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# Assessment of Oil and Seed Extracts of *Moringa oleifera* for Promising Anticandidal Activity in Autistic Children

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*MORINGA OLEIFERA* (*M. oleifera*) is a plant with a high nutritional value and an impressive range of medicinal uses. Plants of the *Moringaceae* family contain a wide range of secondary metabolites with biological activities, with an essential role in the pharmaceutical industry. To the best of our knowledge, this is the first study to report the anti-candidal, antioxidant properties and chemical constituents of *M. oleifera* oil and its seed extracts with its *in vivo* toxicological assessment as herbal medicine. The results showed that *M. oleifera* oil showed the highest antifungal activity against six strains of *Candida* species isolated from the stool samples of the autistic children, which included *C. albicans*, *C. dulblinesis*, *C. glabarata*, *C. kefyr*, *C. krusei*, and *C. lusitania*. The *M. oleifera* oil (1.0%) exhibited the highest growth rate reduction and antioxidant activity. The GC/MS of *M. oleifera* oil revealed 17 compounds with methyl ester, stigmasterol, tetraphenyl and vitamin E as significant components. Moreover, an *in vivo* toxicological assessment confirmed the safety of *M. oleifera* oil. Therefore, *M. oleifera* oil can serve to be a promising therapy for improving autistic gastrointestinal problems.

**Keywords**: Autistic children, Intestinal candidiasis, *Moringa oleifera* oil.

## Introduction

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by language, cognitive disabilities, social interaction problems, communication skills and repetitive childhood routines (Fond et al., 2015; Marotta et al., 2020). ASD children have severe gastrointestinal (GI) problems (Adams et al., 2011). The etiology of GI issues is unknown. However, they could be linked to gut flora (dysbiosis) (Srikantha & Mohajeri, 2019).

The yeast species colonize the mucosal surface of the gastrointestinal tract in an asymptomatic manner. However, ASD children develop the symptoms rapidly and violently, without causing unpleasant symptoms (Burrus, 2012), which are linked with their abnormal autistic behaviors (Klingelhoefer & Reichmann, 2015). When *Candida* grows, it releases ammonia and toxins that cause long-term severe immune system disruption and invade the brain (Foster & Neufeld, 2013; Borre, 2014). *C. albicans* are the most common pathogenic organisms in the intestinal tract, followed by *C. glabrata* (Brunke & Hube, 2013).

Although antifungal medications improve autistic children's candidiasis, most clinically used antifungals have several disadvantages of toxicity, drug–drug interactions and absence of fungicidal effectiveness, cost and the emergence of resistant strains. Polyenes (Nystatin and Amphotericin B) are the widely used antifungals and azoles (Itraconazole and Fluconazole) (Kantarcioglu et al.; 2016). Therefore, new antifungal agents are in high demand, justifying the search for new drugs that are more powerful and less harmful than those currently in use (Matsubara et al., 2016). In recent years, the development of safer antifungal drugs from the natural plant products such as extracts and essential oils has been essential. Additionally, the different secondary metabolites isolated from the plants were considered to have antifungal activities without any side effects on adults and

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children (Nuzhat & Vidyasagar, 2014).

*M. oleifera* is the most commonly recognized and naturalized species in the monogenetic family of *Moringaceaeae* (Ramachandran et al., 1980). *M. oleifera* is a 2.5 to 10m high shrub and a deciduous tree called the "tree for life." The plant parts (leaves, fruits, seeds, roots, bark and flowers) have a variety of therapeutic and nutritional benefits in humans. It has been demonstrated that bioactive compounds in the plant leaves, fruits and seeds contain variety of active constituents that have a wide spectrum of biological impacts and pharmacological uses (Nesamani & Plants, 1999; Jacques et al., 2020; Ouf et al., 2022). Numerous studies have identified *M. oleifera* oil as a potent anti-inflammatory drug (Ezeamuzle et al., 1996). Additionally, its seeds have antimicrobial properties (Eilert et al., 1981), treating diseases, such as typhoid fever, diarrhea, elevated blood sugar and gastrointestinal disorders (Kumar et al., 2010). Our study aimed to investigate the valuable aspects of *M. oleifera* and its potential role as therapy for the treatment of autistic children.

