Indian Journal of Natural Products and Resources
Vol. 12(1), March 2021, pp. 68-73

Evidence-based antifungal potential of some traditional medicinal plants, from the Bechar region (Southwest Algeria)

Naima Fatehi[1*], Houcine Benmehdi[2], Hocine Allali[1], Nafissa Sahel[3] and Nawal Oulednecir[3]

[1]Department of Chemistry, Faculty of Sciences, Abou Bekr Belkaïd University, P.O. Box 119, Tlemcen 13000, Algeria

[2]Laboratory of Chemistry and Environmental Sciences, Tahri Mohamed University, P.O. Box 417, Bechar 08000, Algeria

[3] Department of Biology, Faculty of Natural and Life Science, TAHRI Mohamed University, P.O. Box 417, Bechar 08000, Algeria

Received 24 January 2019; Revised 25 December 2020

The development of more effective and less toxic antifungal agents is required for the treatment of several ailments. In this research, the antifungal activity of the crude aqueous and hydromethanolic extracts of nine medicinal plant, frequently used in the local traditional medicine in the Bechar region (southwest Algeria), was evaluated, using the radial growth method on solid medium, against seven fungal pathogens isolated from local wheat, toasted and green Coffee beans. The results of the antifungal potency revealed that the hydromethanolic extract of *Rhus tripartita* and the aqueous extract of *Traganum nudatum* were the best to suppress the growth of *Aspergillus nidulans* (77 and 66% respectively), followed by the hydromethanolic extract of *Haloxylon scoparia* red (63%). Whereas, the aqueous extract of *Traganum nudatum* was found to be the best to inhibit the growth of *Penicillium oxalicum* (60%) compared to the other extracts. Lesser activities were recorded for the hydromethanolic extract of *Andropogon nardus* (0%) and the aqueous extract of *Globularia vulgaris* (1%) against *Aspergillus nidulans* and *Aspergillus ochracus* respectively. The selected plant extracts can serve as potential sources of new antifungal agents that may be of great use for the development of pharmaceutics against various diseases.

Keywords: Antifungal activity, Bechar (Southwest Algeria), Fungal identification, Fungal isolation, Medicinal plants.

IPC code; Int. cl. (2015.01)-A61K 36/00, A61P 31/00

Introduction

Despite the advancements made in modern medicine, many populated groups in developing countries still depend on traditional medicine for preventing and treating various ailments. This is due to cultural beliefs, low cost, and effectiveness[1]. Natural products derived from plants have importance as they provide an amazing source of new drugs and new chemical entities for drug development[2]**.** Using these kinds of products as potential antifungal agents are promising, as they have been proven to be able to inhibit the synthesis of the fungal cell wall, sphingolipids, and protein[3,4].

The south of Algeria is richly endowed with a wealth of medicinal plants, but very few studies have looked into exploiting their constituents for pharmacological potency and thus necessitate studies in this regard.

As part of our continuing work to investigate and biologically evaluate the folkloric medicinal plants from the Bechar region (Southwest Algeria), the present study aimed to check the efficacy of the crude aqueous and hydromethanolic extracts of nine medicinal plant, frequently used in the local traditional medicine, against seven fungal pathogens isolated from local wheat, toasted and green coffee beans.

Materials and Methods

Plants extraction preparation

Samples of nine medicinal plants were collected during March 2015, from different districts of Bechar province (Southwest Algeria). The exsiccates of the collected plants were verified and identified by the Department of Biology, Faculty of Naturel and Life Science, Mohamed Tahri University, Bechar, Algeria; and a local herbalist Mr Laid Hemzaoui, a specialist in the local traditional alternative medicine, Bechar, Algeria. The samples were deposited in the Herbaria of the Biology Laboratory (Mohamed Tahri University, Bechar, Algeria).

