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Ultra-sound assisted extraction, anti-oxidant, cytotoxic and anti-microbial studies of *Moringa oleifera* flowers

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Abstract: The study was undertaken to determine antioxidant, anticancer and antimicrobial activities of *Moringa oleifera* flowers. We used three different solvents, hexane (MOF-H), ethyl acetate (MOF-EA) and methanol (MOF-M), to extract the flowers of *M. oleifera* using the ultrasound-assisted extraction (UAE) approach. Disc diffusion method was used to test for antimicrobial activity. In cytotoxicity research, cell lines derived from breast cancer (MCF-7) and ovarian cancer (ES-2) were used. IC_{50} values for DPPH, ABTS and Nitric oxide for MOF-EA are 33.54±1.13, 29.86±0.08 and 49.7±1.12µg/mL, respectively, making it the most effective antioxidant in terms of scavenging free radicals. The order of suppression of bacterial growth by the methanolic extract was *E. coli>P. aeruginosa>S. aureus*, making it the most effective antibacterial agent tested. MOF-H, MOF-EA and MOF-M had fungal inhibition zones of 5.6mm, 7mm and 10.7mm, respectively, compared to DMSO. It was found that MOF-EA had potent antioxidant, cytotoxic and antibacterial capabilities that could be employed as an alternate treatment therapy following clinical trials.

Keywords: *Moringa oleifera* flowers, ABTS, DPPH, nitric oxide, cancer cell lines, *E. coli, C. albicans.*

INTRODUCTION

Uncontrolled cell division is a hallmark of cancer, resulting in an exponential rise in the number of cells (López-Lázaro, 2018). Currently, it is the world's second-leading cause of death after heart disease. Many degenerative diseases, including cancer, diabetes and Alzheimer's, have been related to an increase in reactive oxygen species such as hydroxyl radicals (OH), superoxide (SO), hydrogen peroxide (H_2O_2) and singlet oxygen. They are well known for their role in neutralizing free radicals, injuring cells in the body. They are well-known for their ability to neutralize free radicals, which are harmful to live cells and eliminated from the body. Oxidative stress ensues when this delicate balance is thrown off (Sharifi-Rad *et al*., 2020).

Polyphenolic compounds are secondary plant metabolites produced from phenylalanine or a related precursor, shikimic acid. Many studies have shown the antibacterial and antiviral activities of phytochemicals found in flowers, including scavenging oxidative agents, increasing immunity and regulating cell proliferation and gene expression (Lin *et al*., 2014; Srinivasulu *et al*., 2018). Because of this, recent years have seen a significant increase in the search for plants with antioxidant properties that humans can consume.

Moringa oleifera is a member of the *Moringaceae* family, which includes plants native to South Asia, specifically the foothills of the Himalayas in India (Sujatha and Patel, 2017). There have been reports of *M. oleifera* plant parts demonstrating anti-inflammatory and immunomodulatory capabilities, as well as antioxidant and hepatoprotective characteristics, in addition to other beneficial effects (Vergara-Jimenez *et al*., 2017). The many metabolites found in the plant, such as quercetin, kaempferol, zeatin, campesterol and sitosterol, have been shown to provide various medical benefits in animal studies (Abd Rani *et al*., 2018). Bioactive secondary metabolites are linked to these identified pharmacological actions. The least-polar solvents are recommended for secondary metabolite extraction over other polar solvents because of their proper polarity, safe propensity for human eating and environmental safety.

The ultrasound-assisted extraction (UAE) procedure is favouring as an alternative to conventional extraction (Kumar *et al*., 2021). The UAE is an excellent extraction technique in terms of energy consumption, processing time and resource recovery. Ultrasonic extraction is a more environmentally friendly and sustainable method for phenolic compound extraction because of these characteristics (Sharma *et al*., 2020). *M. oleifera* flowers were extracted *in vitro* using an ultrasound method with a solvent. Antioxidant, cytotoxic and antibacterial activities were then determined *in vitro*.

