ahmed, M.N. Bankole, R.K. Shittul, C.L. Saalu, Ashiru. 2007. Antibacterial and Antifungal Activities of Essential Oils of Crude Extracts of *Sesame Radiatum* against Some Common Pathogenic Micro-Organisms Biol. Sci**.,** Vol. 13, pp. 1023-1029**,** 2007.

[25] R. A. Sharma, P. Chandrawat, S. Sharma, D. SHARMA, B. Sharma and D. Singh Ethnomedicinal, pharmacological properties and chemistry of some medicinal plants of Boraginaceae in India. Vol.5 (3)**,** pp. 441 – 444, 2010.

[26] M. Zaker, and H. Mosallanejad. Antifungal activity of some plant extracts on *Alternaria*

alternata, the causal agent of alternaria leaf spot of potato. Pak J Biol Sci. Vol. 21, pp. 1023-9, 2010.

[27] E.E. Thomloudi, P.C. Tsalgatidou, E. Baira, K. Papadimitriou, A. Venieraki, P. Katinakis Genomic and Metabolomic Insights into Secondary Metabolites of the Novel *Bacillus halotolerans* Hil4, an Endophyte with Promising Antagonistic Activity against Gray Mold and Plant Growth Promoting Potential. Microorganisms.;9 (12):2508, 2021.

[28] M. Anandaraj and N.K. Leela, Toxic effect of some plant extracts on *Phytophthora capsici*, the foot root pathogen of black pepper. Indian Pathology, Vol. 49(2): pp.181-184, 1996.

[29] A.M. Metwaly, M.M. Ghoneim, I.H. Eissa, I.A. Elsehemy, A.E. Mostafa, W.M. Hegazy, M.M. Afifi, D. Dou. Traditional ancient Egyptian medicine: A review. Saudi J Biol Sci.; Vol. 28(10), pp. 5823-5832, 2021.

[30] Z. Wang, L. Wang, F. Xiao, Q. Chen, L. Lu, J. A. Hong Traditional Chinese Medicine Traceability System Based on Lightweight Blockchain. J Med Internet Res.;23(6): e25946, 2021.

[31] I. H. Lee, H. Han, Y. H. Koh, I. S Kim, S.W. Lee, and D. Shim, Comparative transcriptome analysis of *Pinus densiflora* following inoculation with pathogenic (*Bursaphelenchus xylophilus*) or non-pathogenic nematodes (*B. thailandae*). *Sci. Rep*. Vol. 9, pp.1–11, 2019.

[32] K.E. Adewole, A.F. Attah, J.O. Adebayo *Morinda lucida Benth* (Rubiaceae): A review of its ethnomedicine, phytochemistry and pharmacology. J Ethnopharmacol.;276:114055, 2021.

[33] A. Przybylska-Balcerek, T. Szablewski, L. Szwajkowska-Michałek, D. Świerk, R. Cegielska- Radziejewska, Z. Krejpcio, E. Suchowilska, Ł. Tomczyk, K. Stuper-Szablewska *Sambucus Nigra* Extracts-Natural Antioxidants and Antimicrobial Compounds. Molecules.; Vol. 26(10), pp.2910, 2021.

[34] G. Yadav, M. Meena 2021. Bioprospecting of endophytes in medicinal plants of Thar Desert: An attractive resource for biopharmaceuticals. Biotechnol Rep (Amst).;30: e00629, 2021

[35] A. Mukherjee, S. Bhowmick, S. Yadav, M.M. Rashid, G.K. Chouhan, J.K. Vaishya, J.P.Verma Re-vitalizing of endophytic microbes for soil health management and plant protection. 3 Biotech. Vol.11(9), pp.399, 2021

[36] B.K. Dash, H.M. Faruquee, S.K. Biswas, M.K. Alam, S.M. Sisir and U.K. Prodhan Antibacterial and Antifungal Activities of Several Extracts of *Centella asiatica* L. against Some Human Pathogenic Microbes Life Sciences and Medicine Research, Volume: LSMR-35, 2011

[37] A.J. Pirzada, W. Shaikh, K. Usmanghani and E. Mohiuddin. Antifungal activity of *Dodonaea viscose* extract on pathogenic fungi isolated from superficial skin infection. Pak. J. Pharm. Sci., Vol.23, No.3, July, pp.337-340, 2010.

[38] R. Ali, M. Rooman, S. Mussarat, S. Norin, S. Ali, M. Adnan, S.N. Khan. A Systematic Review on Comparative Analysis, Toxicology, and Pharmacology of Medicinal Plants Against *Haemonchus contortus*. Front Pharmacol.; Vol.12, pp. 644027, 2021

[39] H. Ahmed-Laloui, H. Zaak, A. Rahmani, I. Kashi, S. Chemat, M.D. Miara, N. Cherb, M. Derdour Assessment of artemisinin and antioxidant activities of three wild Artemisia species of Algeria. Nat Prod Res. Vol (1)-pp.9, 2022

[40] M. Pavel and F. Alecu. Antifungal activity of *Thymus serpyllum* essential oil against *Candida albicans* and Candida non-albicans clinical isolates.18th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID)19.04.2008 - 22.04, 2008.

[41] S.A. El-Shatoury, F.M. Mahmoud, and W.M. El-Kazzaz. Bioactivities of endophytic actinobacteria inhabiting Artemisia herba-alba emphasizing differences from free-living strains. Folia Microbiol Vol. 67, pp. 81–89 (2022).

[42] S. A. Adegoke, O. M. Opata and J. E. Olajide. Antimicrobial activity of the aqueous, methanol and chloroform leaf extracts of *Cissus multistriata*. African Journal of Biotechnology Vol. 9 (8), pp. 1168-1172, 2010.

[43] A. Cosoveanu, R. Cabrera. Endophytic Fungi in Species of *Artemisia*. J Fungi (Basel).; Vol.4(2), pp.53.2018

[44] A.T. Aziz, M.A. Alshehri, C. Panneerselvam, K. Murugan, S. Trivedi, J.A. Mahyoub, M.M. Hassan, F. Maggi, S. Sut, S. Dall'Acqua, A. Canale, G. Benelli. The desert wormwood (*Artemisia herba-alba*) - From Arabian folk medicine to a source of green and effective nanoinsecticides against mosquito vectors. J Photochem Photobiol B.; Vol.180, pp.225-234, 2018