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Research article

ANTIMICROBIAL ACTIVITIES AND EVALUATION OF GENETIC EFFECTS OF MORINGA PEREGRINA (FORSK) FIORI USING MOLECULAR TECHNIQUES

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ABSTRACT: *Moringa peregrina* (Forsk) *Fiori,Moring aceaeused in* folk medicine. In the present work, ethanol extracts of different parts of this plant (leaves, seed coat and seed endosperm) along with some standard antibiotic were assessed against three Gram+ve bacteria *(Bacillus subtilius, Micrococcus luteus, Staphylococcus aureus*) and three Gram-ve bacteria (*Escherichia coli, Pseudomonas aeruginosa & Klebsiella pneumoniae*).The effect magnitude of these extracts on tested bacteria strains were as follows: leaves extract>seed coat >seed endosperm respectively. Results showed a strong effect of all these parts on *Staphylococcus aureus*. The leaf ethanol extract exhibited remarkable antifungal activities on the tested fungi in order of sensitivity as *C. albicans>A.flavus>A.fumigatus>A.niger C.* albicans was the most sensitive to all used *M.peregrina* parts. Antimicrobial activities of standard antibiotics showed an inhibitory effect on all tested bacteria. However the ciproflaxocin is more effective than streptomycin. The antifungal activities of standard antibiotics showed that the amphotericin is less effective than nystatin. The results of our antimicrobial assay revealed that the different parts extract of *M. peregrina* showed higher inhibitory activity on tested organisms than the standard antibiotics. The antimicrobial effect of *M.* peregrina leafs extract were explored at the molecular level, using random amplification of polymorphic DNA (RAPD) extracted from the control andtreated *Staphylococcus aurueus* and Candida albicans. The results demonstrate polymorphic band pattern for most treated microbes compared with the untreated strains. The present study suggested that *M. peregrina* leafs could have wide antimicrobial activity spectrum.

Key words: Medicinal plants,*Moringa peregrina*,antimicrobial, mutagenicity, RAPD, crude extract.

INTRODUCTION

Medicinal plants are from the most advantageous source of life saving drugs for humans, animals and plants. Bioactive compounds usually extracted from plants are used as medicines, food additives, dyes, insecticides, cosmetics, perfumes and fine chemicals. In some Asian and African countries, 80% of the populationare reliant on medicinal plants to maintain their health and to cure their ailment [41]. Synthetic antibiotics, known to enhanceantibiotic resistance, which is not the case with natural agents [40].The *Moringaceae* family consists of 10species [37] or 12 [29] belong to only one genus called *Moringa*.[31] reported that the most common species are *Moringaperegrina (forsk) fieri (*syn. *M.arabica* (Lam.) Pens., *M. arborea verde* (Kenya) *Moringazeylanica Sieb;* Balanus myrepsica Blackm), *Morin gastenopetala* Cufod, *Moringaborziana* Mattel, *Morin galongituba* Engl, *Moringaconcanensis* NImmo, *Morin gaovalifolia* DInter and A.Berger, *Morin gadrouhardii, Morin gahildebrantii.* All plant parts of *Morin gaceae* familyare used in folk medicine. But however a precise elucidation of the beneficial properties this family has not been completed. Leaves of *Moringa* species known to have various biological activities, including antitumor, antioxidant, anti-inflammatory/diuretic, anti-hepatotoxic properties, hypotensive, hypercholesterolemia and hypoglycemic actions [38]. Previous studies have reported that various parts of *Moringa* roots, flowers, bark, and stem including seeds possess antimicrobial properties [26, 6]. Seed extracts of *Moringaoleifera* found to have antimicrobial properties [21, 22]. However, a little review is available on the antimicrobial properties of *Moringaperegrina*. Thus, the present study try to explore the antimicrobial activities of different parts extracts of this species on some bacterial and fungal species and evaluate its genetic effects, at the molecular level using molecular genetics techniques.

