ORIGINAL ARTICLE

Efficacy of *Ferula Caspica* Dried Sap by Extracting its Essential Oil Against the Clinical Yeast Isolates of *Candida Albicans*

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Abstract

Introduction: The aim of this experiment is to study the efficacy of *Ferula caspica* dried sap by extracting its essential oil against the clinical yeast isolates of *Candida albicans*, along with the comparison of standard antifungal chemical agents used in the therapy. This study also focused on a rapid gas chromatogram–mass spectrometric analysis for the phytochemical constituents of *F. caspica* dried sap essential oil extract to know exactly its content as many literature mentioned about its rich chemical contents contributing 4–20% of volatile oil, 20–25% latex gum, and the remaining 40–65% of the resin. **Materials and Methods:** The clinical sample from the patients was collected by employing the aseptic technique and was inoculated on a sterile Rose Bengal Agar plate by streak plate method and incubated at 37°C for 24–48 h to observe the yeast oval colonies. **Results and Discussion:** The interpretation of the observation and results for the *F. caspica* essential oil extract has shown the rejoicing study results regarding its efficacy as potential antifungal agents when compared to that of the standard synthetic chemical agents used against the clinical isolates of *C. albicans.* **Conclusion:** This study, thus, suggests that these types of natural essential oils can also be employed for the treatment of many infectious agents in the future as an alternative medicine.

Key words: Asafoetida dried sap, *Candida albicans*, clinical yeast isolates, essential oil, *Ferula caspica*, Ibn Sina, phytochemicals

INTRODUCTION

Ibn Sina the Persian noble scholar popularly known in the west as Avicenna regarded for his valuable contributions in the field of medicines has stated in his novel book of medicine about the medicinal values of many herbs to cure the infectious diseases and one such herbal product during his era was *Asafoetida*.[1] He termed *Asafoetida* has "Dog Dung" due to its pungent smell due to its rich content of sulfur and applied in his practice as a good antimicrobial substance.[1,2] Asafoetida was obtained from the taproot in the form of dried oleo resin gum from the different species of *Ferula* such as *Ferula asafoetida* and *F. asafoetida* with its relative species of *Ferula communis*, *Ferula caspica*, and *Ferula conocaula* belonging to the plant family of *Umbelliferae*.[3]

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The plants belonging to this family are perennial herbs found abundantly in the Persian region.[4] This herb is used mostly in the Indian culinary, especially in the southern parts of India, though it is not the origin of India.[2] *F. caspica* dried sap procured from the local market was used for this study against the *C. albicans* clinical yeast isolates to enumerate and evaluate the efficacy of this herbal sap toward the fungus.[5] The fungal yeast *C. albicans* clinical samples were collected from the immunocompromised patients with a prolonged exposure to the steroid therapies.

The aim of this experiment is to study the efficacy of *F. caspica* dried sap by extracting its essential oil against the clinical yeast isolates of *C. albicans* along with the comparison of standard antifungal chemical agents used in the therapy.[5-7] This study also focused on a rapid gas chromatogram–mass spectrometric (GC–MS) analysis for the phytochemical constituents of *F. caspica* dried sap essential oil extract to know exactly its content as many literatures mentioned about its rich chemical contents contributing 4–20% of volatile oil, 20–25% latex gum, and the remaining 40–65% of the resin.[8,9] The focus of the GC–MS study is focused mainly on the volatile oil content of the *F. caspica* dried sap as many literatures were already studied the strong sulfur content of this sap.[10] The resin portion of the sap was also studied much as it composes of major constituents of as a resinotannol as esters of the ferulic acid is also one. The abundantly sesquiterpene quantity of the galbanic acid presents in the resin portion along with another major key constituent known as umbelliferone.[7,11-14] The studies on the gum fraction of the sap suggest that it comprises abundant quantities of sugar glucose, rhamnose, galactose, 1- arabinose, and glucuronic acid. The studies of the volatile oils of the sap obtained from the distillation process have shown that it compromises mostly the abundance of the polysulphides, along with the versatile terpenoidal compounds.[6,12,13] The other major components present in the sap were the phenolic compounds such as vanillin and 3,4-dimethoxycinnamyl-3-(3,4-diacetoxyphenyl) acrylate, and acetylenes such as falccarinolone, along with the diterpenes such as the 7-oxocallitrisic acid, picealactone C, and 15-hydroxy-6-en-dehydroabietic with other miscellaneous compounds such as β-sitosterol and oleic acid.[6,12,13] These chemical components, especially the presence of the phenolic compounds, act as a major source for its antimicrobial efficacy. Hence, the study was conducted to evaluate and enumerate the efficacy *F. caspica* dried sap by extracting its essential oil against the clinical yeast isolates of *C. albicans*.[15,16]

MATERIALS AND METHODS

Materials

F. caspica dried sap, clinical samples from the patient, Saraud's dextrose agar, Rose Bengal Agar plates, dinitrogen and sodium sulfate, Gram's Stain Kit, Sheep's Serum, Whatman No. 1 filter paper, Corn Meal Agar, KOH, lactophenol, and potato dextrose broth were used. The dried saps of *F. caspica* were procured from Jeddah local market. All the chemicals used during this research were of analytical grade. Standard antibiotics and standard HiMedia were used.