The fresh plant samples were cut into pieces, ambient dried in shade, then ground. A total of 50 g of each plant material was exhaustively refluxed with distilled water and 80% water-methanol mixture separately for 3 hours. The extracts were filtered out,

*Correspondent author
Email: fthnaima@gmail.com

evaporated, and dried under reduced pressure using a rotatory vacuum evaporator

Pathogenic fungi associated with wheat and coffee beans

Collection of test samples

Wheat and coffee beans are subjected to various operations of contamination by microorganisms during growth (while seeds are on the trees), after harvesting (when seeds are de-hulled, washed and stored) and during storing. Three samples were investigated in this study: local wheat, roasted and green coffee beans, collected randomly from local markets in Bechar province in February 2016 and the experiments were carried out for three months (February, March, and April) in 2016 at Biology Laboratory, Tahri Mohammed University, Bechar, Algeria. The samples were homogenized and then divided into three equal sub-samples and labelled.

Isolation of fungal strains

The dilution method (or indirect method) was employed for the isolation of fungal strains from local wheat, roasted and green coffee beans[5]; suspensions (5 g of each sample + 45 mL of physiological water + a few drops of Tween 80) was diluted up to 10^{-5}. The aliquots were cultured for fungus on Potatoes Dextrose Agar acidified (PDAa) and Dichloran Rose Bengal Chloramphenicol (DRBC) media. For primary isolation, Rose Bengal (30 mg/L) was also added to the medium[6]. Three plates from each sample were incubated for 5 to 7 days at 25±2 °C and each morphologically unique fungal colony was sub-cultured and purified using standard techniques.

Identification and characterization of fungal strains

The fungal species were identified and characterized based on their morphological characters (colony growth (length and width), presence or absence of aerial mycelium, colony colour, presence of wrinkles and furrows, pigment production, etc.) and microscopic analysis by using taxonomic guides and standard procedures[7-11]. The confirmation of genera was realized by the microculture method described by Barnett & Hunter[10], whereas, the confirmation of species was carried out by the Single Spore method described by Pitt[12] and Ramirez[13], using three cultures media: Czapek Dextrose Agar (CDA), Czapek Yeast Agar (CYA) and Malt Extract Agar (MEA).

***In vitro* antifungal assay**

Investigated fungal strains

Out of the twenty-isolated fungal strains, seven pathogenic species (*Aspergillus flavus, A. nidulans A. niger, A. ochracus, Penicillium chrysogenum, P. digitatum,* and *P. oxalicum*) were used to evaluate the antifungal activity of the selected medicinal plants. All fungi were stored on the sabouraud dextrose agar slants in the refrigerator at 4 °C prior to use.

***In vitro* antifungal activity evaluation**

The antifungal activity was determined by using the radial growth method on solid medium[14-16]. Exactly 1 mL of 100 mg/mL (w/v) of each plant extract was introduced in tubes containing 19 mL of sterile acidified potato dextrose agar (PDAa). After agitation, the mixture was poured into different Petri dishes and allowed to solidify. The mycelial felt (0.5 cm diameter) of each pathogenic fungus was transferred aseptically to the centre of Petri dishes. A control experiment was performed without the extracts. Petri plates were incubated for 7 days at 25±2°C. The inhibition percentage of mycelial growth of each extract was calculated using the following formula:

$$(PI = ((D_T - D)/D_T) \times 100)$$

where D_T is the diameter of mycelial growth in control and D is the diameter of mycelial growth in treatment[17,18].

Statistical analysis

Three samples of each plant extract were independently analyzed, and all of the determinations were carried out in triplicate. The results are expressed as means±standard deviations.

Results

Many investigations have been carried out to discover plant products that inhibit the fungi like *Aspergillus* sp. and *Penicillium* sp[1,19]. These two species can produce highly toxic mycotoxins (Aflatoxins and ochratoxins) that cause common diseases in humans which are difficult to control effectively[20], hence, plant products that inhibit their growth without harming the host represent potential therapeutic agent[1]. In the present study, nine different medicinal plants belonging to different families (Table 1), used traditionally by the native people of the Bechar region (Southwest Algeria). These were collected from different places in Bechar province and extracted with water and Methanol (80%, v/v). Then, their antifungal activities were detected using the radial growth method on a solid medium against seven pathogenic fungal strains, isolated from local wheat, toasted and green coffee beans.