MATERIALS AND METHODS

Collection and authentication of plant

The flowers of *Moringa oleifera* (*Moringaceae*) was collected from the local areas of Bangalore, Karnataka and the taxonomical identification of the plant was made by Dr. N. M. Ganesh Babu, Associate Professor, Heading

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Centre of Herbal Gardens. The Voucher specimen is maintained in the same university. The University of Tans-Disciplinary health Sciences and Technology, Bangalore, Karnataka, India.

Extraction of M. oleifera flowers

Extraction was carried out using Ultrasound aided extraction (UAE) at frequencies between 20 and 50kHz for one hour at room temperature on 4kg of *M. oleifera* (MOF) flowers. Three different solvents were used to extract the flowers. First in order of decreasing polarity is hexane (MOF-H), then ethyl acetate (MOF-EA) and finally methanol (MOF-M). The extracts were filtered and evaporated using vaccum at a reduced pressure of 100 bar at a temperature of 40±5°C (Buchi Rotavapor R-210). It was determined how much each extract yielded and stored in a desiccator for subsequent analysis/studies.

Phytochemical Screening of the M. oleifera flower extracts

We used the conventional standard methods for determining the presence of different secondary metabolites in all three crude extracts of *M. oleifera* flowers (MOF-H, MOF-EA and MOF-M). There is much evidence (Harborne, 1984).

Determination of total phenolic content (TPC) in M. oleifera extracts

In order to determine the total phenolic content, we employed the Folin Ciocalteu reagent. A solution of Folin-Ciocalteu reagent and 0.5ml of the extracts (MOF-H, MOF-EA and MOF-M) was prepared. After 10 minutes at 25°C, 8mL of water and 2mL of a 7.5% Na2CO3 solution were added and the mixture was allowed to sit for 2hours. In both the test and standard solutions (Gallic acid), we used a 725nm spectrophotometer to determine absorbance (UV-1800 UV-Spectrophotometer, Shimadzu Corp. Japan). Gallic acid equivalents (µg/g) were used to measure Total Phenolic Content (TPC) (Wali *et al*., 2020).

Determination of total flavonoid content (TFC) in M. oleifera extracts

After that, 4mL of distilled water was added, along with a 0.3 percent NaNO2 solution. It took 5 minutes to add 10 percent AlCl3, followed by 2mL of 1M NaOH, then thinned out to final concentration using distilled water. Shimadzu Corporation Japan's UV-1800 UV-Spectrophotometer was used to evaluate the solution's absorption at 510 nanometers (nm). A known quantity of quercetin was employed as a reference standard for the calibration curve. Quercetin equivalents per gramme of MOF-H, MOF-EA and MOF-M samples were used to calculate the total flavonoid content (TFC) (Wali *et al*., 2020). TFC of the extracted compounds (MOF-H, MOF-EA and MOF-M) was calculated using colourimetric tests.

FTIR (Fourier transform infrared spectrometry) analysis of m. oleifera extracts

FTIR was performed to detect the functional groups in *M. oleifera* flower extracts. Diffuse reflectance sampling was used to identify functional groups on the Cary 630 FTIR spectrometer (Agilent Technologies, USA). Agilent Resolution Pro software was used to collect and analyze the data. At a minimum resolution of 8cm-1, FTIR spectra with a wavelength range of 4000-600cm-1 were captured.

Pharmacological screening of M. oleifera flower extracts

Antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and Nitric oxide radical scavenging techniques were used to assess the antioxidant activity of three *M. oleifera* flower extracts (MOF-H, MOF-EA and MOF-M) (Olaoye *et al*., 2021).

DPPH radical scavenging activity

Using the radical scavenging technique DPPH (2, 2-diphenyl-1, picrylhydrazyl), we evaluated the efficacy of MOF-H, MOF-EA and MOF-M extracts to counteract oxidative stress. Methanolic extracts ranging from 50 to 250µg/mL of MOF-H, MOF-EA and MOF-M were mixed before 2mL of the DPPH solution (0.1mM) was added. In a Shimadzu UV-1800 UV-Spectrophotometer, DPPH and methanol were employed as a blank and the absorbance at max 517nm was measured for 30 to 45 minutes against that of the mixtures (Wali *et al*., 2019). The radical scavenging percentage of DPPH was determined using the following equation:

$$\%Inhibition = \left(\frac{A_0 - A_1}{A_0} \right) \times 100 \quad (1)$$

Where A_o=absorbance of the control and A_1=absorbance of the test extracts.

