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The effect of plant extracts of *Moringa Oleifera* on Some Pathogens Isolated from the oral cavity of Diabetic mellitus type 2 Patients

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Abstract

The present study seeks to assess the biological activity of Moringa oleifera plant extracts in the Samawa governorate. The aerial parts of the plants were extracted using a Soxhlet extractor with two solvents of varying polarity: methanol and hexane. Active compounds were identified through Gas Chromatography Mass Spectrometry (GC-MS). The gas chromatography results for the Moringa extract using methanol revealed 9 active chemicals, but the extract using hexane exhibited 24 active compounds.

The antibacterial efficacy of the crude extracts is evaluated against three species of pathogenic bacteria and fungi, including two gram-positive bacteria. Streptococcus mutans Utilize a disc-diffusion experiment to evaluate Enterococcus faecalis and Candida albicans, and ascertain the Minimum Inhibitory Concentration (MIC) for four distinct extract concentrations of each organism. Concentrations: (0, 25, 50, 75 mg/ml). Did the results of the inhibitory activity of the extracts alter based on the type of extract and the various bacteria tested? Moringa oleifera extracts exhibit significant inhibitory effects against all bacteria and fungus, particularly the hexane and methanol extracts.

Keyword: Moringa oleifera, S. mutants, Enterococcus feacalis ,Candida albicans, the GC-MC

1-Introduction

Medicinal plants serve as a significant source of secondary metabolites, owing to their pharmacological qualities and potential components for product development in the food, visual, and pharmacological sectors [1].

The Moringa plant (Moringa Oleifera) has been extensively studied for its diverse applications and recognized antibacterial properties.MO is a rapidly expanding tropical fruit-bearing tree. This plant has been utilized for ages and is extensively prescribed in traditional medicines; it was referenced in ancient Egyptian, Roman, and Greek texts. It is currently disseminated and produced as crop in numerous nations in Africa, Asia, Latin America, and the Caribbean.

Diabetes mellitus derives from the Greek term 'Diabetes,' signifying 'siphon' or 'to pass through,' and the Latin word 'mellitus,' meaning 'sweet.' It is an endocrinological condition caused by an anomaly in insulin secretion or action. Diabetes mellitus (DM) is a metabolic condition that can impact the oral micro biome and result in problems such as periodontitis. [5].

The mouth cavity harbours around 700 species of bacteria and other microbes, including fungus, parasites, and viruses, collectively known as the oral macrobiotic. Streptococcus mutans is an oral bacteria that can induce dental caries. Enterococcus faecalis and Candida albicans are identified as oral pathogens that may contribute to these problems [8].

2-Materials and Methods:

Collection and classification of plants:

Moringa oleifera leaves were collected and identified.

Preparation of plant samples: The leaves were dried, ground into powder, and stored for later use.

Preparation of plant extracts: The powdered leaves were extracted using two solvents (hexane and methanol) with a Soxhlet apparatus [9].

Preparation of stock solution and dilutions: The extracts were dissolved in water to prepare concentrated solutions. These solutions were further diluted to obtain different concentrations for testing [10].

Disk diffusion method: This method was used to test the effectiveness of the plant extracts against bacterial and fungal pathogens. The extracts were loaded onto discs placed on agar plates containing the pathogens. The presence of clear zones around the discs indicates inhibition of pathogen growth by the extracts. [11].

Identification of Components by GC-MC: The mass spectrum interpretation of GC-MS was conducted utilising the National Institute of Standards and Technology (NIST) database, which has over 62,000 patterns. The mass spectrum of the unidentified component was juxtaposed with the spectra of the known components archived in the NIST library.

Collection of bacterial isolates: Bacteria (*Streptococcus mutans, Enterococcus faecalis)* and fungus (*Candida albicans*) were isolated from mouth swabs collected from diabetic patients.