Detection, isolation, and identification of fungal strains

Wheat and coffee seeds could be attacked by several economically important post-harvest fungal

Table 1 — List of selected traditional medicinal plants

Scientific name (Voucher specimen no.)	Family	Local name
Andropogon nardus L. (BCH/BIOLAB/2015/49)	Poaceae	Ledkhir
Andropogon schoenanthus L. (BCH/BIOLAB/2015/50)	Poaceae	Lemmad
Globularia vulgaris L. (BCH/BIOLAB/2015/68)	Globulariaceae	Tasselgha
Hammada scoparia Pomel. (BCH/BIOLAB/2015/72)	Chenopodiaceae	Remth lahmer
Hammada scoparia Pomel. (BCH/BIOLAB/2015/73)	Chenopodiaceae	Remth lakhder
Periploca laevigata Ait. (BCH/BIOLAB/2015/112)	Asclepiadaceae	Lhellab
Rhus tripartita R. Sch. (BCH/BIOLAB/2015/132)	Anacardiaceae	Djedari
Tamarix gallica L. (BCH/BIOLAB/2015/146)	Tamaricaceae	Fersig
Traganum nudatum Del. (BCH/BIOLAB/2015/151)	Chenopodiaceae	Damran

pathogens under storage condition[21]. In this study, more than 50 fungal isolates were obtained from the analyses of three investigated samples (local wheat, toasted and green coffee beans) through the dilution method. All fungal isolates were obtained in pure cultures by using standard techniques (Fig. 1). The photomicrographs, presented in Fig. 2, were taken to help in the identification of the fungal isolates.

The cultural characteristics and the sporulating structures of these isolates are presented in Fig. 3[ref 2-24].

***In vitro* antifungal assay**

Out of the twenty isolated fungi, seven pathogenic strains (*A. flavus, A. nidulans A. niger, A. ochracus, P. chrysogenum, P. digitatum,* and *P. oxalicum*) were used to evaluate the antifungal activity of the selected medicinal plants, via calculating the inhibition percentage of mycelial growth of each extract (Table 2). The results of the antifungal potency revealed that the hydromethanolic extract of *R. tripartita* and the aqueous extract of *T. nudatum* were the best to suppress the growth of *Aspergillus nidulans* (77 and 66% respectively) compared to the control, followed by the hydromethanolic extract of *H. scoparia* red (63%). The hydromethanolic extracts of *G. vulgaris*, *T. nudatum* as well as the aqueous extract of *H. scoparia* green also inhibited *A. nidulans* growth (60% each). The aqueous extracts of *A. nardus, G. vulgaris* and *R. tripartita* suppressed the growth of *P. digitatum* (49, 47, and 43% respectively), whereas the aqueous extract of *T. nudatum* was found to be the best to inhibit the growth of *P. oxalicum* (60%) compared to the other extracts. Moderate activity was recorded against *A. niger, A. ochracus, P. chrysogenum*, and *P. soxalicum* by remaining plant extracts. Lesser activities were recorded for the hydromethanolic extract of *A. nardus* (0%) and the aqueous extract of *G. vulgaris* (1%) against *A. nidulans* and *A. ochracus* respectively, followed by the low activity recorded by the hydromethanolic extracts of *A. schoenanthus* and

