Pak. J. Bot., 49(1): 353-359, 2017.

PRELIMINARY PHYTOCHEMICAL SCREENING, ANTIFUNGAL AND CYTOTOXIC ACTIVITIES OF LEAVES EXTRACT OF *MORINGA OLEIFERA* Lam. FROM SALT RANGE, PAKISTAN

MUHAMMAD MAQSOOD[1], RAHMATULLAH QURESHI[1*], MUHAMMAD ARSHAD[1], MUHAMMAD SHEERAZ AHMED[2] AND MASROOR IKRAM[3]

[1]*Department of Botany, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.* [2]*Department of Biochemistry, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan,* [3]*Department of Physics and Applied Mathematics, Pakistan Institute of Engineering and Applied Sciences, Nilore, Islamabad, Pakistan*
[*]*Corresponding author's email: rahmatullahq@yahoo.com, rahmatullahq@uaar.edu.pk*

Abstract

Moringa oleifera Lam. is a miraculous plant that endowed with variety of medicinal properties and traditionally used as herbal drug as well as nutraceutical agent. There is sporadic information on phytochemical and antifungal activity of various solvents based leaves extracts. Therefore, the present study was designed to explore *In vitro* antifungal activity of *M. oleifera* leaves against *Aspergillus fumigatus*, *A. niger* and *Candida albicans* at four different concentrations (50-300 mg/ml) by ager well diffusion method. Leaves of this plant were collected from the Thal Desert, Pakistan, dried under shade, powdered and kept in air tight sterilized bottles. Polarity based solvent extraction of powdered samples were carried out with different solvents. The ethanolic and methanolic extracts were found the most effective against all selected fungal strains. The maximum zone of inhibition was recorded in the case of methanolic leaves extract (16 mm) against *A. niger* at a concentration of 300 mg/ml, which was at par to the standard antibiotic. Methanolic extract showed the highest MIC value (70 mg/ml) against *A. niger*. There was very strong activity in terms of IC_{50} against MCF-7, INS-1, RG2 and HeLa (<5 μgmL^{-1}). The quantitative phytochemical analysis revealed that leaves possessed high amount of flavonoids (21.76±0.68), followed tannins (14.3±0.26), saponins (12.56±0.51) and alkaloids (2.4±0.85). This piece of research would be used as benchmark to carry-out further detailed study ranging from isolation, characterization, pharmacological diagnosis and clinical trials prior to launching marketable drug.

Key words: *Moringa oleifera,* Medicinal properties, Phytochemicals, Antifungal activity, Leaves extracts.

Introduction

Moringa oleifera Lam. locally known as *Suhanjana*, belongs to family Moringaceae (David *et al.*, 2015), mostly cultivated in the Sindh province and irrigated plains as well as arid areas of the Pakistan (Iqbal & Bhanger, 2006; Qaiser, 1973). This species is distributed in India, Pakistan, Bangladesh and Afghanistan (Fahey, 2005). *M. oleifera* is a shrub or small tree of 2.5-10 m in height (Vlahof *et al.*, 2002). This is a dynamic plant with rich medicinal properties. The entire plants have protein, vitamins, mineral and carbohydrates, seeds have oil content, while the coagulant of seeds is used for the treatment of the wastewater (Foidl *et al.*, 2001).

Various parts of *M. oleifera* are used as vegetables and for treatment of variety of human ailments in the folk medicine (Sastri, 1962; Nadkarni & Nadkharni, 1976). In Ayurvedic system of medicine, its root, root bark, leaves, flowers and unripe pods are reported as antipyretic, abortificient, galactogogue and anthelmintic. The bark is used as an emmenagogue in Siddha and Unani system of medicine and root decoction is used as gargle, abortifacient, rubefacient, counter-irritant in rheumatic cases (Chopra, 1938). Several studies reported that it has some pharmacological activities including antibacterial (Eilert *et al.*, 1981; Dayrit *et al.*, 1990, Elumalai *et al.*, 2015), anti-inflammatory (Ezeamuzie *et al.*, 1996), hepatoprotective (Pari & Kumar, 2002), anti-hypertensive (Faizi *et al.*, 1995) and anti-tumor (Murakami *et al.*, 1998), antispasmodic, diuretic, antioxidant (Kumbhare *et al.*, 2012) and antifungal activities (Caceres *et al.*, 1992).

Leaves have been used for wound healing, as diuretic, antiulcer and anti-inflammatory (Caceres *et al.*, 1992; Udupa *et al.*, 1994; Pal *et al.*, 1995; Jaiswal *et al.*, 2009). The leaves aqueous extract possess antifertility activity (Shukla *et al.*, 1981), ethanolic extract have shown activity against a number of pathogenic fungi (Chuang *et al.*, 2007), whereas leaf methanolic extract has a strong depressant action on central nervous system (Pal *et al.*, 1996). The seeds of *M. oleifera* possess strong anti-microbial and coagulative properties (Eilert *et al.*, 1981). Since most of the work is reported from various parts of the world, however no detailed study is reported from Pakistan, therefore present work was planned to screen phytochemicals, antifungal and cytotoxic activities of leaves extracts of *M. oleifera*.