MATERIALS AND METHODS

Collection and processing of plant materials

Moringa peregrina leaves and seeds were collected in May 2012 from Alboware, (23°56' 39.36"N 39°15'36.37"E) 80 km. northern Almadinah, Saudi Arabia.

Species statusof this plant was fervid at Faculty of Sciences Herbarium, King Abdulaziz University, Jeddah. The samples were brought to the laboratory, thoroughly washed in running tap water to remove debris and dust particles and then rinsed in distilled water for five minutes, then air-dried under room temperature until constant weight.

Extracts preparation

Ten grams of dried *Moringiaperegrina* parts (leaves and seeds) were catted into small pieces by blender 1-2 mm. Extraction was done by adding 100 ml of ethanol (1:10W/V) left under cold conditions for 48h, then the extracts were filtered through a whatman filter paper(No.1). The extracts solutions were evaporated under reduced pressure at 40°C until dryness, subsequently; the extract was diluted by dimethyl sulf. Oxide (DMSO) and stored under 20°C until analysis according to [8].

Bacterial and fungal strains cultures were prepared for in vitro antibacterial assay of the six Bacteria strains, three Gram positive:

Bacillus subtilis (ATCC11774); *S.aureus* (ATCC29213) and Micrococcus luteus (ATCC4698) and three Gram negative *Escherichia coli* (ATCC8739); *Klebsiellapneumonia* (ATCC700603) and *Pseudomon-as aeruginosa* (ATCC27853).

Those strains were provided by Microbiologics® USA. Tested organisms were subcultured on nutrient agar (Oxoid laboratories, UK) slopes. For the antifungal assay, four fungi (*Aspergillus flavus* (ATCC200026); *Aspergillus fumigatus* (ATCC204305); *Aspergillus niger* (ATCC1015) and *Candida albicans* (ATCC10231) were used. The tested organisms were subcultured on nutrient agar medium (Oxoid laboratories, UK) slopes for bacteria and Saboroud dextrose agar slopes (Oxoid laboratories, UK) for fungi were the media used. These stock cultures stored in the dark at 4°C until used.

Antimicrobial activity

Antimicrobial activity was determined using the agar well diffusion assay method as described by Holder and Boyce [19]. DMSO was used asa negative control.

The plates were organized in triplicate. Bacterial cultures were incubated at 37°C for 24 h

Antimicrobial activity was determined by measuring the inhibition zone [4].

DNA extraction and RAPD – PCR amplification conditions.

Genomic DNA was prepared from 18h cultures in exponential phase in Luria–Bertani medium (1000 mL deionized water ,10 g Bactotryptone, 5 g Bacto yeast, 5 g NaCl several drops, 5 M NaOH several drops and 1 M HCl) . Aliquots of 10 ml of each bacterial culture were harvested by centrifugation at 12.000 rpm for 15 min at 4 °c and washed once in sterile distilled water. The pellets were re suspended in 400 µl of lysis buffer containing 2% glucose, 50 m M Tris – HCl (pH 8.0), 25 m M EDTA, 3 mg / ml lysozyme and 200mg / ml RNase. The cell suspension was incubated for 1h at 37ºC. Further DNA extraction was performed as described by Sambrook [34]. PCR amplification was carried out in a DNA thermocycler (Biomatra, Germany) for 30 cycles each. The PCR reaction was carried out in a final volume of 25 µL with 1X PCR buffer containing 10 mM Tris – HCl, 25 mM MgCl2, 1 ml of Template DNA, 0.2 mM deoxy nucleoside triphosphate, 1 to 2 µM (each) primer and 0.5 u of Taq DNA polymerase (Promega). PCR conditions consisted of initial denaturation at 95ºc for 2min followed by 95ºc for 1min, annealing to primers at 37ºc for 1 min, and extension at 72ºc for 1 min with a final extension step at 72ºc for 5 min. PCR – amplified products were separated using agarose gel electrophoresis in 1% TBE buffer (Tris base 108 g, boric acid 55 g, 0.5M EDTA 20 ml and H2O2 to 1 L) dilute accordingly for 1X stock and stained with 0.2 µg /ml ethidium bromide according to Sambrook [34]. Amplified fragments were detected and photographed under UV light. Two random primers were synthesized from University of British Colombia (UBC), Vancouver, Canda (UBC 16, 28 and 89) and their sequences are presented in Tabulation. The random primer names and sequences that used for RAPD analysis are as follows Williams [42].

