ORIGINAL ARTICLE

Exploration the Impact of *Morenga Oleifera* leaves as anti-bacterial and tumor inhibitor and Phytochemical profiling by GC-Mass analysis

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ABSTRACT

Plant-derived medications remain an attractive resource to fight many diseases especially in developing countries, *Morenga oleifera* is called (miracle tree) due to its potential use in many diseases treatment traditionally. After cultivation of *Morenga* locally, alcoholic leaves extract has prepared by maceration. Authors intended to provide a phytochemical profiling for leaves of *Morenga* via GC-Mass analysis. Then directed fifteen bioactive compounds that investigated within alcoholic leaves extract to explore their ability to inhibit the growth of several tested pathogens and to inhibit proliferation of breast cancer cell line by using MTT assay. Authors hypothesized that plant extract was good candidate as safe and alternative therapy against infections caused by *Pseudomonas aurogenosa*, *Streptococcus* sp. and *Candiad albicans* after calculating (22± 0.002, 20± 0.002, 23± 0.003)mm. Inhibition zones by plant extract against the previous pathogens respectively as compared with synthetic antibiotics. The decrease of absorbance values were detected when treat MCF7 cell line with different concentration of plant extract as compare with un treated cancer cells after follow- up periods especially after 48 hr. incubation. This indicates that Morenga leaves could be a potential drug to eliminate breast cancer incidences.
Keywords: Morenga Oleifera, phytochemical, GC-Mass analysis, Iraq

INTRODUCTION

Morenga oleifera plant is one of fourteen species related to morengaceae family, a native tree cultivated in various regions of the worldwide including India, Africa, Asia as well as Arab regions[1], this tree characterized with size ranged from small to medium with height from 5 to 12m, have a straight trunk hold an open umbrella-like crown, stabilizes in soil with a tuberous tap root. Depending on climate its leaves appearing in evergreen or deciduous s colo[2].

Traditionally *M. oleifera* plant naming 'Miracle Tree' due to its economic significance and adaptability[3] due to its high nutritional value, this tree is broadly used as food product as well as considers one of the potent plants possess a variety of therapeutic applications[4]. *M. oleifera* is a tropical tree widely applied as conventional therapy of several diseases , Various parts of this tree calculated to posses some therapeutic properties, Paliwal et al.,2011[5] reported that flowering parts and seed exhibited anti-hypertensive and hypolipidemic properties, While root and flower exhibited anti-inflammatory properties, also anti-ulcer properties have reported for bark. Traditionally, different parts have been used as cancerous treatment either in powdered form or as aqueous and alcoholic extract[6].

Herbal mediated treatment is one of the most distributed strategy till now for basic health care nearly 65-80% of the world's populations , particularly in developing regions, which resulted as good fitness of the individual body, well cultivation appropriateness as well as less common adverse impacts. The medicinal values of medicinal plants explaining by owing different bioactive compounds which produce unique physiological effectiveness which positively affect on the human body[7].

During this study, researchers were attempt to give insight for using the leaves of *M. oleifera* as promising therapy by applied of different concentrations of leaves extract were prepared by alcoholic maceration against infections caused by several pathogens including bacteria and *Candida* isolated from various clinical sources, and directed the extract as anti-proliferative agent on breast cancer cell line (MCF7). These suggestions have based on exploration of the main phytochemicals with their biological activities within *M.oleifera* leaves extract via GC-Mass analysis.

MATERIALS AND METHODS

Plant description and extract preparation: The researchers have grown *M. oleifera* plant during February in 2019, classified by a plant taxonomists in Department of biology/ Mustansiriyah university/Iraq. After that the leaves of *M. oleifera* harvested during November to make crude extract which employed for exploration of phytochemicals and screening of bioactive properties.

Figure 1: photograph of *Morenga oleifera* tree cultivated by the researchers

Plant leaves have been dried at 37 C, grinded and extracted in alcohol (methanol) by maceration of leaves powder applying method reported by (8)(Priadarshini *et al*.,2013). The leaves powder 5gm added in to the conical flask of 100ml methanolic extract, agitated for six hours and preserved quietaly for the next 18 hours. After that, the mixture was get and separated using whatman No.1 filter paper. The resulted liquid part then added to the clean beaker and dried in oven about 400C. The dried powder was measured and stock solution was prepared to obtain concentration (25 mg/ml). Finally, these dried powder preserved in the dark in refrigerator until applied for further study.