## Materials and Methods

*Plant materials*

The *M. oleifera* seeds and oils were obtained from the Egyptian Scientific Society of Moringa, National Research Centre, Dokki, Giza, Egypt.

*Preparation of M. oleifera seed extracts*

The seeds of *M. oleifera* were air-dried at room temperature (28°C). According to Price (2000), the dried *M. oleifera* seeds were selected by removing their coats and powdering them using a blender. The aqueous seed extracts of *M. oleifera* were prepared by dissolving 100g of the crushed seeds in 500mL of distilled water for 15 min in a water path at 70°C, filtered. The filtrate was frozen and extracted for 96h using the freeze drier apparatus. The supernatant was carefully collected in a clean jar. For solvent extraction, 100g of powder was taken for extraction using 500 ml acetone and ethanol, respectively, for 48h at room temperature and filtered using Whatman's filter paper. The filtrate was concentrated at 35°C in a rotary evaporator (Djeussi et al., 2013). The yield was estimated by dividing the grams of extracted material by the grams of seeds charged in the jar (Palafox et al., 2012). All extracts were dissolved in dimethyl sulphoxide (DMSO) (<1%) to obtain the crude extracts (100µg/mL). Until further use, all crude extracts were kept at 4°C.

*Preparation of different concentrations of M. oleifera oil*

The different concentrations of *M. oleifera* oil (0.5%, 1.0%, 1.5% and 2.0%) were prepared by adding 40 µl of tween 80 to each concentration to disperse the oil in the medium (El-Shouny et al., 2016).

*Microbial strains*

Six strains of *Candida* (*C. albicans*, *C. dulblinesis*, *C. glabarata*, *C. kefyr*, *C. krusei* and *C. lusitania*) were obtained from our previous study (El-Shouny et al., 2016). The strains were isolated from the stool specimens of autistic children, referred by the Clinical Genetics Department, National Research Centre, Giza, Egypt. The parents of the autistic children provided written informed consent. The strains were stored at −70°C. According to the guidelines, the identification of phenotypes was confirmed with the API *Candida* method (BioMérieuxVitek, Hazelwood, MO, USA). Every strain was frozen, stocked in glycerol, sub cultured overnight on Sabouraud's dextrose broth (SDB; Ifco Laboratories, Detroit, MI, USA) and incubated for 24h at 37°C.

*Inoculum preparation*

Each strain of *Candida* was sub cultured overnight in the SDB and suspended in sterile saline solution. The fungal concentration was $1\times 10^7$ colony-forming unit/ml (CFU/ml) (Bemer & Drugeon, 2001). The inoculum of each isolated *Candida* was prepared to study its susceptibility against standard antifungals.

*Antifungal activity of standard antifungal agents*

The inhibitory activity of the standard antifungals was detected using the disc diffusion method (El Zawawy & Hafez, 2017), as disks of 50 µg of (Metronidazole, Nystatin, Fluconazole, Amphotericin B, ketoconazole and Itraconazole) were placed aseptically on the surface of agar plates inoculated with each *Candida* strain ($1\times 10^7$) separately and left at room temperature for 15 min for complete diffusion. The plates were incubated at 37°C for 18h. The diameter of the inhibition zone was measured and compared with the clinical and laboratory standards. The susceptibility test results were interpreted according to the National Committee for Clinical Laboratory Standard using the disk diffusion method (NCCLS, 2003). The inhibition zone diameters (mm) were measured

as the mean of three replicates. The synthetic antifungal agent of the highest inhibition zone was taken as a positive control.

*Antifungal activity of M. oleifera seed extracts and oil*

The activity of *M. oleifera* oil and seed extracts was monitored using the well diffusion method (El-Shouny et al., 2016). The wells (8mm diameter) were made using a sterile borer in the SDA plates, previously inoculated with 100 µl of each strain ($1 \times 10^7$), separately. Each well was aseptically filled with 100 µl from the prepared extract suspension and different concentrations of oils (0.5%, 1%, 1.5% and 2%). DMSO (<1%), tween 80 and distilled water were used as negative control and incubated for 48 h at 37°C. After incubation, the inhibition zones diameters (mm) were measured as mean inhibition zones of three replicates in millimeters and the results were compared with the negative and the positive control (Nystatin).