RAPD Primers	Primer Sequences (5-3)
OPA-05	AGGGGTCTTG
OPA-06	GGTCCCTGAC
OPB-06	TGCTCTGCCC

RESULTS AND DISCUSSION

Antibacterial activity

The antibacterial activity of ethanolic extracts of leaves, internal and external seeds of *Moringa peregrine* was assessed against three Gram +ve bacteria (*B.subtilis, M.lutues and S.aureus*) , three Gram – ve bacteria (*E.coli, K.pneumonia and P. aeruginosa*) at concentrations 0f 200mg/ml, the leaves extract showed the highest activity against tested organisms followed by the external > enternal seeds respectively (Table 1). It can be deduced that *Moringa peregrine* leaf ethanol extract had the broadest spectrum of activity on the tested bacteria.

The magnitude of its inhibition zones against the tested bacteria was as follows: *S. aurueus* (27.67mm), *M.lutues* (23.67 mm),, B.subtilis (20.00mm), *K.peneumonia* (26.67 mm), P.aeruginosa (20.67mm) and *E.coli* (19.67 mm) were more sensitive at 200 µl /plates concentration. Napoleon [32] also reported *Enterobacter* spp, *S.aureus, P.aeruginosa, S. Typi* and *E.colito* be sensitive to *Moringa oleifera* leaf ethanol extract at the same concentration .This is justified by the reports of Bukar [10] who indicated that Moringa oleifera leaf ethanol extract had activitiy against four bacterial isolates Enterobacter spp, S.aureus, P.aeruginosa, and E.coli. Moringa perngina leaf extracts exhibited remarkable antibacterial activities against the tested bacteria in the following sensitivity order *S. aureus> M. lutues> B.subitilus* .within the Gram –ve bacteria sensitivity order was:*K.penumonia> P.aeruginosa> E.coli*. The present results coincided with those of Bukar et al., 2010, whom reported thatM. oleifera leaf extracts were more potent than M. oleifera seeds extracts, which showed high activities against S. aureus (24.00mm) and *P.aeruginosa* (13.33mm) where by the internal seeds extract had the least antibacterial activity compared to other parts. *M.peregrina* internal seed extract was active against three bacterial isolates: M. *lutues (13.33mm), E.coli* (17.67*mm)* and *K.peneumonia* (16.33) but *B.subtilius* ,*S.aurueus* and *P.aeruginosa* were not sensitive. Of note that *P.aeruginosa* has the least sensitive to *M.peregrina indicting* that *M.peregrinamight* have limited effect on the proliferation and activities of *P .aeruginosa* which well-known for its strong resistant [9]. The antibacterial properties of the leaf and seed of *M.peregrina* as shown in the present study corroborate the earlier claims by Foidi [14, 2]. The antimicrobial activity of *M.peregrina* seeds is due to the presence of an array of phytochemicals, but most importantly due to the activity of ashort poly peptide and benzyl- iso thiocyanate [12, 16]. The peptide may act directly on microorganisms and results in growth inhibition by disrupting cell membrane synthesis or synthesis of essential enzymes [36, 39]. Antimicrobial activities of used standard antibiotics showed an inhibitory effect against all tested bacteria, but results showed that the ciprofloxacinwas more effective than streptomycin. This goes in line with Shital [35]. The antifungal activity of different parts of M.peregrina extract compared to standard antibiotics are shown in (table 2). The leaf ethanol extract exhibited remarkable antifungal activities against the tested fungi in the following sensitivity order *C. albicans>A.flavus>A.fumigatus>A.niger. C. albicans* on one hand, was the most sensitive to all *M.peregrinaparts* extracts compared with other tested fungi, on the other hand *A.niger* was the least sensitive fungi. The present results coincided with Dipali [11,17]. The leaves extracts showed the highest activity against testes fungi followed by external.>enternal seeds respectively (Table 2). The antifungal activities of standard antibiotics showed that the amphotericin is less than nystatin as appositive antibiotics [18]. The results of our antimicrobial assay revealed that the extract of different part of *Moringaperegrina* showed better inhibitory activity against tested organisms compared with standard antibiotics [17].