Exploration the phytochemicals of extract via Gas Chromatography-Mass Spectrometry (GC-Mass): The substances have detected via Gas Chromatography-Mass Spectrometry (SHIMADZU—Japan) and used the procedure which was reported in [9] for comparison of our results with NIST library search and authentic standards.

Collection and performance of bioactivity Test of methanolic extracts of *Morenga oleifera* against tested pathogens:

In terms to microbial isolates, authors have collected them from higher graduate laboratory in biology department/college of science/ Al-Mustansiriyah University. Their isolates were derive from different clinical sources. The obtained bacterial strains was re cultivated on nutrient agar slants and kept at 4 °C for further study .While yeasts maintained using Sabouraud dextrose agar (SDA) medium . The bioactivity Test of Three concentrations (25, 12.5, 6.25 mg/ml) of methanolic leaves extracts were tested in vitro by using well diffusion method according to [10].

Antibiotic sensitivity Assay

To compare between prepared extract of Morenga leaves and traditional antibiotics, authors have investigated the efficiency of five different antibiotics (Amikacin, Ticarcillin-Clavulanate , Ciprofloxacin , Cefepime) against tested bacteria and a concentration (10µg/ml) of Clotrimazole against tested *Candida albicans* by using the disc diffusion method [11] .

In Vitro anticancer activity: To achieve this test, four serial dilutions were prepared from stock leaves extract to obtain (12.5, 6.25, 3.125, 1.56 /mg/ml). The methanolic efficiency of plant leaves estimated as a breast cancer cell lines (MCF7) which achieved by the center Biotechnology research center of Al-Nahraine University. The MTT assay was used for colorimetration of cell viability which was evaluated in (10^6 cell/ mL) 96-well tissue culture plate as shown by (Fayyad et al.,2019) [9] and (Freshney, 2012) [12]. The anticancer activity of plant extract have determined by measuring the absorbance using an ELISA reader at 620 nm for each well cancer cell.

RESULTS AND DISCUSSION

Exploration the phytochemicals of extract using Gas Chromatography-Mass Spectrometry: As a result of this analysis, fifteen compounds have been recorded within alcoholic extract of *Morenga oleifera* leaves (Figure 1). These phytochemicals authenticated to possess different biological activities (Table 1). These compounds displayed in ascending manner according to percentage area that the compound have occupy within plant extract. The bioactive properties of these compounds mentioned with details during explaining both Anti -bacterial and Anti -tumor results.

Figure 2: GC-Mass spectrophotometery chromatogram presented the alcoholic extract of *Morenga oleifera* leaves

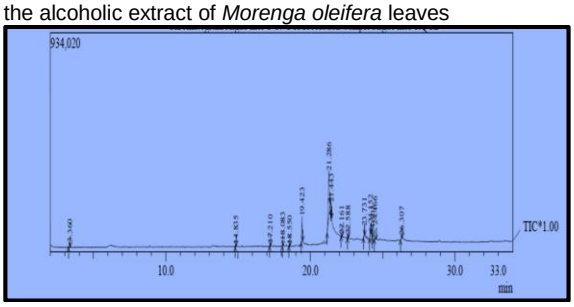
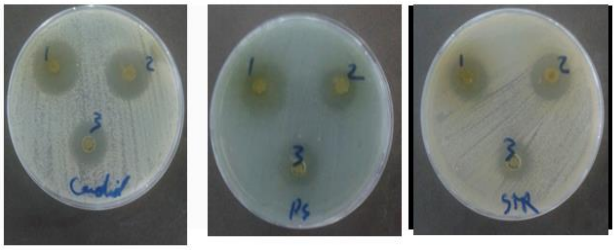


Figure 3: inhibition zones resulted by the three different concentrations of plant leaves extract, well no.1:25 mg/ml , well no.2:12.5 mg/ml, well no.3:6.25 mg/ml



Bioactivity Test of methanolic extracts of *Morenga oleifera* against tested pathogens: As displayed in table 2, the extract gave inhibition zones around tested pathogens culture at different rates and the potency of the plant extract was depend on microbial isolate as well as the dose of plant extract.