Isolation and purification of *C. albicans*

The clinical sample from the patients was collected by employing the aseptic technique and was inoculated on a sterile Rose Bengal Agar plate by streak plate method and incubated at 37°C for 24–48 h to observe the yeast oval colonies.

A loopful of the colony was taken, and Gram's stain was performed to observe the Gram-positive violet-colored oval yeast budding cells.[16,17]

The KOH and lactophenol tests were performed to observe the yeast budding calls under a microscope by employing a wet mount technique.

The laboratory diagnostic test of the *C. albicans* was done by performing the confirmatory technique chlamydospore formation test. In this technique the isolated yeast colonies were inoculated on a corn meal agar and incubated at 37°C for 24–48 h to observe the chlamydospore formation of the yeast. This is an important characteristic of the medically important *C. albican*s and was also confirmed by the germ tube techniques. Here the collected sample was inoculated into the sheep's serum in a test tube and incubated for 2 h to observe the formation of the characteristic germ tube formation of the yeast.[18]

Extraction of *F. caspica* essential oil

The dried saps of *F. caspica* were collected and homogenized into a fine powder using a mechanical grinder separately and hydrodistillated.[4-6] The moisture content of *F. caspica* was enumerated to be 6.81 g/100 g on a dry basis and essential oil yield in the raw material which was 1.916 ml essential oil/100 g of *F. caspica* dried sap.[5,6] The extracted essential oil was filtered using the Whatman's No. 1 filter paper and concentrated with dinitrogen. The concentrate was then dried using anhydrous sodium sulfate and stored at 1°C in amber-colored flasks.

Phytochemical constituents of the *F. caspica* essential oil extract by GC/MS analysis

The GC–MS was applied for the determination of the phytochemical constituents of the *F. caspica* essential oil extract by employing the HP61711A mass selective

detector, along with an HP68170 gas chromatograph with an HP-6MS capillary column (10 m × 0.16 mm; film thickness 0.16 µm).[8,9,19,20] The temperature of the setup was programmed from 60 to 180°C at the rate of 1°C per min. The carrier gas used was helium with a flow rate of 1 ml/min. Injector and detector temperatures were programmed at 180°C. The ionization voltage of 10 eV with an ion source temperature of 110°C, along with the mass range of 16–116, was programmed as the MS operating parameters. The operating software employed here was the MSD Chem Station. The n-alkanes (C_8-C_{11}) were injected after the *F. caspica* essential oil at the same conditions used for the calculation of retention indices to determine the constituents of the extracts by means of the fragmentation process.[8,9,20]

Antimicrobial susceptibility test

The antimicrobial susceptibility test for the isolated clinical specimens of *C. albicans* was evaluated for the efficacy of the standard synthetic chemical antifungal agents by performing the latest rapid e-test methodology, where the clinical isolates were inoculated on potato dextrose agar plates separately[10,17] and e-test plastic strips for the respective antibiotics were impregnated and incubated at 37°C overnight to visualize the zone and ellipse and the results were tabulated by interpreting the observed results for the interaction of the ellipse as the minimum inhibitory concentration (MIC), whereas the zone as the susceptibility of the antibiotic toward the yeast.[10,17,20] The traditional standard antibiotic assay methods such as Kirby–Bauer disc diffusion method were employed to observe the susceptibility of the clinical isolates of *C. albicans* the standard disc prepared from the *F. caspica* essential oil extract, where the yeast isolates were inoculated separately on the potato dextrose agar plates along with the impregnated discs for 24 h at 37°C to observe the zone formation determining the sensitivity of the yeast toward the disc.[20] The results were tabulated and interpreted. The MIC values, along with minimum fungicidal concentration values for the efficacy antimicrobial activity of the *F. caspica* essential oil extract toward the yeast, were estimated by performing the standard tube dilution method, where the clinical isolates were inoculated separately in the different sets of dilutions of the extracts in the peptone water and incubated for 24 h at 37°C to observe the no turbidity determining the sensitivity of the yeast toward the acid. The last dilution with turbidity determines the MIC value of the acid toward the yeast. The results were tabulated and interpreted.[15,16]