Table 1.The antibacterial activity of different parts extract of *Moringa perngina* concentration 100 mg/ml compared to antibiotics against the tested bacteria.

	Gram positive bacteria			Gram negative bacteria			
	B.subtilis	***M.lutues***	***S.aureus***	***E.coli***	***K.penoumonia***	***P.aeruginosa***	
	ATCC11774	ATCC4698	ATCC29213	ATCC8739	ATCC700603	ATCC27853	
Leaves	20.00±0.00	23.67±0.33	27.66±0.33	19.67±0.33	26.67±0.00	20.67±0.33	Between Strains SD=0.57735
Internal seed	0.00±0.00	13.33±0.33	00.00±0.00	17.67±0.33	16.33±0.33	0.00±0.00	
External seed	18.67±0.33	20.33±0.33	24.00±0.00	19.33±0.33	20.67±0.33	13.33±0.00	
Streptomycin	25.00±0.00	23.67±0.33	19.00±0.00	23.00±0.00	25.00±0.00	22.00±0.00	
Ciprofloxacine	34.00±0.00	46.00±0.00	38.00 ±0.00	44.00±0.00	42.00±0.00	42.00±0.00	
Total	19.53	25.40	21.73	24.73	26.13	19.60	
Std.Deviation	11.54	11.36	12.97	10.14	9.02	14.14	

Molecular characterization of *S. aurues* and *C.albicans* by RAPD analysis
Three random primers OPA-05, OPA-06 and OPB-06 were used for RAPD analysis of the tested bacteria.
The genetic effects of the ethanolic leaves extract of *M.peregrina*

Table 2.The antifungal activity of different parts extract of *Moringa perngina* concentration 100 mg/ml compared to antibiotics against the tested fungi.

Plant parts	*A.flavus*	*A.fumigatus*	*A.niger*	C.albicans	Between Strains LSD=0.57735
Leaves	23.33±0.58	22.67±0.58	18.67±0.58	24.67±0.58	
Internal seed	17.33±0.58	21.33±0.58	14.67±0.58	20.67±0.58	
External seed	21.67±1.15	22.33±0.58	17.67±0.58	22.67±0.58	
Amphoteracin	29.00±0.00	25.33±0.58	24.33±0.58	27.33±0.58	
Nystatin	31.67±0.57	27.00±1.00	29.00±1.00	28.33±1.15	
Total	24.60	23.73	20.87	24.73	
Std.Deviation	5.35	2.25	5.34	3.01	

Table3. RAPD analysis of the polymorphic amplified fragments of S.aureus and C.albicans isolates using three primers

Primer name	Fragment No	Fragment Bs	*S.aureus* Control	*S.aureus* Treated	*C.albicans* Control	*C.albicans* Treated
OPA-05	1	1100	-	-	ı	
	2	900	-	-	+	–
	3	700	ı	-	-	-
	4	590	+	-	+	–
	5	480	-	-	+++	+––
	6	400	+	Faint–	-	-
	7	370	-	-	+	–
	8	350	ı		ı	ı ı
	9	300	+	–	-	-
	10	180	+	–	+	–
	11	120	+	–	+	–
Total	11		7	5	8	8
Percentage polymorphic band			63.64%	45.45%	72.73%	72.73%
OPA-06	1	1200	-	-	+	–
	2	890	-	-	ı	
	3	630	-	-	+	–
	4	460	ı		ı	
	5	390	-	-	+	–
	6	430	ı	-	-	-
	7	300	+	–	-	-
	8	290	-	-	-	-
	9	240	ı		ı	
	10	210	+	–	-	-
	11	160	-	-	ı	
	12	110	+	–	-	-
Total	12		6	5	7	7
Percentage polymorphic band			50.00%	41.67%	58.33%	58.33%
OPB-06	1	940	-	-	ı	
	2	780	-	-	+	-
	3	700	-	So faint+	-	-
	4	600	-	-	-	-
	5	500	-	-	ı	-
	6	410	-	-	-	–
	7	370	-	So faint+	-	-
	8	290	ı		ı	
	9	150	+	–	+	–
Total	9		2	4	6	5
Percentage polymorphic band			22.22%	44.44%	66.67%	55.55%