*Determination of minimum inhibitory concentration (MIC)*

The MIC of the extract and oil was calculated using a previously modified method (Ali et al., 2016), using the concentrations of 12.5, 25, 50, 100 and 200 µg/ml for the crude extract and 0.5%, 1.0%, 1.5% and 2.0% for oil. MIC was regarded as the lowest concentration with no growth.

*Antioxidant assay*

The antioxidant efficiency of *M. oleifera* oil (the most effective) was carried out using the DPPH radical scavenging activity (Gülçin et al., 2012). Briefly, a DPPH (0.1mM) solution was prepared in ethanol and 0.5mL of this solution was added to 1.5mL of *M. oleifera* oil solution in different concentrations (20–640 µg/mL). The solutions were incubated in the dark after vortexing thoroughly. DPPH radicals were reduced using a UV visible spectrophotometer at 517nm after 30min. Higher DPPH free radical scavenging activity is suggested by the lower absorbance of the reaction mixture. The antioxidant efficiency was calculated according to the following equation:

Scavenging effect (%)= [(Absorbance of control–Absorbance of sample)/ Absorbance of the control] × 100

*Gas chromatography–mass spectrometry (GC/MS) analysis*

*M. oleifera* oil was analyzed using an Agilent 5973 Network mass selective detector using an Agilent 6890N Network GC (Gas chromatograph) system. The derivatized samples were injected (1µL) into an Agilent HP-5 GC column (5% phenyl-methyl polysiloxane, 30m 0.32mm i.d., 0.25m) with a split ratio (50:1). The heat was used as the carrier gas with a volumetric flow rate of 1.1mL $min^{-1}$. The temperature was increased from 140°C to 190°C at a rate of 25°C$min^{-1}$ and then increased to 240°C at a rate of 4°C $min^{-1}$ for 15min (Palafox et al., 2012).

Qualitative identification of the different constituents was performed by comparing their relative retention times and mass spectra with those of the real reference compounds (Adams et al., 1995). The search software for the probability merge and the search program for NIST MS spectra were used for this function. The relative amounts of the individual oil components were expressed as the percentage of the peak area relative to the peak area as a complete component retention index was calculated for the retention periods (Chuang et al., 2007).

*Toxicological assessment*

*Experimental animals*

The experimental animal procedure was performed in compliance with the guidelines of the Institutional Animal Ethics Committee (IAEC). It was approved by the IAEC, Faculty of Science, Tanta University, Egypt (code: Rec-Sci-Tu-020). Twenty-five albino rats (21± 0.57g/rat) were used as experimental animal models for oral toxicity evaluation of *M. oleifera* oil. The rats were kept for 7 days at the Animal House of Faculty of Science, Tanta University, Egypt, for assimilation prior to oral administration.

*Acute oral toxicity*

Acute oral toxicity was confirmed by a short-term oral toxicological study for 14 days. The toxicity assessment was conducted to determine the *M. oleifera* oil lethal dose 50 ($LD_{50}$) according to the Guidelines of the Organization for International Cooperation and Development (OECD) (OECD. Test No. 425, 2008). $LD_{50}$ is the dose that kills half of the animals of a particular species. To determine the $LD_{50}$, the rats were classified into five groups, each containing five rats. The rats in the control group were administered with 0.5mL of saline solution, whereas different concentrations of *M. oleifera* oil (0.2 to 1.6mL/kg body weight/day) were administered to the other

four classes of rats via oral gavage. For 2 weeks, both treated and control groups were administered once daily. Visual observations were made during the experimental period, including the body weight, exterior appearance and daily activities (Randhawa, 2009).

*Hemolytic activity*

The hemolytic activity assay of *M. oleifera* oil on the rat blood cells was determined according to Ogino et al. (1997). Briefly, 50µL of the blood cell suspension from the healthy albino rats was prepared by diluting the cell pellet to 0.5% with phosphate saline buffered by mixing with each oil concentration separately. During the incubation for 10–30min at 37°C, the suspension was centrifuged and examined for hemolysis with time. Free hemoglobin of the supernatant was measured using a UV visible spectrophotometer (Shimadzu-UV2600, Japan) at 405nm. At each oil concentration, three replicates were performed. The hemolytic activity was demonstrated by an increase in the optical density of the solution due to hemoglobin release over time. Nystatin was used as the positive control and tween 80 as a negative control.

