Journal of Pharmacognosy and Phytotherapy

Volume 7 Number 8 August 2015
ISSN 2141-2502

Academic Journals

ABOUT JPP

The **Journal of Pharmacognosy and Phytotherapy (JPP)** is published monthly (one volume per year) by Academic Journals.

The **Journal of Pharmacognosy and Phytotherapy (JPP)** is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject such as ethnobotany, phytochemistry, ethnopharmacology, zoopharmacognosy, medical anthropology etc.

The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JPP are peer-reviewed.

Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: jpp@academicjournals.org. A manuscript number will be mailed to the corresponding author shortly after submission.

The Journal of Pharmacognosy and Phytotherapy (JPP) will only accept manuscripts submitted as e-mail attachments.

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author.

Journal of Pharmacognosy and Phytotherapy

Table of Contents: Volume 7 Number 8 August 2015

ARTICLES

Research Articles

academicJournals

Vol. 7(8), pp. 132-163, August 2015
DOI: 10.5897/JPP2015.0254
Article Number: 5F9D8B754311
ISSN 2141-2502

http://www.academicjournals.org/JPP

Journal of Pharmacognosy and Phytotherapy

Full Length Research Paper

Analysis of bioactive chemical compounds of *Aspergillus niger* by using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy

Imad Hadi Hameed*, Lena Fadhil Hamza and Sabreen A. Kamal

Department of Molecular Biology, Babylon University, Hilla City, Iraq.

Received 9 June 2015; Accepted 9 July 2015.

Bioactives are chemical compounds often referred to as secondary metabolites. Thirty five bioactive compounds were identified in the methanolic extract of *Aspergillus niger*. The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula. Gas chromatography-mass spectrometry (GC-MS) analysis of *Aspergillus niger* revealed the existence of the 6-Acetyl-ß-d-mannose, 4-[Dichloromethyl]-2-[[2-[1-methyl-2-pyrrolidinyl]ethyl]amino-6-trichloro, 2-Furan-carboxaldehyde,5-methyl, 2,2,2-Trifluoro-N-[2-(1-hydroxy-2,2,6,6-tetramethyl-piperidin-4-yl), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), Tetraacetyl-d-xylonic nitrile, Eicosanoic acid, phenylmethyl ester, Dodecanoic acid, 3-hydroxy, Desulphosinigrin, Glycyl-dl-serine, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, 2,5-Furandicarboxaldehyde, 2H-Oxecin-2-one,3,4,7,8,9,10- hexahydro-4-hydroxy-10-methyl, 6-Acetyl-ß-d-mannose, DL-Leucine, N-glycyl, 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl, I-Gala-l-ido-octonic lactone, 2H-Pyran,tetrahydro-2-(12-pentadecynyloxy), 5-Hydroxymethylfurfural, Strychane,1-acetyl-20α-hydroxy-16-methylene, α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)ß-D-fru, Boroxin, tris(2,3-dimethylbut-2-yl), 16-Nitrobicyclo[10.4.0]hexadecane-1-ol-13-one, 3-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9(2H,10H)-a, Uric acid, 1,2,4-Trioxolane-2-octanoic acid ,5-octyl-,methyl ester, Tetraacetyl-d-xylonic nitrile, 1,2-Cyclopentanedicarboxylic acid, 4-(1,1-dimethylethyl)-,dimethyl, 2-Bromotetradecanoic acid, i-Propyl 11,12-methylene-octadecanoate, 1H-2,8a-Methanocyclopenta[a]cyclopropa [e]cyclodecan-11-one, and Octadecanoic acid. The FTIR analysis of *A. niger* proved the presence of aromatic rings, alkenes, aliphatic fluoro compounds, tetiary amine, C-N stretch, aromatic nitro compounds, ammonium ions and organic nitrate which shows major peaks at 696.30, 744.52, 821.68, 844.82, 900.76, 931.62, 1026.13, 1145.72, 1207.44, 1234.44, 1261.45, 1315.45, 1359.82, 1377.17, 1413.82, 1452.40, 1631.78, 1741.72, 2924.09, 3118.90, 3217.27 and 3271.27. *Datura stramonium* was very active against *A. niger*. Methanolic extract of bioactive compounds of *A. niger* were assayed for *in vitro* antibacterial activity against *Pseudomonas aerogenosa, Escherichia coli*, *Proteus mirabilis, Staphylococcus aureus* and *Klebsiella pneumonia* by using the diffusion method in agar. The zones of inhibition were compared with different standard antibiotics. The diameters of inhibition zones ranged from 0.46±0.1 to 6.52±0.61 mm for all treatments.

Key words: *Aspergillus niger*, bioactive compounds, gas chromatography-mass spectrometry, fourier-transform infrared spectroscopy.

INTRODUCTION

Aspergillus spp are ubiquitous opportunistic moulds that cause both allergic and invasive syndromes. The genus comprises approximately 180 species, of which 33 have been associated with human disease (Segal et al., 1998; Perfect et al., 2001). *Aspergillus niger* is the third most common species associated with invasive pulmonary aspergillosis (Bellini et al., 2003; Anupama et al., 2007). *A. niger* has a great economical and biotechnological interest and is extensively used for production of extracellular enzymes and organic acids such as citric acid (Baker, 2006; Perrone et al., 2007; Mogensen et al., 2010). It also produces fumonisin B2 (FB2) along with OTA. 9, 19, 27. Fumonisins are suspected to cause human and animal toxicoses, and are regarded as carcinogenic (Susca et al., 2010; Chacko et al., 2012; Gebreselema et al., 2013). A culture yielding *Aspergillus* spp, in addition to enabling a diagnosis of invasive aspergillosis, may further define therapeutic options via susceptibility testing or the isolation of a species possessing inherent antifungal resistance; examples of the latter include *Aspergillus terreus* and *Aspergillus nidulans*, which are both resistant to amphotericin B (Walsh, 2004). The main disadvantage of culture is that it is relatively slow (the process takes days), is relatively insensitive, and requires specialized expertise for species determination.

In common with other pathogenic fungi, the ability to grow at 37°C distinguishes *Aspergillus* spp from other nonpathogenic environmental moulds. *Aspergillus* spp can be recovered on most routine solid and liquid microbiological media (example, blood agar, chocolate agar, brain heart infusion broth). A fungal-specific medium example, sabouraud dextrose agar should be included at the time of initial specimen set-up in clinical scenarios in which *Aspergillus* spp (or other moulds) are considered possible pathogens, because of superior yield (Horvath and Dummer, 1995). The addition of antibiotics example, chloramphenicol and gentamicin to the medium is required for the recovery of *Aspergillus* spp from specimens obtained from nonsterile sites, since they prevent bacterial overgrowth. Cycloheximide, a eukaryotic protein synthesis inhibitor, is frequently added to fungal media to inhibit the overgrowth of cultures by non-pathogenic environmental moulds; however, on occasion, cycloheximide may inhibit the growth of *Aspergillus* spp. The aim of this study were analysis of the secondary metabolites and the evaluation of antibacterial and antifungal activity .

MATERIALS AND METHODS

Collection and growth condition

A. niger was isolated from dried fruit and the pure colonies were selected, isolated and maintained in potato dextrose agar slants (Usha and Masilamani, 2013). After the species were identified by the identification key, spores were grown in a liquid culture of potato dextrose broth (PDB) and incubated at 25°C in a shaker for 16 days at 130 rpm.

Production, extraction and determination of metabolites

The metabolites were determined and extracted for gas chromatography (GC) analysis using the method of Siddiquee et al. (2012) with some modifications. The extraction was performed by adding 25 ml methanol to 100 ml liquid culture in an Erlenmeyer flask after the infiltration of the culture. The mixture was incubated at 4°C for 10 min, and then shook for 10 min at 130 rpm. Metabolites was separated from the liquid culture and evaporated to dryness with a rotary evaporator at 45ºC. The residue was dissolved in 1 ml methanol, filtered through a 0.2 µm syringe filter, and stored at 4ºC for 24 h before being used for gas chromatography–mass spectrometry (GC-MS) (Imad et al., 2014a). The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library as well as on comparison of their retention indices either with those of authentic compounds or with literature values**.**

GC-MS analysis

Bioactive compound were examined for the chemical composition using GC-MS (Agilent 789N) equipped with a DB-5MS column (30 m×0.25 mm i.d., 0.25 um film thickness, J&W Scientific, Folsom, CA). The oven temperature was programmed as for the previous analysis (Imad et al., 2015a; Muhanned et al., 2015). Helium was used as the carrier gas at the rate of 1.0 ml/min. Effluent of the GC column was introduced directly into the source of the MS via a transfer line (250°C). Ionization voltage was 70 eV and ion source temperature was 230°C. Scan range was 41 to 450 amu. The constituents were identified after being compared with available data in the GC-MS library in the literatures (Imad et al., 2015b; Mohammed et al., 2013).

Fourier transform infrared spectrophotometer (FTIR)

The powdered sample of the *A. niger* specimen was treated for fourier transform infrared spectroscopy (Shimadzu, IR Affinity 1, Japan). The sample was run at infrared region between 400 nm and 4000 nm.

Determination of antibacterial activity of crude fraction of *A. niger* compounds

The test pathogens (*E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus)* were swabbed in Muller Hinton agar plates. 90µl of fungal extracts was loaded on the bored wells. The wells were bored in 0.5cm in diameter. The plates were incubated at 37°C for 24 h and examined. After the incubation the diameter of inhibition zones around the discs was measured.

*Corresponding author. E-mail: imad_dna@yahoo.com.

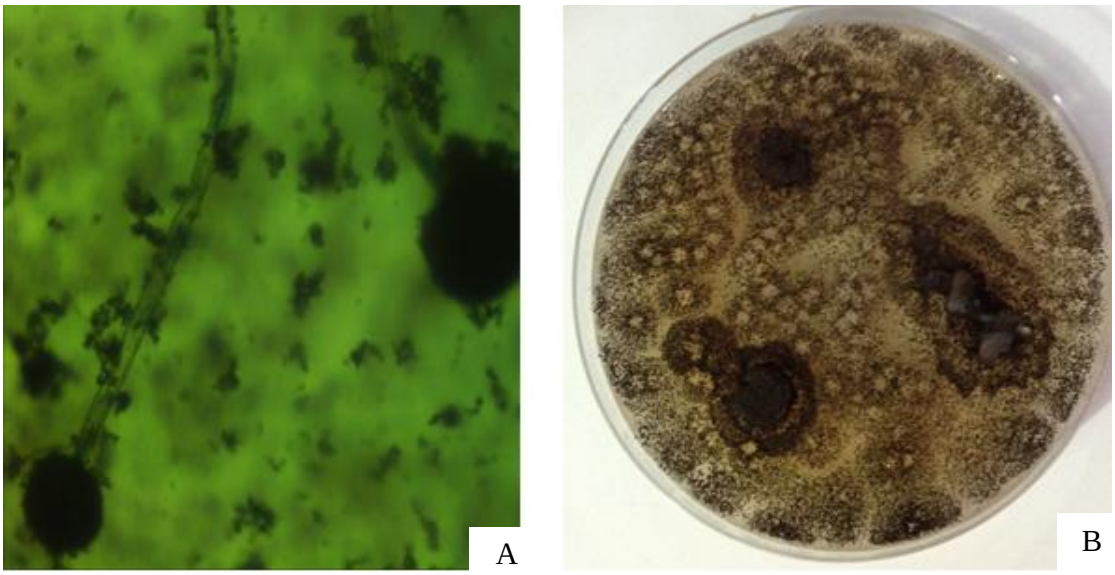


Figure 1. Morphological characterization of *Aspergillus niger*. (B) Microscopic observation (A) colony.

Determination of antifungal activity

A. niger isolate was suspended in potato dextrose broth and diluted to approximately 105 colony forming unit (CFU) per ml. They were "flood inoculated onto the surface of Potato dextrose agar and then dried. Standard agar well diffusion method was followed (Perez et al., 1990; Perez et al., 1999; Erdemogllu et al., 2003; Bagamboula et al., 2004). Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 25 µl of the samples solutions (*Nerium olender, Ricinus communis, Datura stramonium*, *Linum usitatissimum*, *Anastatica hierochuntica* and *Gramineae poaceae*) were delivered into the wells. The plates were incubated for 48 h at room temperature (Huda et al., 2015a; Ameera et al., 2015; Imad et al., 2015c). Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as solvent control. Amphotericin B and fluconazole were used as reference antifungal agent (Anesini and Perez, 1993; Rukayadi et al., 2006; Huda et al., 2015b). The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA), and differences among the means were determined for significance at $P < 0.05$ using Duncan's multiple range test (by statistical package for the social sciences (SPSS) software) Version 9.1 (Imad et al., 2014b).

RESULTS AND DISCUSSION

Isolation of fungi from dried fruit

The fungi were isolated from dried fruit by serial dilution method. Based on morphological, characteristics of fungi was isolated in selective media of potato dextrose agar media. Morphological, Microscopical and microscopical characteristics of fungal strains were determined using specific media light and compound microscope Figure 1.

Production and Identification of secondary metabolites from the methanolic crude extract of *A. niger* by gas chromatography and mass spectrometry and fourier-transform infrared spectroscopy

The 400 ml of fermentation broth (PDA broth) which contain 200 µl of the standardized fugal suspensions were used to inoculate the flasks and incubated at 37°C on a shaker at 90 rpm for 7 days. After fermentation, the secondary metabolites were produced by isolated microorganisms.

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic extract of *A. niger*, as shown in Table 1. The GC-MS chromatogram of the seventeen peaks of the compounds detected was shown in Figure 2. Chromatogram GC-MS analysis of the methanol extract of *A. niger* showed the presence of twenty major peaks and the components corresponding to the peaks were determined as follows. The First set up peak were determined to be 6-Acetyl-ß-d-mannose (Figure 3). The second peak indicated to be 4-[Dichloromethyl]-2-[[2-[1-methyl-2-pyrrolidinyl]ethylamino-6-trichloro (Figure 4). The next peaks considered to be 2-Furan-carboxaldehyde, 5-methyl, 2,2,2-Trifluoro-N-[2-(1-hydroxy-2,2,6,6-tetramethyl-piperidin-4-yl), 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one, HEPES, Tetraacetyl-d-xylonic nitrile, eicosanoic acid, phenylmethyl ester, dodecanoic acid , 3-hydroxy, Desulphosinigrin,

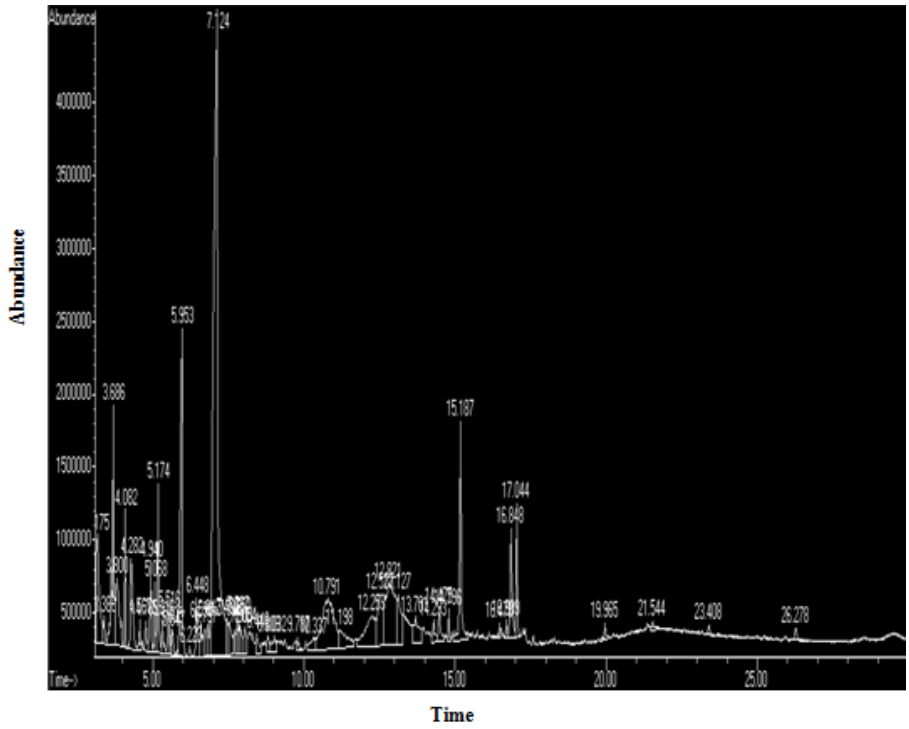


Figure 2. GC-MS chromatogram of methanolic extract of *A. niger.*

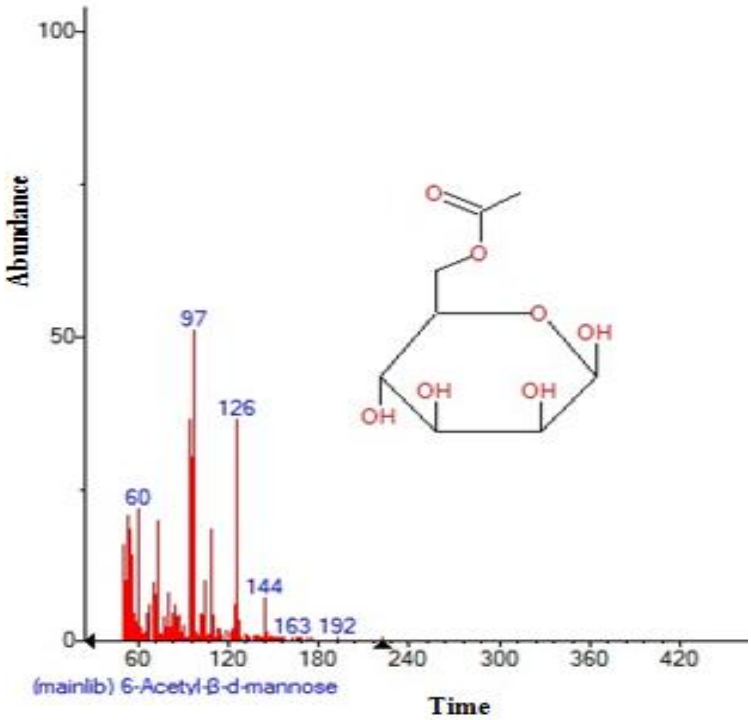


Figure 3. Mass spectrum of 6-Acetyl-ß-d-mannose with Retention Time (RT)= 3.201.

Glycyl-dl-serine, 2,5-Dimethyl-4 -hydroxy-3(2H)-furanone, 2,5-Furandicarboxaldehyde, 2H-oxecine-2-one, 3,4,7,8,9

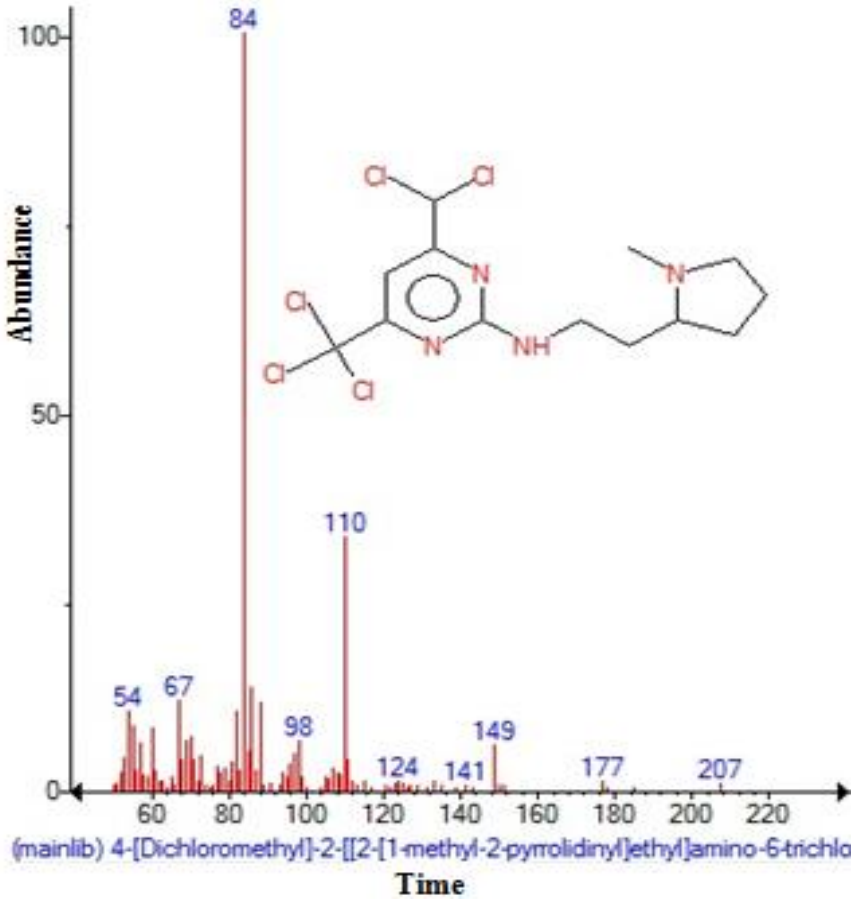


Figure 4. Mass spectrum of 4-[Dichloromethyl]-2-[[2-[1-methyl-2-pyrrolidinyl]ethyl]amino-6-trichloro with Retention Time (RT)= 3.613.

10-hexahydro-4-hydroxy-10-methyl, 6-Acetyl-ß-d-mannose, DL-Leucine, N-glycyl, 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl, l-Gala-l-ido-octonic lactone, 2H-Pyran,tetrahydro-2-(12-pentadecynyloxy), 5-Hydroxymethylfurfural, Strychane,1-acetyl-20α-hydroxy-16-methylene, α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)ß-D-fru, Boroxin, tris (2,3-dimethylbut-2-yl), 16-Nitrobicyclo[10.4.0]hexadecane-1-ol-13-one, 3-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9(2H,10H)-a, Uric acid, 1,2,4-Trioxolane-2-octanoic acid ,5-octyl-,methyl ester, Tetraacetyl-d-xylonic nitrile, 1,2-Cyclopentanedicarboxylic acid ,4-(1,1-dimethylethyl)-,dimethyl, 2-Bromotetradecanoic acid, i-Propyl 11,12-methylene-octadecanoate, 1H-2,8a-Metha-nocyclopenta[a]cyclopropa[e]cyclodecan-11-one and Octadecanoic acid (Figures 5 to 34). Many compounds are identified in the present study. Some of them are biological compounds with antimicrobial activities.

Fourier-transform infrared analysis of dry methanolic extract of *A. niger* proved the presence of aromatic rings, alkenes, aliphatic fluoro compounds, alcohols, ethers, carboxlic acids, esters, nitro compounds, aldehydes, ketones, alkanes, hydrogen bonded alcohols and phenols compounds shows major peaks at 894.97, 927.76, 1024.20, 1236.37, 1317.38, 1608.63, 2306.86, 2850.79, 2922.16, 3184.48, 3277.06 and 3292.49, respectively. (Table 2 and Figure 35).

Antibacterial activity

Four clinical pathogens selected for antibacterial activity namely, *k. pneumoniae, P. aeroginosa, E. coli, S. aeureus.* and maximum zone formation against *k. pneumonia* (Table 3 and Figure 36).

Antifungal activity

Each extract plant showed notable antifungal activities against *A. niger* (Figure 37). In agar well diffusion method the selected medicinal plants (*N. olender, R. communis, D. stramonium*, *L. usitatissimum*, *A. hierochuntica* and *G.*

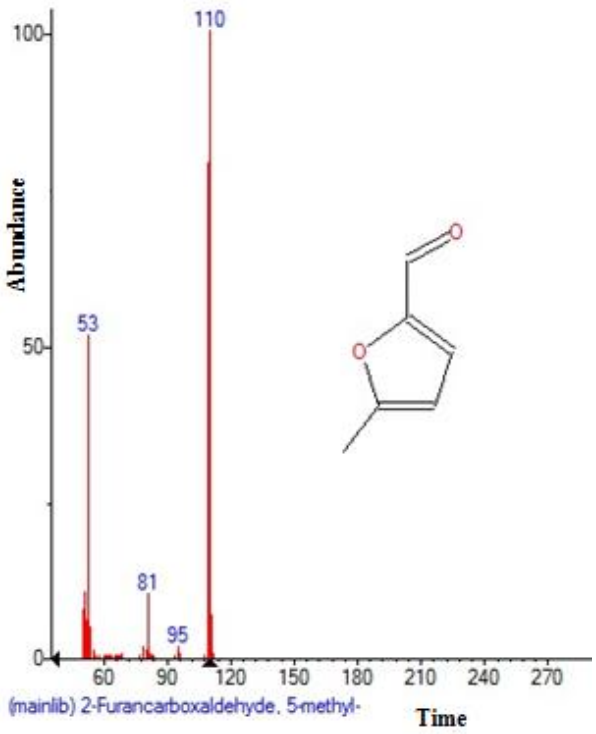


Figure 5. Mass spectrum of 2-Furancarboxaldehyde,5-methyl with Retention Time (RT)= 3.722.

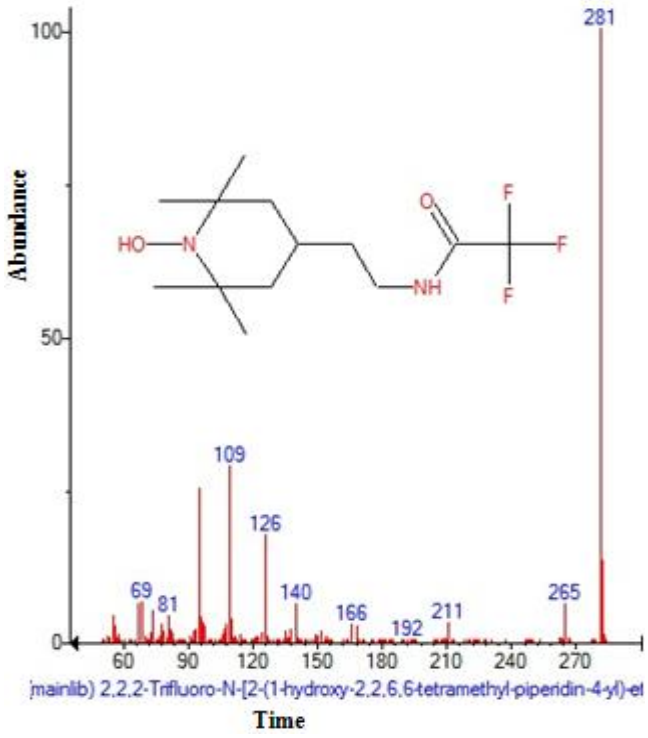


Figure 6. Mass spectrum of 2,2,2-Trifluoro-N-[2-(1-hydroxy-2,2,6,6-tetramethyl-piperidin-4-yl)-el with Retention Time (RT)= 3.779.