Radical scavenging by ABTS radical

The long-lived ABTS radical was quenched by antioxidant molecules utilized in the ABTS technique. Peroxydisulfate oxidation 2, 2′ azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) was the ABTS radical source. In order to make the ABTS radical solution, we used 5mL of an ammonium-aqueous solution containing 7mM ABTS and added to it 88µL of 140mM potassium persulfate, resulting in a dark blue solution after 24 hours. ABTS (1mL) mixed with water (0.5mL) followed by the addition of extracts to diluted ABTS (1ml) and the measured the absorbance (UV-1800 UV-Spectrophotometer, Shimadzu Corp. Japan) at 734nm after 30 min. the first mixing (Wali *et al*., 2019). Equation (1) was used to calculate the percentage inhibition of the test extracts and the standard for their scavenging activity (discussed in the DPPH method).

Nitric oxide radical scavenging method

Extraction from the *M. oleifera* plant was tested to remove nitric oxide (NO) radicals using the method of Sreejayan and Rao, 1997 (Sreejayan and Rao, 1997). When using this method, a Griess reagent quantifies the nitric oxide radicals created by sodium nitroprusside solution. In water, nitrogen oxide radicals produced by sodium nitroprusside spontaneously interact with oxygen to form nitrite ions at healthy pH levels. The formation of nitrite ions is minimized when nitric oxide scavengers operate against oxygen. Flower extracts (50-250µg/mL) in phosphate buffer saline (0.2mmol/L, pH 7.4) were incubated at 25°C for 150 minutes with 3 mL sodium nitroprusside (10mMol/L). At 546nm, the absorbance of the Griess solution (500µL) was measured and the percentage of inhibition was calculated using a UV-1800 UV-Spectrophotometer (Shimadzu Corporation, Japan) (using Equation 1). The low optical densities show a significant scavenging capability for nitric oxide radicals. Methods used in this study generated IC_{50} values (concentration or log concentration) and their relative percentage inhibition on the x-axis, respectively, as a result of these assays.

Cytotoxic activity of M. oleifera extracts

The MTT assay was used to determine the cell viability of *M. oleifera* flower extracts by published directions (Wali *et al*., 2020; Tai *et al*., 2015; Njoya *et al*., 2017). Planting 5000 cells per well in 96-well plates yielded the best results. It took 24 hours to be cultured before treatment with flower extracts (MOF-H, MOF-EA and MOF-M) ranging from 25 to 400µg/mL. Incubation at 37°C and 5% CO_2 for 24 and 48 hours was employed for the testing. Following incubation, the morphology of the cells was examined using microscopy. MTT solution (5mg/mL) was injected into each well for 20µL. When the plates were done incubating, the medium was emptied from them. The formazan crystals were dissolved in 100µL of sample solution using dimethyl sulfoxide (DMSO). Imatinib Mesylate (MCF-7: human breast adenocarcinoma cells) and 5-fluorouracil were the most commonly used drugs in the study (for ES2: ovarian cancer cell line). MCF-7 breast cancer cell lines were utilized to examine the efficiency of the flower extracts in lowering cell viability (Felice *et al*., 2015). In ES-2 cell lines, the extracts were examined for their ability to reduce the viability of ovarian cancer cells. The IC50 values for cancer cell lines were determined by plotting the viability rate vs concentrations at 560nm on the graph. The following formula was used to calculate the inhibition percentage:

% inhibition = [(Ab of test-Ab of blank)/ (Ab of control-Ab of blank)] x 100

For Absorbance control, DMSO is 1% and Absorbance test is the DMSO-treated cells with extracts and the standard.