*Statistical analysis*

The data were presented using the variance analysis using the SPSS software (version 26.0 for Windows, SPSS, Inc., Chicago, USA) and expressed as mean ± SD of three replicates. At $P< 0.05$, statistical differences were considered significant.

## Results and Discussion

*Antifungal activity of standard antifungal agents*

The antifungal susceptibility of the standard agents was tested against the isolated *Candida spp.* from the stool samples of the autistic children and is presented in Table 1. It was observed that nystatin showed the highest zone of inhibition (13–17mm) against all *Candida* strains. The incidence of resistance to the examined antifungal drugs ranged from 0% resistance against nystatin, which inhibits the growth of all tested isolates, followed by metronidazole (16% resistance), which affects five of the six tested isolates, Itraconazole, Amphotericin, Fluconazole (33% resistance). On the other side, 50% of the tested *Candida* strains were resistant to Ketoconazole (50%). All isolates were susceptible to nystatin, which is used as a positive control. This was similar to Kantarcioglu et al. (2016), who reported that yeasts are susceptible to nystatin. Also, Arikan et al. (2002) showed that most *Candida* isolates were inhibited at low concentrations of nystatin.

*Antifungal activity of M. oleifera seed extracts and oil*

On a dry basis, the yield was measured as the grams of extract divided by grams of original powder. For the seed extract, 2.8% of acetone extract, 2.5% of ethanol extract and 1.9% of the aqueous extract.

Antifungal activity of *M. oleifera* seed extracts was performed against the isolated *Candida spp*. from autistic children (Table 2). The acetone seed extracts of *M. oleifera* exhibited the highest inhibition on the isolated *Candida* with inhibition zones ranging from 14 to 24mm, followed by ethanol and aqueous extraction. Similarly, Farooq et al. (2006) found that *Moringa* root acetone extract was reported to be rich in antimicrobial agents. Similar findings showed that *M. oleifera* acetone seed extract had fungicidal and powerful antibacterial effects (Ruckmani et al., 1998). Moreover, Nikkon et al. (2003) found that (N-benzyl, S-ethyl thioformate) isolated from the chloroform fraction of the seed ethanol extract of *M. oleifera* was found to have antimicrobial activities. Fig. 1 showed an inhibitory activity of different concentrations of *M. oleifera* seed acetone extract to detect MIC. The MIC of *M. oleifera* seed acetone extract was 25µg/mL, except with *C. glabarata* and 50µg/mL for all the tested *Candida* isolates. This was similar to Saadabi & Abu (2011), who found no detectable suppression in 5, 10 and 20µg/mL concentrations of *M. oleifera* seed methanolic extract. Table 3 reveals the antifungal activity of *M. oleifera* oil with different dilutions (0.5%, 1%, 1.5% and 2%) against isolated *Candida spp.* from autistic children. The obtained data showed that all oil concentrations inhibited the growth of *Candida* isolates more than seed extract. The inhibition of all isolates increased by increasing the oil concentration. It is noticed that *M. oleifera* oil gave the most significant inhibitory effect against all tested *Candida* isolates as MIC of *M. oleifera* oil was 1%. According to Asgarpanah et al. (2017), who found that *M. oleifera* oil gave high antimicrobial activity. Similarly, Siddhuraju & Becker (2003) showed that *M. oleifera* seed oil was very effective for infectious diseases, inflammation treatment and antimicrobial activities.