Materials and Methods

Collection and identification of plant material: The collection of leaves of *M. oleifera* were carried out from Thal Desert, Pakistan and identified through floristic material (Qureshi, 2012). The voucher specimen (2056) was deposited in the Herbarium of Department of Botany, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi.

Preparation of plant sample: The collected leaves were washed thoroughly with water, chopped, air dried under shade at room temperature, oven dried and ground to powder with electric grinder and stored in airtight jars for further process.

Preparation of plant extract: For the preparation of extracts the powdered samples were extracted in seven polarity based solvents i.e. n-hexane, chloroform, acetone, ethyl acetate, ethanol, methanol and water. Initially, samples were extracted in n-hexane (1:10) and shaken for 24 hours and centrifuged at 10,000 rpm for 15 min. The supernatant was decanted into labeled containers and residue was extracted in next solvent, which was slightly higher in polarity than n-hexane. The evaporation of the all

extracts were carried out under reduced pressure at 40°C and stored at 4°C in refrigerator. For the antifungal assay the dried extracts were dissolved in Dimethylsulfoxide (DMSO) for antifungal assay.

Phytochemicals screening

a. Qualitative identification of phytochemicals: The Leaves extracts of all solvents were subjected to qualitative determination of flavonoids (Trease & Evans, 1996; Kalimuthu *et al.*, 2010), saponins (Tyler, 1994; Hussain *et al.*, 2011), alkaloids (Tyler, 1994; Hussain *et al.*, 2011), phenols (Roopashree *et al.*, 2008) and tannins (Roopasheer *et al.*, 2008).

b. Quantitative determination of phytochemicals: Quantitative determination of major phytochemicals of leaves extracts of *M. oleifera* was carried out as described by Obodoni & Ochuko (2001), Hussain *et al.* (2011) and Sutharsingh *et al.* (2011), Tyler (1994).

Determination of antifungal activity: Different solvent based leaves extract of *M. oleifera* were assessed for its antifungal activity against fungal pathogens viz., *A. niger, A. fumigatus* and *C. albicans* by ager well method. Their clinical isolates were obtained from Pakistan Institute of Medical Sciences (PIMS), Islamabad and used in this study.

Preparation of inoculum: Sabouraud Dextrose Agar (SDA) was used to maintained stock of fungi. A loopful of cells from the stock cultures was transferred to test tubes of sterile Sabouraud Dextrose Broth (SDB) to prepare 10^6 colony forming units (CFU/ml) and tubes were placed at 25°C for 24 hours in shaker incubator.

Agar Well Diffusion method: The antifungal activity was tested by agar well diffusion method (Nejad *et al.*, 2010). SDA media was prepared, autoclaved for 15 minutes at 121°C, cooled and poured in laminar flow cabinet in petri plates. After allowing the media to solidify at room temperature, one hundred microlitres of inoculums (10^6 CFU/ml; 0.5 McFarland) of each tested fungus evenly spread onto separate agar plates using a sterile glass spreader (Fenner *et al.*, 2005; Thongson *et al.*, 2004). The agar plates were dried and punch wells were formed by sterile borer of 6 mm in diameter. The wells were filled with 100 µl of plant extract. The extract were allowed to diffuse into agar media for 2 hours and incubated at 27°C for 72 hours. Dimethyl sulfoxide (DMSO) was used as a negative control and Fluconazol was used as a positive control.

Measurement of zone of inhibition: The antifungal activity was measured as zone of inhibition in diameter (mm) after 72 hours of incubation at 27°C. All readings were taken in triplicates.

Minimum inhibitory concentration (MIC): The minimum inhibitory concentration (MIC) of leaves extracts was carried out by the agar well diffusion method as described by Thongson *et al.*, 2004. The lowest concentration of extract caused complete inhibition of fungal growth was taken as MIC.

Cytotoxicity activity: Dilutions of the stock solution were made in cell culture medium, utilizing, minimum essential medium (MEM) alpha for MCF-7 , Dulbecco's Modified Eagle's Medium (DMEM) for RG2 and RD while Roswell Park Memorial Institute medium (RPMI-1640) for HeLa and INS-1 cells. All media containing phenol red and 2% fetal bovine serum (FBS), 1% L-glutamine (2mM), 1% sodium pyruvate (1mM) and 1% antibiotics penicillin (5000UmL^{-1}) and streptomycin (2500UmL^{-1}) (all from Gibco®, Invitrogen, CA, USA) as supplements. Logarithmic dilutions of the stock solutions were between kept 20 and 0.1 µgmL^{-1} to determine the extract fractions of cytotoxicity (IC_{50} value). Dilutions were kept at 4ºC until future use.

Statistical analysis: All the results were expressed as mean ± SD (standard deviations). Statistical analysis was performed using MSTATC and MS-Excel 2007. The significant difference was considered at α values ≤ 0.05.