Antimicrobial M. oleifera extract

By disc diffusion method

Before the extracts were evaluated for antibacterial action, bacteria and fungi were cultivated in nutrient agar and seeded at 10^7CFU/mL (MOF-H, MOF-EA, MOF-M). After five minutes of soaking in flower extracts (each at 25g/mL) on sterile Whatman No.1 filter paper (5mm), the discs were gently pushed onto seeded plates. Ampicillin and Ketoconazole were the standard antibiotics for bacteria and fungi, both with a 10g/mL concentration. Platters were incubated overnight at 37°C. DMSO-soaked disc served as the "negative" control for our experiment. Each bacterial (*S. aureus, P. aeruginosa* and *E. coli*) and fungal (*C. albicans*) strain were examined in three separate experiments. It was used to quantify antibacterial activity by the mean diameter of the zone of inhibition (mm) (Wali *et al*., 2019; Kamurthy *et al*., 2019).

By minimum inhibitory concentration (MIC)

The *M. oleifera* extracts were tested using the Minimum Inhibitory Concentration (MIC) method in a 96-well microplate (MOF-H, MOF-EA, MOF-M). Bacterial and fungal cultures (each comprising 10µL of nutrient broth) were injected into each well and the total volume of nutrient broth in each well was 90µL. Flower extracts were added to each well in amounts of 200µL, with concentrations ranging from 900 to 6.25mg/mL in each well. For the next six hours, each well was incubated with 40µL of p-iodonitrotetrazolium violet (INT) at 0.4mg/mL after the plate had been incubated overnight at 37°C. Plates were incubated and a colour change from yellow to red to purple was observed as a sign of active bacteria and fungi. This was the lowest concentration of floral extract that did not alter the colour of the sample. Three separate rounds of testing were completed (Wali *et al*., 2019).

STATISTICAL ANALYSIS

All data were displayed with their corresponding means and standard deviations. Significant values were determined using GraphPad Prism Software version 5.0 using Duncan's test to compare the average of each group. $p<0.05$ was used to determine statistical significance.

RESULTS

M. oleifera floral extracts phytochemical screening

In a preliminary phytochemical investigation, *M. oleifera* flower extracts were found to include phenols, flavonoids, alkaloids and tannins (table 1).

Total phenolic and flavonoid contents

According to TPC and TFC results of several *M. oleifera* flower extracts, MOF-EA flower extract had the highest TPC (169.25±1.77mg GAE/g of extract) and the highest flavonoids concentration (129.09±2.03µg QE/g of