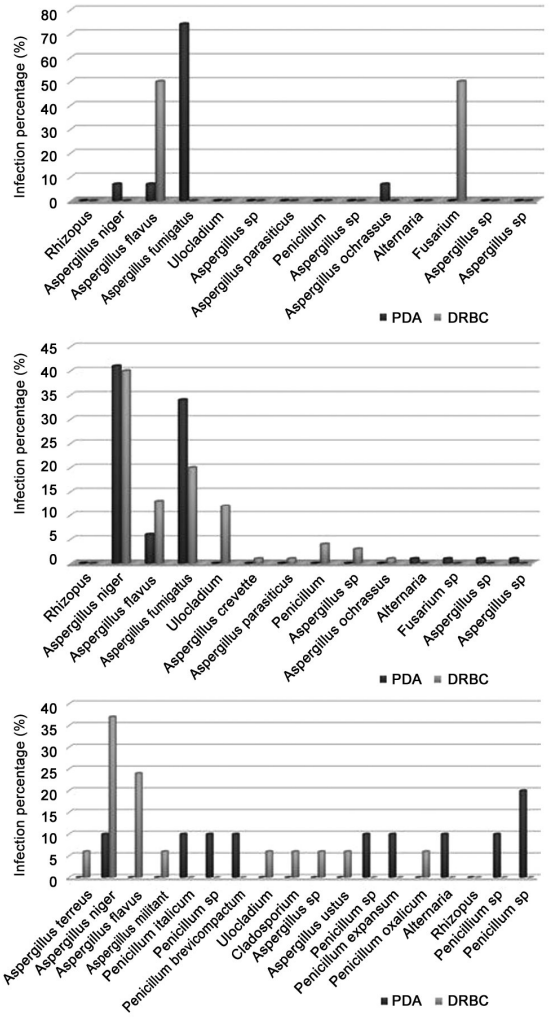

Fig. 1 — Infection percentage of tested samples detected by dilution method.

T. nudatum against *A. niger* (2% each). The maximum mycelial growth inhibition was recorded against *A. nidulans*, which was the most susceptible fungus for all the tested extracts (except for the hydromethanolic extract of *A. nardus*) (Fig. 4).

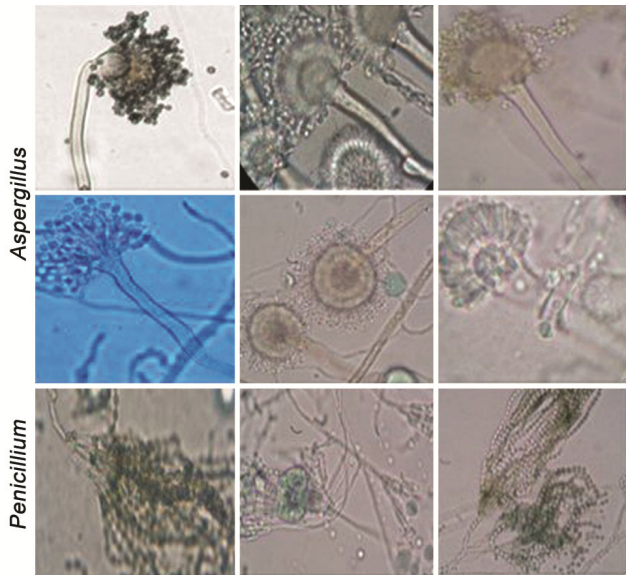

Fig. 2 — Photomicrographs of some fungal strains: *Aspergillus* and *Penicillium* genera.

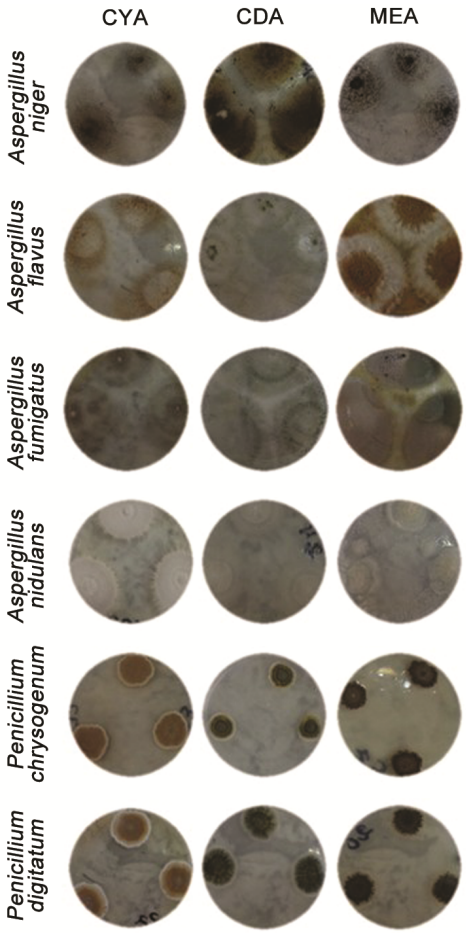

Fig. 3 — Identification of some fungal strains according to Pitt[21], Ramirez[15], and Pitt et Hocking[25].