Results and Discussion

Phytochemical screening

Qualitative analysis of leaves extracts for phytochemicals: Qualitative phytochemicals analysis of Moringa leaves showed the presence of all tested phytochemicals and the results are summarized in Table 1. Ethanol and methanol were found the most effective solvents that isolated all phytochemicals. Various studies discovered that ethanol extract has capability to isolate maximum number of compounds (Tijjan *et al.*, 2009; Bennett *et al.*, 2003). Interestingly Bukar *et al.* (2010) isolated saponins and flavonoids from ethanol extract but failed to separate tannins and alkaloids from it. The absence of later phytochemicals was also reported by Kasolo *et al.* (2010) from ethanol extract.

The water as solvent in the present endeavour isolated alkaloids, phenols and tannin, but unable to detect flavonoids and saponins which contradicts with the study of Kwaghe & Ambali (2009) and Kasolo *et al.* (2010). Quantitatively, phenols and tannins were present in very higher amount in ethanol, methanol and water (Table 1). Tannins are a group of polymeric phenolic substances capable of killing microorganisms (Cowan, 1999; Hausteen, 2005).

Table 1. Qualitative phytochemicals analysis of leaves extracts.

Sr. No	Solvent	Flavonoids	Saponins	Alkaloids	Phenol	Tannins
1.	n-Hexane	—	+	—	—	—
2.	Chloroform	+	+	—	—	—
3.	Acetone	+	—	—	+	—
4.	Ethyl acetate	+	—	—	++	+
5.	Ethanol	++	++	+	+++	+++
6.	Methanol	+++	++	+	+++	+++
7.	Water	—	—	++	+++	+++

Key: Absent = —, Trace = +, moderately present = ++, highly present = +++

The ethyl acetate showed the presence to flavonoids, phenols and tannins; whereas acetone leaves extract isolated phenols and flavonoids which were present in low amount (Table 1). There was a little difference found in ethyl acetate and acetone that the former extract contained tannins in addition to flavonoids and phenols which is in agreement with those of Kwaghe & Ambali (2009). In the case of chloroform, flavonoids and saponins were extracted that corresponds with the finding of Kwaghe & Ambali (2009), however, present study failed to isolate further compounds as mentioned by previous authors. Interestingly, Bukar *et al.* (2010) found alkaloids, saponins and tannins from chloroform leaves extract but unable to detect flavonoids which contradicts with our findings. The n-hexane was found least effective solvent and solely isolated saponins. The present study revealed that both phenols and tannins were richly found, whereas, saponins in moderate amount in methanol and ethanol extract (Table 1).

Quantitative phytochemicals determination of leaves: The data provided in Table 2 shows that phytochemicals composition of leaves were found in the order of flavonoids (21.76±0.68%)> tannins (14.3±0.26%)> saponins (12.56±0.51%)> and alkaloids (2.4±0.85%). Flavonoids were found in higher proportion (21.76%) than rest of chemicals and are reported to be of high value in deriving a wide range of biological activities (Hodek *et al.*, 2002). Similarly, Munazir *et al.* (2015) reported higher amount of flavonoids from *L. pyrtotechnica*. Thus high antifungal activity determined by this study reveals that the activity may be attributed due to presence of high proportion of flavonoids and tannin content. Krishnaiah *et al.* (2009) investigated leaves of medicinal plants for five major groups of phytochemicals and reported tannins (9.2%), saponins (2.3%), flavonoids (0.51%), alkaloids (0.36%) and phenols (0.08%). The present investigation discovered significantly higher proportion compared with former study. There are multiple factors which govern in proportion within chemical composition and concentration in plants. These might be due to environmental, edaphic and nature of plant growth stage, etc. Mustafa *et al*. (2016) carried phytochemical screening of some plants from Cholistan desert.

Table 2. Quantitative estimation of phytoconstituents (%) in *M. oleifera* leaves.

Flavonoids	Saponins	Alkaloids	Tannins
21.76 ± 0.68	12.56 ± 0.51	2.4 ± 0.85	14.3 ± 0.26

Values are expressed as means ± S.D after triplicate analysis

Antifungal activity of leaves extracts: Antifungal activity of different leaves extracts (n-hexane, chloroform, acetone, ethyl acetate, ethanol, methanol and water) of *M. oleifera* was tested against *A. niger, A. fumigatus* and *C. albicans* (Table 3). The extracts showed significant differences in their efficacy. The activity of different leaves extract was in the order of methanol > ethanol > ethyl acetate > water > and acetone (Table 3). It was found that leaves extract showed maximum antifungal activity against *A. niger* (16 mm), followed by *A. fumigatus* (13.83 mm) and *C. albicans* (13 mm). Similar findings are reported by Kekuda *et al.* (2010), who observed that steam distillation of leaves showed more inhibition against *A. niger* (46.51%). Therefore, *A. niger* was the most susceptible fungus among all the tested fungi.