**TABLE 1. Antifungal susceptibility of standard antifungal agents against isolated *Candida* spp.**

| Yeast isolates | Antifungal agents | | | | | |
|---|---|---|---|---|---|---|
| | AP | FLC | IT | KT | MT | NS |
| | Inhibition zone diameter (mm) | | | | | |
| *C. albicans* | 15 ± 0.3 | 15 ± 0.6 | 0.0 ± 0.0 | 0.0 ± 0.0 | 10 ± 0.0 | 16 ± 0.2 |
| *C. dulblinesis* | 16 ± 0.2 | 0.0 ±0.0 | 10 ± 0.0 | 0.0 ± 0. | 8 ± 0.66 | 17 ± 0.1 |
| *C. glabarata* | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0. | 0.0 ± 0. | 11±0.00 | 15 ±0.1 |
| *C. kefyr* | 9 ± 0.01 | 11 ± 0.1 | 13 ± 0.0 | 8 ± 0.60 | 10 ± 0.6 | 14 ± 0.6 |
| *C. krusei* | 0.0 ±0.0 | 11 ± 0.1 | 10 ± 0.0 | 12 ± 0.0 | 10 ± 0.0 | 15 ±0.03 |
| *C. Lusitania* | 9 ± 0.11 | 12 ± 0.6 | 10 ± 0.0 | 8 ±0.32 | 0.0 ±0.1 | 13 ± 0.3 |
| Total mean | 8.7 ± 0.1 | 7 ± 0.21 | 7.7 ± 0.0 | 4 ± 0.13 | 8.1 ±0.1 | 14.7 ±0.2 |
| No. of resistant isolates | 2 | 2 | 2 | 3 | 1 | 0 |
| Resistant percentage % | 33 | 33 | 33 | 50 | 16 | 0 |
| P value | 0.000*** | 0.000*** | 0.002*** | 0.000*** | 0.001*** | 0.007** |

Standard antifungal agents include Amphotericin B (AP), Fluconazole (FLC), Itraconazole (IT), Ketoconazole (KT), Metronidazole (MT), and Nystatin (NS). Each result is the mean of three readings ± standard deviation (SD). (Values are mean inhibition zone (mm) ± SD of three replicates, ns= Insignificant, difference is significant; P > 0.05 is represented by *, P≤ 0.01 is represented by **, P≤ 0.001 is represented by ***).

**TABLE 2. Antifungal activity of different seed extracts of *M. oleifera* against isolated *Candida* spp.**

| Yeast isolate | *M. olifera* seed extracts (100 µg/mL) | | | Nystatin |
|---|---|---|---|---|
| | Aqueous | Acetone | Ethanol | |
| | Zone of inhibition (mm) | | | |
| *C. albicans* | 19 ± 0.01 | 20 ± 0.11 | 17 ± 0.16 | 16 ± 0.2 |
| *C. dulblinesis* | 8 ± 0.14 | 14 ± 0.14 | 16 ± 0.21 | 17 ± 0.1 |
| *C. glabarata* | 11 ± 0.23 | 16 ± 0.11 | 18 ± 0.13 | 15 ± 0.11 |
| *C. kefyr* | 15 ± 0.0 | 21 ± 0.13 | 12 ± 0.0 | 14 ± 0.6 |
| *C. krusei* | 14 ± 0.11 | 22 ± 0.15 | 14 ± 0.11 | 15 ± 0.03 |
| *C. lusitania* | 12 ± 0.21 | 24 ± 0.0 | 9 ± 0.11 | 13 ± 0.3 |
| Total mean | 13.1 ± 0.1 | 19.5 ± 0.1 | 14.3 ± 0.1 | 15 ± 0.2 |
| P value | 0.017* | 0.006** | 0.011* | 0.007** |

Values are mean inhibition zone (mm) ± SD of three replicates, ns= Insignificant, difference is significant; P> 0.05 is represented by *, P≤ 0.01 is represented by **, P≤ 0.001 is represented by ***.

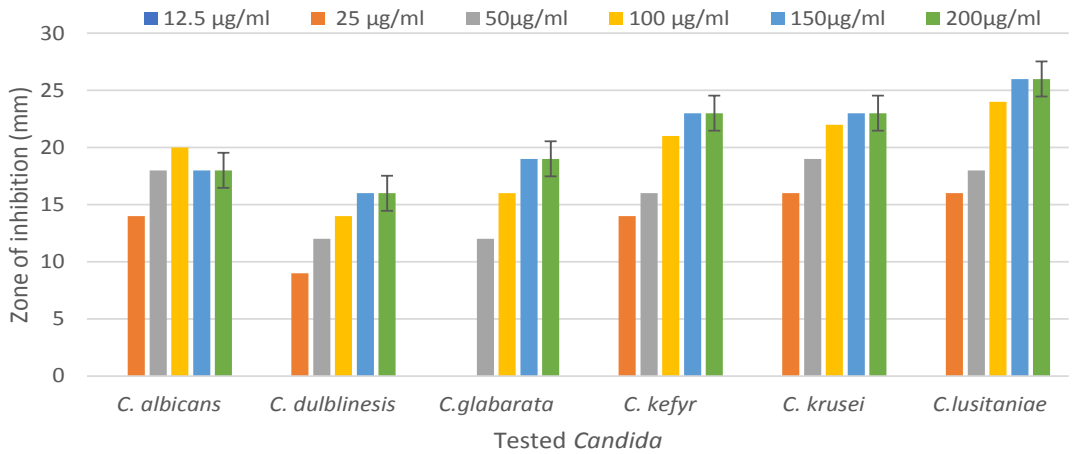


**Fig. 1. Effect of different concentrations of acetone seed extract of M. oleifera against the isolated *Candida* spp.**