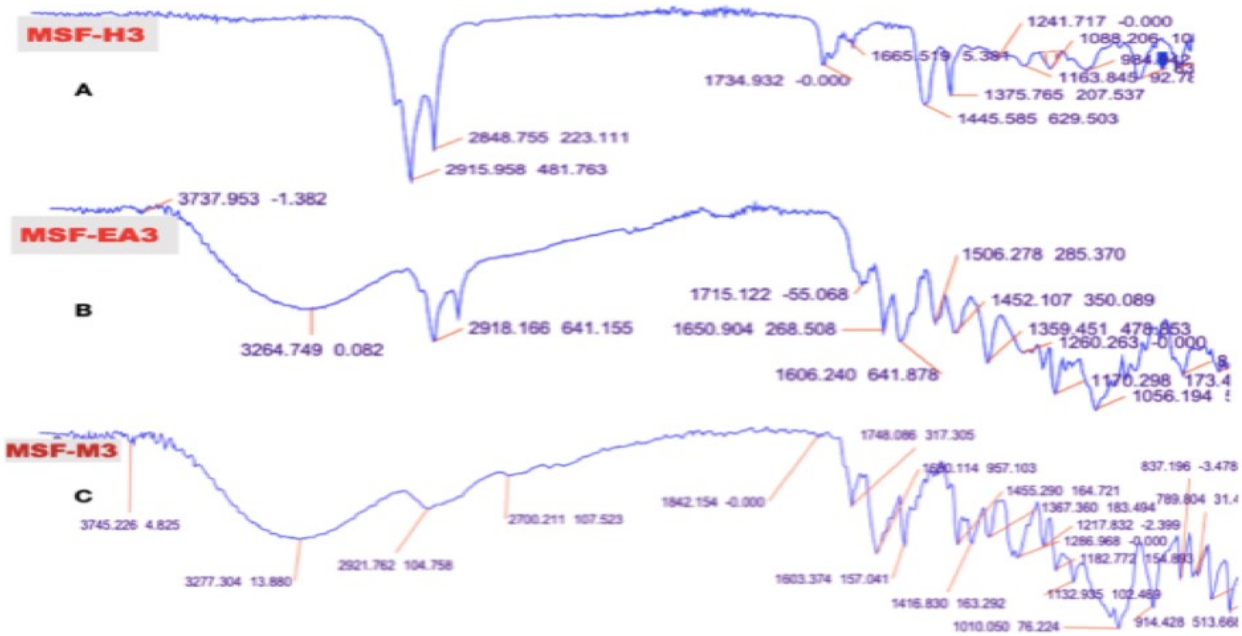

Fig. 1: FTIR spectrum of *M. oleifera* n-hexane (A), ethyl acetate (B) and methanolic (C) flower extract

extract). However, floral extracts of the MOF-M and MOF-H varieties showed (137.87±2.0GAE/g and 76.31±1.74 QE/g) and (119.4±0.95GAE/g and 38.58±0.79 QE/g, respectively) (table 2).

M. oleifera flower FTIR spectroscopy

Fig. 1A, B and C depict the FTIR spectra of *M. oleifera* flower extracts MOF-H, MOF-EA and MOF-M, respectively. Results showed absorption bands at 1734.93cm^{-1} (C=O str) and 1445.58 (C-H bending) and 1375.76cm^{-1} (C-O str for ester group) as well 1241.71, 1163.84cm^{-1} and 1088.206cm^{-1} (C-O str ester) (O-H deformation). Extracted MOF-H FTIR spectra are depicted in fig. 1A, which shows intense bands at 2915.95cm-1 2848.78cm-1 (C-H aromatic and CH2 vibration of aliphatic hydrocarbons).

Extract MOF-EA broad band FTIR spectrum at 3264.74cm^{-1} (fig. 1B) confirms the existence of phenolic (OH) group. Bands at 2918.166cm^{-1} (aliphatic hydrocarbon C-H and CH_2 vibrations), 1715.12cm^{-1} (C=O stretching), 1452.10cm^{-1} (C-H bending), 1359.45cm^{-1} (ester group C-O str), 1260.26cm^{-1}, 1170.29cm^{-1} (tertiary alcohol C-O str) and 1056.19cm^{-1} (C-O str) (C-O stretching primary alcohol).

Broadband at 3277.30cm^{-1} in the FTIR spectrum of the extract MOF-M shows the presence of an OH group, as seen in fig. 1C. The C-H and CH_2 vibrations of aliphatic hydrocarbons were seen at 2921.004 and 2700.211cm^{-1}, respectively, as well as the C-O str COOH at 1748.06cm^{-1}, the C=C str, α, β-unsaturated ketone at 1658.11cm^{-1}, the C-H bending at 1455.29cm^{-1}, the C-O str for ester group at 1367.36cm^{-1} (C-O str primary alcohol), C-O str tertiary alcohol at 1286.68cm^{-1}, 1132.93cm^{-1} and C-O str primary alcohol at 1010.05cm^{-1}.