The all concentrations of ethanol and methanol based *M. oleifera* leaves extracts were found the most effective against all selected fungal strains except 50 mg/ml. The maximum zone of inhibition (16±2 mm) was recorded in the case of methanol leaves extract against *A. niger* at concentration of 300 mg/ml, which was almost equivalent to standard antibiotic (Table 3; Fig. 5). Therefore, this study suggests that methanolic leaves extracts of screened plant would be helpful in treating diseases caused by *A. niger.* At the same concentration *A. fumigatus* and *C. albicans* showed inhibition of 13.83±1.25 mm (Fig. 1) and 13±1 mm respectively. Chuang *et al.* (2007) reported that ethanol extracts showed *In vitro* antifungal activity against *Trichophyton rubrum, T. mentagrophytes, Epidermophyton Xoccosum,* and *Microsporum canis*. The results of present study confirmed that ethanolic and methanolic Moringa leaves extract possess broad spectrum antifungal activity.

It has been observed that inhibition of *A. niger* and *C. albicans* was directly proportional with concentrations. It has been found 15.3±1.52 mm (Fig. 5), 13.41±2.30 mm, 11.17±1.44 mm inhibition zones (Table 3) were produced by ethanol extract against *A. niger* at 300 mg/ml, 200 mg/ml and 100 mg/ml respectively. In the case of *C. albicans* 12.66±1.60 mm, 11.33±2.08 mm and 8.5±1.32 mm inhibition zones recorded at 300 mg/ml, 200 mg/ml and 100 mg/ml respectively. In the case of *A. fumigatus*, there was no change found in inhibition zone when concentrations were kept 200 (Fig. 2) to 300 mg/ml (Fig. 3) and results were same i.e. 13 mm (Table 3; Fig. 4).

The present study reveals that with increase of concentration of extract the antifungal activity also increased which is in agreement with findings of Wanchaitanawong *et al.* (2005), that used various concentration of extract of selected plant on the inhibition of *A. niger, A. oryzae* and *penicillium* spp. The aqueous extracts of Moringa in this investigation exhibited moderate antifungal activity and zones of inhibition were in the order of 12.3 ± 0.57 mm > 12 ± 1 mm against *A. fumigatus* and *A. niger* respectively at 300 mg/ml. However, no activity recorded in the case of *C. albicans* that corresponds to the findings of Caceres *et al.* (1991) (Table 3; Fig. 3). Rahman *et al.* (2009) reported that *M. oleifera* fresh leaves juice showed stronger antibacterial activity and higher zone of inhibitions (15-25 mm) than present findings. The strong antibacterial activity of the same solvent base extract may be attributed due to difference in prokaryotic cell composition.

Table 3. Antifungal activity of leaf extracts of *Moringa oleifera*.

Solvents	Extract conc. mg/ml	Fungal strains diameter of zone of inhibition in mm		
		A. fumigatus	*A. niger*	*C. albicans*
n- hexane	300	_	_	_
	200	_	_	_
	100	_	_	_
	50	_	_	_
Chloroform	300	_	_	_
	200	_	_	_
	100	_	_	_
	50	_	_	_
Acetone	300	10.16 ± 1.25	11.6 ± 0.76	_
	200	8.75 ± 0.66	10.08 ± 0.87	_
	100	_	8 ± 1.5	_
	50	_	_	_
Ethyl acetate	300	11.16 ± 1.52	13.91 ± 1.37	11 ± 1
	200	10 ± 1.73	12 ± 2	10 ± 2
	100	8.75 ± 1.29	9.5 ± 0.86	_
	50		_	_
Ethanol	300	13±2	15.3 ± 1.52	12.66 ± 1.60
	200	13±1	13.41 ± 2.30	11.33 ± 2.08
	100	10.8±1.25	11.17 ± 1.44	8.5 ± 1.32
	50	_	_	_
Methanol	300	13.83 ± 1.25	16 ± 2	13 ± 1
	200	12.75 ± 1.29	14.8 ± 1.89	12.08 ± 1.58
	100	11.16 ± 0.57	12 ± 1	9.5 ± 0.86
	50	_	_	_
Water	300	12.3 ± 0.57	12 ± 1	_
	200	10 ± 2	10.5 ± 1.5	_
	100	_	9.66 ± 0.76	_
	50	_	_	_
Fluconazole	10 µg/ml	15.1 ± 0.86	16.5 ± 0.1	17.2 ± 0.62

Fig. 1. Antifungal activity of ethyl acetate and methanol extract against *A. fumigatus* at 300 mg/ml.

Fig. 2. Antifungal activity of ethyl acetate and water extract against *A. fumigatus* at 200mg/ml.

The ethyl acetate extract was found effective against all tested strains (Table 3). *A. niger* was highly susceptible to extract and inhibited at 13.91±1.37 mm, followed by *A. fumigatus* (11.16±1.52 mm) (Fig. 1) and *C. albicans* (11±1 mm) at the concentration of 300 mg/ml (Table 3). The acetone leaves extract reasonably inhibited *A. niger* (11.16±0.76 mm), followed by *A. fumigatus* (10.16±1.25 mm) at the concentration of 300 mg/ml, while *C. albicans* was found resistant to extract and not inhibited at any concentration (Fig. 3). The result of ethyl acetate extracts authenticates superiority on acetone extracts in terms of antifungal activity that may be due to the isolation of tannins, as confirmed by the phytochemical analysis. Banso & Adeyemo (2007) stated that the tannins from the medicinal plants possess remarkable toxicity against bacteria and fungi and may have pharmacological importance. The tannins from *S. adstringens* have strong ability to inhibit the growth of *C. albicans* (Luiz *et al.*, 2015). The Zn nanoparticles produced from of *M. oleifera* possess antimicrobial potential because of amino acids, alkaloids, flavonoids and phenolics of the leaf extracts which further validate the result of present study (Elumalai *et al.*, 2015).