Each value is Mean ± SD (n=3): Based on the previous data, plant extract exhibited highest inhibition zone against *C.albicans(* 23 ± 0.003 mm. {at 25 mg/ml}) followed by *P. aurogenosa* (22±0.002 mm.{ at 25mg/ml}) . Then (20±0.002mm). {at 12.5 mg/ml}) against *Streptococcus sp.*

Depending on GC- Mass analysis findings, prepared alcoholic extract of *Morenga* leaves containing many antimicrobial compounds as listed below:

1. Acetic acid, 1-methylpropyl ester reported as antibacterial, anti-salmonella, anti-vaginitic and Fungicide[13].
2. Hexadecen-1-ol also reported as antimicrobial agent[9].
3. Pentadecanal also exhibits antibacterial activity[14].
4. Cyclopropanebutanoic acid authenticated as antifungal agent[15].
5. Eicosanoic acid is fatty acid reported as antimicrobial activity[16].

Antibiotic sensitivity Assay: Antimicrobial resistance has become an intensive problem, since synthetic drugs agents like antibiotics have shown a potency toward many infectious diseases , their use most be limited due to the fast incidence of resistant development by pathogens. Thus a significant number of new antibiotics offered to the market are synthesized from semi-synthetic or natural resources[17].

Different antibiotic susceptibility of tested bacteria have registered in current study, data has been displayed in table 3. Notably, not all tested antibiotic caused growth inhibition on tested bacteria. As compared with antibacterial results that recorded by alcoholic extract of *Morenga* leaves, authors hypothesized that plant extract was good candidate as safe and alternative therapy against infections caused by *P. aurogenosa, Streptococcus* sp*. and C. albicans* .To explain that, plant extract gave inhibition zone (22±0.002mm) against *P. aurogenosa* where as Amikacin, Ticarcillin-Clavulanate caused less zone on, and Cefepime gave no inhibition zone on. In case of *Streptococcus* sp., plant extract gave (20±0.002mm) this was equivalent to Ticarcillin-Clavulanate but the bacteria was resists against both Ciprofloxacin and Cefepime. Also for *C. albicans*, (23±0.003mm.) was recorded by *Morenga* extract. This zone diameter was less than that recorded by antifungal (clotrimazole) 21mm.

In Vitro anticancer activity: Findings of current study revealed that leaves of *M. oleifera* could act as potential anti-proliferative drug for breast cancer based on decrease of the absorbance values treated wells as compared to control. This denotes proliferation inhibition of cancer cells after follow up periods especially after (48 hr.) incubation with the four dilutions of *Morenga* leaves extract (Table 4).

Table 1: Major Phyto-components obtained through the GC/MS Study of *M. oleifera leaves extract*

NO. of peak	Compound name	Retention time	Composition Area
9	pentadecen-1-ol	22.161	0.77%
5	Pentadecanal	18.550	0.84%
2	Hexadecanoic acid	14.835	0.89%
3	Tetradecanoic acid	17.210	0.90%
13	Cyclooctene	24.303	0.91%
10	Eicosanoic acid	22.588	1.50%
14	Octadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	24.466	1.97%
4	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	18.083	2.09%
15	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	26.307	2.53%
8	Cyclopropanebutanoic acid	21.443	3.45%
1	Acetic acid, 2-methylpropyl ester	3.360	3.78%
11	14-Methyl-8-hexadecyn-1-ol	23.731	4.78%
12	Oleyl Alcohol	24.152	6.16%
6	Tetradecanoic acid	19.423	14.41%
7	Hexadecatrienal	21.286	55.04%

Table 2: Diameters of inhibition zones formed around tested pathogens cultured with plant extract.