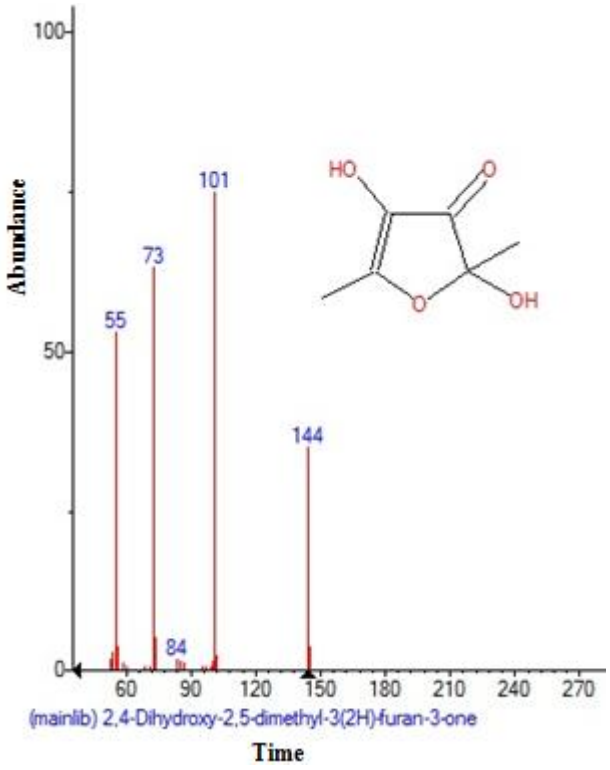


Figure 7. Mass spectrum of 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one with Retention Time (RT)= 4.076.

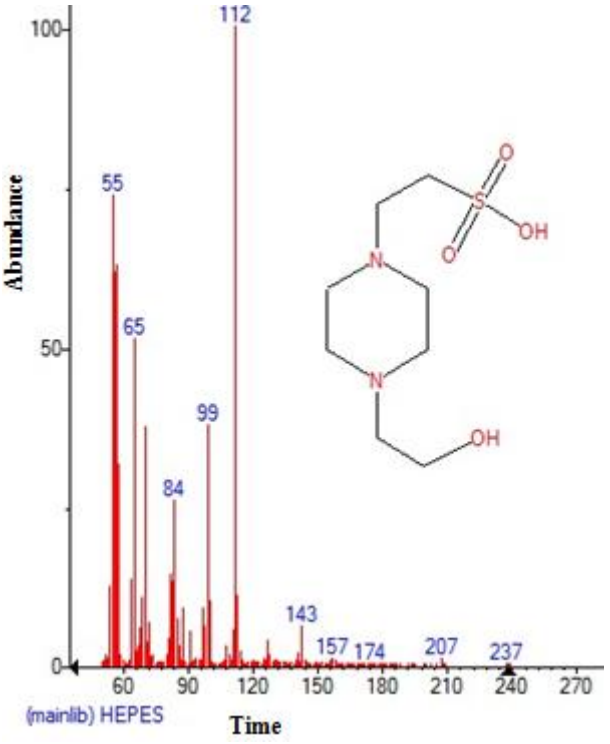


Figure 8. Mass spectrum of 2 HEPES with Retention Time (RT)= 4.271.

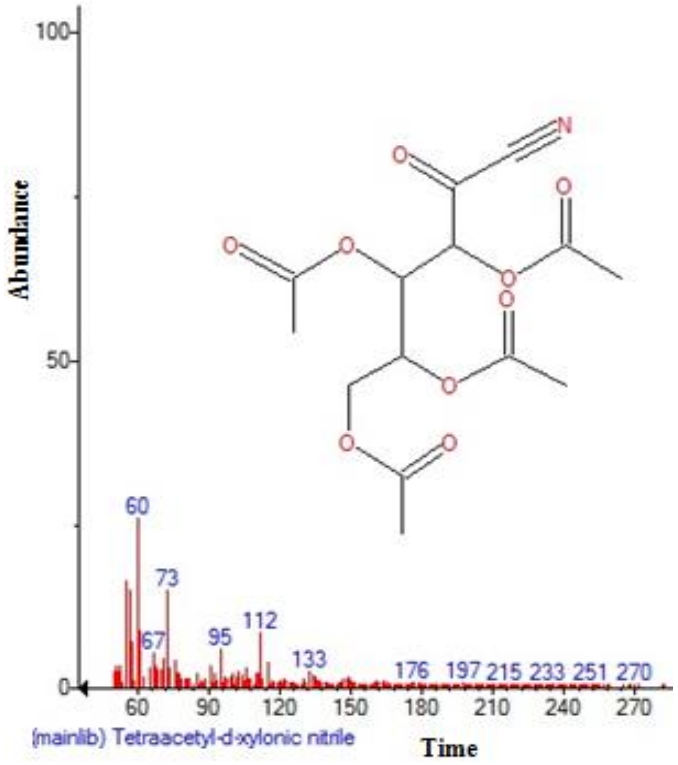


Figure 9. Mass spectrum of Tetraacetyl-d-xylonic nitrile with Retention Time (RT)= 4.465.

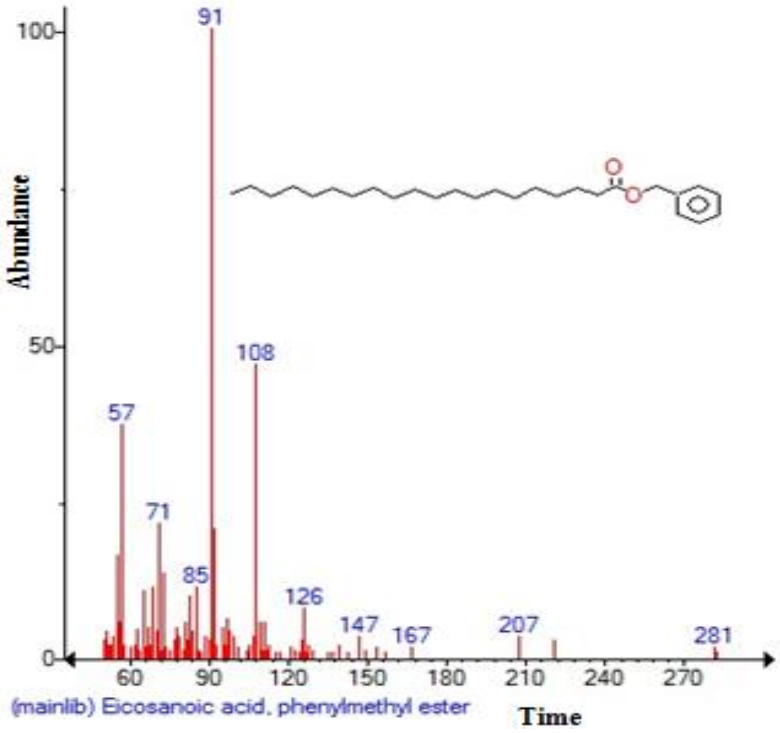


Figure 10. Mass spectrum of eicosanoic acid , phenylmethyl ester with retention time (RT)= 4.546.

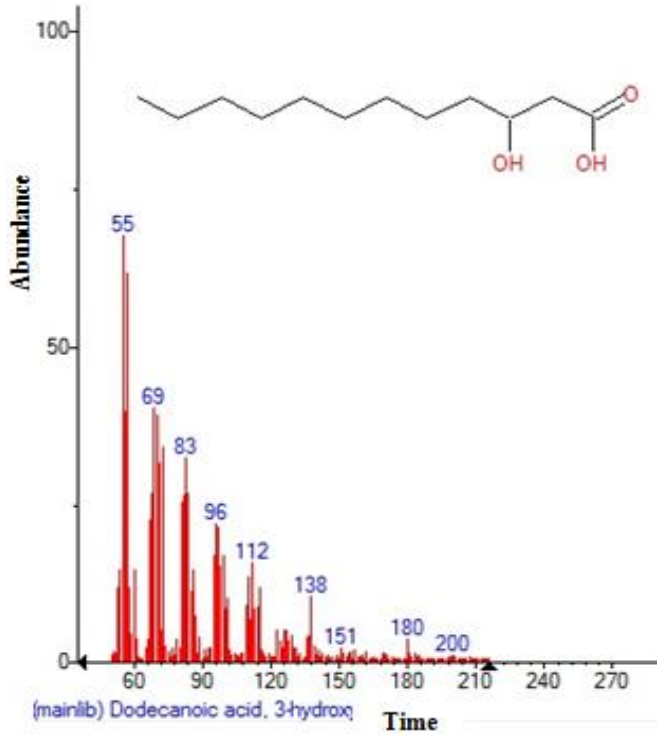


Figure 11. Mass spectrum of dodecanoic acid , 3-hydroxy with retention time (RT)= 4.574.

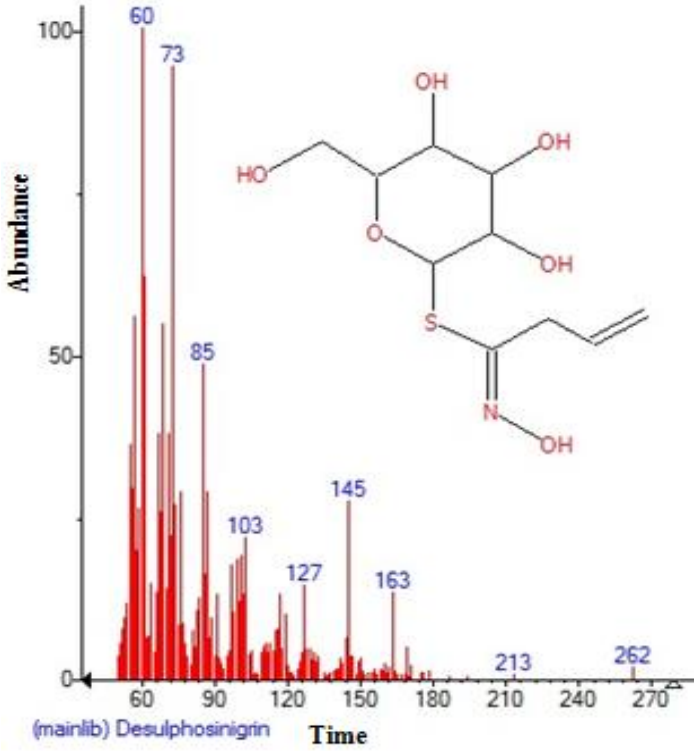


Figure 12. Mass spectrum of desulphosinigrin with retention time (RT)= 4.654.

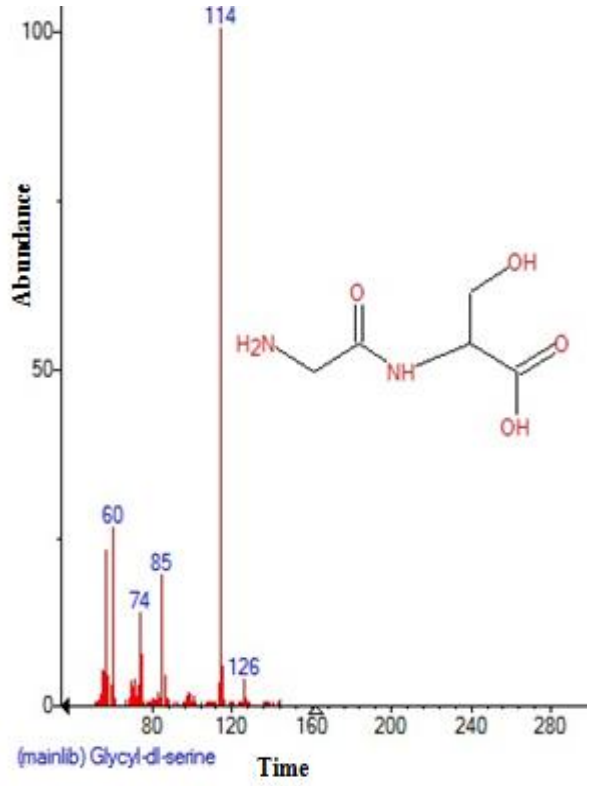


Figure 13. Mass spectrum of Glycyl-dl-serine with retention time (RT)= 4.763.

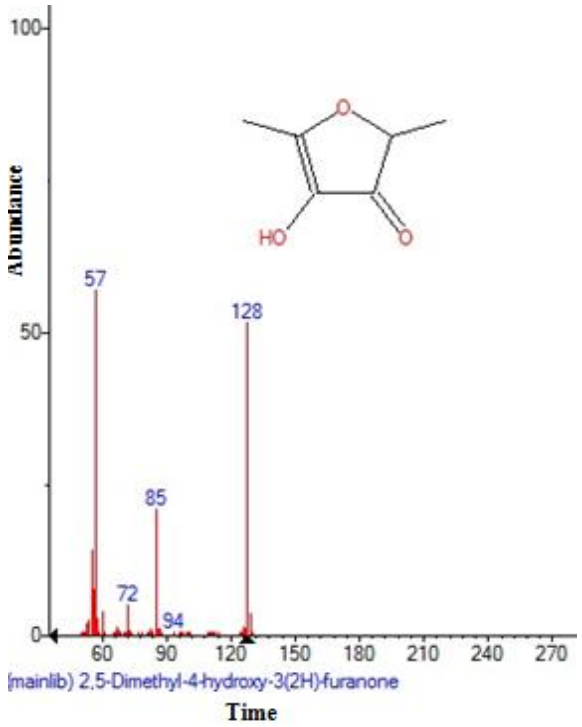


Figure 14. Mass spectrum of 2,5-Dimethyl-4-hydroxy-3(2H)-furanone with retention time (RT)= 4.929.

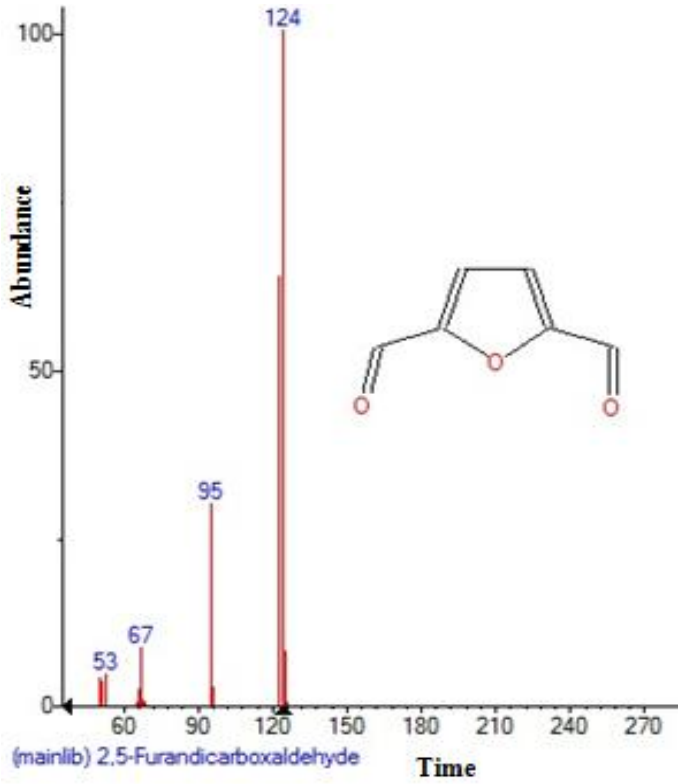


Figure 15. Mass spectrum of 2,5-Furandicarboxaldehyde with retention time (RT)= 5.066.

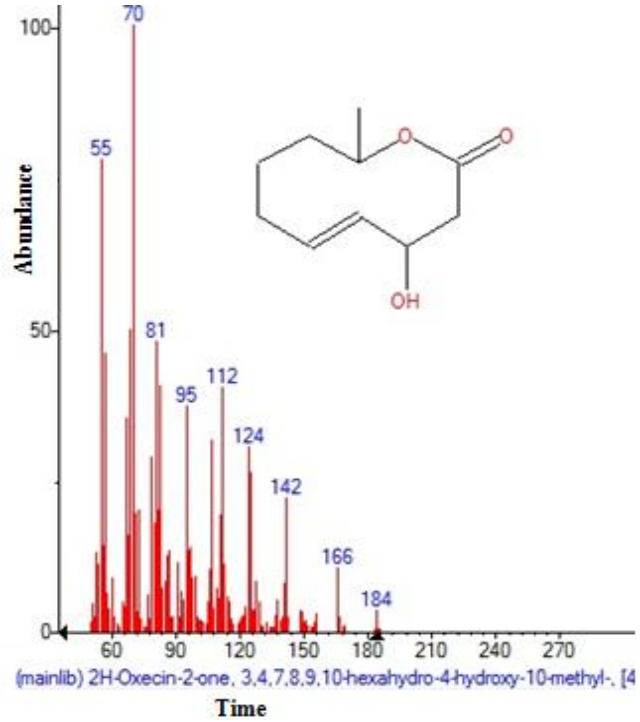


Figure 16. Mass spectrum of 2H-Oxecin-2-one,3,4,7,8,9,10-hexahydro-4-hydroxy-10-methyl-,[4 with retention time (RT)= 5.261.

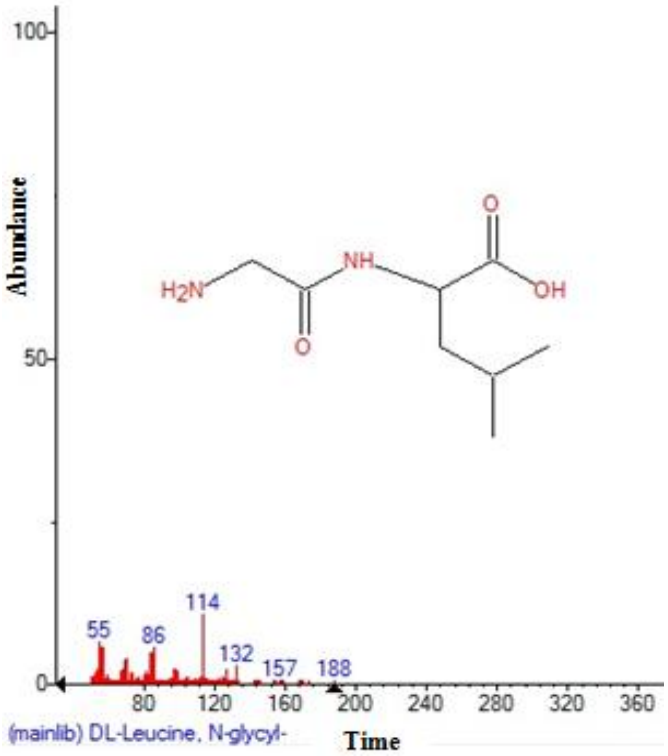


Figure 17. Mass spectrum of DL-Leucine , N-glycyl with retention time (RT)= 5.616.

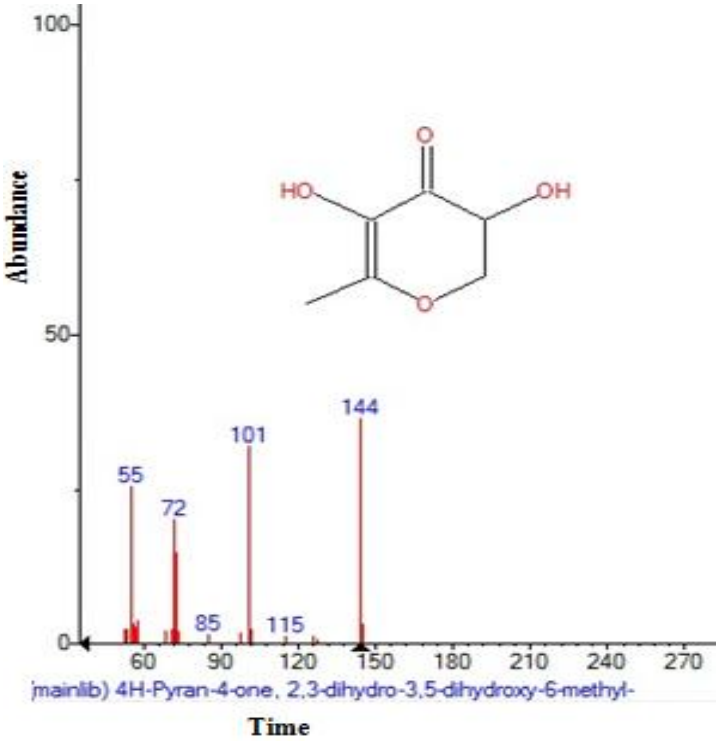


Figure 18. Mass spectrum of 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl with retention time (RT)= 5.942.

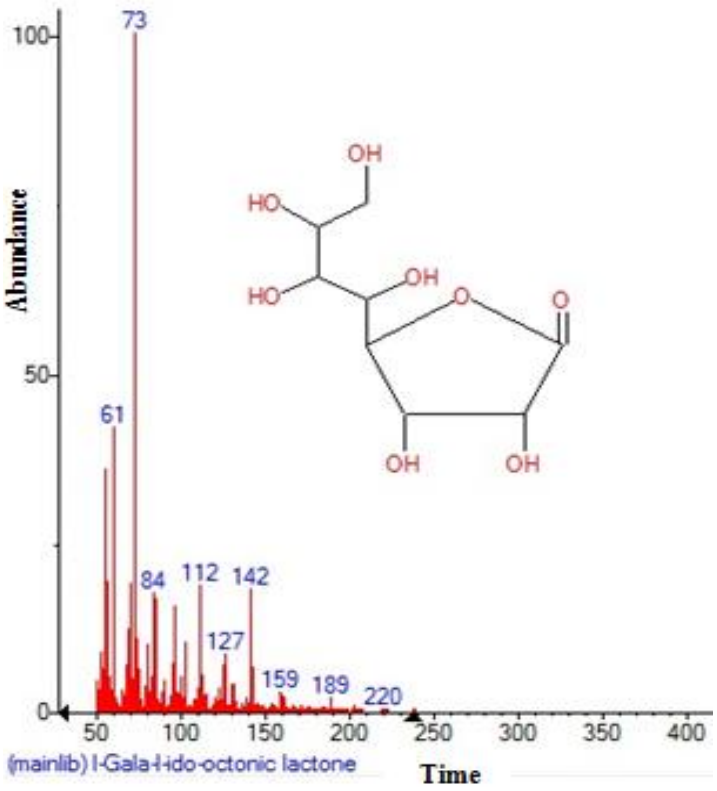


Figure 19. Mass spectrum of l-Gala-l-ido-octonic lactone with retention time (RT)= 6.577.

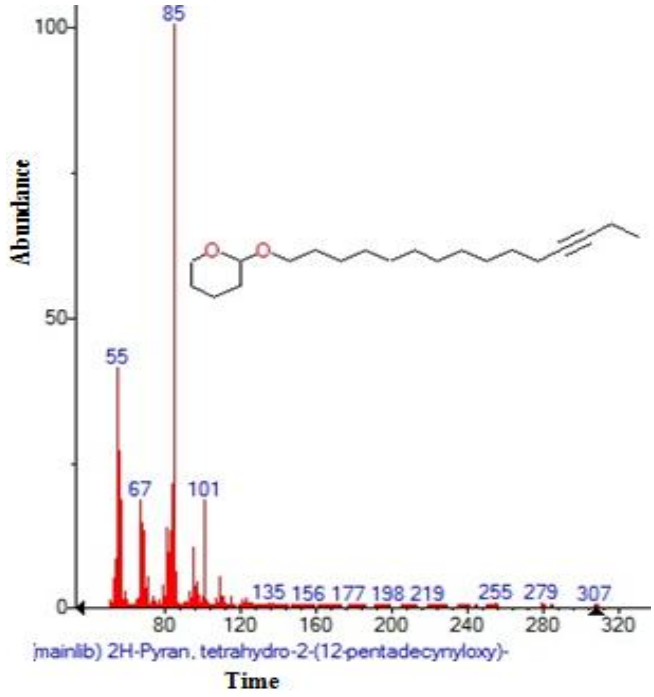


Figure 20. Mass spectrum of 2H-Pyran,tetrahydro-2-(12-pentadecynyloxy) with retention time (RT)= 6.737.

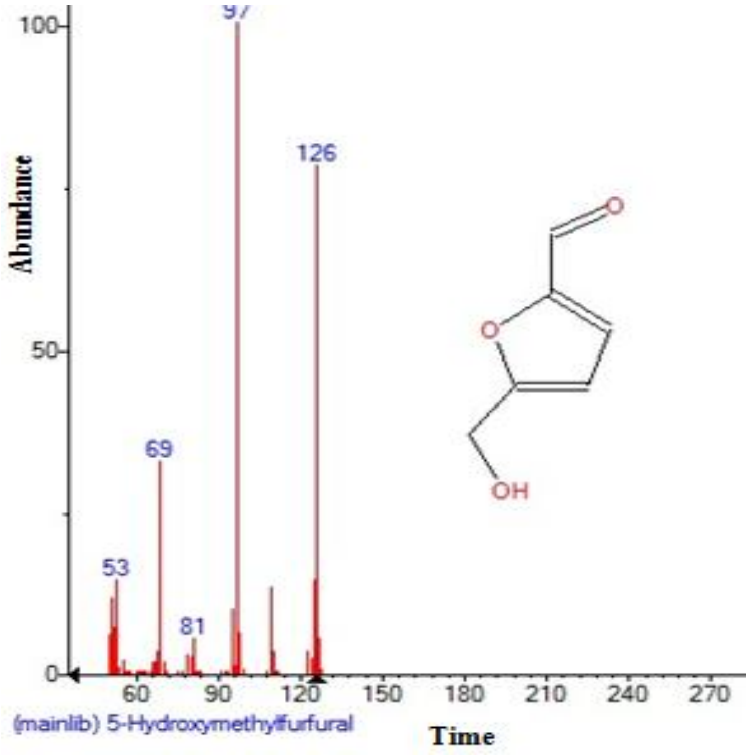


Figure 21. Mass spectrum of 5-Hydroxymethylfurfural with retention time (RT)= 7.120.