The membrane fecal coliform (MFC) was determined by inoculating each dilution of MIC dilutions onto the separate agar plates for each clinical isolates of *C. albicans* for the MIC dilutions separately. The inoculated plates were incubated for 24 h at 37°C to observe the no growth determining the sensitivity of the yeast toward the *F. caspica* essential oil

Table 1: Phytochemical constituents of the *Ferula caspica* essential oil extract by gas chromatogram–mass spectrometric analysis

S. No	Phytochemical constituents	Percentage
1	Myrcene	1.21
2	α-Phellandren	0.90
3	β-Acoradiene	0.61
4	β-Gurjunene	0.95
5	β-Selinene	0.76
6	α-Selinene	0.46
7	Germacrene D	4.11
8	(E)-1-propenyl sec-butyl disulfide	1.21
9	Bis(1-methylpropyl1) disulfide	0.96
10	α-Copaene	0.76
11	β-Elemene	0.41
12	α-Cubebene	1.91
13	(Z)-Caryophyllene	1.41
14	α-Gurjunene	1.81
15	Bis(1-methyl thio) propyl disulfide	1.91
16	β-Gurjunene	1.51
17	α-Elemene	0.96
18	(Z)-1-propenyl propyl trisulphide	1.16
19	(E)-1-propenyl trisulphide	1.91
20	α-Humulene	1.71
21	α-Acoradiene	2.90
22	p-Cymene	0.55
23	Limonene	0.75
24	(Z)-β-ocimene	0.90
25	(E)-β-ocimene	0.88
26	1-Propyl sec-butyl disulfide	1.91
27	(Z)-1-propenyl sec-butyl disulfide	1.11
28	Cuparene	1.80
29	β-Bisabolene	1.28
30	Cadinene	1.36
31	methyl pentyl tetra sulphide	0.70
32	β-Cadinene	1.88
33	Calamenene	1.91
34	(E)-bisabolene	1.90
35	α-Cadinene	0.66
36	Elemol	0.81
37	Germacrene B	11.178
38	α-Amorphene	1.10
39	β-Humulene	0.16
40	Longipinene epoxide	1.91
41	Guaiol	1.75
42	6-epi-1-epi-α-eudesmol	8.08
43	β-Eudesmol	1.88
44	Epi-α-cadinol	13.11
45	α-Pinene	1.90
46	Camphene	0.80
47	Sabinene	0.76
48	1-β-pinene	0.96

Table 2: Efficacy of standard antifungal agents against clinical isolates of *Candida albicans* by e-test

Standard antifungal agents	Specimens									Average zone value
	Otic	Oral	Sputum	Urine	Vaginal	Blood	Catheter	Stool	Abscess	
Nystatin	2 mm R	4 mm R	12 mm I	20 mm S	22 mm S	20 mm S	20 mm S	23 mm S	20 mm S	15.88 mm
Clotrimazole	4 mm R	2 mm R	20 mm S	20 mm S	20 mm S	20 mm S	9 mm I	11 mm I	21 mm S	14.11 mm
Amphotericin B	4 mm R	2 mm R	10 mm I	20 mm S	21 mm S	21 mm S	2 mm R	4 mm R	2 mm R	9.5 mm
Fluconazole	20 mm S	21 mm S	20 mm S	23 mm S	20 mm S	20 mm S	9 mm I	11 mm I	22 mm S	18.44 mm
Itraconazole	9 mm I	11 mm I	21 mm S	20 mm S	20 mm S	21 mm S	20 mm S	10 mm I	20 mm S	16.88 mm
Posaconazole	11 mm I	6 mm R	20 mm S	20 mm S	2 mm R	10 mm I	2 mm R	4 mm R	2 mm R	8.55 mm
Caspofungin	4 mm R	2 mm R	20 mm S	20 mm S	10 mm I	10 mm I	4 mm R	2 mm R	9 mm I	9 mm
Micafungin	4 mm R	2 mm R	9 mm I	11 mm I	20 mm S	20 mm S	9 mm I	11 mm I	21 mm S	11.22 mm
Anidulafungin	20 mm S	20 mm S	10 mm I	10 mm I	9 mm I	20 mm S	20 mm S	10 mm I	21 mm S	16.66 mm
Total sensitives	2	2	5	7	6	7	3	1	6	
Total intermediates	2	1	4	2	2	2	3	5	1	
Total resistance	5	6	0	0	1	0	3	3	2	

extract. The first dilution with no growth determines the MFC of the extract toward the fungal yeast. The results were tabulated interpreted.[15,16]