	Plant extract concentrations (mg/ml)		
isolate	6.25	12.5	25
Pseudomonas aurogenosa	16 ± 0.002mm.	17 ± 0.01mm.	22± 0.002 mm.
Streptococcus sp.	16 ± 0.004mm.	20± 0.02 mm.	19 ± 0.01mm.
Klebsiella pneumonia	8 ± 0.007mm.	10 ± 0.001mm.	10 ± 0.002mm.
Candida albicans	19± 0.002 mm.	23± 0.002 mm.	23 ± 0.003mm.
Staphylococcus aureus	10± 0.002 mm.	10 ± 0.001mm.	12 ± 0.002mm.

Table 3: Findings of antibiotic sensitivity test against tested pathogens

Isolate	*Pseudomonas aurogenosa*	*Streptococcus sp.*	*Klebsiella pneumonia*	*Staphylococcus aureus*	*Candida albicans*
Clotrimazole					21
Amikacin	17	36	20	23	
Ticarcillin-Clavulanate	19	20	R	R	
Ciprofloxacin	30	R	25	28	
Cefepime	R	R	R	R	

Table 4: absorbance values of tested cancer cell line after treatment with different concentrations of plant extract

Concentration	After 24 hr.	After 48 hr.
1.56 mg/ml	0.842±0.2	0.332±0.05
3.125 mg/ml	0.941±0.01	0.509±0.05
6.25 mg/ml	0.826±0.2	0.518±0.07
12.5 mg/ml	0.755±0.06	0.645±0.08
Control	1.2±0.2	

Each value is Mean ± SD (n=3)

Strong cell lines inhibitory effects exhibited by methanolic extract of *Morenga oleifera* leaves in the current study may due to the presence of many chemical compounds recorded to possess many biological activities including anti-tumor, antioxidant effect as described below:

1. Acetic acid, 1-methylpropyl ester that have anticancer, acidulant, Expectorant, Perfumery and Pesticide activity as reported by Ramya et al. 2015[13].
2. N-Hexadecanoic acid that reported as antioxidant, nematicide and pesticide compound by Aparna et al., 2012[18], this chemical compound also has been reported as a selective anti-proliferative effect on human cell lines besides anti-tumor efficiency in mice in vivo as depicted by Harada et al., 2001[19].
3. Tetradecanoic acid, that have anticancer, antioxidant, nematicide, anti-spasmodic, anti-asthmatics activity and used in cosmetics as demonstrated by Khairy and El-Kassas 2010[20].
4. Hexadecen-1-ol that have anticancer, antidiuretic, antioxidant activity Raman et al, 2012[21].
5. Pentadecanal that possess antioxidant activity in addition to using it as nutrient, stabilizers, surfactants and emulsifieras reprted by Jadhav et al., 2014[14].
6. Eicosanoicacid that reported as antioxidant as concluded by Idowu and Ojo 2017[22].
7. Pentadecanoic acid, reported as anti-tumor, anti-abortive and anti-asthmatics chemical compounds Ansarali et al., 2018[23].
8. 9,12-Octadecadienoic acid (Z,Z)-methyl ester that reported as cancer preventative, 5 Alpha reeducates inhibitor, insectifuge and used as perfumery flavor as reported by Diana and Parthipan, 2015[24].

Breast cancer is corresponding to the major cause of death among women worldwide and following cardiovascular diseases in Iraq[25]. Breast cancer rates in Iraq were basically constant during the period from 2000 to 2009, but recent statistic studies from the Iraqi Cancer Registry reported increasing values from 2009 as concluded by Abedalrahman et al., 2019[26].

Patients suffering from cancer use medication strategy involving in plants as complement or sustainable in traditional treatment[27]. Anti- proliferative effects on breast cancer cell line in present paper discussed as prior of Al-Asmari et al., 2015[28], the researcher explained perceivable rising of whole apoptotic cells numbers with dose dependent manner. Also decreasing in cellular motility, colony formation and survival rate for cancer cells have been detected after treated breast cancer cell line with extract of *Morenga oleifera*.