Cytotoxic activity: The crude methanolic extract (CME) of *Moringa oleifera* showed IC_{50} values that was ranged from 0.86 t 10.61 μgmL^{-1} following the incubation period of 48 to 72 hours. The lowest IC_{50} value was recorded as 0.86 μgmL^{-1}, revealing a very strong activity against RG2 after 72 hours (Table 4). Very strong activity in terms of IC_{50} was recorded in the case of MCF-7, INS-1, RG2 and HeLa (<5 μgmL^{-1}) and moderate against RD cells (10-20 μgmL^{-1}) according to the criteria of Wibowo *et al.* (2011).

Table 4. IC_{50} value of *Moringa oleifera* (fruit and leaves) against the selected cell lines

Cell line	Time period (hours)	Leaves IC_{50} (μgmL^{-1})
MCF-7	48	2.58 ± 0.03
	72	3.19 ± 0.02
INS-1	48	3.28 ± 0.03
	72	2.44 ± 0.02
RG2	48	4.13 ± 0.04
	72	0.86 ± 0.06
HeLa	48	1.79 ± 0.02
	72	1.68 ± 0.01
RD	48	10.61 ± 0.09
	72	6.88 ± 0.07

Legend: >100: not active; 20–100: weak; 10–20: moderate; 5–10: Strong; < 5: very strong (Wibowo *et al.*, 2011)

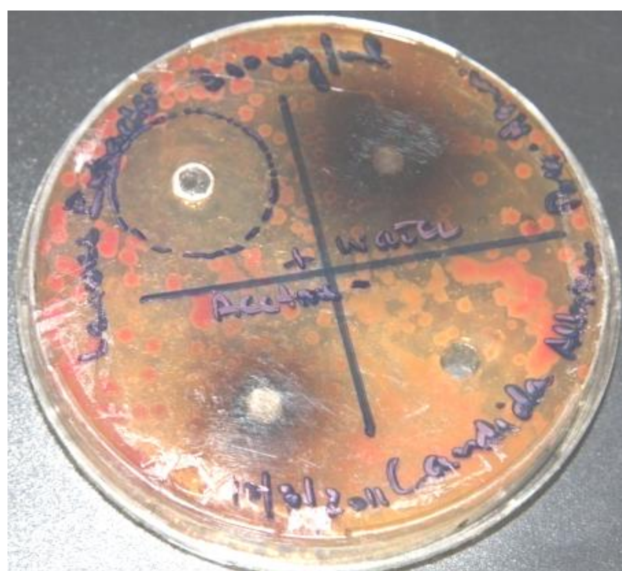

Fig. 3. Antifungal activity of water and acetone leaves extracts against *C. albicans*.

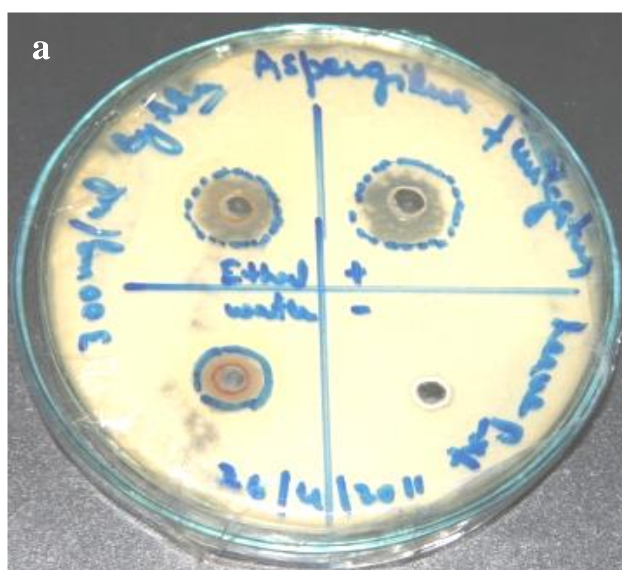

Fig. 4. Comparison of antifungal activity of ethanol leaf extract against *A. fumigatus* at 300 (a) and 200 mg/ml (b).