Bacterial samples identification: The bacteria were identified using standard microbiological methods like Gram staining, microscopy, and culturing on specific media

3-Results and Discussion

Identification of Components of the moringa extraction with methanol by GC-MS:

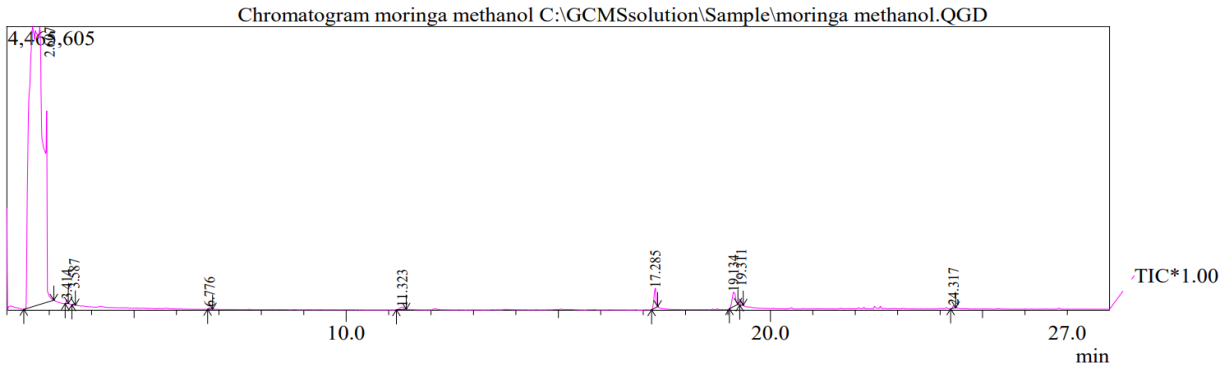

Figure 1 : GC-MS Chromatogram of methanolic extract of Moringa oleifera

The GC-MS analytical results facilitated the identification of many components from the methanol extract of the *Moringa oleifera* plant. The GC-MS chromatogram displayed nine peaks, signifying the existence of 45 chemicals.

Table (1): Phytocomponents identified in the methanolic extracts of *Moringa oleifera*

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%%	A/H	Name
1	2.607	2.400	3.108	101279298	97.06	4408093	84.71	22.97	Dipirartril-tropico
2	3.414	3.375	3.450	105294	0.10	58999	1.18	1.78	Methane sulfonamide
3	3 3.587	3.533	3.617	57142	0.05	24367	0.47	2.38	Hepta-4,6-diyn-2-ol
4	4 6.776	6.733	6.850	84678	0.08	27521	0.53	3.05	N-Nitroso-2-methyl-oxazolidine
5	5 11.32	11.183	11.417	254288	0.24	36375	0.70	6.97	3-Butene-1,2-diol
6	6 17.28	17.200	17.342	1035148	0.99	312778	6.01	3.29	3-Ethylheptanoic acid
7	7 19.13	19.033	19.233	1248698	1.20	226391	4.35	5.48	9-Oxabicyclo[6.1.0] nonane, cis-
8	8 19.31	19.275	19.358	167171	0.16	71605	1.38	2.34	3-Ethylheptanoic acid
9	9 24.31	24.250	24.367	111257	0.11	37873	0.73	2.89	12-Cyclohex-3-enyl-3-methyl-8,9,10,12-tetrahydro-7H-benzo[b][4,7]phenanthrol n-11-one
				104342974	100.00	5204002	100.00		

Peak Report TIC

Identification of Components of the moringa extraction with hexane by GC-MS:

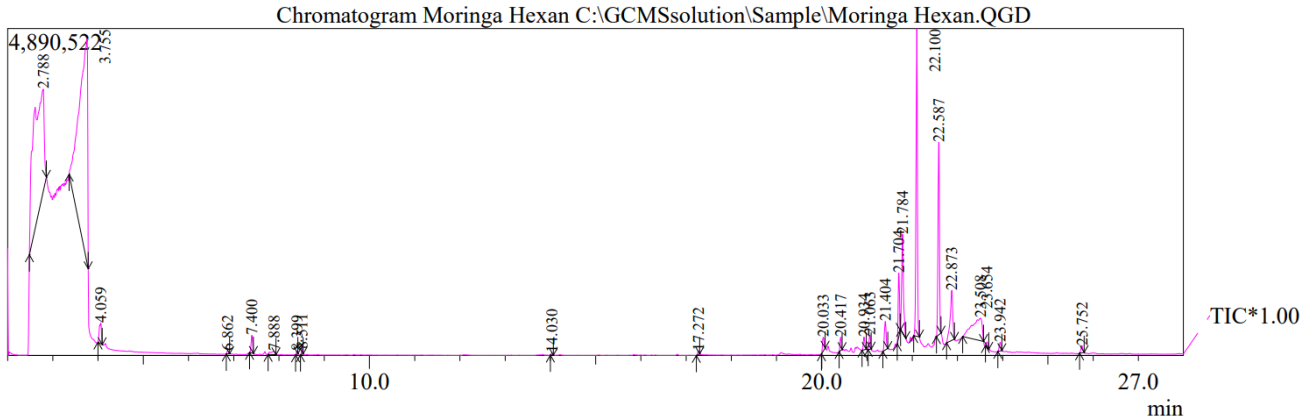

Figure 2: GC-MS Chromatogram of methanolic extract of *Moringa oleifera*

The GC-MS analytical results facilitated the identification of many components from the methanol extract of the Moringa oleifera plant. The GC-MS chromatogram displayed 22 peaks, signifying the existence of 110 chemicals.

4- Microbial effect of *Moringa oleifera* Extract with methanol

The results of the methanol extract of the *Moringa oleifera* plant showed antimicrobial effects at the three concentrations mentioned (25, 50 and 75 %). The extract showed increasing effectiveness with increasing concentrations, indicating a dose-dependent relationship.

The methanol extract of *M. oleifera* showed in (table 4-5) a higher inhibitory effect against *Enterococcus feacalis* with mean of inhibition zone diameter (30.1333 a)mm at concentration (75%) mg/ml followed *candida albicans* with the same concentration the mean of inhibition zone diameter (28.1667 c)mm, *Streptococcus mutans* was recorded with mean of inhibition zone diameter (21.1333 e) mm with the same concentration .

There were high significant differences between the concentration of extract of *M. oleifera* with the highest a mean of

Pathogens	**Conc. Of methanol extract of *M. oleifera* (mg/ml)**				**Mean of total inhibition zone and SD**	**P value**
	0%	**25%**	**50%**	**75%**		
Streptococcus mutant						
Mean	.0000 i	15.0333 h	16.1667 g	21.1333 e	13.0833 c	P < 0.001
Std. Deviation	.00000	.35119	.56862	.61101	8.25446	P < 0.001
Enterococcus feacalis						
Mean	.0000 i	28.0333 c	29.2333 b	30.1333 a	21. 8500 a	P < 0.001
Std. Deviation	.00000	.55076	.11547	.61101	13.15862	P < 0.001
candida albicans						
Mean	.0000 i	20.1000 c f	25.1000 d	28.1667 c	18.3417 b	P < 0.001
Std. Deviation	.00000	.65574	.55678	.85440	11.41181	P < 0.001
Total						
Mean	.0000 d	21.0556 c	23.5000 b	26.4778 a		
Std. Deviation	.00000	5.69344	5.68749	4.10379		

inhibition zone diameter against *E.feacalis* , *S.mutans and c. albicans* and other concentrations, whereas there were generally non-significant differences between a mean of total inhibition zone of *E.feacalis* , *S.mutans and c. a*

Table (2): the mean of inhibition zone diameters (mm) caused by Methanol extract of *Moringa oleifera at* various concentrations against pathogens tested.

lbicans , this was evident in Figure ( ).

Due to the appearance of bioactive compounds with microbial activity in the GC-MC analysis table (4-4), proved that *M. oleifera* by extract methanol it has antifungal and antibacterial properties .

SD: standard deviation; p: Anova ; HS :highly significant at p < 0.001

5-Microbial effect of Moringa *oleifera* Extract with n- Hexane

The hexane extract of *M.oleifera* manifested a very high inhibitory effect against the tested bacterial species and c*andida albicans* at all or most of the concentrations as manifested by the (Table 3), the extract showed increasing effectiveness with increasing concentrations.