**TABLE 3. Antifungal activity of *M. oleifera* oil against the isolated *Candida* spp.**

| Yeast isolates | *M. olifera* oil | | | | Nystatin |
|---|---|---|---|---|---|
| | Zone of inhibition (mm) | | | | |
| | 0.5% | 1% | 1. 5% | 2% | |
| *C. albicans* | 22 ± 0.04 | 25 ± 0.11 | 26 ± 0.66 | 26 ± 0.66 | 16 ± 0.2 |
| *C. dulblinesis* | 19 ± 0.7 | 23 ± 0.32 | 25 ± 0.20 | 25 ± 0.21 | 17 ± 0.1 |
| *C. glabarata* | 21 ± 0.34 | 22 ± 0.21 | 24 ± 0.18 | 24 ± 0.18 | 15 ± 0.11 |
| *C. kefyr* | 22 ± 0.11 | 24 ± 0.01 | 23 ± 0.60 | 23 ± 0.61 | 14 ± 0.6 |
| *C. krusei* | 17 ± 0.00 | 23 ± 0.10 | 24 ± 0.34 | 24 ± 0.34 | 15 ± 0.03 |
| *C. Lusitania* | 21 ± 0.15 | 24 ± 0. 24 | 25 ± 0.21 | 25 ± 0.21 | 13 ± 0.3 |
| Total mean | 20.3 ± 0.22 | 23.5 ± 0.16 | 24.5 ± 0.36 | 24.4 ± 0.3 | 15 ± 0.2 |
| P value | 0.020* | 0.003** | 0.002** | 0.002** | 0.007** |

Values are mean inhibition zone (mm) ± SD of three replicates, ns= Insignificant, difference is significant; P> 0.05 is represented by *, P≤ 0.01 is represented by **, P≤ 0.001 is represented by ***.

*Antioxidant activity of M. oleifera oil*

Table 4 illustrated the evaluation of the antioxidant activity of *M. oleifera* oil against ascorbic acid as a standard. The findings showed a high antioxidant activity of *M. oleifera oil* with $IC_{50}$ = 158.6 µg/ml as the inhibition of the free radicals increased with the increased concentration of the extracts. Similarly, Jahan et al. (2018) found an increase in inhibition of the free radicals with the increase in the concentration of *M. oleifera* extracts.

*GC/MS for M. oleifera oil*

The results showed 17 compounds in *M. oleifera* oil in Table 5 illustrates the major components as follows: Fatty acid esters as (methyl ester 9-Octadecenoic acid 45.04%, Stigmasterol 16.23%) were the highest component followed by phenolic and alkaloids 8.3% and 8.1%, respectively, followed by (Thieno [3,4-C] Pyridine, 1,3,4,7-Tetraphenyl 7.87% and Vitamin E 4.2%). Similarly, Chuang et al. (2007) found that the GC/MS of the essential oil of *M. oleifera* leaf extract revealed a total of 44 compounds with exacosane and (E)-phytol as major compounds. In medicine and the healthcare industry, plant sterols have taken on an increasingly significant role. It can also be very beneficial and appears to be a possible means of stopping the growth and metabolite activities of pathogenic fungi (Mbambo et al., 2012). According to the results, the antifungal activity of *M. oleifera* oil extracts mostly had returned to its active constituents as fatty acid ester, phenolics and alkaloids; this is consistent with Metwally et al. (2022), who showed that the antifungal activity of *P. dioscoridis* leaf extract attributed to its active constituents as phenolics, flavonoids, terpenoids and alkaloids.