As reported by Mohammed Ali et al.,2020[29], who reported that many plant extracts contains antioxidants compounds therefore it can be used as potent cancer treatment agents, and the side effects and toxicity of these compounds are less than synthetic drugs .According to displayed data concerning with anti-microbial and MTT tests, *Morenga oleifera* leaves can be acts as alternative medicine toward breast cancer and to eliminate the infectious diseases caused by the tested serious pathogens and can ultimately make a progress in life quality of the rural area and peri-urban individuals in developing countries.

Therefore, this study provides a phytochemical profiling for methanolic extract of *Morenga* leaves via GC-Mass analysis. Depending on the findings, the constituents of this tree can act as suitable candidates in many pharmaceutical industries due to its properties as Anti-asthmatics, anti-abortive, antidiuretic, antispasmodic, stabilizer, surfactants and emulsifier

CONCLUSIONS

- The detected active constituents of *Morenga* leaves sample could be a source of potential candidates to investigate for active constituents that could lead to expansion of drug industries of both broad antimicrobial spectrum antibiotic and breast cancer therapy.
- However, it should be noted that research on plant derived bioactive compounds at in vitro level might not always culminate with the same efficacy when administered into human body. More in vivo studies and investigations of certain mode of action of phytochemicals are needed.

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REFERENCES

1. Somali, MA., Bajnedi, MA., Al-Faimani, SS. Chemical composition and characteristics of Moringa peregrina seeds and seed oil. *The Journal of the American Oil Chemists' Society*, 1984; 61: 85–86.
2. Khudaer, N., Hassn, ZY., AL-Sammarrae, KW., Ibrahim, NK. Purification and Identification of Total Flavonoids Extracted from Moringa oleifera Leaves in Iraq. *Journal of Biotechnology Research Center*, 2016; 10(2): 73-80.
3. Abe, R., Ohtani, K. An ethnobotanical study of medicinal plants and traditional therapies on Batan Island, the Philippines . Journal of Ethnopharmacology, 2013; 145(2): 554-65. doi: 10.1016/j.jep.2012.11.029.
4. Vinodini, NA., Pratik, KC., Anwar, A., Suman, VB., Sheila, RP. Evaluation Of Liver Functions With Moringa Oleifera Leaf Extract In Cadmium Induced Adult Wistar Albino Rats. International Journal of Plant and Environment Sciences, 2014; 4(3): 103-106.
5. Paliwal, R., Sharma, V., Sharma, PS. Elucidation of free radical scavenging and antioxidant activity of aqueous and hydro-ethanolic extracts of Moringa oleifera pods. Research Journal of Pharmacy and Technology, 2010; 4(4): 566-571.
6. Krishnamurthy, PT., Vardarajalu, A., Wadhwani, A., Patel, V. Identification and characterization of a potent anticancer fraction from the leaf extracts of Moringa oleifera L. Indian Journal of Experimental Biology. 2015; 53(2): 98-103.
7. Mohammed-Ali AN., Fayyad, RJ., Dwaish, AS., Al-aboodi, AK. Cytotoxic effect of methanolic extract of Morenga oleifera leaves on two cancer cell lines in vitro. Bioscience Researches, 2019; 16(2): 1214-1222
8. Priadarshini, A., Pankaj, PP., Varma, MC., Kumar K. Evaluation of the antibacterial potential of Moringa oleifera and Azadirachta indica against some pathogenic microbes: A comparative study. International Journal of Drug Development and Research, 2013; 5(1): 214-218.
9. Fayyad, RJ., Mohammed-Ali, AN., Dwaish, AS, Al-aboodi, AK. Anticancer activity of *spirulina platensis* methanolic extracts L20B and MCF7 human cancer cell lines. Plant Archives, 2019; 19(1): 1419-1426.
10. Clinical and Laboratory Standards Institute (CLSI): 2007. Performance for antimicrobial Susceptibility
11. Sreenivasa, R., Parekh, KS. Antibacterial activity of Indian seaweed extracts. *Botanica Marina,* 1981; 24: 577-582.
12. Freshney, RI. Culture of Animal Cell. 6thEdition.Wily-Liss, 2012. New York