S.mutant and E.feacalis were the most sensitive to the extract at all concentrations, the highest at concentration (75%)mg/ml with a mean of inhibition zone diameter (30.0333 a)mm, followed by *c. albicans* also at all concentrations, was most sensitive at the same concentration(75%)mg/ml with a mean of inhibition zone diameter (27.1000 c)mm.

There are general statistically significant differences between the average diameters of the total inhibition zone for *S.mutant , E.feacalis* and c.albicans where the positive bacteria were the most sensitive to the extract, this was confirmed by (Figure 4-11).

Table (1) GC-MC results for the *Moringa* plant with hexane showed many active chemical compounds with antibacterial and antifungal properties, this proves that the *Moringa* plant with hexane is very useful in treating many different microbial species.

The hexane extract was high effective to all of the tested microorganisms compared with the methanol extract showed low antibacterial activity against (*S.mutants*, *E. feacalis*, and *C. albicans*).

Table (3): the mean of inhibition zone diameters (mm) caused by n-hexan extract of *Moringa* oleifera at various concentrations against pathogens tested .

Pathogens	Conc. Of n-hexane extract of *M. oleifera* (mg/ml)				Mean of total inhibition zone and SD	P value
	0%	25%	50%	75%		
Streptococcus mutants						
Mean	.0000 h	25.1667 e	27.0667 c	30.0333 a	20.5667 b	P < 0.001
Std. Deviation	.00000	.66583	.30551	.25166	12.53811	P < 0.001
Enterococcus feacalis						
Mean	.0000 h	25.9667 d	29.0467 b	30.0333 a	21.2617 a	P < 0.001
Std. Deviation	.00000	.45092	.33247	.45092	12.92027	P < 0.001
candida albican						
Mean	.0000 h	22.1000 g	24.1333 f	27.1000 c	18.5833 c	P < 0.001
Std. Deviation	.00000	.76376	.41633	.45826	11.28997	P < 0.001
Total						
Mean	.0000 d	24.7444c	26.7489 b	29.0556 a		
Std. Deviation	.00000	1.51502	2.16276	1.50674		

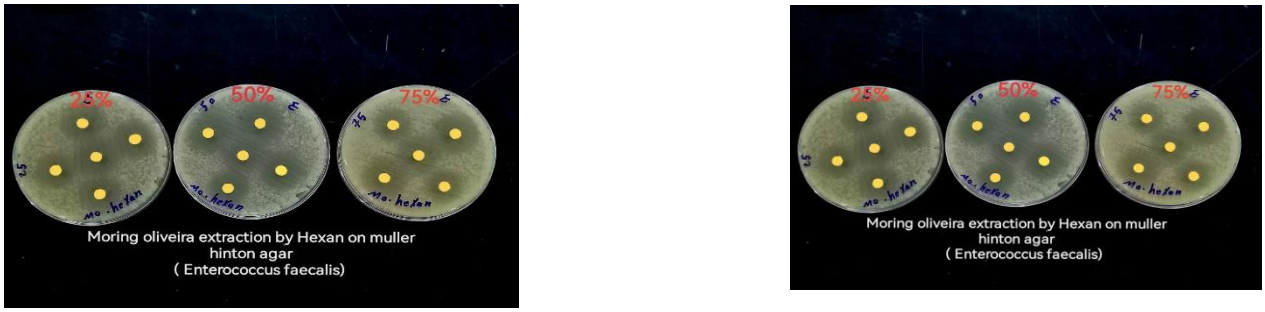

Figure 3 : Inhibition Zone Diameters (Mm) Of Hexane Extract Of *Moringa Oleifera* Activity Against Tested Bacterial And Fungal

6-Conclusion

This study investigated the biological activity of *Moringa oleifera* extracts from the Samawa governorate. Both methanol and hexane extractions yielded bioactive compounds, with hexane extracts showing a greater number (24 vs. 9 for methanol).*Moringa oleifera* extracts, particularly the hexane extract, exhibited potent inhibitory effects against all tested bacteria (*Streptococcus mutans, Enterococcus faecalis*) and the fungus *Candida albicans*. The inhibitory activity increased with higher extract concentrations (0, 25, 50, 75 mg/ml).

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