RESULTS AND DISCUSSION

The phytochemical constituents of *F. caspica* essential oil extract by GC/MS analysis[19,20] shown around 48 components comprising the sap such as myrcene, α-phellandrene, β-acoradiene-gurjunene, β-selinene, α-selinene, germacrene D, E-1-propenyl sec-butyl disulfide, bis(1-methylpropyl1) disulfide, α-copaene, β-elemene, α-cubebene, Z-caryophyllene, α-gurjunene, bis (1-methyl thio) propyl disulfide, β-gurjunene-elemene, Z-1-propenyl propyl trisulphide, E-1-propenyl trisulphide, α-humulene, α-acoradiene, p-cymene, limonene, Z- β-ocimene, E- β-ocimene--propyl sec-butyl disulfide, Z-1-propenyl sec-butyl disulfide, cuparene, β-bisabolene-cadinene, methylpentyl tetra sulfide-cadinene, calamenene, E- -bisabolene, α-cadinene, elemol, germacrene B, α-amorphene, β-humulene, longipinene epoxide, guaiolepi-1-epi-α-eudesmol, β-eudesmol, epi-α-cadinol, α-pinene, camphene, sabine, and 1-β-pinene. The presence of phenolic and other miscellaneous constituents in the *F. caspica* essential oil extract contributes to its rich antimicrobial content and has shown promising results in this study as well.[9,15] The efficacy of the *F. caspica* essential oil extract against clinical isolates[9] of *C. albicans* was in line with that of the standard antifungal agents used in therapy and has shown excellent results when compared. The average value of the zone of inhibition susceptibility value of the *F. caspica* essential oil extract against clinical isolates of *C. albicans* was 21.77 mm for all the samples compared to the standard antifungal agents values of 15.88 mm for nystatin, 14.11 mm for clotrimazole, 9.5 mm for amphotericin B, 18.44 mm for fluconazole, 16.88 mm for itraconazole, 8.55 mm posaconazole, 9 mm for caspofungin, 11.22 mm for micafungin, and 16.66 mm for anidulafungin, respectively, for all the samples enumerated. The average MIC value of the *F. caspica* essential oil extract against clinical isolates of *C. albicans* was 1.25 µ/ml for all the samples compared to the standard antifungal agents values of 1.11 µ/ml for nystatin, 1.16 µ/ml for clotrimazole, 1.27 µ/ml for amphotericin B, 1.33 µ/ml for fluconazole, 1.61 µ/ml for itraconazole, 1.83 µ/ml for posaconazole, 2 µ/ml for caspofungin, 2.19 µ/ml for micafungin, and 2.22 µ/ml for anidulafungin, respectively, for all the samples enumerated. The MFC test was also performed[9,17] for the *F. caspica* essential oil extract against clinical isolates of *C. albicans* which was 1.5 µ/ml for all the samples. The details of all the antimicrobial susceptibility test assays were tabulated [Tables 1-4] for the reference. A comparative study chart [Figure 1] was prepared to project the efficacy of *F. caspica*

Table 3: MIC values of standard antifungal agents against clinical isolates of *Candida albicans* by e-test

Standard antifungal agents	Specimens								Average MIC values
	Otic	Oral	Sputum	Urine	Vaginal	Blood	Catheter	Stool	Abscess	
Nystatin	2.25 µ/ml	1 µ/ml	1 µ/ml	1.25 µ/ml	1.25 µ/ml	0.5 µ/ml	1.25 µ/ml	0.75 µ/ml	0.75 µ/ml	1.11 µ/ml
Clotrimazole	2.25 µ/ml	1.25 µ/ml	1.25 µ/ml	1.25 µ/ml	1.25 µ/ml	0.5 µ/ml	1.5 µ/ml	0.75 µ/ml	0.75 µ/ml	1.16 µ/ml
Amphotericin B	2.25 µ/ml	1.25 µ/ml	1.25 µ/ml	1.25 µ/ml	1.25 µ/ml	0.75 µ/ml	1.5 µ/ml	1 µ/ml	1 µ/ml	1.27 µ/ml
Fluconazole	2.25 µ/ml	1.5 µ/ml	1.25 µ/ml	1.5 µ/ml	1.25 µ/ml	0.75 µ/ml	1.5 µ/ml	1 µ/ml	1 µ/ml	1.33 µ/ml
Itraconazole	2.25 µ/ml	1.75 µ/ml	1.25 µ/ml	1.5 µ/ml	1.25 µ/ml	1 µ/ml	2.25 µ/ml	1.5 µ/ml	1.5 µ/ml	1.61 µ/ml
Posaconazole	2.5 µ/ml	2. µ/ml	2.25 µ/ml	1.5 µ/ml	1.25 µ/ml	1 µ/ml	2.25 µ/ml	1.75 µ/ml	1.75 µ/ml	1.83 µ/ml
Caspofungin	2.5 µ/ml	2.25 µ/ml	2.25 µ/ml	2.25 µ/ml	1.5 µ/ml	1.0 µ/ml	2.25 µ/ml	2.0 µ/ml	2.0 µ/ml	2 µ/ml
Micafungin,	2.5 µ/ml	2.5 µ/ml	2.25 µ/ml	2.5 µ/ml	1.5 µ/ml	1.25 µ/ml	2.5 µ/ml	2.25 µ/ml	2.5 µ/ml	2.19 µ/ml
Anidulafungin	2.5 µ/ml	2.25 µ/ml	2.5 µ/ml	2.5 µ/ml	1.75 µ/ml	1.25 µ/ml	2.5 µ/ml	2.5 µ/ml	2.75 µ/ml	2.22 µ/ml