13. Ramya, B., Malarvili, T., Velavan, S. GC-MS Analysis of Bioactive Compounds in Bryonopsis Laciniosa Fruit Extract. *International Journal of Pharmaceutical Sciences and Research*, 2015; 6(8): 3375-3379.
14. Jadhav, V., Kalase, V., Patil, P. GCMS analysis of bioactive compounds inmethanolicextract of Holigarnagrahamii(wight) Kurz. *International Journal of Herbal Medicine*, 2014; 2 (4): 35-39.
15. Hussein, HJ., Hadi, YM., Hameed, IH. Study of chemical composition of Foeniculum vulgare using Fourier transform infrared spectrophotometer and gas chromatography - mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*, 2016; Vol. 8(3), pp. 60-89
16. Fayyad, RJ., Numan, RS., Hamdn, NT., Hameed, RS., Maliki, SAJ. Assessment of antagonistic effect of alcoholic extract from cyanophyta (Spirulina platensis) against several human and plant derived pathogenic fungi. *Indian Journal of Forensic Medicine and Toxicology*, 2020,14(1): 703-709.
17. Devendra, BN., Srinivas, N., Prasad, VS., Swarn, P. Antimicrobial Activity of Moringa Oleifera Lam., leaf extract against selected bacterial and fungal strains. *International Journal of Pharma and Bio Sciences*, 2011; 2(3): 13-18.
18. Aparna, V., Dileep, KV., Mandal, PK., Karthe, P, Sadasivan, C., Haridas, M. Anti-inflammatory Property of nhexadecanoic acid: Structural Evidence and kinetic Assessment. *Chemical Biology and Drug Design*, 2012; 80: 434–439.
19. Harada H, Yamashita U, Kurihara H, Fukushi E, Kawabata J, Kamei Y. Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. *Anticancer research,* 2001; 22 (5):2587–90.
20. Khairy, HM., El-Kassas, HY. Active substance from some blue green algae species used as antimicrobial agents. *African journal of Biotechnology*, 2010; 9(19): 2789-2800.
21. Raman, BV., La, S., Saradhi, MP., Rao, BN., et al. Antibacterial, Antioxidant Activity and GC-MS analysis of Eupatorium odoratum. *Asian Journal of Pharmaceutical and Clinical Research*, 2012; 5(2): 99-106
22. Idowu, OT., Ojo, OA. Antimicrobial Activity and Fatty Acids from Ipomea ochraceae. Medicinal Chemistry, 2017; 7:10. DOI: 10.4172/2161-0444.1000471
23. Ansarali, S., Manikandan, S., Lakshmanan, GM. Identification of biological components from potential bone healer medicinal plants. *Journal of Drug Delivery and Therapeutics,* 2018; 8(3): 32-41.
24. Diana, H., Parthipan, Y. Compounds Identification from Hypersaline Oscillatoria Salina Using GC-MS Analysis. *International Journal of Research Studies in Biosciences*, 2015; 3(9): 6-10.
25. Alwan NAS. Family history among Iraqi patients diagnosed with breast cancer. *International Journal of Scientific Research*, 2017; 6: 869–873.
26. Abedalrahman, SK., Ali, BM., Al-Khalidy, NA., Al-Hashimi, AS. Risk factors of breast cancer among Iraqi women. *Journal of Contemporary Medical Sciences*, 2019; 5(3): 149–153
27. Njoroge, GN., Kibunga, JW. Herbal medicine acceptance, sources and utilization for diarrhoea management in a cosmopolitan urban area (Thika, Kenya). *African Journal of Ecology*, 2007; 45(1): 65-70
28. Al-Asmari, AK., Albalawi, SM., Athar, MT., Khan, AQ., et al. Moringa oleifera as an Anti-Cancer Agent against Breast and Colorectal Cancer Cell Lines. *PLoS ONE*, 2015: 10(8): e0135814. doi: 10.1371/journal.pone.0135814.
29. Mohammed-Ali, ANM., Saeed, NAAAH., Omear, HA. The Anticancer Properties of Artemisia aucheri Boiss Extract on HT29 Colon Cancer Cells. *Journal of Gastrointestinal Cancer*, 2020 Jan 6. doi: 10.1007/s12029-019-00354-2. Epub ahead of print. PMID: 31907764.