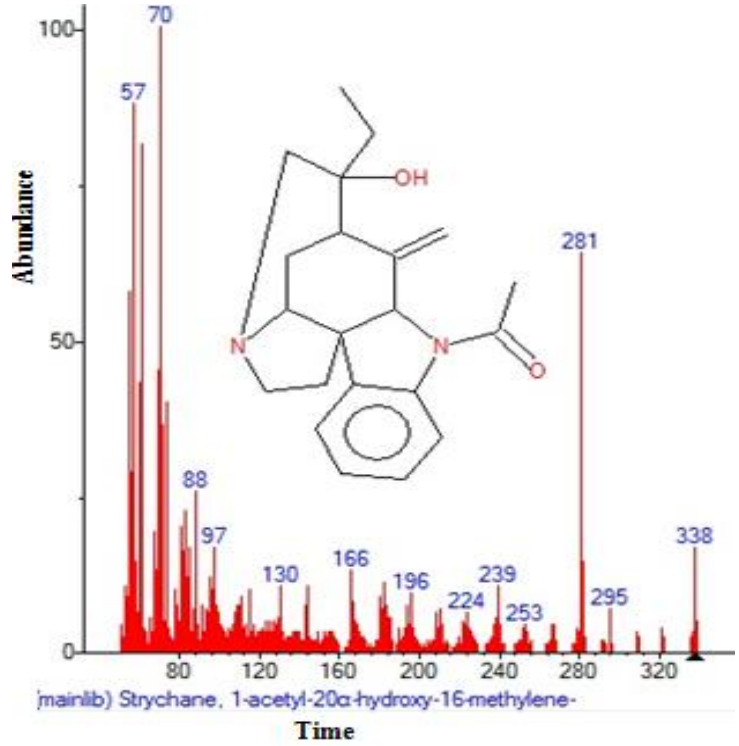


Figure 22. Mass spectrum of Strychane,1-acetyl-20α-hydroxy-16-methylene with retention time (RT)= 8.053.

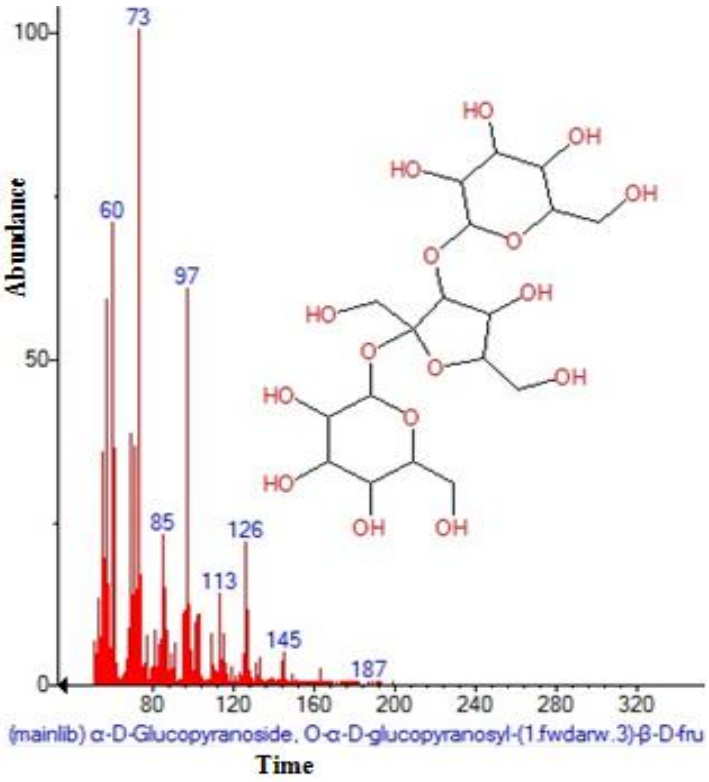


Figure 23. Mass spectrum of α-D-Glucopyranoside ,O-α-D-glucopyranosyl-(1.fwdarw.3)ß-D-fru with retention time (RT)= 7.836.

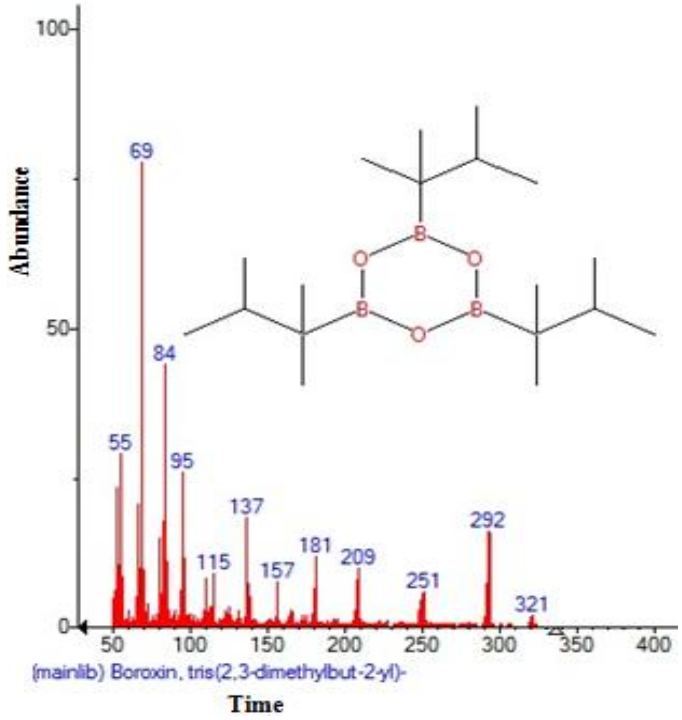


Figure 24. Mass spectrum of Boroxin , tris(2,3-dimethylbut-2-yl) with retention time (RT)= 8.442.

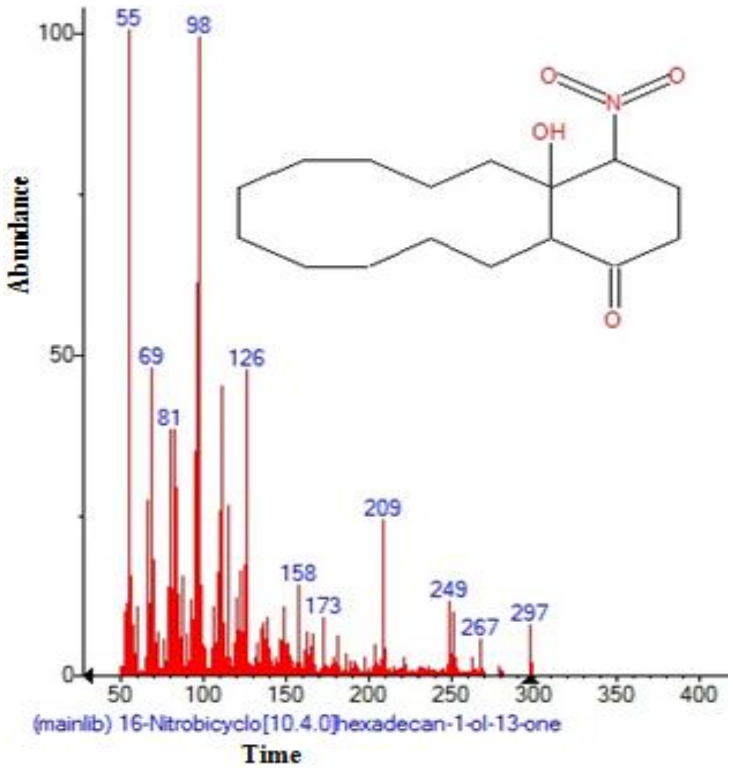


Figure 25. Mass spectrum of 16-Nitrobicyclo[10.4.0]hexadecane-1-ol-13-one with retention time (RT)= 8.797.

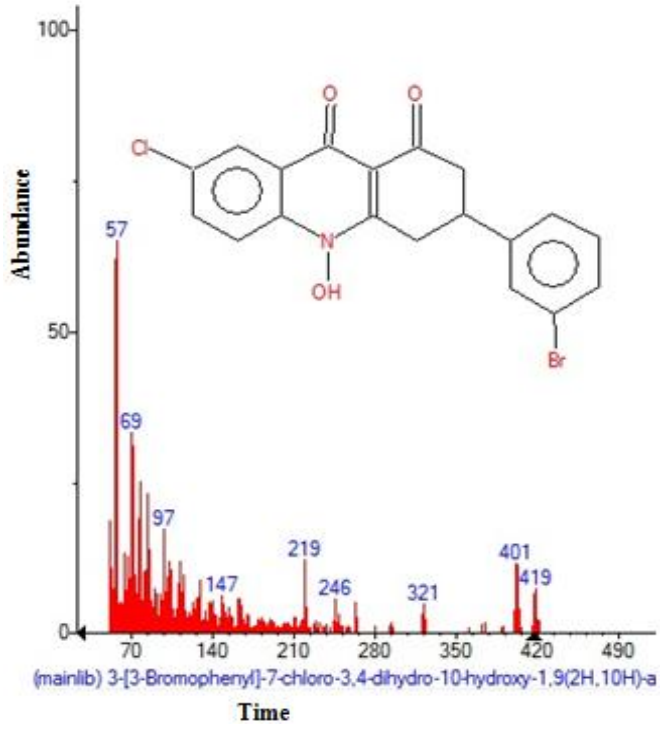


Figure 26. Mass spectrum of 3-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9(2H,10H)-a with retention time (RT)= 9.043.

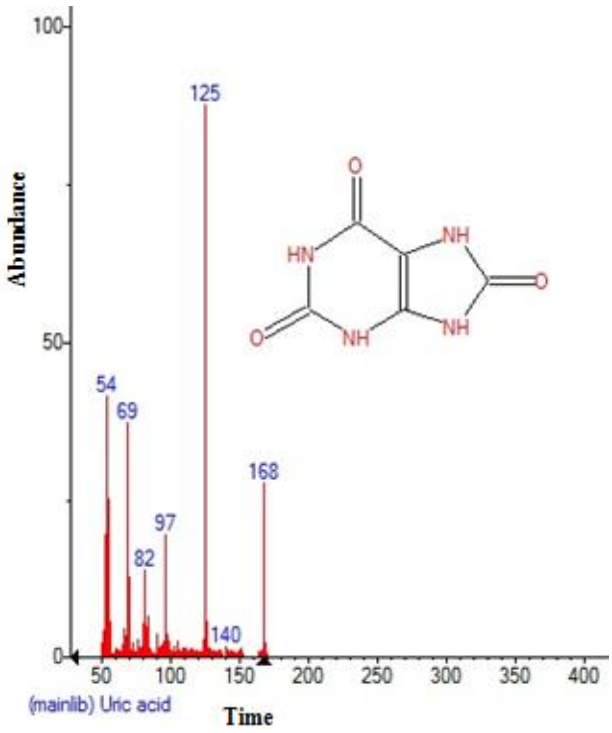


Figure 27. Mass spectrum of uric acid with retention time (RT)= 9.672.

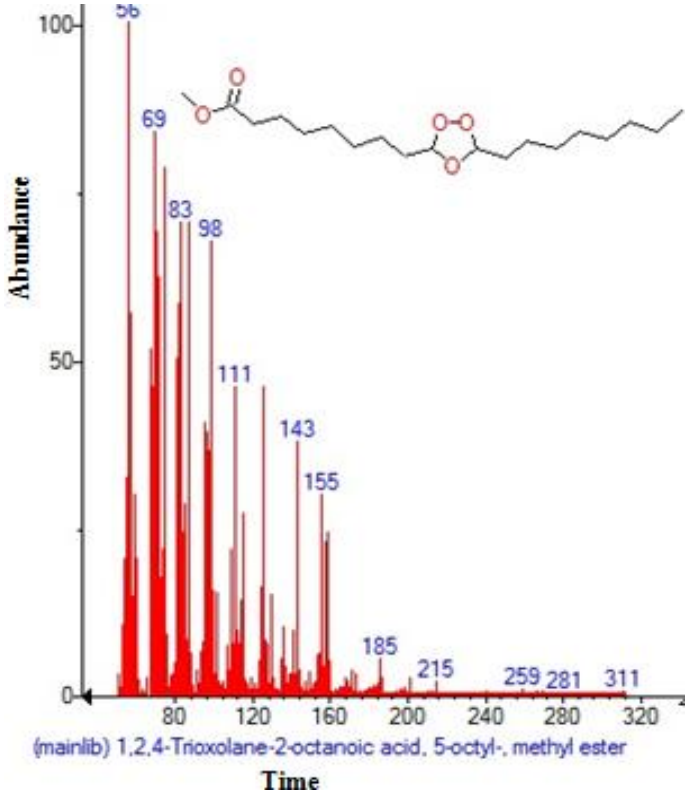


Figure 28. Mass spectrum of 1,2,4-Trioxolane-2-octanoic acid ,5-octyl-,methyl ester with retention time (RT)= 11.320.

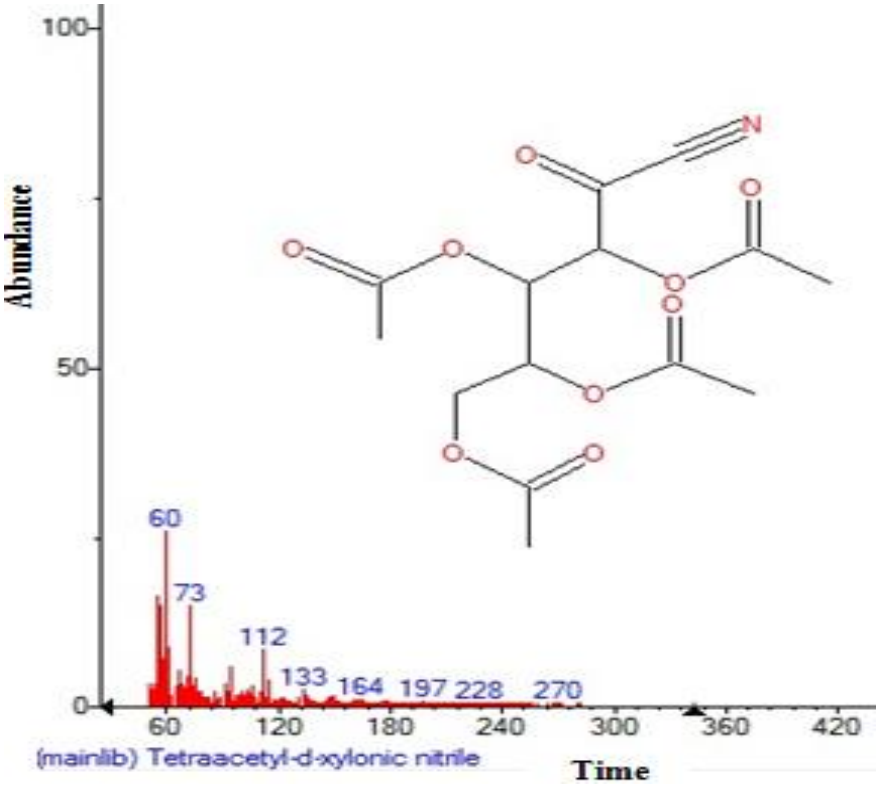


Figure 29. Mass spectrum of Tetraacetyl-d-xylonic nitrile with retention time (RT)= 12.276.

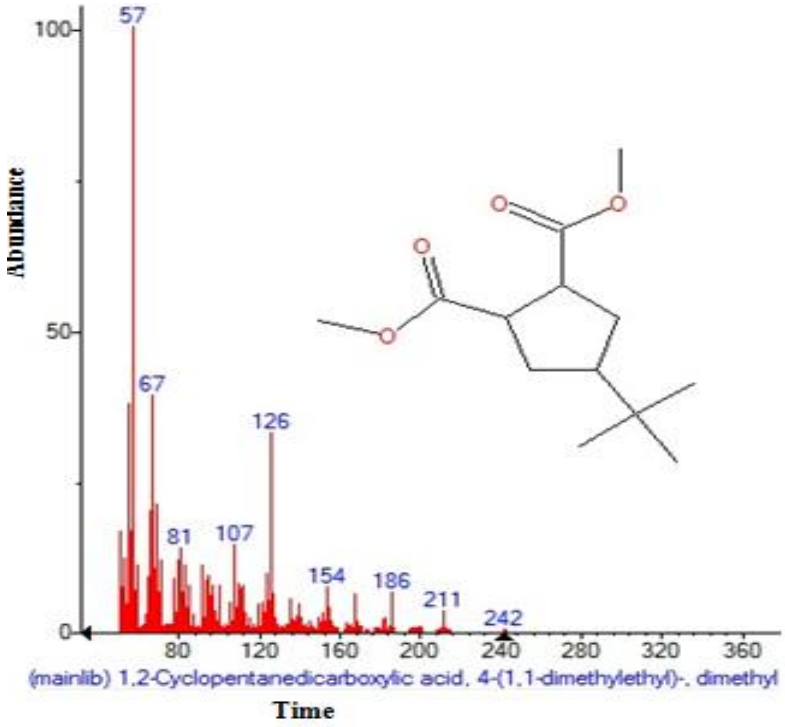


Figure 30. Mass spectrum of 1,2-Cyclopentanedicarboxylic acid ,4-(1,1-dimethylethyl)-,dimethyl with retention time (RT)= 13.975.

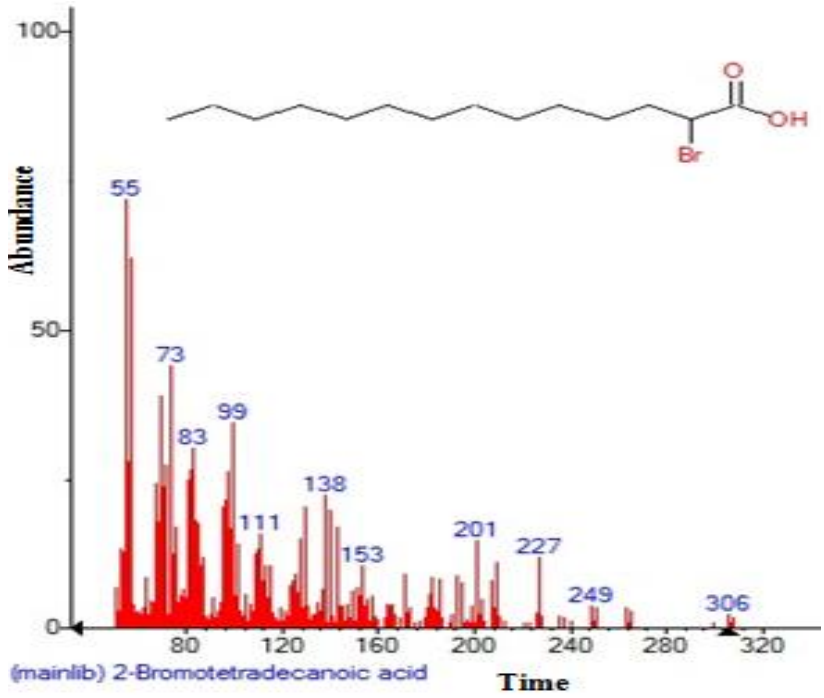


Figure 31. Mass spectrum of 2-Bromotetradecanoic acid with retention time (RT)= 14.771.

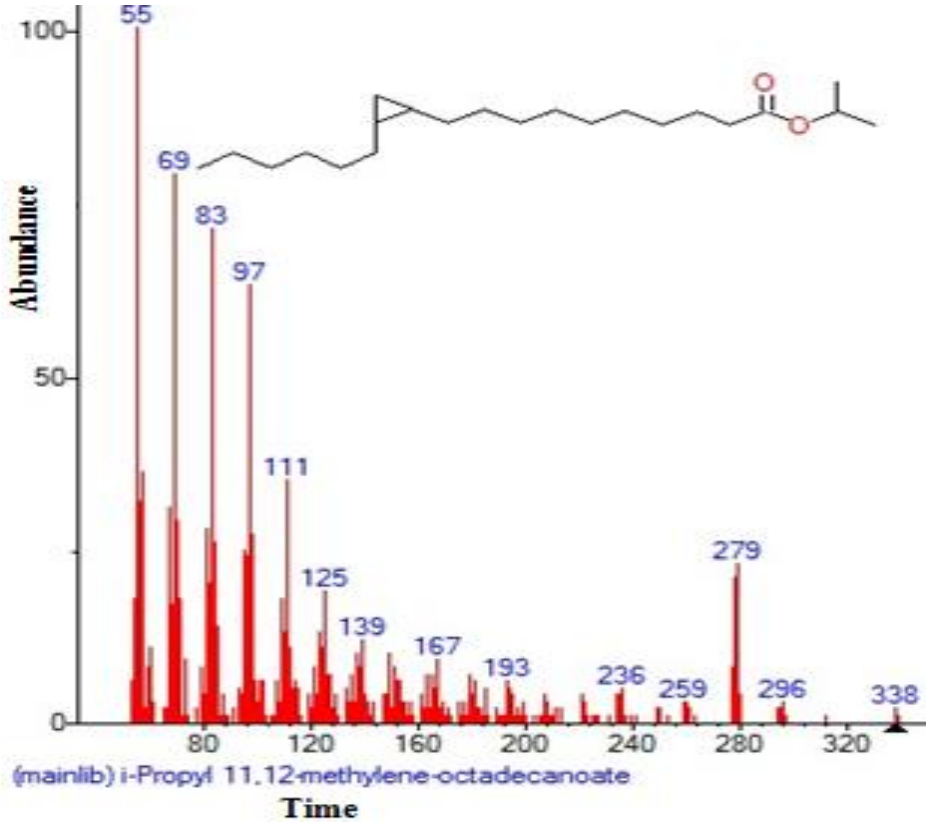


Figure 32. Mass spectrum of i-Propyl 11,12-methylene-octadecanoate with retention time (RT)= 15.022.

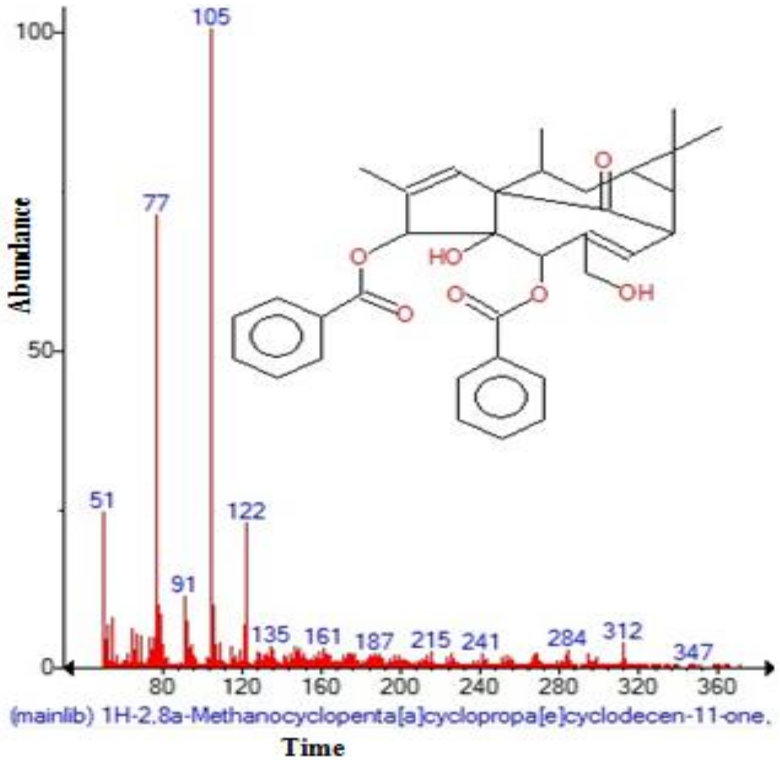


Figure 33. Mass spectrum of 1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecan-11-one with retention time (RT)= 17.214.

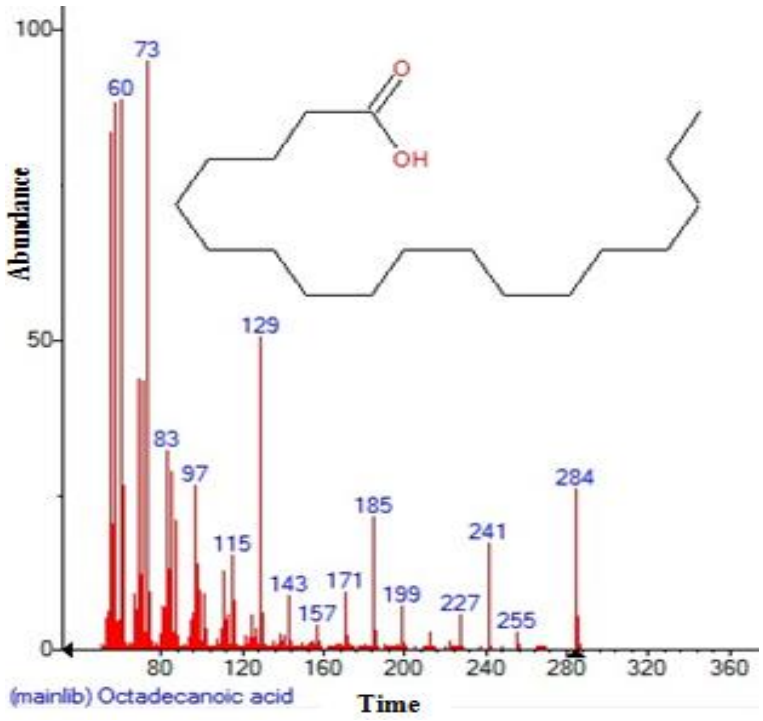


Figure 34. Mass spectrum of octadecanoic acid with retention time (RT) = 17.048.