Discussion

Plant-derived compounds are of interest in this context because they comprise safer or more effective substitutes for synthetically produced antimicrobial agents[25]. Many plant extracts used in folkloric medicine in Algeria were investigated for their antifungal activity and their use to treat pathogenic fungi[26-34]. In the present investigation, *R. tripartita* and *T. nudatum* showed excellent activity compared to other investigated plants.

All the studied plant extracts have proven to be one of the most important antimicrobial agents successfully used against at least three investigated fungi. The low values recorded for some plant extracts may be attributed to the fact that the extracts being in crude form, contain very small amounts of bioactive compounds.

Secondary metabolites produced by plants possess several interesting biological activities and are a source of pharmacologically active principles against pathogenic microorganisms. Useful antimicrobial phytochemicals, such as phenolics, flavonoids, tannins, coumarins, terpenoids, and alkaloids plus other compounds, are abundantly found in plant species used in this study according to our previous investigations, and they may be responsible for this significant activity against the tested fungi.

Several studies have been conducted to understand the mechanism of action of plant extracts; however, it is still unclear. Possible action mechanisms by which mycelial growth may be reduced or completely inhibited have been proposed[35-39].

Several researchers suggested that the mechanism of actions may include enzyme inhibition by the oxidized compounds, and act as a source of stable free radical and often leading to inactivation of the protein and loss of function. They can form a complex with extracellular and soluble proteins and to form a complex with microbial cell walls and disrupt microbial membranes[40]. Some extracts may have the ability to intercalate with DNA, the formation of ion channels in the microbial membrane, competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors[41]. It is also commonly accepted that it is the toxic effects of some phytochemical components and extracts on the functionality and structure of the cell membrane that is responsible for the aforesaid activity[42]. The different results obtained using several species as bio-fungicides extracts suggest that there are many substances, which can still be exploited for the management of pathogens.

These substances can be further subjected to isolation of the therapeutic antimicrobials and carry out a further

Table 2 — Antifungal inhibitory activity of the plants extracts using radial growth method on solid medium

		A. nardus	*A. schoenanthus*	*G. vulgaris*	*H. scoparia green*	*H. scoparia red*	*P. laevigata*	*R. tripartita*	*T. gallica*	*T. nudatum*
		Mycelial growth inhibition (%)								
Aspergillus genus	*A.flavus* Aq. Ext	26.6±0.5	9.0±0.0	13.3±1.1	19.0±6.3	24.8±1.1	20.0±3.4	21.2±2.8	45.4±0.0	23.0±0.0
	Hm. Ext	36.3±4.3	9.0±2.0	24.8±2.3	9.0±5.2	9.6±2.5	22.7±3.5	50.3±2.5	18.1±3.0	10.9±0.0
	A.nidulans Aq. Ext	48.0±1.4	50.6±2.3	34.6±4.5	60.0±0.0	28.0±3.6	49.3±2.0	44.0±0.0	48.0±1.7	66.6±1.5
	Hm. Ext	0.0±4.0	58.6±0.5	60.0±0.0	52.0±2.8	62.6±1.1	30.6±3.6	77.3±1.1	40.0±0.0	60.0±0.0
	A.niger Aq. Ext	13.7±4.1	17.4±2.8	10.6±1.5	4.9±1.1	16.5±0.7	18.1±3.5	7.4±4.0	11.8±4.3	22.5±2.3
	Hm. Ext	18.7±5.1	2.0±4.9	21.2±4.8	23.7±3.7	23.7±1.1	23.1±1.7	19.9±7.0	4.3±3.0	2.4±2.0
	A.ochracus Aq. Ext	16.6±3.0	9.0±1.5	0.6±2.3	5.5±0.5	17.3±4.0	20.1±2.8	7.6±1.1	27.7±4.5	6.9±0.5
	Hm. Ext	40.9±2.8	20.8±2.6	11.8±2.5	29.1±4.9	4.1±2.6	6.9±0.5	4.8±2.0	6.9±0.5	4.1±1.0
Penicillium genus	*P.chrysogenum* Aq. Ext	18.9±0.0	25.6±2.8	39.1±0.0	22.9±3.6	29.7±2.5	18.9±0.0	18.9±0.0	25.6±2.8	22.9±1.4
	Hm. Ext	39.1±0.0	21.6±1.1	39.1±0.0	32.4±2.8	22.9±1.7	29.0±3.5	32.4±2.8	32.4±2.8	25.6±2.8
	P.digitatum Aq. Ext	48.7±4.6	1.8±2.5	45.6±1.0	13.1±4.3	6.2±4.0	14.3±4.0	42.5±3.5	34.3±3.0	21.2±2.7
	Hm.Ext	11.8±2.6	15.6±5.0	21.8±2.8	14.3±1.1	8.1±1.4	10.9±3.5	10.9±3.5	15.6±0.0	15.6±2.0
	P.oxalicum Aq. Ext	11.7±0.0	32.3±4.3	26.4±1.5	14.7±1.1	22.7±2.1	36.7±3.1	36.7±1.1	11.7±0.0	60.2±1.7
	Hm. Ext	36.7±1.1	42.6±0.0	35.2±3.7	19.1±1.1	19.1±1.5	36.7±1.1	11.7±0.0	7.3±0.0	22.0±2.5