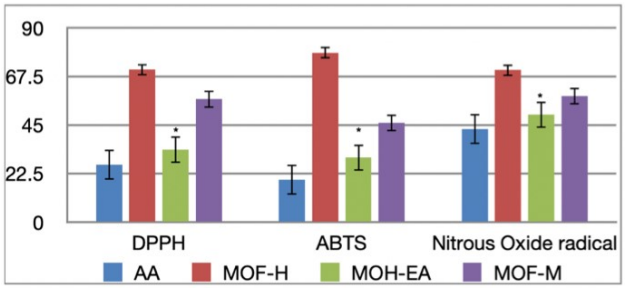

Fig. 2: Effects of *M. oleifera* flower extracts for antioxidant activity

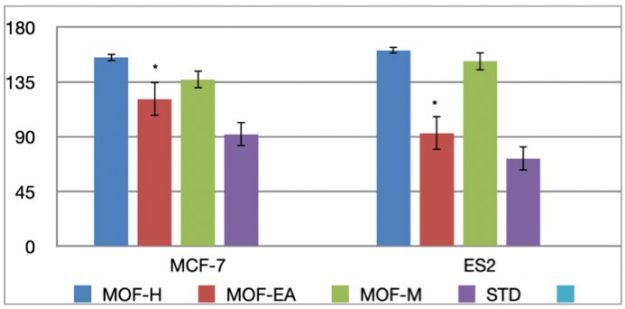

Fig. 3: Effects of *M. oleifera* flower extracts on the growth of MCF-7 and ES2 tumour cell lines

Antioxidant activity of M. oleifera extracts

DPPH radical scavenging activity

A typical ascorbic acid showed antioxidant activity of 26.4±0.79µg/mL, but the different extracts had a DPPH inhibition percentage of 70.65±1.02, 33.54±1.13 and 56.95±0.77µg/mL for MOF-H, MOF-EA and MOF-M, respectively (fig. 2). M. oleifera extracts IC50 values

Table 1: Phytochemical Screening of the *Moringa Oleifera* flower extracts

S. No.	Extract	MOF-M	MOF-EA	MOF-H
1	Alkaloids	+	+	+
2	Terpenoids	+	-	+
3	Flavonoids	+	+	+
4	Phenolic	+	-	-
5	Tannins	+	+	+
6	Saponins	+	+	-
7	Carbohydrates	+	-	+
8	Glycosides	+	-	+

'+'= Present, '-'= Not-present

Table 2: Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Method of Extraction	Extracts	Total phenolic content (mg GAE*/g of extract)	Total flavonoids content (µg QE**/g of extract)
Ultrasound-assisted solvent extraction	MOF-H	119.4 ± 0.95	38.58 ± 0.79
	MOF-EA	169.25 ± 1.77	129.09 ± 2.03
	MOF-M	137.87 ± 1.9	76.31 ± 1.74

Table 3: Antibacterial and antifungal activity of *M. oleifera* flowers extracts by disc diffusion method

Extracts	Diameter of Zone of Inhibition (mm)				
	B. subtilis	*S. aureus*	*P. aeruginosa*	*E. Coli*	*C. Albicans*
MOF-H	8.2 ± 0.95	9.3 ± 0.37	10.6 ± 0.60	11.5 ± 0.85 *	5.6± 0.85
MOF-EA	9.7 ± 0.73	10.5 ± 1.14	11.7 ± 0.99 *	12.8 ± 1.00 *	7 ± 0.74 *
MOF-M	11.6 ± 1.99 *	12.7 ± 1.85 *	13.7 ± 0.14 *	14.5 ± 1.21 *	10.7 ± 1.14 *
Ampicillin	15 ± 0.11 *	15 ± 0.30 *	15 ± 0.62 *	17 ± 0.12 *	ND
Ketoconazole	ND	ND	ND	ND	15 ± 0.45 *
Control (DMSO)	0.00	0.00	0.00	0.00	0.00

The data represent the mean ± SD (n=3), significantly different at *p<0.05 compared with control group. ND-Not determined: Ampicillin and Ketoconazole (10 µg/mL) are the standard antibiotic medication concentrations. All plant extracts are inoculated at 25 µg/mL (Stock solution: 200 µg/mL).

were found using linear regression. Higher levels of antioxidant activity may be due to flavonoid components found in this extract than in methanolic or hexane extracts (Rahman *et al*., 2015).