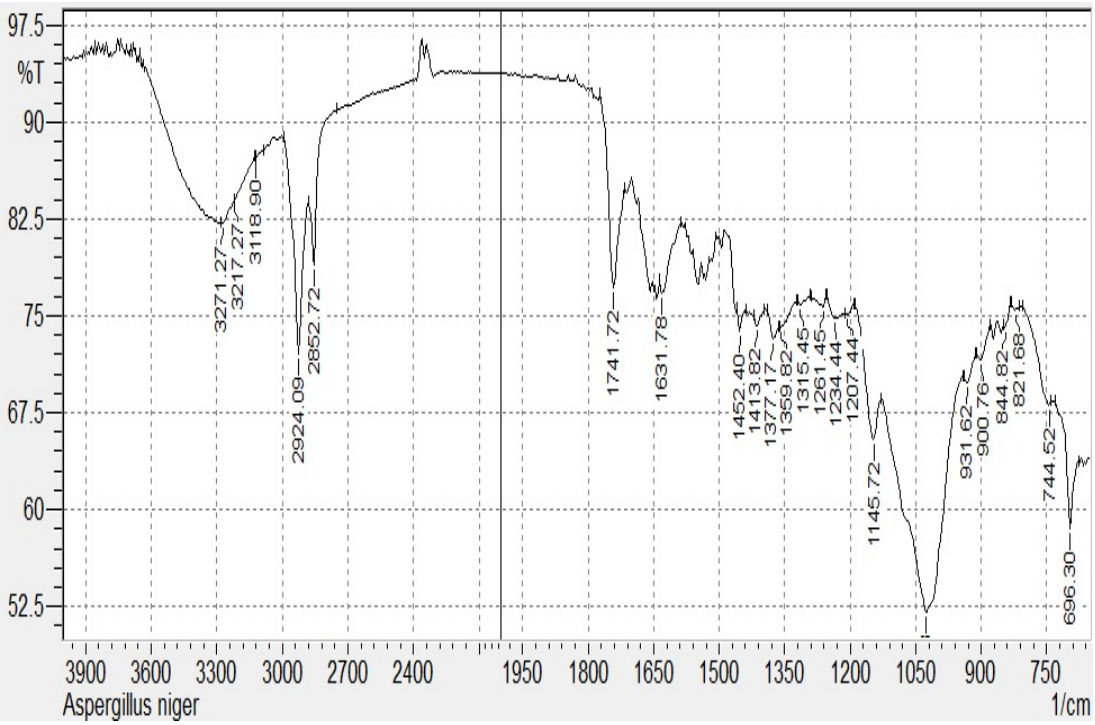


Figure 35. Fourier-transform infrared spectroscopy peak values of *A. niger*

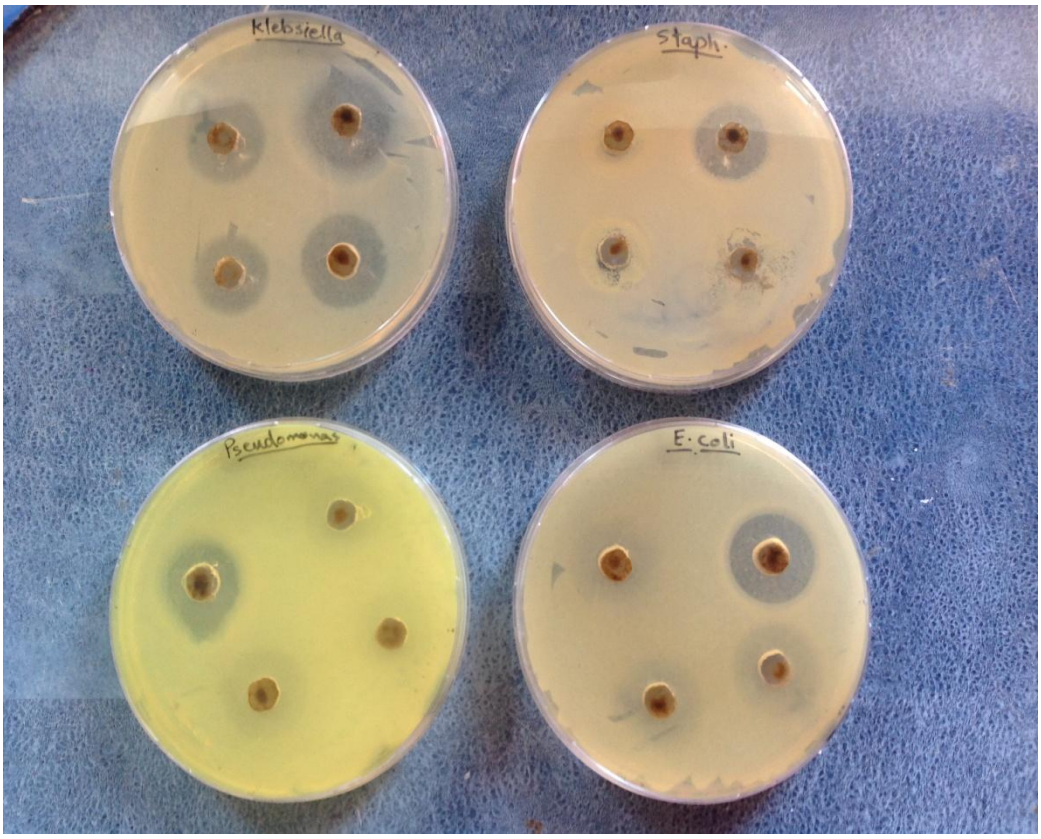
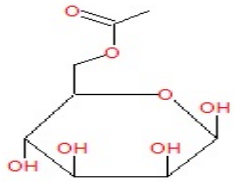
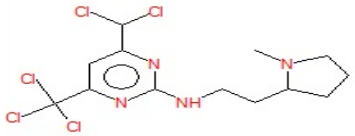
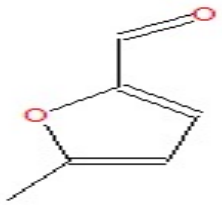
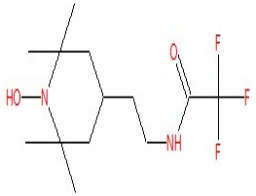


Figure 36. Antimicrobial activity of *A. niger.*

Table 1. Major bioactive chemical compounds identified in methanolic extract of *Aspergillus niger*.

S/N	Phytochemical compound	RT (min)	Formula	Molecular weight	Exact mass	Chemical structure	MS Fragment- ions
1	6-Acetyl-ß-d-mannose	3.201	$C_6H_{14}O_7$	222	222.073953		60, 97, 126, 144, 163, 192
2	4-[Dichloromethyl]-2-[[2-[1-methyl-2-pyrrolidinyl]ethyl]amino-6-trichloro	3.613	$C_{13}H_{17}Cl_5N_4$	403	403.989586		54, 67, 84, 98, 110, 124, 141, 149, 177, 207
3	2-Furancarboxaldehyde,5-methyl	3.722	$C_6H_6O_2$	110	110.0367794		53, 81, 95, 110
4	2,2,2-Trifluoro-N-[2-(1-hydroxy-2,2,6,6-tetramethyl-piperidin-4-yl)	3.779	$C_{13}H_{23}F_3N_2O_2$	296	296.171164		69, 81, 109, 126, 140, 166, 192, 211, 265, 281

poaceae) were effective against *A. niger* (Table 4). *D. stramonium* was very highly active against *A. niger*. *A. niger* was found to be sensitive to all test medicinal plants, and mostly comparable to the standard reference antifungal drug amphotericin B and fluconazole to some extent.

CONCLUSION

The results of this study showed that *A. niger*

Table 1. Contd.

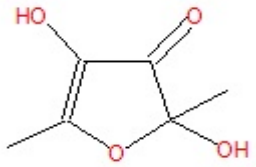
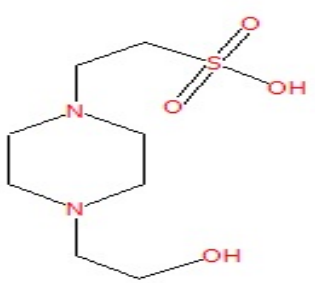
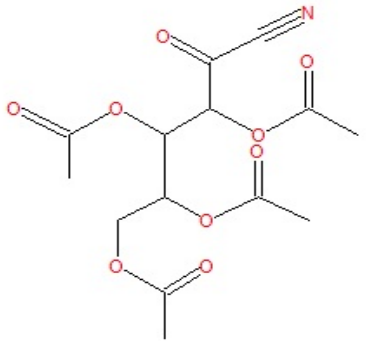
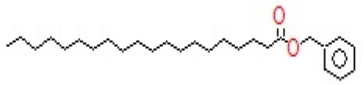
5	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	4.076	$C_6H_8O_4$	144	144.042258		55, 73, 84, 101,144
6	HEPES	4.271	$C_8H_{18}N_2O_4S$	238	238.098728		55, 65, 84, 99, 112, 143, 157, 174, 207, 237
7.	Tetraacetyl-d-xylonic nitrile	4.465	$C_{14}H_{17}NO_9$	343	343.090332		60, 67, 73, 95, 112, 133, 176, 197, 215, 233, 251, 270
8	Eicosanoic acid , phenylmethyl ester	4.546	$C_{27}H_{46}O_2$	402	402.349781		57, 71, 85, 91, 108, 126, 147, 167, 207, 281

Table 1. Contd.

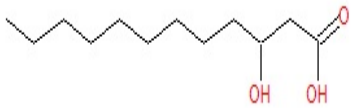
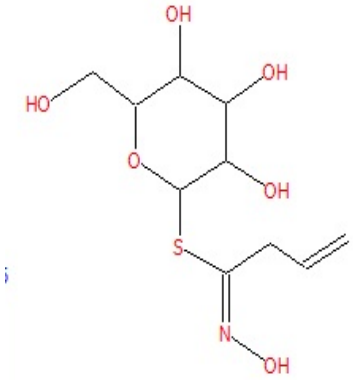
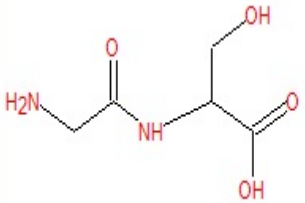
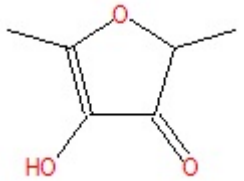
9	Dodecanoic acid , 3-hydroxy	4.574	$C_{12}H_{24}O_3$	216	216.125445		55, 69, 83, 96, 112, 138, 151, 180, 200
10	Desulphosinigrin	4.654	$C_{10}H_{17}NO_6S$	279	279.077658		60, 73, 85, 103, 127, 145, 163, 213, 262
11	Glycyl-dl-serine	4.763	$C_5H_{10}N_2O_4$	162	162064056		60, 74, 85, 114, 126
12	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	4.929	$C_6H_8O_3$	128	128.047344		57, 72, 85, 94,128

Table 1. Contd.

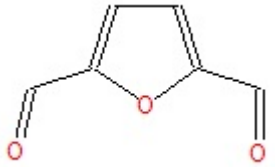
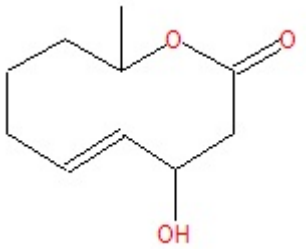
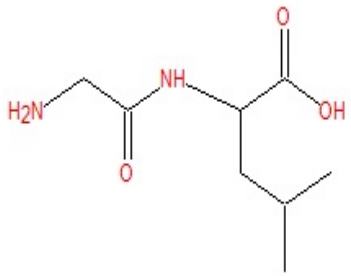
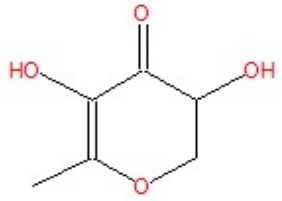
13	2,5-Furandicarboxaldehyde	5.066	$C_6H_4O_3$	124	124.016044		53, 67, 95, 124
14	2H-Oxecin-2-one,3,4,7,8,9,10-hexahydro-4-hydroxy-10-methyl-,[4	5.261	$C_{10}H_{16}O_3$	184	184.109944		55, 70, 81, 95, 112, 124, 142, 166, 184
16	DL-Leucine , N-glycyl	5.616	$C_8H_{16}N_2O_3$	188	188.116093		55, 86, 114, 132, 157, 188
18	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	5.942	$C_6H_8O_4$	144	144.042258		55, 72, 85, 101, 115, 144

Table 1. Contd.

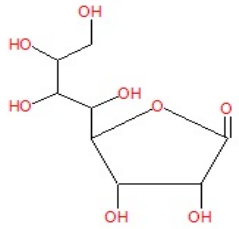
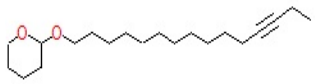
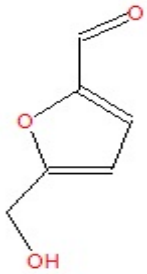
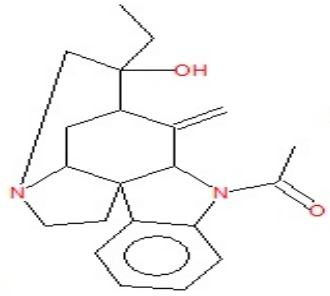
19	l-Gala-l-ido-octonic lactone	6.577	$C_8H_{14}O_8$	238	238.068868		61, 73, 84,112, 127, 142, 159, 189, 220
20	2H-Pyran,tetrahydro-2-(12-pentadecynyloxy)	6.737	$C_{20}H_{36}O_2$	308	308.27153		55, 67, 85, 101, 135, 156, 177, 198, 219, 255, 279, 307
21	5-Hydroxymethylfurfural	7.120	$C_6H_6O_3$	126	126.031694		53, 69, 81, 97, 126
22	Strychane,1-acetyl-20α-hydroxy-16-methylene	8.053	$C_{21}H_{26}N_2O_2$	338	338.199429		57, 70, 88, 97, 130, 166, 196, 224, 239, 253, 281, 295, 338

Table 1. Contd.

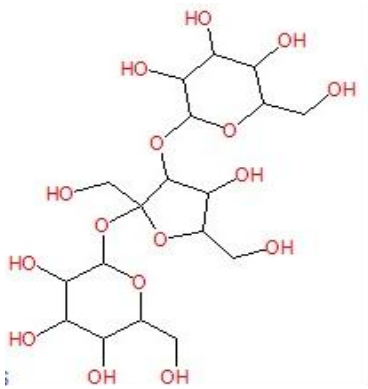
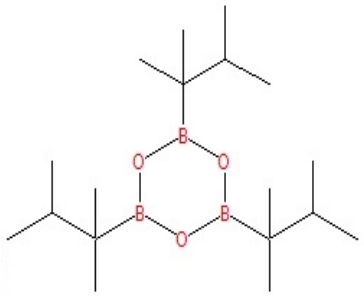
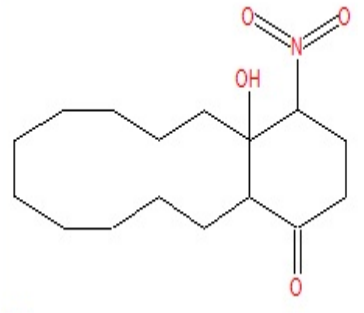
23	α-D-Glucopyranoside ,O-α-D-glucopyranosyl-(1.fwdarw.3)ß-D-fru	7.836		$C_{18}H_{32}O_{16}$	504	504.169035		60, 73, 85, 97,113, 126, 145, 187
24	Boroxin , tris(2,3-dimethylbut-2-yl)	8.442	$C_{18}H_{39}B_3O_3$		336	336.317837		55, 69, 84, 95, 115, 137, 157, 181, 209, 251, 292, 321
25	16-Nitrobicyclo[10.4.0]hexadecane-1-ol-13-one	8.797	$C_{16}H_{27}NO_4$		297	297.194008		55, 69, 81, 98, 126, 158, 173, 209, 249, 267, 297

Table 1. Cont'd.

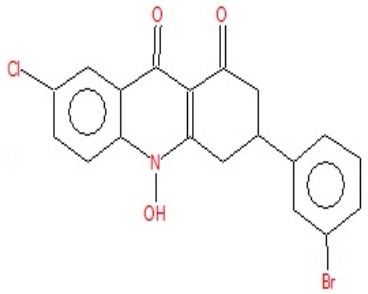
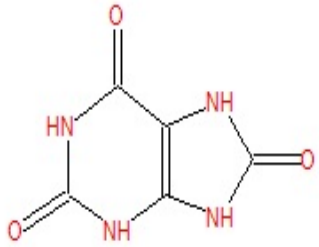
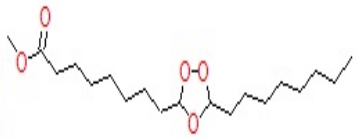
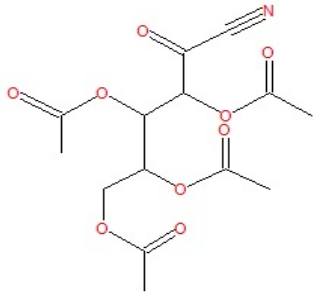
26	3-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9(2H,10H)-a	9.043	$C_{19}H_{13}BrClNO_3$	416	416.976732		57, 69, 97, 147, 219, 246, 321, 401, 419
27	Uric acid	9.672	$C_5H_4N_4O_3$	168	168.02834		54, 69, 82, 97, 125, 140, 168
28	1,2,4-Trioxolane-2-octanoic acid ,5-octyl-,methyl ester	11.320	$C_{19}H_{36}O_5$	344	344.256275		56, 69, 83, 98, 111, 143, 155, 185, 215, 259, 281, 311
29	Tetraacetyl-d-xylonic nitrile	12.276	$C_{14}H_{17}NO_9$	343	343.090332		60, 73, 112, 133, 164, 197, 228, 270

Table 1. Cont'd.

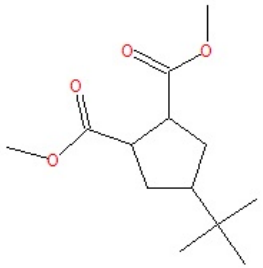
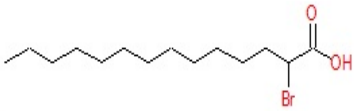
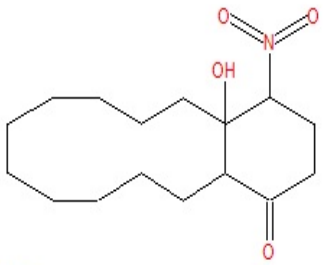
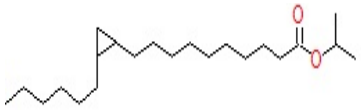
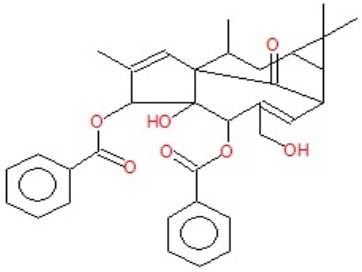
30	1,2-Cyclopentanedicarboxylic acid ,4-(1,1-dimethylethyl)-,dimethyl	13.975		$C_{13}H_{22}O_4$	242	242.151809		57, 67, 81, 107, 126, 154, 186, 211, 242
31	2-Bromotetradecanoic acid	14.771	$C_{14}H_{27}BrO_2$		306	306.119442		55, 73, 83, 99, 111, 138, 153, 201, 227, 249, 306
32	16-Nitrobicyclo[10.4.0]hexadecane-i-1-ol-13-one	14.908	$C_{16}H_{27}NO_4$		297	297.194008		55, 69, 81, 98, 126, 158, 173, 209, 221, 249, 267, 297
33	i-Propyl 11,12-methylene-octadecanoate	15.022		$C_{22}H_{42}O_2$	338	338.318481		55, 69, 83, 97, 111, 125, 139, 167, 193, 236, 259, 279, 296, 338
34	1H-2,8a-Methanocyclopenta[a]cyclopropa[e]cyclodecan-11-one	17.214		$C_{34}H_{36}O_7$	556	556.246105		51, 77, 91, 105, 122, 135, 161, 187, 215, 241, 284, 312, 347

Table 1. Cont'd.

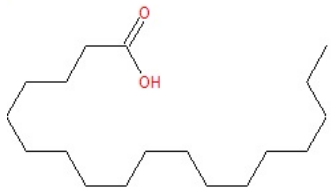
35	Octadecanoic acid	17.048	$C_{18}H_{36}O_2$	284	284.27153		60, 73, 83, 97, 115, 129, 143, 157, 171, 185, 199, 227, 241 ,255, 284

Table 2. Fourier-transorm infrared spectroscopy peak values of *A. niger.*

S/N	Peak (Wave number cm-ˡ)	Intensity	Bond	Functional group assignment	Group frequency
1	696.30	58.479	C-H	Aromatic rings	690-900
2	744.52	68.028	C-H	Alkenes	675-995
3	821.68	75.498	C-H	Alkenes	675-995
4	844.82	74.141	C-H	Alkenes	675-995
5	900.76	71.557	C-H	Alkenes	675-995
6	931.62	69.887	C-H	Alkenes	675-995
7	1026.13	52.098	C-F stretch	Aliphatic fluoro compounds	1000-10150
8	1145.72	65.416	C-F stretch	Aliphatic fluoro compounds	1000-10150
9	1207.44	75.125	C-H	Tetiary amine, C-N stretch	1150-1207
10	1234.44	74.798	-	Unknown	-
11	1261.45	75.761	-	Unknown	-
12	1315.45	75.890	-	Aromatic nitro compounds	1310-1390
13	1359.82	74.081	-	Aromatic nitro compounds	1310-1390
14	1377.17	73.205	-	Aromatic nitro compounds	1310-1390
15	1413.82	74.198	-	Ammonium ions	1390-1430
16	1452.40	73.841	-CH3	Methyl-CH. asym	1430-1470
17	1631.78	76.752	-	Organic nitrate	1620-1640
18	1741.72	77.128	-	Unknow	-
19	2852.72	78.925	-	Methylene-CH. asym	2840-2860
20	2924.09	72.033	-	Methylene-CH. asym	2915-2935
21	3118.90	87.299	-	Unknown	-
22	3217.27	83.936	O-H	Normal polymeric O-H stretch	3200-3400
23	3271.27	82.140	O-H	Normal polymeric O-H stretch	3200-3400

Table 3. Zone of inhibition (mm) of test bacterial strains to *A. niger* bioactive compounds and standard antibiotics.

Fungal products Antibiotics	Bacteria				
	Klebsiella pneumonia	***Pseudomonas eurogenosa***	***Staphylococcus aureus***	***Proteus mirabilis***	***Escherichia coli***
Fungal products	6.52±0.61	4.71±0.52	6.16±0.42	5.51±0.62	6.30±0.43
Rifambin	1.12±0.1	1.10±0.1	1.21±0.5	0.60±0.1	0.81±0.2
Streptomycin	1.25±0.3	1.11±0.3	1.30±0.5	1.73±0.2	1.34±0.6
Kanamycin	0.82±0.3	0.53±0.4	0.60±0.2	0.46±0.1	0.92±0.1
Cefotoxime	1.29±0.5	1.50±0.1	1.27±0.1	1.22±0.6	1.25±0.3

Table 4. Zone of inhibition (mm) of test different bioactive compounds and standard antibiotics of plants to *A. niger*.

S/N	Plant	Zone of inhibition (mm)
1	*Nerium olender* (Alkaloids)	4.19±0.25
2	*Ricinus communis* (Alkaloids)	4.70
3	*Datura stramonium* (Alkaloids)	7.81±0.61
4	*Linum usitatissimum* (Crude)	7.60±0.50
5	*Anastatica hierochuntica* (Crude)	3.52±0.09
6	*Gramineae poaceae* (Crude)	7.50±0.13
7	Amphotericin B	5.0±0.20
8	Fluconazol	13.0±0.00
9	Control	0.00

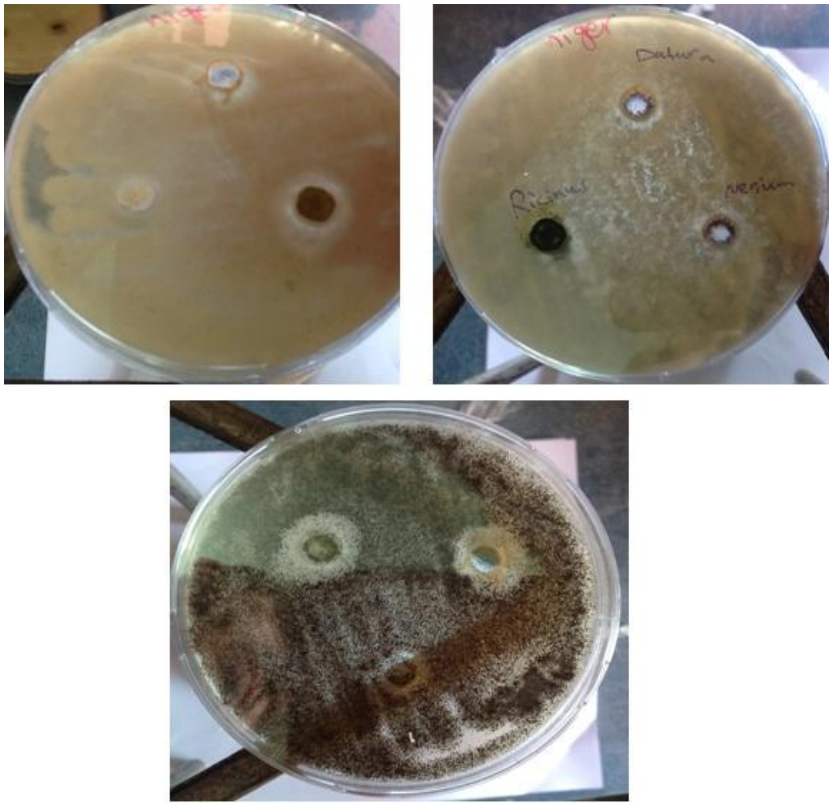


Figure 37. Antifungal activity of extract plant on *A. niger.*

produce many important secondary metabolites with high biological activities. Based on the significance of employing bioactive compounds in pharmacy to produce drugs for the treatment of many diseases, the purification of compounds produced by *A. niger* can be useful.

ACKNOWLEDGEMENTS

The author's sincere thanks go to Dr. Ali Al-Marzuqi for providing them with the opportunity to work on this study. They also would like to thank Zainab Al-Habubi from the Department of Biology for her guidance and help in the laboratory work.

Conflict of interest

Authors have none to declare.

REFERENCES

Ameera OH, Imad HH, Huda J, Muhanned AK (2015). Determination of Alkaloid Compounds of *Ricinus communis* by gas chromatography-mass spectroscopy (GC-MS). J. Med. Plant. Res. 9(10):349-359.

Anesini C, Perez C (1993). Screening of plants used in Argentine folk medicine for antimicrobial activity. J. Ethnopharmacol. 39:119-128.

Anupama M, Narayana KJ, Vijayalakshmi M (2007). Screening of streptomyces perpuofucus for antimicrobial metabolites. Res. J. Microbiol. 2:992-994.

Baker SE (2006). *Aspergillus niger* genomics: past, present and into the future. Med. Mycol. 44:17–21.

Bellini C, Antonini P, Ermanni S (2003). Malignant otitis externa due to *Aspergillus niger*. Scand. J. Infect. Dis. 35(4):284-288.