**TABLE 4. Antioxidant activity of *M. oleifera* oil compared to ascorbic acid**

| *M. olifera* oil conc. (µg/mL) | DPPH scavenging % | Reference standard conc. (µg/mL) (Ascorbic acid) | DPPH scavenging % |
|---|---|---|---|
| 640 | 89.45 | 40 | 92.48 |
| 320 | 69.91 | 35 | 87.53 |
| 160 | 50.36 | 30 | 80.65 |
| 80 | 29.09 | 25 | 77.41 |
| 40 | 16.09 | 15 | 54.86 |
| 20 | 7.82 | 5 | 11.78 |
| 0 | 0 | 0 | 0 |

**TABLE 5. Major components of *M. oleifera* oil by GC/MS analysis**

| Compound name | RT (min) | Peak area % | Compound nature other names | Molecular formula | Molecular weight | Medicinal uses and Ref. |
|---|---|---|---|---|---|---|
| 9-Octadecenoic acid (Z)-, methyl ester | 36.95 | 45.04 | (Oleic acid methyl ester) (fatty acid methyl ester) | $C_{19}H_{36}O_2$ | 296 | Antimicrobial, Nematicidal Chandrasekharan et al., 2008; Lima, 2011) |
| Stigmasterol | 60.25 | 16.23 | Stigmastern | $C_{29}H_{48}O$ | 412 | Antihypercholestrolemic, cytotoxicity, antiosteoarthritic, antitumor, antioxidant hypoglycemic, antimutagenic, anti-inflammatory and CNS effects. (Kaur et al., 2011) |
| Thieno[3,4-C] pyridine,1,3,4,7-tetraphenyl | 59.89 | 7.87 | | $C_{31}H_{21}NS$ | 439 | |
| Vitamin E | 58.14 | 4.20 | | $C_{29}H_{50}O_2$ | 430 | Keep the immune system strong against microbes. Antioxidant, can prevent cancer, heart disease, dementia, liver disease. Share in the formation of red blood cells, helps the body use vitamin K. Also helps widen blood vessels and keep blood from clotting inside them. (Mason, 2016; Salwen, 2017) |
| Hexadecanoicacid,methyl ester | 32.29 | 3.94 | Palmitic acid methyl ester (fatty acid methyl ester) | | | Antifungal, Antibacterial and antioxidan, (Agoramoorthy, 2007) |

*Toxicological assessment*

Toxicological experiments were conducted in the laboratory animals to determine the possible toxicity and safety of phytochemical constituents. Fig. 2 showed the toxicological assessment of *M. oleifera* oil after oral administration in albino rats. The oral dose of *M. oleifera* oil is administered up to the peak concentration of 1.6mL/kg. There were no clinical symptoms and no mortality. At the end of the experimental period, there was an increase in body weight between *M. oleifera* oil-treated groups and the control group, as shown in Fig. 2A. The acute lethal dose ($LD_{50}$), expressed as milliliters of oil per unit weight of albino rat (ml/kg) tested, is based on the data and assumed to be greater than 1.6mL/kg. It implies that depending on the scale of Hodge and Sterner, this oil could usually be considered nontoxic. Moreover, for treated albino rats, an increase in body weight was considered normal since body weight is the most sensible predictor of an adverse effect (Hodges et al., 1995). Also, the hemolytic activity was an alternative approach for easy cytotoxicity evaluation. As recorded in Fig. 2B, there was no significant hemolytic activity of red blood cells of rats treated with *M. oleifera* oil at concentrations of 0.2 to 1.6 ml/kg compared with Nystatin as a positive control, which promotes its safety. Other studies have reported toxicological assessments of various plants using an *in vitro* hemolytic activity assay (Awodele et al., 2018; Gupta et al., 2018). The toxicological assessment using albino rats promotes the safety of *M. oleifera* for oral use. Further assessment should be conducted to identify the safe doses for human use.