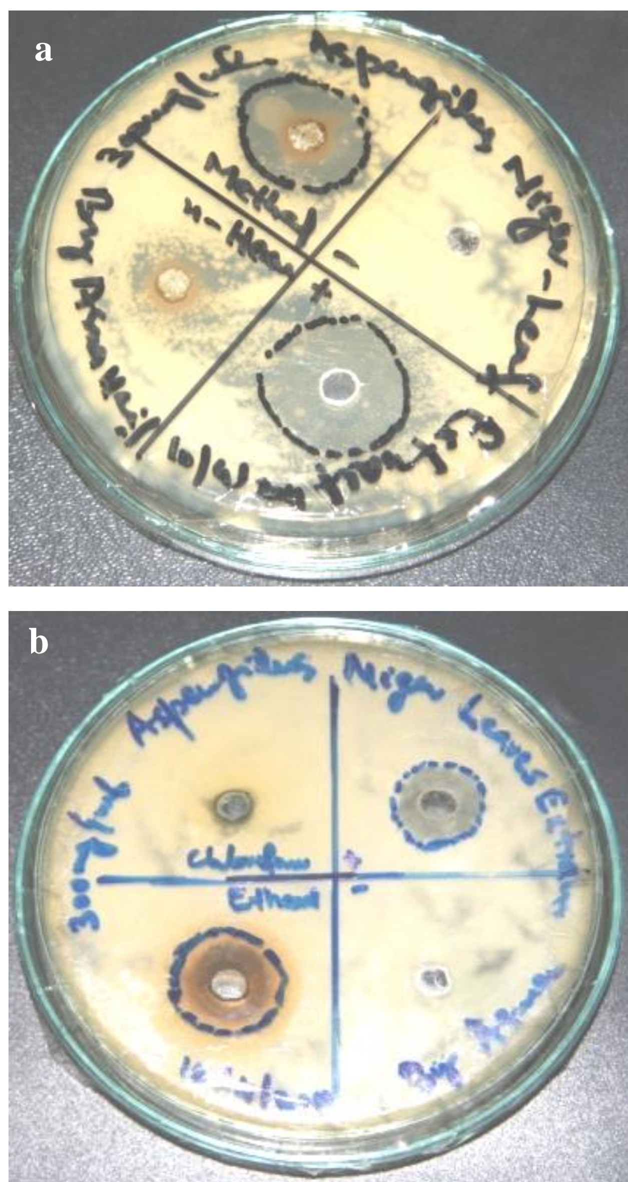

Fig. 5. Antifungal activity of methanol (a) and ethanol (b) leaves extract against *A. niger* at 300 mg/ml.

Conclusion

M. oleifera is a highly medicinal plant. The phytochemical analysis revealed it is rich in alkaloids, saponins, phenolics and flavonoids. The ethanol and methanol are the best solvents to isolate phytochemicals and their extracts are most effective against *A. niger* and *A. fumigatous*. Besides, CME of leaves showed lowest IC_{50} vlues against some cancer cell lines such as MCF-7, INS-1, RG2 and HeLa.Therefore, *M. oleifera* can be a potential candidate for drug delivery programme.

Acknowledgement

The paper is extracted from PhD thesis of the first author.

References

Banso, A. and S.O. Adeyemo. 2007. Evaluation of antibacterial properties of tannins isolated from *Dichrostachys cinerea. Afri. J. Biotechnol.,* 6(15): 1785-1787.

Bennett, R., F. Mellon, J. Pratt, M. Dupont, L. Pernins and P. Kroon. 2003. Profiling glucosinolates and phenolics in vegetative and reproductive tissues of multi-purpose trees *Moringa oleifera* L. (horseradish tree) and *Moringa stenopetal* L. *J. Agric. Food Chem.,* 51: 3546-5553.

Bukar, A., A. Uba and T.I. Oyeyi. 2010. Antimicrobial profile of *Moringa oleifera* Lam. extracts against some food borne microorganisms. *Bajopas*, 3(1): 43-48.

Caceres, A., A. Saravia, S. Rizzo, L. Zabala, E.D. Leon and F. Nave. 1992. Pharmacologie properties of *Moringa oleifera*. 2: screening for antispasmodic, anti-inflammatory and diuretic activity. *J. Ethanopharmacol.*, 36(3): 233-237.

Chopra, R.N. 1938. *Indigenous drugs of India, their medicinal and economic aspects*, Art Press, Calcutta.

Chuang, P.H., C.W. Lee, J.Y. Chou, M. Murugan, B.J. Shieh and H.M. Chen. 2007. Anti-fungal activity of crude extracts and essential oil of *Moringa oleifera* Lam. *Bioresource Technol.,* 98: 232-236.

Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clinical Microbiol. Rev.,* 12: 564-582.

David, M., Fernandes, Raquel M.F. Sousa, Alberto de Oliveira, Sérgio A.L. Morais, Eduardo M. Richter and Rodrigo A.A. Muñoz. 2015. *Moringa oleifera*: A potential source for production of biodiesel and antioxidant additives. *Fuel*, 146: 75-80.

Dayrit, F.M., A.D. Angela and I.M. Villasena. 1990. Studies on *Moringa oleifera* seeds. Part I. The antibiotic compound and its deactivation in aqueous solution. *Phillipp. J. Sci*., 119: 23-32.

Eilert, U., B. Wolters and A. Nadrtedt. 1981. The antibiotic principle of seeds of *Moringa oleifera* and *Moringa stenopetala*. *Planta Med*., 42: 55-61.