Chacko S, Vijay S, Ernest D (2012). A comparative study on selected marine actinomycetes from pulicat, Muttukadu, and Ennore estuaries. Asian Pac. J. Trop. Biomed. 2(3):S1827-S1834.

Gebreselema G, Feleke M, Samuel S, Nagappan R (2013). Isolation and characterization of potencial antibiotic producing actinomycetes from water and sediments of lake Tana, Ethiopia. Asian Pac. J. Trop. Biomed. 3(6):426-435.

Horvath JA, Dummer S (1996). The use of respiratory-tract cultures in the diagnosis of invasive pulmonary aspergillosis. Am. J. Med. 100:171-178.

Huda J, Ameera OH, Imad HH, Muhanned AK (2015a). Characterization of alkaloid constitution and evaluation of antimicrobial activity of *Solanum nigrum* by using (GC-MS). J. Pharmacogn. Phytother. 7(4):56-72.

Huda J, Imad HH, Muhanned AK (2015b). Analysis of alkaloid phytochemical compounds in the ethanolic extract of *Datura stramonium* and evaluation of antimicrobial activity. Afr. J. Biotechnol. 14(19):1668-1674.

Imad H, Ameer I, Mohammed A, Cheah Y, Aamera J (2014a). Haplotypes and variable position detection in the mitochondrial DNA coding region encompassing nucleotide positions 10,716–11,184. Mitochondrial DNA 26(4):544-9.

Imad H, Mohammed A, Aamera J, Ameer I, Cheah Y (2014b). Haplotype data of mitochondrial DNA coding region encompassing nucleotide positions 11,719–12,184 and evaluate the importance of these positions for forensic genetic purposes in Iraq. Mitochondrial DNA 4:1-4.

Imad HH, Huda J, Muhanned AK, Ameera OH (2015c). Alkaloid constitution of *Nerium oleander* by using gas chromatography- mass specroscopy (GC-MS). J. Med. Plant. Res. 9(9):326-334.

Imad HH, Israa A, Hawraa J (2015a). Gas chromatography mass spectrum and fouriertransform infrared spectroscopy analysis of methanolic extract of *Rosmarinus oficinalis* leaves. J. Pharmacogn. Phytother. 7(6):90-106.

Imad HH, Mohammed AJ, Muhanned AK (2015b). Forensic analysis of mitochondrial DNA hypervariable region HVII (encompassing nucleotide positions 37 to 340) and HVIII (encompassing nucleotide positions 438-574) and evaluate the importance of these variable positions for forensic genetic purposes. Afr. J. Biotechnol. 14(5):365-375.

Mogensen JM, Frisvad JC, Thrane U, Nielsen KF (2010). Production of fumonisin B2 and B4 by Aspergillus niger on grapes and raisins. J Agric. Food Chem. 58:954-8.

Mohammed A, Imad H (2013). Autosomal STR: From locus information to next generation sequencing technology. Res. J. Biotechnol. 8(10):92-105.

Muhanned AK, Ameer IA, Imad HH, Mohammed AJ (2015). A New Polymorphic Positions Discovered in Mitochondrial DNA Hypervariable Region HVIII From Central and North-Central of Iraq. Mitochondrial DNA 23:1-5.

Perfect JR, Cox GM, Lee JY (2001). The impact of culture isolation of Aspergillus species: a hospital-based survey of aspergillosis. Clin. Infect Dis. 33:1824-1833.

Perrone G, Susca A, Cozzi G, Ehrlich K, Varga J, Frisvad JC (2007). Biodiversity of Aspergillus species in some important agricultural products. Stud. Mycol. 59:53-66.

Rukayadi Y, Yong D, Hwang JK (2006). In vitro anticandidal activity of xanthorrhizol isolated from Curcuma xanthorrhiza Roxb. J. Antimicrob. Chemother. 57:1231-1234.

Segal BH, DeCarlo ES, Kwon-Chung KJ, Malech HL, Gallin JI, Holland SM (1998). *Aspergillus nidulans* infection in chronic granulomatous disease. Medicine (Baltimore) 77:345-54.

Susca A, Proctor RH, Mule G, Stea G, Ritieni A, Logrieco A (2010). Correlation of mycotoxin fumonisin B2 production and presence of the fumonisin biosynthetic gene fum8 in Aspergillus niger from grape. J. Agric. Food Chem. 58:9266-7922.

Usha NS, Masilamani SM (2013). Bioactive compound produced by streptomycin strain. Int. J. Pharm. Pharm. Sci. 5(1):0975-14.

Walsh TJ (2004). Detection of galactomannan antigenemia by enzyme immunoassay for the diagnosis of invasive aspergillosis: variables that affect performance. J. Infect. Dis. 190:641-49.

academicJournals

Vol. 7(8), pp. 164-182, August 2015
DOI: 10.5897/JPP2015.0355
Article Number: 4426F5554312
ISSN 2141-2502

http://www.academicjournals.org/JPP

Journal of Pharmacognosy and Phytotherapy

Full Length Research Paper

Ethnobotanical survey of healing medicinal plants traditionally used in the main Moroccan cities

Messaoudi Moussi I.*, Filali, H., Tazi, A. and Hakkou, F.

Laboratory of Pharmacology and Toxicology, Faculty of Medicine and Pharmacy of Casablanca, University Hassan II. Morocco.

Received 6 June 2015; Accepted 13 July 2015

The present study is a survey conducted to indicate the healing medicinal plants, traditionally used by the Moroccan population, and especially to select the healing plants mainly used in the form of essential oils. This survey allowed an inventory of 59 species of healing plants belonging to 37 families. The results showed that the whole plant is the most commonly used part (31%), followed by the leaves (29%). Most plants are Moroccan and are used as a single powder and as essential oils. The most common frequency of use is 2 times per day and the treatment duration depends mainly, on the pathological field and the nature of the wound. Also, the results revealed that plants whose leaves are the most used as essential oils are: *Rosmarinus officinalis*, *Lavandula angustifolia* and *Artemisia tridentata.* This study showed that medicinal plants play an important role in healing practices. It's a very valuable source of information for the studied areas and for national medicinal flora. So, it could be a database for further research in the field of herbal medicine, pharmacology and in order to manufacture new drugs based on medicinal plants.

Key words: Medicinal plants, pharmacological properties, healing, traditionnal medicine, ethnobotanical survey, Morocco.

INTRODUCTION

Since ancient times, man has always used medicinal plants to treat himself; and for fifteen years, researches have increased worldwide and have sought to investigate the pharmacological activities of these plants. Indeed, it is estimated that 80% of the population of developing countries, use traditional medicine (Bousta and Ennabili, 2011). These countries include Morocco, whose knowledge of phytotherapy and traditional medicine is transmitted by the culture through generations. These knowledge are developed, and enriched, thanks to the strategic geographic position of the kingdom.

The geographical position of Morocco provides a remarkable range of bioclimates, a great biodiversity, a good wealth of medicinal and aromatic plants and a variety of traditional knowledge. Indeed, the Moroccan flora is composed of over 4200 species and subspecies belonging to a large botanical known family, with 130 families and 940 species represented by: *Ateraceae*, *Fabaceae*, *Poaceae*, *Brassicaceae*, *Caryophylaceae*, *Lamiaceae*, *Apiaceae*, *Scrophulariaceae* and other families (Bellakhdar, 1998). Medicinal plants are therefore a precious heritage for humanity and especially for Morocco.

Wounds have always had a considerable impact on

*Corresponding author. E-mail: messimane38@yahoo.fr.

Figure 1. Map of Morocco showing the surveyed cities, Source: http://www.diplomatie.gouv.fr/fr/dossiers-pays/maroc/.

health especially those related to chronic skin wounds constitute a major health burden in developing countries (Agyarea et al., 2009). Subsequently, surgical wounds present in Morocco, a problem of drug therapy.Inflammation,swelling, pain and infection due to these wounds are closely related properties and involved in a big number of skin trauma.

To complete the partial and fragmentary studies conducted everywhere in Morocco, ethnobotanical surveys help to gather a very valuable source of information, ready to be scientifically exploited. In this context, the present study is part of the latter surveys. It aims to make a thorough inventory of medicinal plants with healing properties, traditionally used in Morocco and to choose the healing plants that are likely to be used in subsequent studies.

MATERIALS AND METHODS

Area of the study

Morocco is located at the north western tip of the African continent, separated from Spain by the 14 km of the Strait of Gibraltar. It is bathed in the west by the Atlantic Ocean and in the north by the Mediterranean Sea, both of which give it two coastlines spanning nearly 3500 km. Constituting a North-South passage area, Morocco has a surface of 710,850 km² and belongs to both the Mediterranean and the Sahara worlds (Bellakhdar, 1998). Due to its strategic location, Morocco has a rich flora. Changes in the climate and terrain are the major factors that can explain this floral wealth. Indeed, it has two coastlines and therefore receives rain streams from the Atlantic Ocean, which accumulate against the mountain barriers of the Atlas. This leads to heavy rainfalls in the cities of Rabat, Casablanca, Fez and the formation of snow in the high peaks of the Atlas, while the south and east stay arid.Moreover, the botanical interest of Morocco is intense and contains at least 2.5 million hectares of forest (about 15% of the total area) which contains cedars, palms, date palms, fig trees, olive trees, almond trees, acacias, fruit trees, cork oaks, pines, eucalyptus, and endemic plant that is the Argan tree, which is found nowhere else in the world, but in Morocco. (Bellakhdar, 1998).

Among the big and great cities in this country, we find Casablanca, Marrakech, Agadir, Fez, Tetouan, Tangier and Oujda, in addition to the towns of Mohammedia, Salé, Kenitra and Taroudant in the Agadir area. The study chose to carry out its investigation in these cities because they belong to the most populated regions of Morocco. They are not only best known for their richness on medicinal plants, climate and biodiversity, but also for their cultural diversity. They are a home from the numerous herbalists who live there. The surveyed cities (framed in red) are illustrated in Figure 1.

METHODOLOGY

This study was conducted to establish an inventory, as complete as possible, of medicinal plants traditionally used because of their healing properties by herbalists located in the main Moroccan cities to treat their patients. The study investigation lasted about five months (from 21 September, 2014 to 16 January, 2015). The total number of herbalists contacted is 202 whose distribution is represented in Table 1.

To have an overview of the local traditional uses and the pharmacopoeia's floristic diversity of these Moroccan cities, the ethnobotanical survey was conducted using series of direct and telephonic interviews with herbalists of the cities cited in Table 1. It was carried out using a predetermined questionnaire with specific questions about the healing plant: its local name, its origin (country,

Table 1. Number of herbalists contacted per city.

Surveyed cities	Number of herbalists	Surveyed cities	Number of herbalists
Casablanca	80	Marrakech	20
Fès	20	Tanger	18
Kenitra	8	Tétouan	5
Mohammedia	5	Agadir	10
Rabat	18	Taroudant	3
Salé	12	Oujda	3

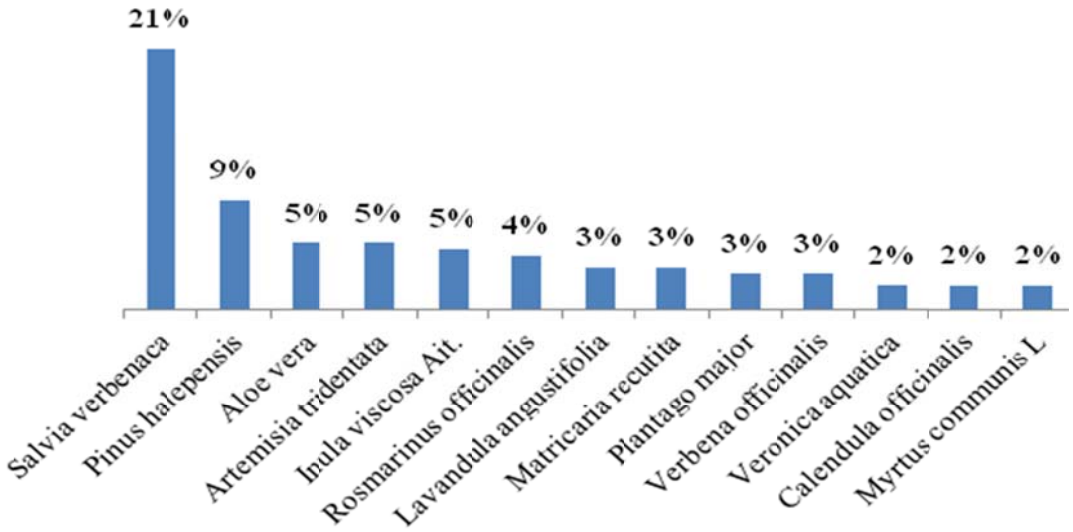


Figure 2. Distribution of collected healing plants.

region or city), its harvest season, its parts used, its form of preparation, the administration way of the plant, the frequency of use per day, the duration of the treatment, and if the plant is used alone or in combination with other plants.

Interviews were conducted in the dialect language of the country. The identification of the scientific names of the medicinal plants was carried out by a Professor Pharmacognosist at the High School of Technology (EST) in Casablanca, and with reference to the book of traditional medicinal plants (Bellakhdar, 1998). The study was able to collect 211 questionnaires in relation with the healing plants. Among the 202 herbalists contacted, 152 accepted to answer to the questions, while the others either refused to respond to our request, or were not specialized in medicinal plants or were unreachable.

The study was especially interested to find a relationship between the criteria of the plants that were collected during the investigation, in order to highlight information that will be used for the selection of healing plants for further experimental studies.

Statistical analysis

The counting of the results was carried out by an established mask on Statistical Package for the Social Sciences (SPSS) software, version 21. The relationship between the variables was evaluated by the test of independence Chi-square (χ^2) with a confidence level of 95% and by calculating the *p*-value. The results are considered significant when the *p*-value is less than 0.05.

RESULTS

Floristic screening

Scientific name of the listed plants

The survey allowed an inventory of 59 species of healing medicinal plants. So, the analysis identified several plants but the most represented are: *Salvia verbenaca* at a frequency of 21%, *Pinus halepensis (*9%), *Aloe vera* (5%)**,** *Artemisia tridentata* (5%), *Inula viscosa ait.* (5%), *Rosmarinus officinalis* (4%) and *Lavandula angustifolia* of 3%. The classification of these species by their frequency is represented in Figure 2. All the indexed healing plants and their corresponding properties are listed in Table 6 and 7,

Families of healing plants

The recorded healing medicinal plants belong to 37 families four of which are predominant: *Lamiaceae* (34%), *Asteraceae* (18.7%), *Pinaceae* (8.9%) and

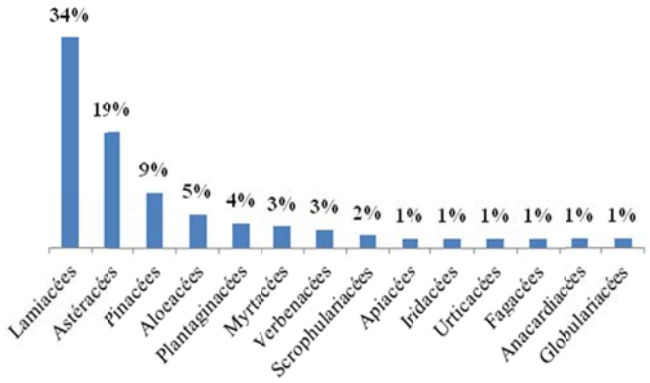


Figure 3. Distribution of healing plants families.

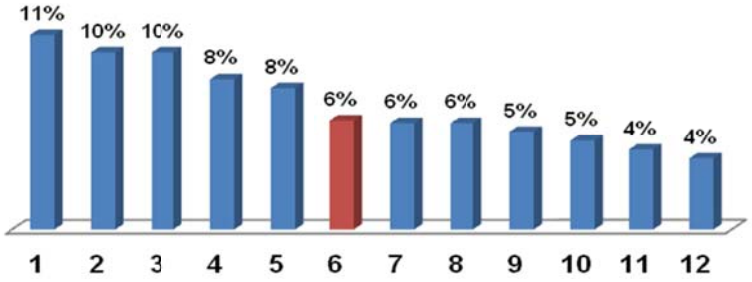


Figure 4. Distribution of healing plants based on Moroccan regions, (1) Souss-Massa-Draa, (2) Marrakech-Tensift-Al Haouz, (3) Gharb-Chrarda-BeniHssen, (4) Meknes-Tafilalet, (5) Tangier- Tetouan (6) No Moroccan, (7) Taza-Al Hoceima-Taounate, (8) Fez-Boulemane, (9) Tadla-Azilal, (10) Oriental, (11) Doukkala-Abda, (12) Rabat-Sale-Zemmour- Zaër.

Aloaceae (5.4%). The rest of results are shown in Figure 3.

Origin of plants

The plants were classified according to their original area and where they are most prevalent. Most of Moroccan healing plants are distributed in the region of Souss-Massa-Draa (11%), followed by the region of Marrakech-Tensift-Al Haouz and the region of Gharb-Chrarda-Beni Hssen with 10%, while 6% of the plants are not Moroccan Figure 4.

Plants season

The availability and distribution of plants depend on the season and climate. The study obtained information on the harvest season for each plant. Spring is the season with high percentage of plants (50%), followed by summer (14%), winter (9%) and autumn (5%). Figure 5 illustrates the obtained results.

Ethnobotanical and pharmacological screening

Used parts of plant

The used parts of plants were collected and classified according to their order of importance. The whole plant is the most commonly used form with a frequency of 31%, followed by leaves with 29%, flowers (12%), bark (7%) and seeds (7%). The term "Other" refers to other parts of the plant found, as mucilage for *Aloe vera* species and stigma for *Crocus sativum* species. Figure 6 includes all these results.

Form of use

To facilitate the use of medicinal plants, herbalists recommend several types of preparations: powder, decoction, infusion and cold water, use as essential oils and many others. The most widely used form is a single powder of 57%, followed by the form of essential oils of 22% and by other forms (11%), like the application of the plant directly on the wound, or formed into a wound

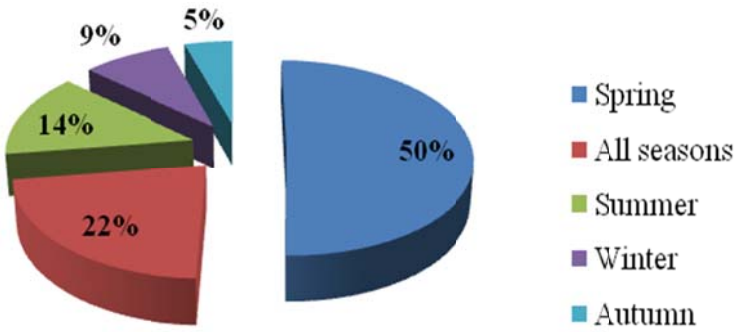


Figure 5. Distribution of healing plants by season.

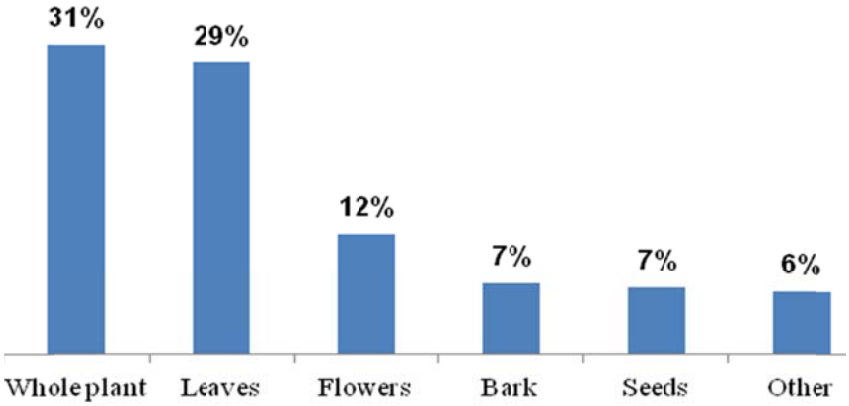


Figure 6. Distribution's frequency of used parts of healing plants.

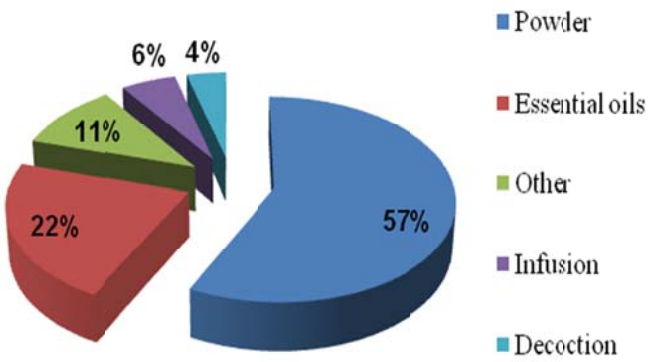


Figure 7. Dosage form of recorded healing plants.

dressing or applied as a cream Figure 7.

Way of administration

The most common ways of drugs administration are dermal and oral. In this study, the extreme majority staffs for dermal administration (90.5 %), 16% for a combination of dermal and oral administration and 2% for the oral way were found out.

Frequency and duration of use

According to each herbalist, medicinal plants are applied one to five times a day and the diagnosis may sometimes

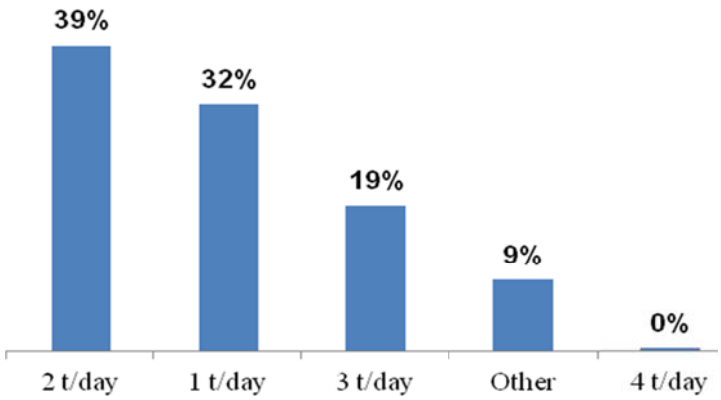


Figure 8. Frequencies of use of healing plants.

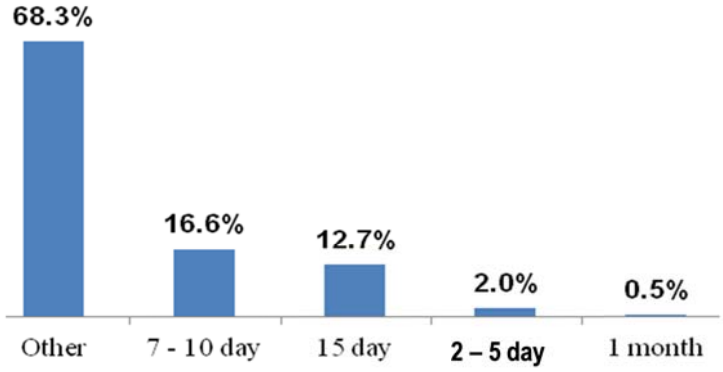


Figure 9. Distribution's frequency of the treatment's duration.

vary depending on the wound**.** The treatment period for its part, can extend to a month, but that depends on the pathological field (case of the patient) and on the depth of the wound. In this study, 39% for a frequency use of 2 times a day (morning and evening), 32% for 1 time a day (especially at night), 19% for 3 times a day, and 9% represents the frequency that depends on the wound and the treated patient's case were obtained (Figure 8). For the duration of treatment, the majority of herbalists were unable to inform us about the exact duration of therapy because it depends on the wound, the patient's healing duration, the response to the treatment and the effectiveness of this latter. The study got a major frequency of 68.3% for the criterion "Other" (depending to the wound, to the patient's case and the response of the latter to the treatment), 16.2% for a period of 1 week to10 days and 12.7% for a period of 2 weeks (Figure 9).

Plants association

According to herbalists, there are plants that can be associated with other species or mixed with vegetable or essential oils from other plants. The study investigation revealed that the predominant part (66.4 %) is for a single use of plants without any association with other species and the rest (33.6 %) is for the association of plants with others. Names and frequencies of associated plants are listed in Table 2.

Evaluation of the independence of variables with Chi-square test

Let X and Y be two qualitative variables with l and k modalities, respectively. The study test that X and Y are independent (H_0). The Chi-square variable is associated with a number of degrees of freedom (v) calculated including: $v = (k-1)\ (l-1)$ (Mountassir, 2014). The study also based on the calculation of p value to test the independence of two variables. The test is significant and H_0 is rejected when the p value is less than 0.05.

Crossing between used parts and form of use

Consider X as the qualitative variable that represents the part of the plant used and Y qualitative variable that

Table 2. List and frequencies of associated plants.

Healing plant	Associated medicinal plants
Aloe vera	*Myrtus communis L.*(1)
Arnica L.	*Artemisia absinthium* (1)
Artemisia tridentata	*Lavandula angustifolia* (3)*, Myrtus communis L.* (1), *Matricaria recutita* (1)*, Thymus vulgaris* (1)*, Artemisia absinthium* (1)*, Quercus faginea lamk* (1)*, Rhus albidum schousb.* (1)*, Tea melaleuca* (1)
Calendula officinalis	*Lavandula angustifolia (1), Rosmarinus officinalis (1)*
Inula viscosa ait.	*Argania spinosa* (2), *Matricaria rectutita* (1)
Saponaria vaccaria L.	*Argania spinosa* (1)
Globularia alypum L.	*Salvia verbenaca* (1)
Lavandula angustifolia	*Argania spinosa* (1)
Marrubium vulgare	*Artemisia abstinthium* (1)
Rosmarinus officinalis	*Calendula officinalis* (1), *Thymus vulgaris* (1)
Salvia verbenaca	*Plantago major* (6), *Verbena officinalis* (9), *Pinus halepensis* (6), *Thymus vulgaris* (1), *Plantago psyllium* (1), *Inula viscosa ait.* (1), *Argania spinosa* (1), *Myrtus communis L* (1), *Artemisia tridentata* (1), *Marrubium vulgare* (2), *Lavandula angustifolia* (1)
Thymus vulgaris	*Rosmarinus officinalis* (1)
Laurus nobilis L.	*Argania spinosa* (1)
Smilax aspera L.	*Matricaria recutita* (1)
Pinus halepensis	*Salvia verbenaca* (2), *Verbena officinalis* (2), *Artemisia tridentata* (2), *Myrtus communis L.* (1), *Marrubium vulgare* (1), *Plantago major* (1)
Plantago major	Salvia verbenaca (1), Verbena officinalis (1)
Urtica dioica	Thymus vulgaris(1), Rosmarinus officnalis(1)
Verbena officinalis	Salvia verbenaca (1), Plantago major (1), Papaver rhoeas L. (1)
Curcuma longa.	Artemisia tridentata (1), Marrubium vulgare (1), Allium cepa (1)

(Frequency).