Radical scavenging activity by ABTS radical

ABTS free radicals are generated when oxidized with PPS (absorption maximum at 734nm). Antioxidants' capacity to quench the ABTS+ radical cation is the basis for this approach. Mould extracts of the flowers were found to be fast and potent ABTS radical scavengers (fig. 2), with activity comparable to that of ascorbic acid (IC_{50}, 19.54±0.38µg/mL). Methanolic extract had moderate activity (IC_{50}=45.9±1.02µg/mL) whereas hexane extract had the lowest activity (IC_{50}=78.5±0.98µg/mL) when tested using the ABTS radical-scavenging method. Interestingly, MOF-EA had the highest antioxidant potential (IC_{50}=29.86±0.08µg/mL).

Nitric oxide radical inhibition method

The nitric oxide radical scavenging method was also used to assess several *M. oleifera* flower extracts (MOF-H, MOF-EA and MOF-M). Fig. 2 shows the extract and standard ascorbic acid NO Scavenging activity data. MOF-EA inhibited nitric oxide radicals at a rate of 49.7±1.12µg/mL, followed by MOF-M at 58.25±0.87µg/mL and MOF-H at 70.42±0.45µg/mL; however, the highest percentage of nitric oxide radical inhibition was shown by MOF-EA.

Cytotoxic activity of M. oleifera flower extracts

The cytotoxic efficacy of *M. oleifera* flower extracts on MCF-7 and ES-2 cell lines was evaluated using MTT cell viability assays. MCF-7 and ES-2 cells treated with MOF extracts revealed an increase in inhibition rate that was dosage dependent. On MCF-7 and ES-2 cells, MOF-EA and MOF-M had the maximum cytotoxicity, followed by MOF-H ($p<0.05$) (fig. 3). After 24h of the incubation study, MOF-EA exhibited the lowest IC_{50} values of 121.34±1.22µg/mL and 93.14±1.85µg/mL respectively in MCF-7 and ES-2 cells, followed by MOF-M (137.33 ±1.11µg/mL & 152.21±0.96µg/mL) and least activity was shown by MOF-H (155.25±1.45µg/mL & 131.49± 0.83µg/mL) ($p<0.05$). The *in vitro* model Breast cancer

Table 4: Minimum inhibitory concentration (MIC, µ/mL) of *M. oleifera* flower extracts against selected bacterial and fungal strains

Extracts	Microorganism Organism (Presence/absence of growth)																							
	S. aureus						*P. aeruginosa*						*E. coli*						*Candida Albicans*					
	200	100	50	25	12.5	6.25	200	100	50	25	12.5	6.25	200	100	50	25	12.5	6.25	200	100	50	25	12.5	6.25
MOF-H	-	-	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	+	-	-	-	+	+	+
MOF-EA	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	+	+
MOF-M	-	-	-	+	+	+	-	-	-	+	+	+	-	-	+	+	+	+	-	-	-	+	+	+
Ampicillin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	×	×	×	×	×	×
Ketoconazole	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×	-	-	-	-	-	-
Control (DMSO)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+'= Present, '-'= Not-present

(MCF-7) cell line and ovarian cancer (E.S. 2) cell line results revealed that MOF-EA showed the highest anticancer activity. Imatinib mesylate and 5-FU were used as standard drugs.

Antibacterial and antifungal activities of M. oleifera flowers extract

Disc diffusion was used to investigate the antibacterial and antifungal properties of *M. oleifera* flower extracts in various solvents. All infections were effectively cured by the extracts (fig. 3). Antibacterial and antifungal activity was found in all of the extracts tested. The ethyl acetate extract had the highest antibacterial activity against all strains, while the n-hexane extract had the lowest. The inhibition zones for MOF-H, MOF-EA and MOF-M were 11.5mm to 14.5mm (table 3). Extracts inhibited bacteria in this order *E. coli>P. aeruginosa>S. aureus.* The MOF-H (5.6mm), MOF-EA (7.7mm) and MOF-M (10.1mm) inhibited *C. albicans* more than the negative control (DMSO). Ampicillin has the 17mm most expansive bacterial zone of inhibition for *E. coli. Candida albicans* growth was suppressed by Ketoconazole, a typical antifungal drug (table 3). All solvent extracts successfully stopped bacterial and fungal growth at the highest dose (table 4). Compared to other solvent extracts and DMSO, MOF-M was the most effective at inhibiting bacterial and fungal growth at 50, 100 and 200mg/mL. The antibacterial and antifungal drugs ampicillin and Ketoconazole worked in all doses tested.