Aq. ext: Aqueous extract Hm. ext: Hydromethanolic extract

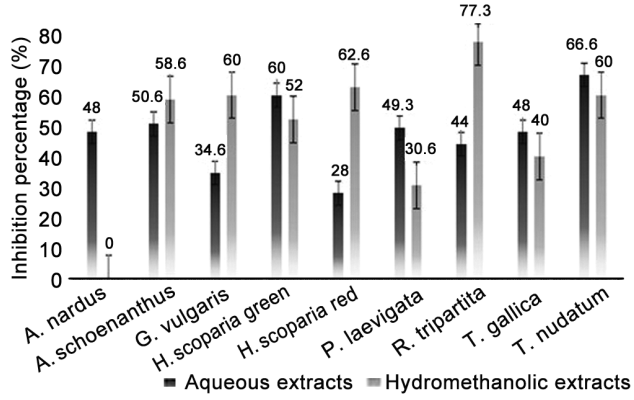

Fig. 4 — Mycelial growth inhibition of *Aspergillus nidulans* by the selected plant species.

pharmacological evaluation to resolve the problems of fungal pathogens[43].

Conclusion

The study concludes that great attention should be paid to the therapeutic potency of some plants used in traditional medicine, which are found to have plenty of pharmacological properties that could be sufficiently better when considering natural food and feed additives to improve human health. Further studies are needed to determine the antifungal compounds in such plant extracts (isolation, separation, and identification) as well as their formulation to be applied as an alternative method to be used in the treatment of fungal diseases.

Conflict of interest

The authors declare no known conflict of interest.

References

1 Makhuvele R, Naidu K, Gbashi S, Thipe V C, Adebo OA, *et al.*, The use of plant extracts and their phytochemicals for control of toxigenic fungi and mycotoxins, *Heliyon*. 2020; **6**(10), e05291.

2 Tabassum N and Vidyasagar G M, Phytochemical analysis and antifungal activity of some medicinal oil plants against human pathogens causing skin infections, *Int J ChemTech Res,* 2017, **10**(3), 171–177.

3 Silva F D S, Landell M F, Paulino G V B, Coutinho H D M and Albuquerque U P, Antifungal activity of selected plant extracts based on an ethno directed study, *Acta Bot Brasilica*, 2020, **34**(2), 442–448.

4 Phuna Z X, Yu J K E, Tee J Y, Chuah S Q, Tan N W H, *et al.*, *In vitro* evaluation of nanoemulsions of curcumin, piperine, and tualang honey as antifungal agents for candida species, *J Appl Biotechnol Reports*, 2020, **7**(3), 190–198.