Table 3. Contingency table: Used parts*form of use.

Form of use/ Part	Leaf	Flower	Roots	Bark	Fruit	Seeds	Whole plant	Other	Total
Powder	2.98	0.21	0.00	2.86	0.06	0.14	4.96	0.64	11.85
Decoction	0.70	0.34	0.32	0.03	0.52	0.64	0.97	0.01	3.53
Infusion	4.70	0.51	0.19	0.94	0.59	0.07	2.71	0.47	10.18
Essential oils	1.65	1.85	0.79	1.73	0.33	0.01	1.76	0.51	8.64
Other	0.00	0.00	0.43	1.83	0.64	0.09	1.58	5.37	9.95
Total	10.05	2.91	1.74	7.40	2.14	0.95	11.97	6.99	44.15

represents the use form of the plant. The study want to test that X and Y are independent (H_0), for a significance level of 95% using a contingency table with double entry. Table 3 summarizes the results of this test. According to this table, $v = 28$, $\chi^2_{obs}=44.15$ and $\chi^2_{v;\alpha}=16.93$ so $\chi^2_{obs} > \chi^2_{v;\alpha}$. Therefore, H_0 is rejected.

Crossing between used parts and use form as essential oils

Let the qualitative variable X: Used parts, and Y: Use form as essential oils. According to the contingency Table 4, $v=7$, $\chi^2_{obs}=69.1$ and $\chi^2_{v;\alpha}=2.17$ so $\chi^2_{obs} > \chi^2_{v;\alpha}$. There-

Table 4. Contingency table: used parts*use as essential oils form.

Form of use	Leaves	Flower	Roots	Bark	Fruit	Seeds	Whole plant	Other	Total
Use as essential oils	47.81	1.36	5.88	5.88	2.91	0.98	1.36	2.91	69.10

fore H_0 is rejected.

In order to exactly know the part of the plant which is most often used in the form of essential oils, or in other words, the part of the plant which is most often used as essential oils form, the study has made the crossing between using form as essential oil and every part of the plant (leaf, flower, fruit, root, bark, seeds and whole plant) (Table 5). From Table 5, the *p* value of the Chi-square test of 0.003 (less than 0.05) made the study to conclude that the use in the form of essential oils depends on the leaves.

For the second crossing, the *p* value: 0.116 is higher than 0.05, the study doesn't reject H_0, and reached the conclusion that the use form of essential oils doesn't depend on the flowers. The *p* value of the third crossing (0.214) higher than 0.05 doesn't reject H_0 and conclude for an independence between the use form as essential oils and roots (at a confidence level of 95%). For the fourth crossing, the *p* value (0.06) is higher than 0.05; therefore study didn't reject H_0 and conclude that the use form as essential oils is independent of the barks. For the fifth crossing, the *p* value (0.663) is higher than 0.05, so the study didn't reject H_0 and conclude that the use form as essential oils doesn't depend on the fruit. Then, the *p* value (0.567) of the sixth crossing is higher than 0.05, so the study did not reject H_0 and conclude that the use form as essential oils does not depend on seeds. Finally, according to the Chi-square of the seventh crossing, the *p* value is less than 0.05. So the study rejects H_0 and concludes that the use in the form of essential oils depends on the whole plant.

Crossing between use form of essential oil and the healing plant

Here, the study wants to test if the use in the form of essential oils form depends on the plant species. According to Table 5, the *p* value is less than 0.05 (0.000). These results are significant and the H_0 hypothesis is rejected.

Crossing between use form of essential oil, leaf-part and healing plant

The study wants to show through this crossing that the use of the leaves in the form of essential oils depends on the healing plant species. Indeed, Table 5 gives a *p* value less than 0.05. Furthermore, plants whose leaves are more often used in the form of essential oils are: *R. officinalis*, *L. angustifolia*, *A. tridentata*, *M. communis L* and *T. vulgaris* (Figure 10)

DISCUSSION

The phytotherapy is frequently used by the Moroccan population. In fact, according to some study, 70 to 80% of Moroccan people use medicinal plants to heal: 60% of them are female, and more than 50% are illiterate (Zeggwagh et al., 2013). 55 to 90% of people use plants to treat chronic diseases of which 16.8% are for the dermatological affections and wounds in Fez (Zeggwagh et al., 2013). Moreover, in the region of Ksar Lakbir, 11.6% of plants are employed to treat skin diseases (Merzouki et al., 2000), 15% in the region of Essaouira (Mehdioui et al., 2007), 17% in Ifran (Rhafouri et al., 2014) and 12.5% in the region of Zaêr (Lahsissene et al., 2010). On the other hand, medicinal plants are used for dermatological affections by 27% in the region of El Hajeb (EL Amri el al., 2014), by 11.80% in the region of Agadir (El Hafian et al., 2014), by 10% in Kenitra (Salhi et al., 2010), by 12% in the region of Haut Atlas Oriental (Belamdini et al., 2014) and by 16% in the region of Meknes-Tafilalet (Fadil et al., 2014).

Indexed healing medicinal plants

A number of 59 species of healing medicinal plants has been inventoried in this study, of which majority are: *S. verbenaca*, *P. halepensis*, *A. vera*, *A. tridentata*, *I. viscosa* ait , *R. officnalis*, *L. angustifolia*. In parallel, the national literature review enabled us to screen 59 Moroccan healing plants including: *S. verbenaca*, *P. halepensis, I. viscosa ait., A. tridentata* and *A. vera, in addition to 54 other healing plants* (Bellakhdar, 1998). Another national ethnobotanical study shows that *A. vera, L. officinalis, M. recutita, M.s communis, C. officinalis* and *T. vulgaris* have wound healing properties (Sijelmassi, 2011). This shows the proximity of the results of this study with those found in the literature.

On the other hand, an ethnobotanical survey confirmed that *S. verbenaca*is has been employed by the local population to facilitate the wound healing, in the region of Zaër (Lahsissene et al., 2009), Haut Atlas Oriental (Belamdini et al., 2014) and of Settat (Bammi et al., 2002). Moreover, it has been shown that *S. verbenaca* and *I. viscosa ait*. have a healing potential on wounds,

Table 5. Chi-square test: use as essential oils and used part of plants.

Parameter		Pearson Chi-square			Fisher's exact test		Interpretation
Crossing		Value	DOF	P value	Signification exacte (bilateral)	Exact significance (unilateral)	Significance
1	Use as essential oils form*Leaves	8.787	1	0.003	0.004	0.003	S
2	Use as essential oils form*Flowers	2.466	1	0.116	0.168	0.092	NS
3	Use as essential oils form*Roots	1.541	1	0.214	0.293	0.195	NS
4	Use as essential oils form*Bark	3.391	1	0.066	0.075	0.052	NS
5	Use as essential oils form*Fruit	0.190	1	0.663	0.705	0.457	NS
6	Use as essential oils form*Seeds	0.327	1	0.567	0.556	0.380	NS
7	Use as essential oils form*Whole plant.	7.204	1	0.007	0.008	0.005	S
8	Use as essential oils form*Name ofplant.	128.265	55	0.000	-	-	S
9	Use as essential oils form*Leaf-part*Name of plant.	8.787	1	0.003	0.004	.003	S

*DOF: Degree Of Freedom; NS: No Significant; S: Significant.

Table 6. Inventory of healing plants recorded in our ethnobotanical survey.

Family	Scientific name	Local name	% of plant	Used part	Usedform	Additive	Frequency of use	Treatment duration	Administration way	Content in EO	Properties	Plants association	Corresponding References
Aloeaceae	*Aloevera*	الصبار	5.7%	Mg (4), Pu (2), L (2), Fl (1), W (2), Ba (1)	Di (6), Cr (1), Po (1), Dr (1), EO (3)	Olive oil (2), Argan oil (2)	1t/d (4), 2t/d (6), 3t/d (2), Other (1)	2 We (4), Other (8)	Cu (10)	Yes (4), No (3)	[1]	Yes	Sijelmassi (2011), Bellakhdar (1998)
Amaryllidaceae	*Allium cepa*	البصلة	0.5%	F (1)	Dr (1)	Honey (1)	1t/d (1)	3 D (1)	Cu (1), Or (1)		[2]	No	El Hafian et al. (2014)
	Pistacialenticus L.	الضرو	0.5%	F (1)	Po (1), EO (1)	-	4 t/d (1)	Other (1)	Cu (1)	Yes (1)	[3]	No	Daoudi et al (2015)
Anacardiaceae	*Pistaciaterebinthus L.*	ايك البطم	0.5%	F (1)	Po (1)	-	3t/d (1)	2 We (1)	Cu (1)	No (1)	[4]	No	-
	Rhusalbidums chousb.	الزواية	0.5%	Ba (1)	Po (1) Dec (1)	-	2 t/d (1) 3t/d (1)	2 We (1)	Cu (1)	No (1)	[5]	No	-
	Carum carvi L.	الكروية	0.5%	S (1)	Po (1)	-	2t/d(1)	Other(1)	Cu (1) Or (1)	-	[6]	No	-
Apiaceae	*Centellaasiatica*	القسط الهندي	0.5%	Ba (1), R (1)	Po (2)		1 t/d (1), 2 t/d (1)	Other (2)	Cu (2)	No (1)	[7]	No	-
	Coriandrum sativum	القزبر	0.5%	L (1), R (1) S	Po (1)		3 t/d (1)	1 We (1), 2 We (1)	Cu (1), Or (1)	Yes (1)	[8]	No	-

Table 6. Contd.

Aristolochiaceae	*Aristolochia long.*	برزطم	0.5%	Ro (1), Bo (1)	Po (1)	Honey (1)	1t/d (1)	1 Mo (1), Other (1)	Cu (1)	No	[9]	No	Bellakhdar (1998)
	Arnica L.	الحلحال	0.9%	L (1), Fl (1) W (1)	Po (1), Dec (1), EO (2), Dr (1)	Salt (1)	2 t/d (1), 3 t/d (2)	Other (2)	Cu (2)	Yes (2)	[10]	Yes	-
	Artemisia tridentata	الشيح	5.2%	L (6), Fl (2), W (2), S (2), Ro (1), F (1)	Po (7), Dec (3), Inf (3), EO (5), with honey, Di (1)	Argan oil (2), Honey (2), Grease (1)	1 t/d (3), 2 t/d (7), 3 t/d (4), 4 t/day (1), Other (1)	1 We (2), 2 We (2), Other (7)	Cu (11), Or (1)	Yes (9)	[11]	Yes	Salhi et al. (2010), Fakchich and Elachouri (2014)
	Calendula officinalis	الجمرة	1.9%	Fl (4) L (1)	Po (1) Dec (1) Inf (1) EO (1) Oil (1) Mac (2)		1 t/d (2) 2 t/d (3) 3 t/d (2)	2 We (1) Other (3)	Cu (4) Or (2)	Yes (2) No (2)	[12]	Yes	Sijelmassi (2011), Bellakhdar (1998)
	Chamaemelumnobile	البابونج	0.5%	L (1) R (1)	Po (1)	Oil (1)	1 t/d (1), 2 t/d (1)	Other (1)	Cu (1)	Yes (1)	[13]	No	Tahri et al. (2012)
Asteraceae	*Inulaviscosa ait.*	مكرمان الترهل	5.2%	L (12)	Po (11), Dec (1), Inf (1), EO (1), Di (2),	Water (1), Resin (1), Argan oil (2), Olive oil (3), Cider vinegar (1,) Propolis (1)	1 t/d (9), 2t/d (3), Other (2)	1 We (1), Other (10)	Cu (11), Or (1)	Yes (9), No (1)	[14]	Yes	Tahri et al. (2012), Salhi et al. (2010)
	Matricaria recutita	البابونج الألماني	3.3%	W (2), Fl (3), F (1), L (1), R (1),	Po (2), Inf (2), EO (5)	Vegetal oil (2)	1t/d (4), 2 t/d (4), 3 t/d (1)	2 We (1), Other (6)	Cu (7), Or (2)	Yes (7)	[15]	No	Sijelmassi (2011), Fakchich et al. (2014), Merzouki et al. (2000)
	Pulicaria arabica	العطازة	0.5%	F (1)	EO (1)	Jojoba oil (1)	1t/d (1)	Other (1)	Cu (1)	Yes (1)	[16]	No	-
	Saussurea coctus	القسط البحري	0.5%	R(1)	Po(1)		1t/d (1)	Other (1)	Cu (1)	No (1)	[17]	No	-
	Tanacetum parthenium	البابونج الكبير	0.5%	L (1) R (1)	Po (1)	Oil (1)	1 t/d (1), 2 t/d (1)	Other (1)	Cu (1)	Yes (1)	[18]	No	-

Table 6. Contd.

Cactaceae	*Opuntia ficus-indica*	الكرموص الهندي	0.5%	F (2) S	EO (1), Other (1)		1t/d (2)	1 We (1), Other (1)	Cu (2)	Yes (1)	[19]	No	Fakchich et al. (2014)
Caryophyllaceae	*Saponaria vaccaria L.*	الصابونية	0.5%	L (1)	Po (1), Mac (1)	Oilve oil (1), Argan oil(1)	1t/d (1)	Other (1)	Cu (1)		[20]	Yes	Bellakhdar (1998)
Cesalpiniaceae	*Cassia absus L.*	حبة البركة	0.5%	F (1)	Po(1) EO (1)		1t/d (1)	Other (1)	Cu (1)	Yes (1)	[21]	No	-
Chenopodiaceae	*Chenopodium L.*	المخينزة	0.5%	L (1)	Di (1), EO (1)		2t/d (1)	Other (1)	Cu (1)	Yes (1)	[22]	No	Bellakhdar (1998)
Fabaceae	*Vicia sativa L.*	عين الأرنب	0.5%	L (1)	Po (1)	Honey (1)	1t/d (1)	Other (1)	Cu (1)	Yes	[23]	No	-
Fagaceae	*Quercus faginea lamk.*	العفصة	1.4%	Nu (2), F (2)	Po (3)		2 t/d (3), 3 t/d (1)	1 We (1), 2 We (1), Other (1)	Cu (3)	No (1)	[24].	No	Fakchich et al. (2014), Merzouki et al. (2000)
Geraniaceae	*Geranium L.*	لمعطرشة	0.9%	W (1), Fl (1)	Po (1), EO (1)	Oil (1)	1 t/d (1), 2t/d(1)	Other (2)	Cu (2)	Yes (2)	[25]	No	Fadil et al. (2014)
Globulariaceae	*Globularia alypum L.*	تسلغا	1.4%	L (2), Fl (1)	Po (3), EO (1)	Almond oil (1), Olive oil (1)	1 t/d (1), 2t/d (3) 3 t/d (1)	1 We (1), 2 We (1), Other (1)	Cu (3)	Yes (2), No (1)	[26]	Yes	-
Hypericaceae	*Hypericum perforatum*	يوفارقون	0.9%	Fl (2), F (1)	Po (2), EO (2)	Honey (1)	1t/d (2), 2 t/d (1)	Other (2)	Cu (2)	Yes (2)	[27]	No	Bammi and Douira (2002)
Iridaceae	*Crocus sativum*	زعفران لحر	1.4%	Sg (2), L (1)	Po (2), Dr (1), Di (1)	Honey (1), Argan oil (1)	1t/d (3)	1 We (2), Other (1)	Cu (3)	No (3)	[28]	No	-
Lamiaceae	*Lavandula angustifolia*	الخزامة	3.3%	L (4), Fl (1), W (3)	Po (4), EO (6), Dec (1)	Honey (1), Vaselin (1), Butter (1), Argan oil (1), Vegetal oil (1), Cider vinegar (1), Lavender water (1)	1 t/d (3), 2t/d (3), 3t/d (2)	1 We (1), 1 Mo (1), Other (5)	Cu (7)	Yes (7)	[29]	Yes	Sijelmassi (2011), Tahri et al. (2012)

Table 6. Contd.

Marrubium vulgare	مريوت	1.4%	W (2) L (1)	Po (1), Di (1), Dr 1)	Salt (1), Olive oil (1)	1 t/d (1), 2 t/d (1), 3 t/d (1)	2 We (1), Other (2)	Cu (3)	Yes (2)	[30]	Yes	Fakchich et al. (2014), Lahsissene et al. (2009), Daoudi et al. (2015)
Mentha x piperita L.	النعناع	0.9%	L (2)	Inf(1), EO (1), Mac (1), Dr (1)	Vaselin (1), Oil (1),Alcohol (1)	1 t/d (2), 2 t/d (1)	Other (2)	Cu (2), Or (1)	Yes (2)	[31]	No	-
Ocimum basilicum	الحبق	0.9%	L (1), Fl (2)	Po (1), EO (2)	Honey (1)	2 t/d (1), Other (1)	1 Mo (1), Other (1)	Cu (2)	Yes (2)	[32]	No	-
Origanum vulgare	زعيترة	0.5%	L (1)	Inf Dec (1), EO (1)	Castor oil (1)	2 t/d (1)	2 We (1)	Cu (1)	Yes (1)	[33]	No	-
Rosmarinus officinalis	اليازير	4.3%	L (5), Fl (1) F (1), W (3)	Po (3), Inf (2), Dec (2), EO (7)	Honey (2), Vegetal oil (3)	1 t/d (2), 2 t/d (6), 3t/d (2)	Other (5), 1 We (3)	Cu (7), Or (2)	Yes (8)	[34]	Yes	Fakchich et al. (2014), El Amri et al. (2014),Lahsissene et al. (2009), Salhi et al. (2010), Bellakhdar (1998)

and are used by the population of Settat's province (Tahri et al., 2012). Indeed, *S. verbenaca, I. viscosa ait., A. tridentata* and *R. officinalis* are recorded as healing medicinal plants by a survey in the region of Gharb of Morocco (Mechraâ Bel Ksir) (Benkhnigue et al., 2011), whereas it was found that *A. tridentata*, *I. viscosa*, *R. officinalis* and *S. verbenaca* have healing properties against wounds, in Kenitra (Salhi et al., 2010).

Families of healing plants

The study has indexed 37 families of which*Lamiaceae*, *Asteraceae*, *Pinaceae* and *Aloaceae* are predominant. In fact, several studies have confirmed the dominant presence of these families. An ethnobotanical survey in Fez (Zeggwagh et al., 2013), at Kenitra (Salhi et al., 2010) and in the region of Haut Atlas Oriental (Belamdini et al., 2014) showed that *Lamiceae*, *Asteraceae* and *Apiaceae* are the most represented. Another one, has demonstrated that *Lamiaceae*, *Fabaceae* and *Apiaceae* are the dominant families at the region of Agadir (El Hafian et al., 2014), Whereas, two other research teams found that *Lamiaceae*, *Asteraceae* and *Liliaceae* are the major families used by the local population of Ifran (Rhafouri et al., 2014) and Essaouira (Mehdioui et al., 2007). Finally, the national study of medicinal plants found that *Asteraceae, Fabaceae* and *Poaceae* are the predominant families in Morocco, which explains this similar distribution through regions (Bellakhdar, 1998).

Origin of plants and season

The plants were found in most parts of Souss-Massa-Draa, followed by the region of Marrakech-Tensift-Al Haouz and the region of Gharb-Chrarda-Beni Hssen. This distribution can be explained by climate changes, soil and reliefs type from one region to another (Bellakhdar, 1998), and also by the cultural outcome of each region which has its own traditional use of medicinal plants. On the other hand, spring is the season of flowering aromatic plants and it's an ideal bioclimatic period for them, that is why the study

Table 6. Contd.

	Salvia verbenaca	الخياطة	21.3%	W (37), L (3), R (2), Fl (2), F (3), Ro (1)	Po (44), Inf (2), EO (1)	Olive oil (2), Honey (2), Butter (1), Cider vinegar (2), Alcohol (1), Water (1)	1t/d (19), 2 t/d(13), 3 t/d (8), 5t/d (1), Other (8)	1 We (9,) 2 We (5), Other (32)	Cu (43), Or (12)	Yes (6), No (2)	[35]	No	Fakchich et al. (2014), Lahsissene et al (2009), Merzouki et al. (2000), Daoudi et al. (2015), Belamdini et al. (2014), Bammi and Douira.(2002), Tahri et al. (2012), Salhi et al. (2010), Bellakhdar (1998)
	Thymus vulgaris	الزعتر	1.4%	L (1) W (1)	EO (2), Inf (1)	Vegetal oil (1)	2 t/d (2), 3 t/d (1)	1 We (1), Other (1)	Cu (2), Or (1)	Yes (2)	[36]	Yes	Sijelmassi (2011), Bellakhdar (1998)
Lauraceae	*Laurus nobilisL.*	ورق سيدنا موسى	0.5%	L (1)	Po (1), EO (1)	Argan oil (1)	1 t/d (1), 2 t/d(1)	Other (1)	Cu (1)	Yes (1)	[37]	Yes	-
Liliaceae	*Cinnamomumverum*	القرفة	0.5%	Ba (1)	Po (1), EO (1)	Oil (1)	2t/d (1)	1 We (1)	Cu (1), Or (1)	Yes (1)	[38]	No	-
	Smilax aspera L.	ورق العليق	0.5%	L (1)	Po(1)	Cider vinegar(1) Propolis (1)	1t/d(1)	Other(1)	Cu (1)	Yes(1)	[39]	Yes	-
Lythraceae	*Lawsonia inermis*	لحنا	0.5%	L	Po (1), Dec(1), Inf(1)	Honey (1)	2t/d (1)	2 We (1)	Cu (1)	No (1)	[40]		Fakchich et al. (2014), Semwal et al. (2014), Hseini and Kahouadji (2007), ;Zeggwagh (2013), Lahsissene and Kahouadji (2010), Bellakhdar (1998)
Myristicaceae	*Myristica fragrans*	الوردالمسكي	0.5%	Fl (2), L (1)	EO(2), Po (1)	-	1t/d (2), 2t/d (1)	Other (2)	Cu (2)	Yes (2)	[41]	No	-

found the majority of aromatic plants in spring. Also, the season of harvesting can influence the composition of the plant on several essential metabolites.

Used parts of plant

The high frequency use of the whole plant can be explained by the facility of the manipulation of the plant. In fact, another study showed that people are more likely to pick the whole plant and to use it instead of choosing a specific desired part (Tahri et al., 2012). This can probably explain the high use of the whole plant in this study. The high use of the leaves can be explained by the fact that they are the seat of several reactions (photosynthesis for example) and they contain several metabolites that are primordial for the pharmacological properties of the plant (Salhi et al., 2011). Also, their performances are better compared to other parts of the plant. Indeed, other studies have shown that essential oils yield of leaves of some plants is higher compared to flowers (Bassole et al., 2001).

Table 6. Contd.

	Melaleucatea	اتاي	1.4%	S (2)	Po (3)	Castor oil (1) Almond oil (1)	1t/d (2), 2t/d (2)	Other (3)	Cu (3)	Yes (1)	[42]	No	-
Myrtaceae	*Myrtus communis L.*	الريحان لحلموس	1.9%	L (2), Fl (1), W (1)	Po (1), EO (3)	Vegetal oil (1), Almond oil (1), Castor oi l(1)	1 t/d (1), 2 t/d (2), 3t/d (1), Other (1)	Other (3), 2 We (1)	Cu (4)	Yes (4)	[43]	No	Sijelmassi (2011), Wahid (2013), Tahri et al. (2010), Bellakhdar (1998)
Papaveraceae	*Papaver rhoeas L.*	بلعمان	0.5%	Fl (1)	Po (1) Di (1)		1t/d (1)	Other (1)	Cu (1)	No (1)	[44]	No	Fakchich et al. (2014)
Pédaliaceae	*Sesamum indicum*	السمسم	0.5%	S (1)	EO (1)		1t/d (1)	Other (1)	Cu (1)	Yes (1)	[45]	No	Zeggwagh et al (2013)
Pinaceae	*Pinus halepensis*	التايدة	9.0%	W (7), R (2), Ba (4), Fl (1)	Po (14), Inf (1)	Olive oil (1), Rancid butter (1), Cider vinegar (1)	1t/d (7), 2 t/d (6), Other (3)	1 We (3), 2 We (1), Other (11)	Cu (14), Or (2)	Yes (1), No (4)	[46]	Yes	Bammi and Douira. (2002)
Plantaginaceae	*Plantago major*	المصاصة	2.8%	W (1), F (1), L (1), Fl (1)	Po (2), Di (1)	-	1 t/d (1), 2 t/d (1), 3 t/d (1)	Other (3)	Cu (3), Or (1)	Yes (1), No (1)	[47]	Yes	Sijelmassi (2011), ElAmri et al. (2014), Bellakhdar (1998)
	Plantago psyllium	زرقطونة	0.9%	W (1)	Po (1)	-	1t/d (1)	Other (1)	Cu (1)	-	[48]	No	Bellakhdar (1998)
Poaceae	*Avena sativa L.*	الخرطال	0.5%	S (1)	Po (1)	Lemon juice (1)	1t/d (1)	Other (1)	Cu (1)	No (1)	[49]	No	-
Rhamnaceae	*Rhamnus alaternus L.*	مليلس	0.5%	L (1)	Po (1), EO (1)		1t/d (1)	1 Mo (1)	Cu (1)	Yes (1)	[50]	No	-
Ranunculaceae	*Clematis cirrhosa L.*	ايكودي	0.5%	R (1)	Mac (1)	Milk juice (1)	1t/d (1)	Other (1)	Cu (1)	No (1)	[51]	No	-
Salicaceae	*PopulusL.*	أسفساف	0.5%	L (1)	Po (1)	-	2t/d (1)	2 We (1)	Cu (1)	Yes (1)	[52]	No	-
Sapotaceae	*Argania spinosa*	أركان	0.9%	F (3)	EO (1), Other (2)	-	1t/d (2), Other (1)	1 We (1), Other (2)	Cu (3)	-	[53]	No	Zeggwagh et al. (2013)
Solanaceae	*Capsicum annuum*	التحميرة	0.9%	W(2)	Po (2)	-	1t/d(2)	Other (2)	Cu (2)		[54]	No	

Table 6. Contd.