The genetic effect of *M.peregrina* ethanolic leaves extract on *S. aurues* and *C.albicans* was investigated, as those two species exhibited the highest sensitivity to this extract. This was executed using RAPD analysis. The RAPD analysis results illustrated in (Tables 2 and Figure1) show polymorphic number of genetic bands, which were the electrophoretic products of PCR for treated *S. aurues* and *C.albicans* compared with those of untreated bacteria.

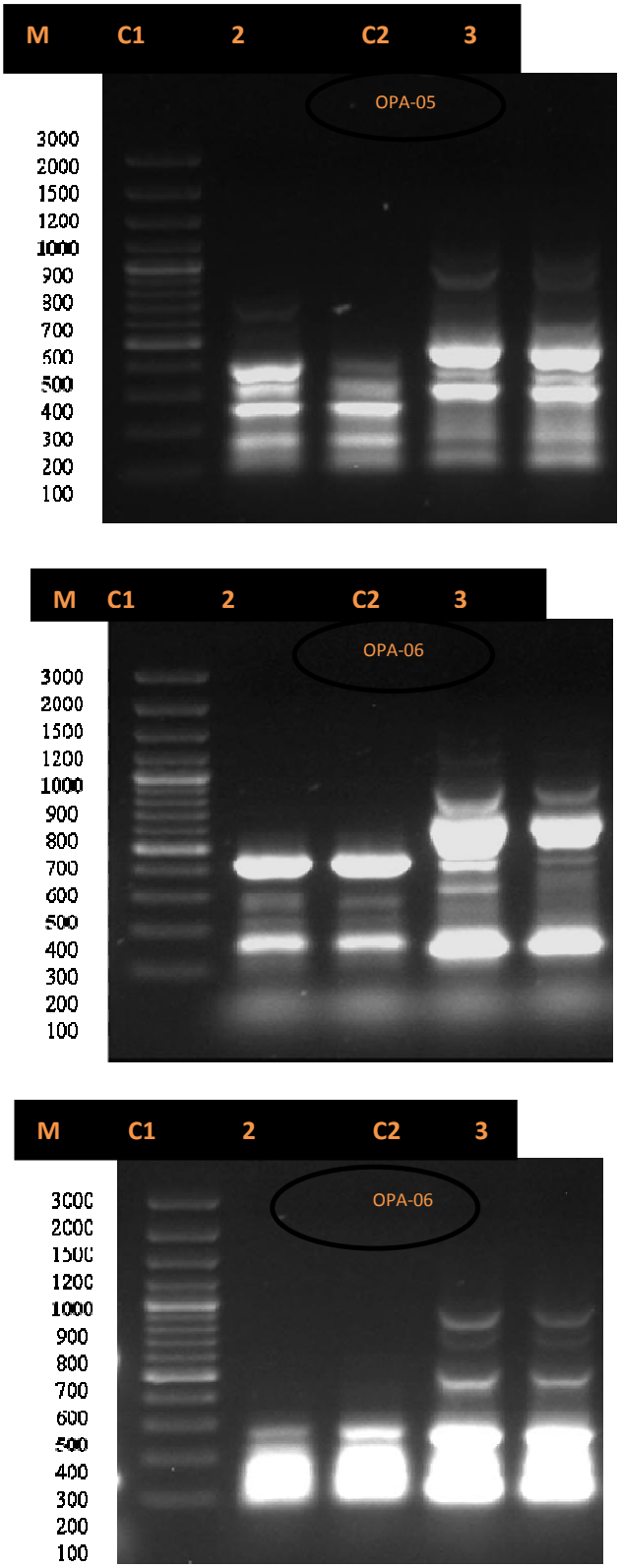

Figure 1: RAPD profile of *S.aureus* and *C.albicans* after the treatment with leaves extract of *Moringa perngina* : whereas;C1 ,untreated *S.aureus*;2,treated *S.aureus* ;C2, untreated *C.albicans* ;3 treated *C.albicans*