Elumalai, K., S. Velmuruganb, S. Ravib, V. Kathiravana and S. Ashokkumara. 2015. Green synthesis of zinc oxide nanoparticles using *Moringa oleifera* leaf extract and evaluation of its antimicrobial activity. *Spectrochim Acta A.*, 143: 158-164.

Ezeamuzie, I.C., A.W. Ambadederomo, F.O. Shode and S.C. Ekwebelem. 1996. Anti-inflammatory effects of *Moringa oleifera* root extract. *Int. J. Pharmacognosy*, 34: 207-212.

Fahey, J.S.W. 2005. *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. *Trees for Life Journal*, 1: 5.

Faizi, S., B. Siddiqui, R. Saleem, S. Siddiqui, K. Aftab and A. Gilani. 1995. Fully acetylated carbamate and hypotensive thiocarbamate glycosides from *Moringa oleifera*. *Phytochem*., 38: 957-963.

Fenner, M., S.M. Sortinob, R. Ratesa, S. Agnola and B. Zacchino. 2005. Antifungal activity of some Brazilian Hypericum species. *Phytomedicine*, 12: 236-40.

Foidl, N., H.P.S. Makkar and K. Becker. 2001. The potential of *Moringa oleifera* for agricultural and industrial uses. In: *The Miracle Tree The Multiple Attributes of Moringa fuglie* L. J. (Eds). USA. p: 45-76.

Hausteen, B.H. 2005. The Biochemistry and medical significance of the flavonoids. *Pharmacol. Therapeutics J.,* 96: 67-202.

Hodek, P., P. Trefil and M. Stiborova. 2002. Flavonoids-Potent and versatile biologically active compounds interacting with cytochrome P450. *Chem. Biol. Int.,* 139(1): 1-21.

Hussain, I., M.U.R. Khattak, Riaz Ullah, Z. Muhammad, N. Khan, F.A. Khan, Z. Ullah and S. Haider. 2011. Phytochemicals screening and antimicrobial activities of selected medicinal plants of Khyberpakhtunkhwa Pakistan. *Afri. J. Pharmacy and Pharmacol*., 5(6): 746-750.

Iqbal, S. and M.I. Bhanger. 2006. Effect of season and production location on antioxidant activity of *Moringa oleifera* leaves grown in Pakistan. *Journal of Food Composition and Analysis,* 19: 544-551.

Jaiswal, D., P.K. Rai, A. Kumar, S. Mehta and G. Watal. 2009. Effect of *Moringa oleifera* Lam. leaves aqueous extract therapy on hyperglycemic rats. *Journal of Ethnopharmacology*, 123: 392-396.

Kalimuthu, S., P. Rajesh, V.R. Kannan, B. Balamurugan and T.M. Chandrasekar. 2010. Antiulcer activity of methanolic extract of *Acalypha indica* Linn. (Euphorbiaceae) by pylorous ligture and swim stress induced ulceration. *J. Pharm. Res.,* 3(11): 2779-2783.

Kasolo, J.N., S. Gabriel, Bimenya, O. Lonzy, O. Joseph and J. W. Ogwal-Okeng. 2010. Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. *J. Med. Pl. Res*., 4(9): 753-757.

Kekuda, T.R.P., N. Mallikarjun, D. Swathi, K.V. Nayana, M.B. Aiyar and T.R. Rohini. 2010. Antibacterial and Antifungal efficacy of steam distillate of Moringa oleifera Lam. *J. Pharm. Sci. & Res.*, 2(1): 34-37.

Kumbhare, M.R., V. Guleha and T. Sivakumar. 2012. Estimation of total phenolic content, cytotoxicity and *In vitro* antioxidant activity of stem bark of *Moringa oleifera*. *Asian Pacific Journal of Tropical Disease*, pp. 144-150.

Krishnaiah, D., T. Devi, A. Bono and R. Sarbatly. 2009. Studies on phytochemical constituents of six Malaysian medicinal plants. *J. Med. Pl. Res*., 3(2): 067-072.

Kwaghe, A.V. and A.G. Ambali. 2009. Preliminary phytochemical screening of fresh and dried *Moringa oleifera* leaves and that of chloroform, ethylacetate and n-butanol fractions. *Sahel J. Vet. Sci.,* 8(2): 21: 1-24.

Luiz, R.L.F., T.V.M. Vila, J.C.P. Mello, C.V. Nakamura, S. Rozental and K. Ishida. 2015. Proanthocyanidins polymeric tannin from *Stryphnodendron adstringens* are active against *Candida albicans* biofilms. *BMC Complement. Altern. Med.,* 15(68): 2-11.

Munazir, M., R. Qureshi and M. Munir. 2015. Preliminary phytochemical screening of roots and aerial parts of *Leptadenia pyrotechnica*. *Pak. J. Bot*., 47(2): 659-664.

Murakami, A., K. Kitasono, S. Jiwajinda, K. Koshimizu and H. Ohigashi. 1998. Niaziminin, a thiocarbamate from the leaves of *Moringa oliefera*, holds a strict structural requirement for inhibition of tumor-promoter-induced Epstein–Barr virus activation, *Planta Medica*, 64: 319-323.