Scrophulariaceae	*Veronica aquatica*	الحريكةالملسة	1.9%	W (2) L (1)	Po (2), Inf (3), EO (1), Dr (1)	-	2 t/d(2), 3 t/d (2)	1 We (1), Other (2)	Cu (3), Or (3)	Yes (2), No (1)	[55]	No	-
Urticaceae	*Urtica dioica*	الحريكة	1.4%	W (2), L (2), R (1), Bo (1)	Po (3), Inf (3), Dec (1), EO (1), Mac (1)	Alcohol (1), Cider vinegar (1)	1 t/d (1), 2 t/d (2), 3t/d (2)	1 We (1), Other (3)	Cu (4), Or (3)	Yes (4)	[56]	Yes	-
Verbenaceae	*Verbena officinalis*	بايموت	2.8%	W (3) Ro (1)	Po (4)	-	1 t/d (1), 2 t/d (1), 3 t/d (2), Other (1)	1 We (1), Other (3)	Cu (4)	-	[57]	Yes	Bellakhdar (1998)
Zingiberaceae	*Curcuma longa*	الخرقوم	0.5%	W (1)	Po (1)	Honey (1), Butter (1)	Other (1)	Other (1)	Cu (1)	-	[58]	Yes	-
Zygophyllaceae	*Peganum harmala L.*	الحرمل	0.5%	S (1)	Po (1)	-	2t/d (1)	1 We (1)	Cu (1)	Yes (1)	[59]	No	Fakchich et al. (2014), Bellakhdar (1998)

(Frequency) ; Ba: Barks; Bo: Boughts; Bu: Bulb; Cu: Cutaneous; Cr: Cream; Dec: Decoction; Di: Direct application; Dr: Dressing; D: Day; EO: Essential oils; F: Fruit; Fl: Flowers; Inf: Infusion; L: Leaves; Mac: maceration; Mo:Month; Mu: Mucilage; Nu: Nuts; Po: Powder; Pu: Pulp; Ro: Rods; R: Roots; S: Seeds; W: Whole plant; We: Week. t/d: time/day.
*Other: Depending the pathological field and to the wound.

Form of use

The high use of the plant as a powder form, according to herbalists, can be explained by the facility and the rapidity of the operation, the nature of the herbalist's profession and his experience. But it can also be explained by the effectiveness of this method on the healing of skin wounds. This was confirmed by other regional studies which found that the use of plant in powder form is frequently used by the local population of Essaouira (25%) (Mehdioui et al., 2007), Ifran (22%) (Rhafouri et al., 2014), Kenitra (25.9%) (Salhi et al., 2010), in the region of Zaêr (11.5%), Atlas Oriental (27.92%) (Belamdini and Douira, 2002) (Lahsissene et al., 2010), and in the region of Haut.

The important use of essential oils can be explained by the fact that they contain several essential compounds, and active ingredients responsible for the plant's activities and its effectiveness.

Way of administration

The important frequency of use of dermal way can be explained by the nature of the disease, which is the skin healing, and also because the effectiveness of treatment is direct and more important by dermal way.

Frequency and duration of use

The frequency and the duration of use changes from one herbalist to another because everyone has its personal diagnosis of the pathological field and of the patient's case. However, majority of herbalists recommend using the plant 2 twice a day in the morning and at the night, but they were not able to adjudicate about the treatment duration. They estimate that they have to examine thepatient before giving a diagnosis decision.

Plants association

These plant associations are explained by the fact that the efficiency of the plant increases when it associated with one or more other plants and that the wound healing time decreases. For example, consider *S. verbenaca* which is the main inventoried species. The herbalists interviewed

Table 7. Inventoried properties list of recorded healing plants.

Plant	Properties
Aloe vera	Healing, antidiabetic, anticancer, against burns, against redness, for hair and face care, against kidney problems, against stretch marks and varicose veins
Allium cepa	Healing, against cold
Pistacia lenticus L	Healing, against skin abscesses
Pistacia terebinthus L.	Healing, against abscesses, hair care
Rhusalbidumschousb.	Healing, anti diarrhea and abscesses
Carum carvi L.	Healing
Centella asiatica	Healing
Coriandrum sativum	Healing, antidiuretic, antiseptic, antispasmodic, anti diarrhea, carminative
Aristolochia long.	Healing, against pimples
Arnica L.	Healing, antiseptic, antidiabetic, antiinflammatory
Artemisia tridentata	Healing, hemostatic, anticancer, antiseptic, antidiabetic, antibiotic, draining, wormer, against sinusitis, against cold, digestive, against stomach problems, acne, eczema
Calendula officinalis	Healing, anti-hepatitis, anticancer, antifungal, assists delivery
Chamaemelum nobile	Healing
Inula viscosa ait.	Healing, against stomach ulcers, against burns, against anal fissures
Matricaria recutita	Healing, antipyretic, anti-inflammatory, antispasmodic, antiallergic, soothing, softening, black marks
Pulicaria arabica	Healing
Saussurea coctus	Healing
Tanacetumparthenium	Healing, antispasmodic, antiseptic, antiviral, for breathing and digestive apparatus, calming
Opuntia ficus-indica	Healing, against stretch marks, anti wrinkles
Saponaria vaccaria L.	Healing, against constipation
Cassia absus L.	Healing
Chenopodium L.	Healing, antipyretic, antirheumatic
Vicia sativa L.	Healing. against stomach problems
Quercus faginea lamk.	Healing, anti-haemorrhagic, anti diarrhea, blackening
Geranium L.	Healing
Globularia alypum L.	Healing, antidiabetic, against burns
Hypericum perforatum	Healing
Crocus sativum	Healing, antiseptic, against herpes, against eyes problems
Lavandula angustifolia	Healing, toning, strengthens immunity, antiseptic
Marrubium vulgare	Healing, antirheumatic, against pimples and abscesses
Mentha x piperita L.	Healing, against burns, against stomach problems, against cold, against headaches
Ocimum basilicum	Healing, antiseptic, calming
Origanum vulgare	Healing
Rosmarinus officinalis	Healing, hypertensive, antioxidant, antiseptic, anti-hepatitis, antifungal, Antibiotic, detoxifying, energizing, antioxidant, enhances memory
Salvia verbenaca	Healing, antidiabetic, antispasmodic, against pimples, against stomach problems, against bad cold, against bloating, anti acne
Thymus vulgaris	Healing, antibiotic, antiseptic, strengthens immunity, digestive, hair care
Laurus nobilisL.	Healing, against facial tasks
Cinnamomum verum	Healing, antiseptic, antirheumatic, constipating, against gases
Smilax aspera L.	Healing
Lawsonia inermis	Healing, antimycosic, antidiarrhea, against abscess, hair care
Myristica fragrans	Healing, face care
Melaleuca tea	Healing
Myrtus communis L.	Healing, antispasmodic, antiseptic, hair care

Table 7. Cont'd.

Papaver rhoeas L.	Healing.
Sesamum indicum	Healing, anti tasks, against sunlight
Pinushalepensis	Healing, antiseptic, analgesic, against burns, against digestive problems
Plantago major	Healing, antiinflammatory, analgesic, against urinary and digestive problems
Plantago psyllium	Healing, antiseptic.
Avena sativa L.	Healing
Rhamnus alaternus L.	Healing, against black tasks
Clematis cirrhosa L.	Healing, against black tasks
PopulusL.	Healing
Argania spinosa	Healing, against sunburn, face care, anti wrinkles, anti tasks, anti eczema
Capsicum annuum	Healing, antiseptic
Veronica aquatica	Healing, antihemorrhagic, against pimples, anticancer, kidney problems, anemia, diarrhea, remineralizing, against anemia
Urtica dioica	Healing, anti-haemorrhagic, antirheumatic, antidiuretic, against hair loss, against hemorrhoids
Verbena officinalis	Healing, antiinflammatory, activates immune system
Curcuma longa	Healing
Peganum harmala L.	Healing

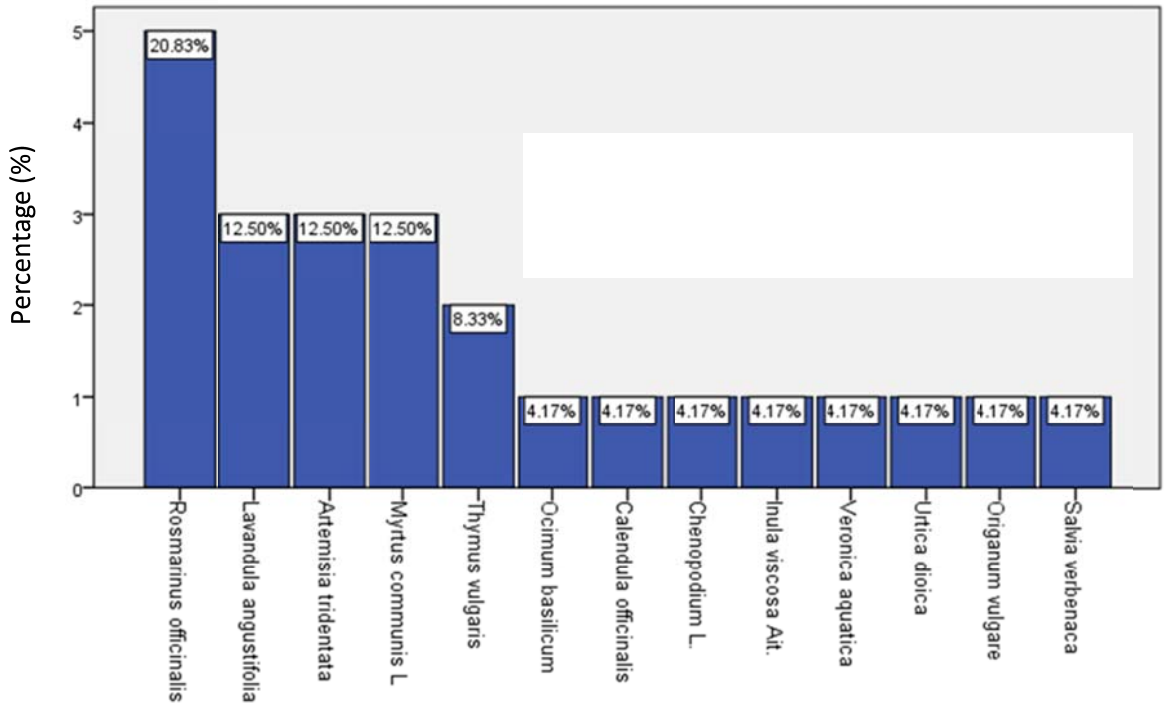


Figure 10. Workforce of healing plants whose leaves are used as essential oils.

said that *S. verbenaca* is used most often when it is associated with other plants, and its efficiency increases exceeding 80% if it is associated with *P. halepensis* or *V. officinalis*. Indeed, this result was confirmed by another ethnobotanical study, which showed that *S. verbenaca* is often associated with these plants (Bellakhdar, 1998).

Crossing between used parts and form of use

From this crossing, the study obtained that $\chi^2_{obs} > \chi^2_{v;\ \alpha}$. Therefore, H_0 is rejected. The study concluded that the used form of the plant depends on the used parts.

Crossing between used parts and use as essential oils

The study found out, through this crossing, the use in the form of essential oils depends on the leaves and on the whole plant but it is independent of the other parts like: flowers, roots, barks, fruit and seeds. The dependence of the use in essential oils to the whole plant confirms the results of the Chi-square test of Table 4. So, the study can conclude from the results, that the use of essential oils depends on part of the plant which is used and more specifically on the leaves. This can be explained by the fact that leaves contain all the essential plant metabolites including essential oils.

Crossing between use form of essential oil with healing plant

The statistical results of the crossing (Table 5) allowed us to conclude that the use of essential oils form actually depends on the healing plant species.

Crossing between use form of essential oil, leaf-part and healing plant

This crossing enabled us to conclude that the use of the leaves as essential oils depends on the nature of the healing plant. Furthermore, the study found that *R. officinalis*, *L. angustifolia*, *A. tridentata*, *M. communis L* and *T. vulgaris* are the plants whose leaves are more often used in the form of essential oils. Based on these results, the study has chosen to use these plants in subsequent experimental studies, as part of of the study research work, to develop and manufacture a phytomedicine.

CONCLUSION

The use of medicinal plants has been in existence for decades; it has been and remains until today requested by the Moroccan population. Furthermore, phytotherapy is a discipline that is changing these days, and people are more likely to go to the natural products to heal and for treatments of wounds.

Indeed, this study was conducted in order to make the most complete inventory of the healing medicinal plants used in Morocco and to gather all the necessary information about the therapeutic uses of these plants and their specific properties. Thus, the ethnobotanical survey allowed us to reveal a large number of information. It allowed the identification of 59 species of healing plants belonging to 37 families, whose *S. verbenaca* is the major species. It is presented in this study that among these families, *Lamiaceae* is the most represented family in the surveyed citie

On the pharmacological side, the whole plant is the most used part; powder is the most common used form. There is a relationship between the form of use and the used part of the plant; especially for essential oils use form. It was also discovered that the leaves which are the most commonly used part as essential oils, and that, of course, depends on the nature of the plant. The indexed plants are distributed specifically in the Souss-Massa-Draa region and their distribution varies from one region to another. Plants are more available in spring, they are used most often 2 times per day and the duration of the use varies depending to the wound and the pathological field.

This study is the result of a series of ethnobotanical surveys made with herbalists and traditional healers in the cities of Casablanca, Mohammedia, Rabat, Salé, Kenitra, Fez, Marrakech, Agadir, Taroudant, Tangier, Tetouan and Oujda. Using direct and telephonic interviews, the survey was carried out over a period of five months, and allowed the study to know the healing medicinal plants used by the Moroccan population of the main cities of Morocco. It revealed the wealth of floral and plant heritage and much other relevant information. It is therefore, necessary to expand the field/population of this study to other regions of the Kingdom, to include all the healing medicinal plants traditionally used, and also to safeguard this precious plant heritage.

These conclusive results allowed the study to justify (as preliminary information gathered in the field) the choice of healing plants that will be used in subsequent experimental studies, to manufacture a new wound healing product.

AKNOWLEDGMENTS

The authors of this article thank the University Hassan II for financing the publication costs. We are very grateful to AIT FARAJI S., from the Direction of Studies and Financial Forecasts, of Ministry of Economy and Finance in Rabat, for his help in the statistical analysis of the results. They are also grateful to Messaoudi Moussi A., from the Moroccan-Libyan Chamber for his assistance in interviews with herbalists, and to traditional herbalists of surveyed cities for their availability and their assistance.

Conflicts of interest

The authors have none to declare.

REFERENCES

Agyarea C, Asase A, Lechtenberg M, Niehues M, Deters A, Hensel A (2009). An ethnopharmacological survey and in vitro confirmation of ethnopharmacological use of medicinal plants used for wound healing in Bosomtwi-Atwima-Kwanwoma area, Ghana. J. Ethnopharmacol, 125:393-403.

Bammi J, Douira A (2002). Les plantes médicinales dans la forêt de l'Achach (Plateau central, Maroc). Acta Botanica Malacitana. 27:131-145.

Bassole IHN, Ouattara AS, Nebie R, Ouattara CAT, Kabore ZI, Traore SA (2001). Composition chimique et activités antibactériennes des huiles essentielles des feuilles et des fleurs de *Cymbopogon proximus* (stapf.) et d'*Ocimum canum* (sims). Pharm. Med. Trad. Afr. 11 : 37-51.

Belamdini N, El Hafian M, Rochdi A, Zidane L (2014). Etude floristique et ethnobotanique de la flore médicinale du Haut Atlas oriental (Haute Moulouya). J. Appl. Biosci. 78:6771-6787.

Bellakhdar J (1998). La Pharmacopée marocaine traditionnelle : Médecine arabe ancienne et savoirs populaires. Mazars Guy Revue d'histoire de la pharmacie 30:86-84.

Benkhnigue O, Zidane L, Fadli M, Elyacoubi H, Rochdi A, Douira A (2010). Etude ethnobotanique des plantes médicinales dans la région de Mechraâ Bel Ksiri (Région du Gharb du Maroc). Acta Botanica Barcinonensia *53*:191-216.

Bousta D, Ennabili A (2011). L'Institut national des plantes médicinales et aromatiques au service du développement de la phytothérapie au Maroc. Phytothérapie 9:297-303.

Daoudi A, Bammou M, Zarkani S, Slimani I, Ibijbijen J, Nassiri L (2015). Etude ethnobotanique de la flore medicinal de la commune rurale d'Aguelmouss province de Khénifra. Phytothérapie 1-9.

El Amri J, El Badaoui K, Zair T, Bouharb H, Chakir S, Alaoui TEM (2014). Ethnobotanical study of medicinal plants in the region El Hajeb (Central Morocco). J. Res. Biol. 4(8):1568-1580.

El Hafian M, Benlamdini N, El Yacoubi H, Zidane L, Rochdi A (2014). Etude floristique et ethnobotanique des plantes médicinales utilisées au niveau de la préfecture d'Agadir-Ida-Outanane (Maroc). J. Appl. Biosci. 81:7198-7213.

Fadil M, Farah A, Haloui T, Rachiq S. (2014). Diagnosis of the aromatic and medicinal plant sector in Morocco: Case of the cooperatives and associations of the Meknès-Tafilalt area. Lazaroa (35):155-166.

Fakchich J, Elachouri M (2004). Ethnobotanical survey of medicinal plants used by people in Oriental Morocco to manage various ailments. J. Ethnopharmacol. 154:76-87.

Hseini S, Kahouadji A (2007). Etude ethnobotanique de la flore médicinale dans la région de Rabat (Maroc occidental). Lazaroa 28:79-93.

Lahsissene H, Kahouadji A, Tijane M, Hseini S (2009). Catalogue des plantes médicinales utilisées dans la region de Zaër (Maroc occidental). Lejeunia 186-192.

Lahsissene H, Kahouadji A (2010). Usages traditionnels des plantes médicinales dans le Maroc occidental : cas de la région de Zaër. Phytothérapie 8(4):210-217.

Mehdioui R, Kahouadji A (2007). Etude ethnobotanique auprès de la population riveraine de la forêt d'Amsittène: cas de la Commune d'Imi n'Tlit (Province d'Essaouira). Bulletin de l'Institut scientifique, Rabat, section Sciences de la vie. 29 :11-20.

Merzouki A, Derfoufi FEd, Mesa M (2000). Contribution of the knowledge of Rifian traditional mediane: II: Folk medicine in Ksar Lakbir district (NW Morocco). Fitoterap 71:278-307.

Mountassir M (2014). Probabilités et statistique : Cours, Exercices, Etudes de cas et Modules technologiques. Afr. Orient. pp. 357-360.

Rhafouri R, Aafi A, Zair T, Strani B, El Omari M, Ghanmi M, Bentayeb A (2014). Ethnobotanical study of medicinal plants in Ifran's National Park (Morocco). J. Mater. Environ. Sci. 6 (3):619-630.

Salhi S, Fadli M, Zidane L, Douira A (2010). Etudes floristique et ethnobotanique des plantes médicinales de la ville de Kénitra (Maroc). Lazaroa 31:133-143.

Semwal RB, Semwal DK, Combrinck S, Cartwright-Jones C, Viljoen A (2014). *Lawsonia inermis L*. (henna): Ethnobotanical, phytochemical and pharmacological aspects. J. Ethnopharmacol. 155(1):80-103.

Sijelmassi A (2011). Les plantes médicinales au Maroc. Editions le Fennec. Casablanca, Morocco. p 285.

Tahri N, El Basti A, Zidane L, Rochdi A, Douira A (2012). Etude Ethnobotanique Des Plantes Medicinales Dans La Province De Settat (Maroc). Kastamonu Üniversitesi Orman Fakültesi Dergisi. 12(2):192-208.

Wahid N (2013). Perspectives de la valorisation de l'usage et de la culture du Myrtus communis L. au Maroc. Phytothérap 11:237-243.

Zeggwagh AA, Lahlou Y, Bousliman Y (2013). Enquête sur les aspects toxicologiques de la phytothérapie utilisée par un herboriste à Fès, Maroc. Pan Afr. Med. J. 14:125

academicJournals

Vol. 7(8), pp. 183-188, August 2015
DOI: 10.5897/JPP2015.0353
Article Number: 7E43C2654313
ISSN 2141-2502

http://www.academicjournals.org/JPP

Journal of Pharmacognosy and Phytotherapy

Full Length Research Paper

Preliminary screening of anti-inflammatory effect of phytochemicals on chemotaxis of human neutrophils

Uthayashanker R. Ezekiel and Rita M. Heuertz

Department of Biomedical Laboratory Science,Doisy College of Health Sciences,Saint Louis University, 3437 Caroline Street,Saint Louis, USA.

Received 24 May, 2015; Accepted 23 July, 2015

Neutrophils are leukocytes that are actively recruited to sites of tissue infection and/or injury by directed movement (chemotaxis). *In vitro* assessment of inhibition of neutrophil chemotaxis is a physiologic indicator of anti-inflammatory potential. To identify nontoxic, anti-inflammatory agents, plant-derived compounds (curcumin, resveratrol, rosmarinic acid and piperine) were assessed for effects on *in vitro* neutrophil movement. Effects were determined on directed migration (chemotaxis) towards the potent chemoattractant of bacterial cell wall origin, f-met-leu-phe (fMLP). Curcumin significantly inhibited neutrophil chemotaxis in a concentration-dependent manner with statistically significant inhibition at 50 and 100 µM. Similarly, resveratrol (25, 50, 100 µM) and rosmarinic acid (100 µM) significantly inhibited fMLP-induced chemotaxis in concentration-dependent manners. Piperine had no effect on neutrophil chemotaxis. These results indicate that curcumin, resveratrol and rosmarinic acid have the potential to elicit anti-inflammatory effects.

Key words: Neutrophils, phytochemicals, chemotaxis, inflammation.

INTRODUCTION

An important *in vivo* biological event is chemotaxis, which is directed cell movement towards a chemical or biological agent. Chemotaxis occurs in many cell types with examples being leukocyte influx into an inflammatory nidus, migration of endothelial cells for blood vessel formation during angiogenesis, development of an embryo and cancer cell metastasis. Chemotaxis is regularly assessed in immune cells where their mobilization and deployment to sites of inflammation are integral parts of the immune response (Kruger et al., 2015).

One immune cell type, the polymorphonuclear neutrophil (PMN), has been well studied for its chemotactic behavior relevant to its important role in innate immunity and inflammation (Headland and Norling, 2015). Recruitment of neutrophils to sites of inflammation or injury involves the following commonly recognized steps: tethering, rolling, adhesion, crawling and endothelial transmigration (Kolaczkowska and Kubes, 2013). Neutrophil chemotaxis is induced by chemoattractants such as chemokines (interleukin-8: IL-8), bacteria-derived agents (f-met-leu-phe: fMLP) and

*Corresponding author. E-mail: messimane38@yahoo.fr.

vasculature-derived components (complement proteins such as C5a) (McNeely et al., 1993). Regardless of the cue, neutrophils in circulation first recognize chemotactic signals in the endothelium close to an inflammatory site, roll along the endothelium and extravasate into the tissue where they fight infections and interact with other cells of the immune system. Neutrophils are the first cells to arrive at an inflammatory site and they do that in massive numbers (Bardoel et al., 2014).

Therefore, assessment of neutrophil chemotaxis is an important early function in acute inflammation. Noteworthy is the fact that impaired recruitment or inappropriate or excessive activation of neutrophils can lead to disease states such as rheumatoid arthritis, inflammatory bowel disease and chronic obstructive pulmonary disease (den Broeder et al., 2003; Kaur and Singh, 2013; Larmonier et al., 2011). It is for this reason that neutrophils have become targets for pharmaceutical approaches to functional regulation. Constant concerns about an ever-expanding population of patients with inflammation-mediated diseases coupled with lack of nontoxic yet efficacious anti-inflammatory agents are sufficient reasons to continue the search for other therapeutic options and strategies. One alternative strategy is the use of plant compounds that have anti-inflammatory properties.

Reports indicate anti-inflammatory properties of rosmarinic acid (derived from rosemary), resveratrol (from skins of red grapes), curcumin (derived from turmeric) and piperine (derived from black pepper). Rosmarinic acid caused susbstantial reduction in inflammation in three different rat models of inflammation, local (carrageenin-induced paw edema) and systemic (liver ischemia/reperfusion injury, thermal injury) (Rocha et al., 2015). Additionally, it reduced inflammation (example, neutrophil infiltration) in LPS-challenged horses (Pearson et al., 2012) and reduced number of inflammatory cells in the airways of an experimental model of respiratory allergy (Costa et al., 2012). Resveratrol inhibited inflammation, with reduced neutrophil infiltration as a marker, in a rat cerebral ischemia/reperfusion model of stroke (Fang et al., 2015) and inhibited airway inflammation and hyperreactivity in mice (Zang et al., 2015). Curcumin, administered as oral doses, inhibited neutrophil infiltration into lavage fluid in mouse models of extrinsic (Hemophilus influenza-induced) and intrinsic (tumor-induced) airway inflammation (Moghaddam et al., 2009), and significantly inhibited neutrophil infiltration and inflammatory cytokine production in a murine model of asthma (Narumoto et al., 2012). Piperine inhibited inflammation in a rat periodontitis model (Dong et al., 2015) and reduced proinflammatory cytokine levels, infarct volume and neuronal loss in a rat model of stroke (Vaibhav et al., 2012).

Given the cited results, plant-derived compounds with anti-inflammatory potential, specifically curcumin, resveratrol, rosmarinic acid and piperine, were selected for assessment of effects on chemotaxis of human neutrophil. These compounds were assessed by the leading front method of chemotaxis to determine their effects on fMLP-induced chemotaxis of human neutrophils (Kinane et al., 1989). Other reports document anti-inflammatory potential of each one of these plant compounds (Budhiraja and Dhingra, 2014; Hassan-Khabbar et al., 2010; Nonose et al., 2014; Sabina et al., 2011) as well as nontoxic host effects (Wang et al., 2015; McCrea et al., 2015).

MATERIALS AND METHODS

Isolation of human neutrophils

Heparinized venous blood was obtained from normal healthy, medication-free human donors after receiving informed consent following a protocol approved by the Saint Louis University Institutional Review Board. Neutrophils were isolated by dextran sedimentation and Ficoll-Hypaque density gradient centrifugation (Boyum, 1968). Contaminating erythrocytes were removed by hypotonic lysis. Cell purity was determined by differential counts of Wright-Giemsa stain (Sigma-Aldrich, St. Louis, MO) of cytospin preparations (routinely >95%) and neutrophil viability was determined by trypan blue (Sigma-Aldrich) exclusion (routinely >98%).