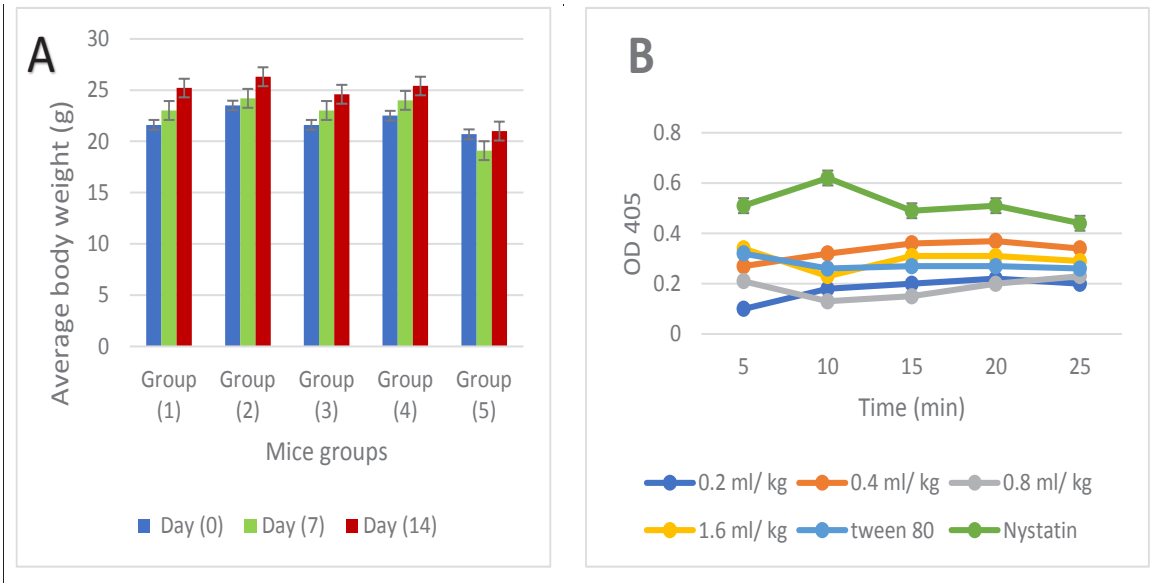


**Fig. 2. *In vivo* evaluation of the toxicity of *M. oleifera* oil after oral administration in albino rats**

## Conclusion

*M. oleifera* oil has the potential to be a safe therapeutic alternative against gastrointestinal candidiasis in autistic children. Additionally, it has a valuable antioxidant property. The clinical trials are highly recommended for further research with confirmatory investigations for the safety of *M. oleifera* oil and its purified component as a medication for autistic children.

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*Competing interests:* The authors report no conflicts of interest regarding this work.

*Authors' contributions*: M.A.M: Experimental design, Reviewing and Editing; N.A.E: Experimental design, Methodology, Investigation and Writing original draft; S.E.H: Experimental design, Methodology, Data curation, Investigation and Writing original draft, Reviewing and Editing.

*Ethics approval:* Not applicable.

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## تقييم زيت ومستخلص البذور من المورينجا أوليفيرا للنشاط المضاد للفطريات لدى الأطفال المصابين بالتوحد

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نبات المورينجا ذو قيمة غذائية عالية وله مجموعة رائعة من الاستخدامات الطبية. نباتات عائلة *Moringaceae* تحتوي على مجموعة واسعة من المستقلبات الثانوية ذات الأنشطة البيولوجية التي تمنحها دورًا مهمًا في صناعة الأدوية. على حد علمنا، هذا هو التقرير الأول لتحديد الخصائص المضادة للأكسدة والمضادات الحيوية لزيت *M. oleifera* ومستخلصات بذوره مع تقييم السمية في الجسم كأدوية عشبية. أظهرت النتائج أن زيت *M. oleifera* له أعلى نشاط مضاد للفطريات ضد ستة سلالات من الفطريات المعزولة من عينات براز الأطفال المصابين بالتوحد وهي *Candida krusei, C. kefyr* , *C. glabarata, C. dulblinesis, C. albicans,* *C. lusitania*, تليها مستخلص الأسيتون للبذور. تركيز 1.0٪ من زيت *M. oleifera* سجل أعلى انخفاض في معدل النمو مع تركيز MIC ونشاط عالي كمضاد للأكسدة. أوضح تحليل GC / MS لزيت المورينجا أوليفيرا إجمالي 17 مركب مع ميثيل استر ، ستيغماستيرول ، تيترافينيل وفيتامين E كمكونات رئيسية. علاوة على ذلك، فإن التقييم السمي في الجسم الحي يمكن أن يؤكد سلامة زيت *M. oleifera* للاستخدام. وفى النهاية، يمكن استنتاج أن زيت *M. oleifera* أداة واعدة لتحسين مشاكل الجهاز الهضمي لدى أطفال التوحد.