Table (1) showed that the highest number of polymorphic bands among treated *S. aurues* was generated in reactions with the primers OPA-05and OPA-06 which was five genetic bands and represented 45.45% of the total bands. While , among treated *S. aurues* and *C.albicans*, the reaction with the primers OPA-05 resulted in the highest number of polymorphic bands (five and eight)that represent 45.45% and 72.73% of the total obtained bands (11) Table (2). The results of RAPD analysis revealed a polymorphic banding pattern when comparing the untreated S. aurues and *C.albicans* with those treated by *Morin gaperegrina* leaves extract (Figs.1 and 2). This observation gives agood evidence to the ability of *Morin gaperegrina* leaves extract to induce point mutation as aresults of deletion compromising at least one nucleotide as revealed by disappearance of many genetic bands and change in restriction endonucleases sites as compared with untreated *S. aurues* and *C.albicans*. These results suggested molecular changes as a deletion in one or more loci which affect gene expression and interruption in biochemical pathways of DNA and protein synthesis, consequently as Alkaloids in *Morin gaperegrina* leaves extract often do. These results are consistent with the results obtained by [1, 30, 15]. Some of the components of R. stricta may act as intercalation agent or generates free radicals which are interacted with plasmid DNA to account for the observed deletions, as suggested by similar results obtained by Ansah [5, 7] in their study with *Cryptolepis sangvinolehta* and *Aspergillus terreus*. However, the interpretation of the molecular events responsible for differences in the RAPD patterns is not an easy task since different DNA alterations can induce similar type of changes. [23]. Exposure of an organism to these plant extracts may result in the formation of covalently bound adducts between the chemical or its metabolites and the DNA; faulty repair of these adducts often results in mutations and, sometimes, cytogenetic changes. In this contest, present results showed that the fragments were displayed in the control and disappeared in the treated bacteria strains. Therefore it can be stated that RAPD could be of great help in the explanation of the antibacterial mode of action of ethanol extract of *Morin gaperegrina* leaves extract on DNA synthesis. The effects seen in the present study may reflect a chemical and physiological changes in tested *S. aurues* and *C.albicans* strains, this change of gene expression clearly showed the effect of change in nucleic acid, and may also reveals anew genotypes [28]. Therefore more rigors investigation is needed in this matter. Disappearing bands are likely to be due to changes in oligonucleotide priming sites, originated from rearrangements and less likely from point mutations and DNA damage in the primer binding sites [33, 24, 1, 25]. Total or partial changes in DNA sequence, due to mutation and/or large deletions create in new priming sites can be induced by effect-induced genotoxicity eg, see Enan [13] and Maryam [27]. Our findings support this claim that DNA polymorphisms detected by RAPD can be considered as a powerful biomarker assay for detection of the level of DNA damage in various treated *S. aurues* and *C.albicans* strains to ethanol extracts of *Morin gaperegrina* leaves, as suggested by similar results obtained by Adel [3] in their study on the effect of *Rhazya stricta* leaf extract and [20] in their study with Conocarpus erectus.

REFERENCES

[1] Adam SE, AL–Farhan AH, AL–Yahya A 2000. Effect of combined Citrullus colocyn this and *Rhazga stricta* use in Najdi sheep. Am. J. Chin. Med. 28:385-390.

[2] Akttar, M., Hassany, S.M., Bhanger, M.I., Iqbal, S. 2006. Absorption potential of *M. oleifera* pods for the removal of organic pollutants from aqueous solutions. Journal of Hazardous Materials. In print.