MIC: Minimum inhibitory concentration

Table 4: Efficacy of *Ferula caspica* essential oil extract against clinical isolates of *Candida albicans*

Specimen	*Ferula caspica* Essential oil extract		
	Disc diffusion	MIC	MBC
Otic	19 mm S	1.75 µ/ml	2.25 µ/ml
Oral	18 mm S	1.5 µ/ml	2 µ/ml
Sputum	23 mm S	1.5 µ/ml	1.75 µ/ml
Urine	22 mm S	1.25 µ/ml	1.5 µ/ml
Vaginal	26 mm S	0.75 µ/ml	1.0 µ/ml
Blood	26.5 mm S	1 µ/ml	1.25 µ/ml
Catheter	19.5 mm S	1.25µ/ml	1.5 µ/ml
Stool	20.5 mm S	1.25 µ/ml	1.5 µ/ml
Abscess	22 mm S	1 µ/ml	1.25 µ/ml
Average value	21.77 mm S	1.25 µ/ml	1.5 µ/ml

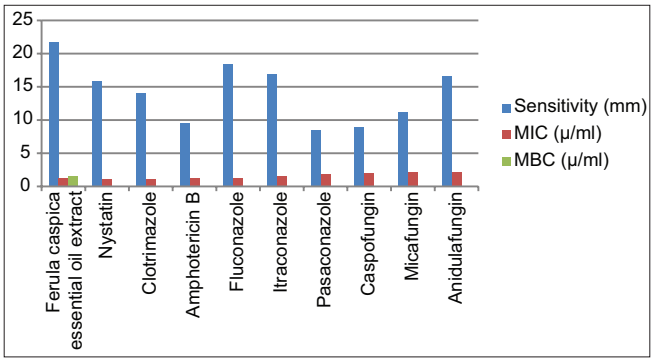

Figure 1: Efficacy of *Ferula caspica* essential oil extract versus standard antifungal agents against clinical isolates of *Candida albicans*

essential oil extract versus standard antifungal agents against clinical isolates of *C. albicans*. The average value of the zone of inhibition susceptibility value of the *F. caspica* essential oil extract against clinical isolates of *C. albicans* was 21.77 mm for all the samples compared to the best standard antifungal agents tested values of 18.44 mm for fluconazole. The average MIC value of the *F. caspica* essential oil extract against the clinical isolates of *C. albicans* was 1.25 µ/ml for all the samples compared to the best standard antifungal agents tested values of 1.33 µ/ml for fluconazole. The MFC test value was also performed for the *F. caspica* essential oil extract against clinical isolates of *C. albicans* which was also reasonable with 1.5 µ/ml for all the samples. The best antifungal drug available in the market is Fluconazole and with no surprise that has

shown the best antifungal efficacies of all the agents used, but the *F. caspica* essential oil extract has surpassed the test values far more better.

CONCLUSION

The interpretation of the observation and results for the *F. caspica* essential oil extract has shown the rejoicing study results regarding its efficacy as potential antifungal agents when compared to that of the standard synthetic chemical agents used against the clinical isolates of *C. albicans* immunocompromised patient's samples. This study, thus, suggests that these types of natural essential oils can also be employed for the treatment of many infectious agents in the future as an alternative medicine.

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CONTRIBUTION OF AUTHORS

All authors have made a substantial contribution to the work and approved it for publication.

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