DISCUSSION

M. oleifera is well-known for the wide variety of therapeutic and nutritional properties. The antioxidant, cytotoxic and antibacterial activities of *M. oleifera* flowers extract were investigated in the present study and the results were published. All three solvents used in this study, hexane (MOF-H), ethyl acetate (MOF-EA) and methanol (MOF-M), yielded a high percentage yield of extract using the ultrasound-assisted extraction (UAE) technique (MOF-M). According to the findings, MOF-EA possessed antioxidant solid, cytotoxic and antibacterial properties that might be used as an alternative therapeutic therapy following the completion of the clinical studies.

Extracts of plants have been shown to have a biological effect due to activated metabolites (Jain *et al*., 2021; Jain *et al*., 2020; Rao *et al*., 2016). Several flavonoids and phenolic chemicals, including quercetin and kaempferol, are found in *M. oleifera* flowers (Tan *et al*., 2015). These compounds were found in significant amounts in the *M. oleifera* flowers. Researchers have reported similar chemicals to ours (Doughari *et al*., 2007; Krishnaiah *et al*., 2009). Therefore, our findings are not surprising. Several chronic diseases can be avoided or postponed by free radical scavengers (antioxidants). This action method is crucial for anti-lipid peroxidation efficacy and can be used to assess antioxidant activity in various circumstances (Mariutti *et al*., 2008). A recent study found *M. oleifera* to have the highest antioxidant activity ever discovered at 49.7±1.12µg/mL (MOF-EA). Plant antioxidant activity is directly related to phenolic content (Adefegha *et al*., 2016; Molehin *et al*., 2014).

Studies by Reddy *et al*., 2005 and Lako *et al*., 2007 have demonstrated that *M. oleifera* exhibits high antioxidant activity (Reddy *et al*., 2005; Lako *et al*., 2007). According to one study, the leaves of *M. oleifera* contain high levels of polyphenols and radical scavengers known as DPPH (Pathak *et al*., 2020). In both DPPH and ABTS

experiments, the samples demonstrated free radical scavenging activity, as well as nitric oxide scavenging activity. Free radical scavenging was shown to be dependent on the concentration of the samples.

Our research revealed that MOF-EA outperformed DPPH, ABTS and Nitric oxide in antioxidant radical scavenging capability. Several features of our findings matched those of Atawodi *et al*. (2010), albeit there were variances in species, organs and geographic areas (Atawodi *et al*., 2010). The root barks of *M. oleifera* demonstrated the highest antioxidant activity, followed by the leaves and stems. However, the researchers did not test the flowers.

The methanolic extract had the most potent antibacterial effect against all tested microorganisms. Various investigations have demonstrated that *M. oleifera* is antibacterial against pathogens like *S. aureus, B. subtilis, S. pyogenes, S. albus, Salmonella gallinarum, P. aeruginosa and E. coli* (Thilza *et al*., 2010; Dewagan *et al*., 2010; Kumar *et al*., 2012; Jain *et al*., 2019). To our knowledge, no prior research has been done on flower extract's antibacterial activity against the bacterial and fungal species listed below. The total antibiotic susceptibility test findings matched the extract's antibacterial activity. Various solvents have differing amounts of extraction capacity (Ashok *et al*., 2014; Majorie, 1999; Srinivasan *et al*., 2001). The data suggest that changes in the chemical composition of the extracts studied may explain the variances in antibacterial effectiveness.

CONCLUSION

The *M. oleifera* flower's antioxidant, anticancer and antibacterial properties were all enhanced by UAE, as evidenced by the study's findings. As a result, this work demonstrated a sustainable method for extracting considerable natural antibacterial and antioxidant bioactive chemicals from *M. oleifera* flower using ultrasound-assisted extraction, which can be used in food processing with less time and energy usage. The plant's inhibition zones, which closely match the inhibition zones of antibiotics, can be exploited to produce new chemical compounds that can be used to treat a wide range of infections.

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