5 Multon L, Méthodes de reference pour le dosage de l'eau dans les grains et graines, In: Multon JL, editor. Conservation et Stockage des Grains et Graines, Paris: Lavoisier-TEC et Doc-APRIA, 1982.

6 Larpent J P, Moisissures Utiles et Nuisibles Importance Industrielle, 2nd edn Paris, Masson, 1990.

7 Botton B, Breton A, Fevre M, Gauthier S, Guy P H, *et al.*, Moisissures utiles et nuisibles, importance industrielle, 2nd edn Paris: Masson, 1990.

8 Domsch K H, Gams W, Anderson T H, *Compendium of soil fungi*, (London: Academic press), 1980.

9 Ellis M B, Dermatacious Hyphomycetes, Kew, Surrey: Commonwealth Mycological Institute, 1976.

10 Barnett H L and Hunter B B, *Illustrated Genera of Imperfect Fungi*, vol 38, (APS Press. Minneapolis Burgress: Publishing Company), 1972.

11 Gilman J C, *A manual of soil fungi*, 2nd edn, (Oxford: IBH publishing Co.), 1944.

12 Pitt J I, An Appraisal of identification methods for Penicillium species : Novel taxonomic criteria based on temperature and water relations, *Mycol Soc Am*, 1973, **65**, 1135–1157.

13 Ramirez C, *Manual and atlas of the Penicillium*, (Amsterdam: Elsevier Biomedical Press), 1982.

14 Bajpai V K, Rahman A and Kang S C, Chemical composition and anti-fungal properties of the essential oil and crude extracts of *Metasequoia glyptostroboides* Miki ex Hu, *Ind Crops Prod*, 2007, **26**(1), 28–35.

15 Banso A, Adeyemo S O and Jeremiah P, Antimicrobial properties of *Vernonia amygdalina* extract, *J Appl Sci Manag*, 1999, **3**(1), 9–11.

16 Zambonelli A, D'Aulerio A Z, Bianchi A and Albasini A, Effects of essential oils on phytopathogenic fungi *In Vitro*, *J Phytopathol*, 1996, **144**(10), 491–494.

17 Pandey D K, Tripathi N N, Tripathi R D and Dixit S N, Fungitoxic and phytotoxic properties of the essential oil of *Hyptis suaveolens*, *J plant Dis Prot*, 1982, **89**(6), 344–349.

18 Singh P, Kumar A, Dubey N K and Gupta R, Essential oil of Aegle marmelos as a safe plant-based antimicrobial against postharvest microbial infestations and aflatoxin contamination of food commodities, *J Food Sci*, 2009, **74**(6), 302–307.

19 Aboody M S A and Mickymaray S, Anti-fungal efficacy and mechanisms of flavonoids, *Antibiotics*, 2020, **9**(2), 45.

20 Pitt J I, The current role of *Aspergillus* and *Penicillium* in human and animal health, *J Med Vet Mycol*, 1994, **32**(1), 17–32.

21 Pétriacq P, López A and Luna E, Fruit decay to diseases: Can induced resistance and priming help?, *Plants*, 2018, **7**(4), 1–16.

22 Harrigan W F and McCance M E, *Laboratory methods in food and dairy microbiology*, (London: Academic Press), 1976.

23 Oteng-Gyang K, *Introduction à la microbiologie alimentaire dans les pays chaud*, (Paris: Lavoisier), 1984.

24 Guiraud J P, *Microbiologie alimentaire*, (Paris: Dunod), 1998.

25 Dupuis G, Johri B, Bandoni R J and Towers G H, Cinnamylphenols as inhibitors of fungal growth, *Can J Microbiol*, 1972, **18**(1), 929–932.

26 Lakhdari W, Biological control of *Fusarium oxysporum* F. sp. *Radicis lycopersici* by using aqueous extracts of medicinal plants of wadi righ region, *SDRP J Plant Sci Biol*, 2017, **2**(1), 1–8.

27 Moghtet S, Menad N, Meddah B and Moussaoui A, Anvillea radiata (Aerial parts): Antifungal effect on mycotoxigenic fungi, *Int J Pharm Sci Rev Res*, 2017, **43**(1), 32–4.