Phytochemical compounds

The phytochemicals, curcumin, resveratrol, rosmarinic acid and piperine were obtained commercially (Sigma-Aldrich). The compounds were solubilized in dimethylsulfoxide (DMSO: Sigma-Aldrich) at 100 mM stock concentrations and stored at -80°C. Phytochemicals used for chemotaxis were assessed at several concentrations (0.01, 0.1, 1, 10, 100 µM: concentrations were generated by diluting stock solutions in chemotaxis buffer (formulation described below)). The highest concentration (0.1%) of DMSO used for solubilization of plant-derived compounds was assessed in the assay as the vehicle control.

Leading front method of neutrophil chemotaxis

Appropriate concentrations of the bacteria-derived chemoattractant, f-met-leu-phe (fMLP: Sigma-Aldrich), was determined empirically by ascertaining concentration response curves of fMLP. Chemotaxis was measured using the leading front method of Zigmond and Hirsch (1973) as modified by Heuertz et al. (1999). Briefly, neutrophils were pretreated (10 min, 37°C) with plant-derived compounds in chemotaxis buffer consisting of Hanks' balanced salt solution (Sigma-Aldrich) containing N-2-hydroxyl-ethyl-piperazine-N'-2-ethanesulfonic acid (Hepes, 10 mM, pH 7.4: Sigma-Aldrich) and bovine serum albumin (1%: Sigma-Aldrich). Chemoattractant (fMLP) or buffer control (random neutrophil movement) was added to wells of the lower chamber in the chemotaxis assembly. A cellulose-nitrate filter (3 µm pore size: Sartorius Filters, Inc., Hayward, CA) was positioned on top of the lower chamber.

Neutrophils (200,000 in 50 µl/well) were then added to wells of the upper chamber and the 48-well chemotaxis apparatus (Neuroprobe, Cabin John, MD) was assembled and neutrophils (200,000 in 50 µl/well) were added to wells of the upper chamber. After set-up, the chemotaxis apparatus was incubated (35 min, 37°C) to allow time for the neutrophils to detect and react to the fMLP. After incubation, filters were removed from the chemotaxis

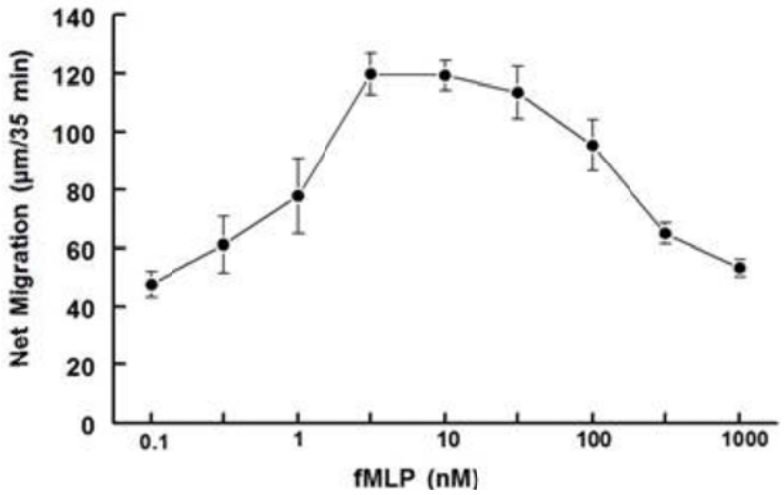


Figure 1. Concentration response curve of fMLP-induced chemotaxis by human neutrophils. The leading front method of chemotaxis was used. n=5.

assembly, fixed in isopropanol and stained with Harris acid hematoxylin (Sigma-Aldrich). Filters were viewed microscopically and neutrophil movement into the filter was quantified by identifying distance traveled by the leading two neutrophils, hence the reason the procedure is called the leading front assay. Net neutrophil migration into the filter was determined using the formula: (distance moved in response to treatment) minus (distance moved in response to buffer) = net migration of neutrophil movement in µm/35 min. A suboptimal concentration of fMLP was used for testing plant-derived compounds in order to determine whether inhibition or augmentation of neutrophil movement resulted.

Statistical analysis

All data were reported as the mean ± SEM. Comparisons of sample means were analyzed using repeated measures ANOVA followed by Dunnett multiple comparisons. Differences with $p < 0.05$ were considered significant.

RESULTS

fMLP concentration response curve

Results of the fMLP concentration response curve indicated that peak neutrophil movement was at 5 nM fMLP (Figure 1). Suboptimal concentrations were 0.5 and 1 nM fMLP. Declining neutrophil chemotaxis was evident at 50 to 1,000 nM fMLP.

Neutrophil chemotaxis after pretreatment with plant-derived compounds

The suboptimal concentration of 0.5 nM fMLP was used for assessment of effects of phytochemical-pretreatment on neutrophils. Several concentrations of phytochemicals (0, 0.01, 0.1, 1, 10, 100 uM) were assessed to determine their effects on neutrophil chemotaxis. Curcumin significantly inhibited fMLP-induced neutrophil chemotaxis in a concentration-dependent manner with statistically significant inhibition at 50 and 100 µM (Figure 2A) whereas vehicle (0.1% DMSO) had no effect on the chemotaxis (data not shown). Resveratrol inhibited fMLP-induced chemotaxis in a concentration-dependent manner with statistically significant inhibition at 25, 50 and 100 µM (Figure 2B). Rosmarinic acid inhibited fMLP-induced neutrophil chemotaxis in a concentration-dependent manner with statistically significant inhibition evident at 100 µM (Figure 2C). Piperine had no effect on fMLP-induced chemotaxis of neutrophils (Figure 2D). Trypan blue viability assays were performed in parallel with chemotaxis assays using the highest concentrations of all agents tested. None of the agents tested had any effect on PMN viability.

DISCUSSION

Neutrophils are actively recruited to sites of tissue infection and/or injury by directed movement (chemotaxis). *In vitro* assessment of inhibition of neutrophil chemotaxis is a physiologic indicator of anti-inflammatory potential (Antonicelli et al., 2004). The concentration response curve of fMLP showed suboptimal (0.5 and 1 nM fMLP), peak (5 nM fMLP) and declining (50 to 1,000 nM fMLP) neutrophil chemotaxis. Use of suboptimal concentrations allowed for interpretation of augmentation as well as inhibition outcomes. Declining chemotaxis at higher fMLP concentrations was not used due to the desensitization of neutrophil surface receptors to fMLP at those concentrations.

Plant-derived compounds (curcumin, resveratrol,

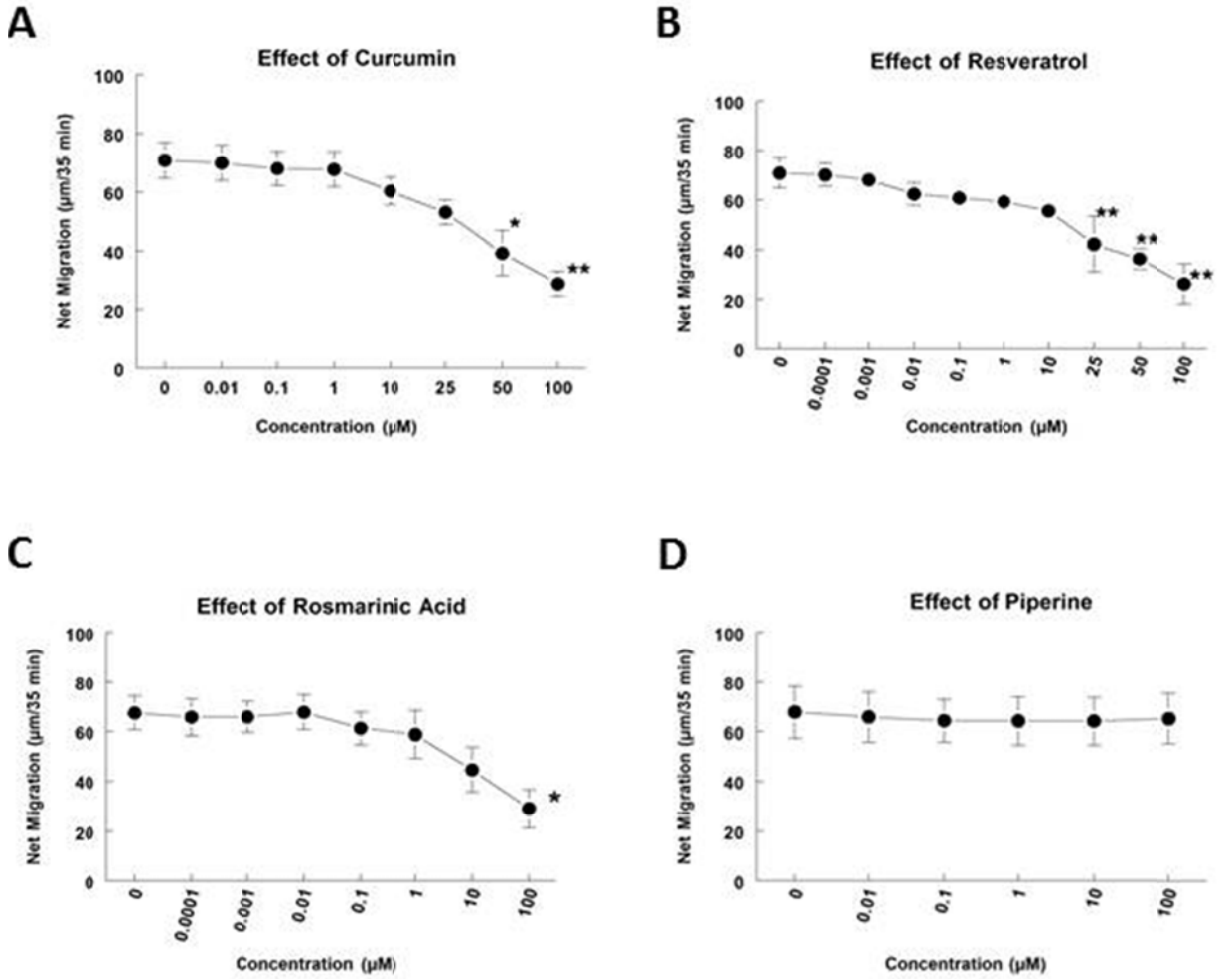


Figure 2. Effect of phytochemicals on fMLP-induced chemotaxis of human neutrophils. Cells were pretreated (10 minutes, 37°C) with indicated compounds and concentrations and then added to the chemotaxis unit for assessment of fMLP (0.5 nM)-induced chemotactic movement by the leading front method. A. Curcumin concentration response curve. n=6 at each concentration. B. Resveratrol concentration response curve. n= 6 at each concentration. C. Rosmarinic acid concentration response curve. n=6 at each concentration. D. Piperine concentration response curve. n = 3 at each concentration. For all panels: * = $p < 0.05$ versus fMLP alone (0 concentration of plant compound). ** = $p < 0.01$ versus fMLP alone (0 concentration of plant compound).

rosmarinic acid, piperine) were assessed for effects on *in vitro* neutrophil movement, that is, on chemotaxis towards the potent chemoattractant of bacterial cell wall origin, f-met-leu-phe (fMLP). Recent studies have shown that curcumin exerts anti-cancer activities on multiple types of cancer (Deguchi, 2015). For this reason, curcumin is one of the most promising phytochemicals that targets cancers and inflammation-mediated diseases. In the present study, curcumin significantly inhibited neutrophil chemotaxis in a concentration-dependent manner with statistically significant inhibition at 50 and 100 µM (Figure 2A). Neutrophil chemotaxis to an IL-8 homologue in mice (MIP-2) proceeds through a signalling pathway that initiates at a G-protein-coupled receptor (CXCR2). Activation of this receptor renders an effect on F-actin polymerization in the lamellar region of the neutrophil through phosphoinositide 3-kinase (PI3K) and phosphatidylinositol(3,4,5)-trisphosphate ($PI(3,4,5)P_3$) pathway which includes protein kinase B (Akt/PKB) and guanosine triphosphatases Cdc42 and Rac 2. Larmonier et al. (2011), showed that curcumin inhibited actin formation at the leading edge of neutrophils during movement, an event that involved PI3K and PI ($3,4,5P_3$). The finding that ERK phosphorylation was not affected indicated that the curcumin effect was not targeted to initial stages of MIP-2/CXCR2 signal transduction in the neutrophil. The inhibitory effect herein reported may be due to regulation of F-actin polymerization through phosphoinositide 3-kinase (PI3K) and phosphatidylinositol(3,4,5)-trisphosphate ($PI(3,4,5)P_3$) in the lamellar region of a migrating neutrophil, all of which are vital components of leading edge formation

(Larmonier et al, 2011).

Resveratrol, the polyphenol present in skins of red grapes and in red wines, has been linked with anti-inflammatory and anti-cancer activities (Inoue and Nakata, 2015; Wang et al., 2015)". It is herein shown that resveratrol inhibited fMLP-induced chemotaxis in a concentration-dependent manner with statistically significant inhibition at 25, 50 and 100 µM (Figure 2B). This result confirms prior reports (Inoue and Nakata, 2015; Wang et al., 2015) that resveratrol has anti-inflammatory activities. Inoue and Nakata state that resveratrol is a phytoalexin indicating that it is an antimicrobic synthesized by plants in response to assault by pathogenic bacteria or environmental stresses with resultant resistance to infection and stresses. Other anti-inflammatory processes ascribed to resveratrol are cyclooxygenase inhibition (Inoue and Nakata, 2015) and prevention of cytokine-induced vascular leakage (Wang, Dabrosin et al. 2015).

Rosmarinic acid from *Rosmarinus officinalis* has been shown to be anti-inflammatory *in vivo* by reducing number of leukocytes that roll, adhere and migrate to an inflamed site after injection of inflammatory agents in a rat model (Nogueira de Melo et al., 2011). Following up on this report, the question was posed as to whether purified rosmarinic acid rendered a similar effect in vitro as rosemary essential oil did in vivo. As shown herein, rosmarinic aid displayed significant anti-chemotactic effect, albeit at a single concentration (100 uM: Figure 2C). To our knowledge, this is the first report of inhibition of chemotaxis of human neutrophils by rosmarinic acid.

While piperine from *Piper nigrum* has been reported to have anti-inflammatory (Mujumdar et al., 1990; Sunila and Kuttan, 2004) and pro-inflammatory properties (Mujumdar et al., 1990; Sunila and Kuttan, 2004), piperine exhibited no effect on fMLP-induced neutrophil chemotaxis at the doses tested (Figure 2D). Mujumdar and Dhuley assessed piperine action in *in vivo* acute and chronic models of inflammation and identified that an anti-inflammatory piperine action was significantly manifested during acute inflammation at early stages. However, Sunila and Kuttan assessed piperine effect on solid tumor development and found that piperine increased total leukocyte number at the tumor site thereby implicating phagocyte recruitment and pro-inflammatory processes at the site. In another study, chemotaxis of macrophages (cell line Raw 264.7 of murine origin) was inhibited by piperine in a dose-dependent manner at concentrations similar to those used in the current study (Woo et al., 2007). It was therefore of interest to define the effect of piperine on chemotaxis of human neutrophils.

The present study identified the use of a well-established and long-used method to assess anti-inflammatory effects of phytochemicals, specifically the anti-inflammatory effect of inhibition of neutrophil chemotaxis. Studies suggest that neutrophil and cancer cell movement share common features such as mechanisms of signal transduction, movement as receptor-mediated events and induction by chemokine chemoattractants (Soon, 2007; Wang, 2009). Therefore, prevention of chemotaxis by phytochemicals has the potential to alleviate disease conditions involving inflammation. As the inflammatory process becomes better elucidated, the list of diseases caused or affected by inflammation grows longer. Interestingly, links between cancer and inflammation have been identified, especially as related to cell movement (Lin and Karin, 2007). For this reason, effects of phytochemicals on cancer cell movement deserve assessment for anti-metastasis potential. Future studies focus on mechanistic actions of these phytochemicals relevant to neutrophil chemotaxis.

CONCLUSION

Plant-derived compounds, such as resveratrol, curcumin and rosmarinic acid, inhibit chemotaxis of human neutrophils. These results indicate that phytochemicals have the ability to inhibit neutrophil recruitment to sites of infection and injury, and therefore have potential as anti-inflammatory agents with mechanistic action targeted at initial step of the innate immune response.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

Add an Acknowledgements section to this paper. The Acknowledgements should state "This work was funded in part by the Saint Louis University President Research Fund to URE".

REFERENCES

Antonicelli F, Brown D, Parmentier M, Drost EM, Hirani N, Rahman I, Donaldson K, MacNee W (2004). Regulation of LPS-mediated inflammation in vivo and in vitro by the thiol antioxidant Nacystelyn. Am. J. Physiol. Lung Cell Mol. Physiol. 286:L1319-1327.

Bardoel BW, Kenny EF, Sollberger G, Zychlinsky A (2014). The balancing act of neutrophils. Cell Host Microb. 15:526-536.

Boyum A (1968). Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand. J. Clin. Lab. Invest. Suppl. 97:77-89.

Budhiraja A, Dhingra G (2014). Development and characterization of a novel antiacne niosomal gel of rosmarinic acid. Drug Deliv. [Epub ahead of print].

Costa RS, Carneiro TC, Cerqueira-Lima AT, Queiroz NV, Alcântara-Neves NM, Pontes-de-Carvalho LC, Velozo Eda S, Oliveira EJ, Figueiredo CA (2012). *Ocimum gratissimum* Linn. and rosmarinic acid, attenuate eosinophilic airway inflammation in an experimental model of respiratory allergy to Blomia tropicalis. Int.

Immunopharmacol.13:126-134.
Deguchi A (2015). Curcumin targets in inflammation and cancer. Endocr. Metab. Immune Disord. Drug Targets. 15:88-96.
den Broeder AA, Wanten GJ, Oyen WJ, Naber T, van Riel PL, Barrera P (2003). Neutrophil migration and production of reactive oxygen species during treatment with a fully human anti-tumor necrosis factor-alpha monoclonal antibody in patients with rheumatoid arthritis. J. Rheumatol. 30:232-237.
Dong Y, Huihui Z, Li C (2015). Piperine inhibit inflammation, alveolar bone loss and collagen fibers breakdown in a rat periodontitis model. J Periodontal Res. [Epub ahead of print].
Fang L, Gao H, Zhang W, Zhang W, Wang Y (2015). Resveratrol alleviates nerve injury after cerebral ischemia and reperfusion in mice by inhibiting inflammation and apoptosis. Int. J. Clin. Exp. Med. 8:3219-3226.
Hassan-Khabbar S, Vamy M, Cottart CH, Wendum D, Vibert F, Savouret JF, Therond P, Clot JP, Waligora AJ, Nivet-Antoine V (2010). Protective effect of post-ischemic treatment with trans-resveratrol on cytokine production and neutrophil recruitment by rat liver. Biochimie 92:405-410.
Headland SE, Norling LV (2015). The resolution of inflammation: Principles and challenges. Semin. Immunol. 27(3):149-160
Heuertz RM, Tricomi SM, Ezekiel UR, Webster RO (1999). C-reactive protein inhibits chemotactic peptide-induced p38 mitogen-activated protein kinase activity and human neutrophil movement. J. Biol. Chem. 274:17968-17974.
Inoue H, Nakata R (2015). Resveratrol targets in inflammation. Endocr. Metab. Immune Disord. Drug Targets. J. Periodont. Res. [Epub ahead of print].
Kaur M, Singh D (2013). Neutrophil chemotaxis caused by chronic obstructive pulmonary disease alveolar macrophages: the role of CXCL8 and the receptors CXCR1/CXCR2. J. Pharmacol. Exp. Ther. 347:173-180.
Kinane DF, Cullen CF, Johnston FA, Evans CW (1989). Neutrophil chemotactic behaviour in patients with early-onset forms of periodontitis (I). Leading front analysis in Boyden chambers. J. Clin. Periodontol. 16:242-246.
Kolaczkowska E, Kubes P (2013). Neutrophil recruitment and function in health and inflammation. Nat. Rev. Immunol. 13:159-175.
Kruger P, Saffarzadeh M, Weber AN, Rieber N, Radsak M, von Bernuth H, Benarafa C, Roos D, Skokowa J, Hartl D (2015). Neutrophils: Between host defence, immune modulation, and tissue injury. PLoS Pathog. 11:e1004651.
Larmonier CB, Midura-Kiela MT, Ramalingam R, Laubitz D, Janikashvili N, Larmonier N, Ghishan FK, Kiela PR (2011). Modulation of neutrophil motility by curcumin: implications for inflammatory bowel disease. Inflamm. Bowel Dis. 17:503-515.
Lin WW, Karin M (2007). A cytokine-mediated link between innate immunity, inflammation, and cancer. J. Clin. Invest. 117:1175-1183.
McCrea CE, West SG, Kris-Etherton PM, Lambert JD, Gaugler TL, Teeter DL, Sauder KA, Gu Y, Glisan SL, Skulas-Ray AC (2015). Effects of culinary spices and psychological stress on postprandial lipemia and lipase activity: results of a randomized crossover study and *in vitro* experiments. J. Transl. Med. 13:7.
McNeely MC, Lawley TJ, Harvath L (1993). Monoclonal antibody modulates human neutrophil chemotaxis to N-formyl-methionyl-leucyl-phenylalanine (fMLP). J. Invest. Dermatol. 101:377-382.
Moghaddam SJ, Barta P, Mirabolfathinejad SG, Ammar-Aouchiche Z, Garza NT, Vo TT, Newman RA, Aggarwal BB, Evans CM, Tuvim MJ, Lotan R, Dickey BF (2009). Curcumin inhibits COPD-like airway inflammation and lung cancer progression in mice. Carcinogenesis 30:1949-1956.
Mujumdar AM, Dhuley JN, Deshmukh VK, Raman PH, Naik SR (1990). Anti-inflammatory activity of piperine. Jpn. J. Med. Sci. Biol. 43:95-100.
Narumoto O, Matsuo Y, Sakaguchi M, Shoji S, Yamashita N, Schubert D, Abe K, Horiguchi K, Nagase T, Yamashita N (2012). Suppressive effects of a pyrazole derivative of curcumin on airway inflammation and remodeling. Exp. Mol. Pathol. 93:18-25.
Nogueira de Melo GA, Grespan R, Fonseca JP, Farinha TO, Silva EL, Romero AL, Bersani-Amado CA, Cuman RK (2011). *Rosmarinus officinalis* L. essential oil inhibits *in vivo* and *in vitro* leukocyte migration. J. Med. Food 14:944-946.
Nonose N, Pereira JA, Machado PR, Rodrigues MR, Sato DT, Martinez CA (2014). Oral administration of curcumin (*Curcuma longa*) can attenuate the neutrophil inflammatory response in zymosan-induced arthritis in rats. Acta Cir Bras 29:727-734.
Pearson W, Fletcher RS, Kott LS (2012). Oral rosmarinic acid-enhanced Mentha spicata modulates synovial fluid biomarkers of inflammation in horses challenged with intra-articular LPS. J. Vet. Pharmacol. Ther. 35:495-502.
Rocha J, Eduardo-Figueira M, Barateiro A, Fernandes A, Brites D, Bronze R, Duarte CM, Serra AT, Pinto R, Freitas M, Fernandes E, Silva-Lima B, Mota-Filipe H, Sepodes B (2015). Anti-inflammatory effect of rosmarinic acid and an extract of Rosmarinus officinalis in rat models of local and systemic inflammation. Basic Clin. Pharmacol. Toxicol. 116:398-413.
Sabina EP, Nagar S, Rasool M (2011). A role of piperine on monosodium urate crystal-induced inflammation--an experimental model of gouty arthritis. Inflammation 34:184-192.
Soon LL (2007). A discourse on cancer cell chemotaxis: where to from here? IUBMB Life 59:60-67.
Sunila ES, Kuttan G (2004). Immunomodulatory and antitumor activity of Piper longum Linn. and piperine. J. Ethnopharmacol. 90:339-346.
Vaibhav K, Shrivastava P, Javed H, Khan A, Ahmed ME, Tabassum R, Khan MM, Khuwaja G, Islam F, Siddiqui MS, Safhi MM, Islam F (2012). Piperine suppresses cerebral ischemia- reperfusion-induced inflammation through the repression of COX- 2, NOS-2, and NF-κB in middle cerebral artery occlusion rat model. Mol. Cell Biochem. 367:73-84.
Wang F (2009). The signaling mechanisms underlying cell polarity and chemotaxis. Cold Spring Harb. Perspect. Biol. 1:4.
Wang Z, Dabrosin C, Yin X, Fuster MM, Arreola A, Rathmell WK, Generali D, Nagaraju GP, El-Rayes B, Ribatti D, Chen YC, Honoki K, Fujii H, Georgakilas AG, Nowsheen S, Amedei A, Niccolai E, Amin A, Ashraf SS, Helferich B, Yang X, Guha G, Bhakta D, Ciriolo MR, Aquilano K, Chen S, Halicka D, Mohammed SI, Azmi AS, Bilsland A, Keith WN, Jensen LD (2015). Broad targeting of angiogenesis for cancer prevention and therapy. Semin. Cancer Biol. pii:S1044-579X(15)00002-4.
Woo HM, Kang JH, Kawada T, Yoo H, Sung MK, Yu R (2007). Active spice-derived components can inhibit inflammatory responses of adipose tissue in obesity by suppressing inflammatory actions of macrophages and release of monocyte chemoattractant protein-1 from adipocytes. Life Sci. 80:926-931.
Zang N, Li S, Li W, Xie X, Ren L, Long X, Xie J, Deng Y, Fu Z, Xu F, Liu E (2015.) Resveratrol suppresses persistent airway inflammation and hyperresponsivess might partially via nerve growth factor in respiratory syncytial virus-infected mice. Int. Immunopharmacol. 28: 121-128.
Zigmond SH, Hirsch JG (1973.) Leukocyte locomotion and chemotaxis. New methods for evaluation, and demonstration of a cell-derived chemotactic factor. J. Exp. Med. 137:387-410.


Journal of
Pharmacognosy and
Phytotherapy
Related Journals Published by Academic Journals
■ African Journal of Pharmacy and Pharmacology
■ Research in Pharmaceutical Biotechnology
■ Medical Practice and Reviews
■ Journal of Clinical Pathology and Forensic Medicine
■ Journal of Medicinal Plant Research
■ Journal of Drug Discovery and Development
■ Journal of Clinical Virology Research
academicJournals