Mustafa, G., S. Ahmed, N. Ahmed and A. Jamil. 2016. Phytochemical and antibacterial activity of some unexplored medicinal plants of Cholistan desert. *Pak. J. Bot.*, 48(5): 2057-2062.

Nadkarni, K.M. and A.K. Nadkharni. 1976. The Indian Materia Medica, Popular Prakashay, Bombay, India, 810 pp.

Nejad, B.S., F. Shiravi, S. Ghanbari, M. Alinejadi and M. Zarrin. 2010. Antifungal activity of *Satureja khuzestanica* (Jamzad) leaves extracts. *Jundishapur J. Microbiol*., 3(1): 36-40.

Obodoni, B.O. and P.O. Ochuko. 2001. Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. *Global J. Pure Appl. Sci*., 8: 203-208.

Pal, S.K., P.K. Mukherjee and B.P. Saha. 1995. Studies on the antiulcer activity of *Moringa oleifera* leaf extract on gastric ulcer models in rats. *Phytotherapy Res.,* 9: 463-465.

Pal, S.K., P.K. Mukherjee, K. Saha, M. Pal and B.P. Saha. 1996. Studies on some psychopharmacological actions of *Moringa oleifera* Lam. (Moringaceae) leaf extract. *Phytotherapy Res.,* 10: 402-405.

Pari, L. and N.A. Kumar. 2002. Hepatoprotective activity of *Moringa oleifera* on antitubercular drug-induced liver damage in rats. *J. Med. Food,* 5: 171-177.

Qaiser, M. 1973. Moringaceae. In: *Flora of West Pakistan*, (Eds.): Nasir, E. and S.I. Ali. No.38. Department of Botany, University of Karachi, Pakistan, p. 1-4.

Qureshi, R. 2012. *The Flora of Nara Desert, Pakistan.* Nova Science Publishers, Inc. Hauppauge, New York, USA. pp. 1-317.

Rahman, M., Mashiar, M.M.I. Sheikh, S.A. Sharmin, M.S. Islam, M.A. Rahman, M.M. Rahman and M.F. Alam. 2009. Antibacterial activity of leaf juice and extracts of *Moringa oleifera* Lam. against some human pathogenic bacteria. CMU. *J. Nat. Sci*., 8(2): 219-227.

Roopashree, T.S., R. Dang, R.H.S. Ran and C. Narendra. 2008. Antibacterial activity of antisporiatic herb *Casia tora, Mimordica chirantia, Clandula officinalis. Int. J. Ap. Res. Nat. Pro.*, 3(1): 20-28.

Sastri, B.N. 1962. The wealth of India, Council of Scientific and Industrial Research, New Delhi, India, 1: 425-429.

Shukla, S., R. Mathur and A.O. Prakash. 1981. Effects of aqueous extract of *Moringa oleifera* Lam. on the periodicity of oestrous cycle in adult intact rats. *Ind. J. Pharmacol*. *Sci*., 49: 218-219.

Sutharsingh, R., S. Kavimani, B. Jayakar, M. Uvarani and A. Thangathirupathi. 2011. Quantitave phytochemical estimation and antioxidant studies on aerial parts of *Naravelia zeylanica*. *International Journal of Pharmaceutical Studies and Research*, 2(2): 52-56.

Tijjani, M., I. Bello, A. Aluyu, T. Olurishe, S. Maidawa, J. Habila and E. Balogun. 2009. Phytochemical and antibacterial studies of root extract of *Cochlospermum tinctorium* a. rich (Cochlospermaceae). *Res. J. Med. Plants*, 3: 16-22.

Thongson, C., P.M. Davidson, W. Mahakarrchanakul and J. Weiss. 2004. Antimicrobial activity of ultrasound assisted solvent-extracted spices. *Lett. Appl. Microbiol.*, 39: 401-406.

Trease, G.E. and W.C. Evans. 1996. *Pharmacognosy*, 12th edition. Baillere Tindall, London, pp. 47-48.

Tyler, V. E. 1994. Phytomedicines in Western Europe: their potential impact on herbal medicine in the United States. *Herbal Gram*, 30: 24-31.

Udupa, S.L., A.L. Udupa and D.R. Kulkarni. 1994. Studies on the anti-inflammatory and wound healing properties of *Moringa oleifera* and *Aegle marmelos*. *Fitoterapia*, 65: 119-123.

Vlahof, G., P.K. Chepkwony and P.K. Ndalut. 2002. 13C NMR characterization of triacylglycerols of *Moringa oleifera* seed oil: An Oleic-Vaccenic acid oil. *J. Agric. Food Chem*., 50: 970-975.

Wanchaitanawong, P., P. Chaungwanit, N. Poovarodom and S. Nitisinprasert. 2005. *In vitro* antifungal activity of Thai herb and spice extracts against food spoilage fungi. *Kasetsart J. Nat. Sci*., 39: 400-405.

(Received for publication 6 December 2015)