28 Terfaya B, Makhloufi A, Mekboul A, Benlarbi L and Abdelouahed D, Antifungal activity of *Juniperus oxycedrus* Tar; growing wild in North-west of Algeria, *Appl Biol Sahar Areas*, 2017, **1**(1), 33–36.

29 Bendifallah L, Tchoulak Y, Djouabi M, Oukili M and Ghezraoui R, Phytochemical study and antimicrobial activity of *Origanum vulgare* L . (Lamiaceae) in boumerdes mountainous region (Algeria), *J Med Biol Eng*, 2015, **4**(6), 2013–2016.

30 Benarba B and Meddah B, Ethnobotanical study, antifungal activity, phytochemical screening and total phenolic content of Algerian *Aristolochia longa*, *J Intercult Ethnopharmacol*, 2014, **3**(4), 150–154.

31 Rahmoun N M, Ziane H and Boucherit-Otmani Z, Antibacterial and antifungal screening of four medicinal plants, *J Coast Life Med*, 2014, **2**(12), 975–979.

32 Tabti L, Dib M E A, Gaouar N, Samira B and Tabti B, Antioxidant and antifungal activity of extracts of the aerial parts of *thymus capitatus* (l.) hoffmanns against four phytopathogenic fungi of *citrus sinensis*, *Jundishapur J Nat Pharm Prod*, 2014, **9**(1), 49–54.

33 Gacem M A, Ould E L Hadj-khelil A and Gacemi B, Evaluation of antifungal effect of organic extracts of Algerian *Citrullus colocynthis* seeds against four strains of *Aspergillus* isolate from wheat stored, *J Med Plants Res*, 2013, **7**(12), 727–733.

34 Amrouche A, Benmehdi H, Moussaoui A, Mebarki K, Chaoufi A, *et al.*, Evaluation of antifungal activity of some oils from Algerian medicinal plants against *Aspergillus flavus* strain produced aflatoxins, *J Appl Pharm Sci*, 2011, **1**(8), 48–53.

35 Cristani M, D'Arrigo M, Mandalari G, Castelli F, Sarpietro M G, *et al.*, Interaction of four monoterpenes contained in essential oils with model membranes: Implications for their antibacterial activity, *J Agric Food Chem*, 2007, **55**(15), 6300–6308.

36 Omidbeygi M, Barzegar M, Hamidi Z and Naghdibadi H, Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste, *Food Control*, 2007, **18**(12), 1518–23.

37 Lucini E I, Zunino M P, López M L and Zygadlo J A, Effect of monoterpenes on lipid composition and sclerotial development of *Sclerotium cepivorum* Berk, *J Phytopathol*, 2006, **154**(7–8), 441–446.

38 Veldhuizen E J A, Tjeerdsma-Van Bokhoven J L M, Zweijtzer C, Burt S A and Haagsman H P, Structural requirements for the antimicrobial activity of carvacrol, *J Agric Food Chem*, 2006, **54**(5), 1874–1879.

39 Sharma N and Tripathi A, Fungitoxicity of the essential oil of *Citrus sinensis* on post-harvest pathogens, *World J Microbiol Biotechnol*, 2006, **22**(6), 587–593.

40 Mishra A K, Mishra A, Kehri H K, Sharma B and Pandey A K, Inhibitory activity of Indian spice plant *Cinnamomum zeylanicum* extracts against *Alternaria solani* and *Curvularia lunata,* the pathogenic dematiaceous moulds, *Ann Clin Microbiol Antimicrob*, 2009, **8**(9), 1–9.

41 Cowan M M, Plant products as antimicrobial agents, *Clin Microbiol Rev*, 1999, **12**(4), 564–582.

42 Sikkema J, de Bont J A and Poolman B, Mechanisms of membrane toxicity of hydrocarbons, *Microbiol Rev*, 1995, **59**(2), 201–222.

43 Abayhne M and Chauhan N, Antifungal activity of various medicinal plants against late blight of potato from Ethiopia, *J Sci Res Reports*, 2016, **12**(5), 1–9.