[3] Adel A. E. El-Tarras, Mohamed M. Hassan and Mohamed A. M. El-Awady. Evaluation of the genetic effects of the in vitro antimicrobial activities of *Rhazya stricta* leaf extract using molecular techniques and scanning electron microscope, African journal of Biotechnology,12(21):3171-3180.

[4] Agwa H, Aly MM, Bonaly R 2000. Isolation and characterization of two Streptomyces species produced non polyenic antifungal agents. J. Union Arab Biol. 7:62-82.

[5] Ansah C, Khan A, Gooderham NJ 2005. *In vitro* genotoxicity of the West African anti-malarial herbal Cryptolepis sanguinolenta and its major alkaloid cryptolepine. Toxicol. 1:208 (1):141-7.

[6] Anwar, F. and U. Rashid. 2007. Physico-chemical characteristics of Moringa Oleifera seeds and seed oil from a wild provenance of Pakistan Pak. J. Bot., 39(5): 1443-1453.

[7] Baeshin NA, Qari SH, Sabir JSM, ALhejin AM 2008. Biochemical and Molecular Evaluation of Genetic Effects of *Rhazya stricta* (Decne) Leafs Extract on *Aspergillus terreus*. Saudi J. of Biol. Sci. 15:25-33.

[8] Boeru .V, Derevici .A 1978. Some chemical and physical data on Romania propolis. Apimondia "propolis" Bucharest pp. 19-26.

[9] Brooks, G.F., Butel, J.S. and Morse, S.A. 2001. Jawetz, Melnick and Adelberg's Medical Microbiology (22ndedn.). Lange Medical Books. Pp. 145-150.

[10] Buker, A;Uba,A and Oyeyi, T.I. 2010. Antimicrobial profile of *Moriga olifera* LAM. Extracts against some food-born microorganisms. Bayero Journal of Pure and Applied Sciences 3(1): 43048.

[11] Dipali YJ, Akshaya K, Sahooo JS, Ghosh R, Ranveer C, Aruna M, Mali M 2010. Phyto chemical Detection and *in vitro* Evaluation of Tamarind Fruit Pulp for Potential anti activity. Int. J. Trop. Med. 5(3):68-72.

[12] Eilert, U., Wolters, B. and Nadrtedt, A. 1981. The antibiotic principle of seeds of *Moringa oleifera* and *Moringa stennopetala*. Planta Med. 42:55–51.

[13] Enan M. R. 2006. Application of random amplified polymorphic DNA (RAPD) to detect the genotoxic effect of heavy metals. Biotechnology and Applied Biochemistry 43: 147-154.

[14] Foidl, N., Makkar, H.P.S. and Becker, K. 2001. The potential of *Moringa oleifera* for agricultural and industrial uses. In: "The miracle tree (Ed Lowell, J.F.) CTA, USA.

[15] Gilani SA, Kikuchi A, Shinwari ZK, Khattak ZI, Watanab KN 2006. Phytochemical, pharmacological and ethnobotanical studies of *Rhazya stricta* Decne. Phytother. Res. 21:301-307.

[16] Guevara, A.P., Vargas, C. and Sakurai, H.1999. An antitumor promoter from *M. Oleifera* Lam.Mutat. Res.440:181 – 188.

[17] Gumgumjee, N. M. and Hajar , A. S. 2012. Antimicrobial efficacy of *Casuarina equisetifolia* extracts against some pathogenic microorganisms. Journal of Medicinal Plants Research. 6(47): 5819-5825.

[18] Gumgumjee, N. M; Khedr; A and Hajar , A.S 2012. Antimicrobial activities and chemical properties of *Tamarindus indica* L. leaves extract. African Journal of Microbiology Research Vol. 6(32), pp.6172-6181.

[19] Holder IA and Boyce ST 1994. Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. Burns, 20: 426-429.

[20] Hajar,A. S .and Gumgumjee, N. M. 2013. Antibacterial Efficiency and DNA Impairment Unveil in some Bacteria strains treated with Conocarpus erectus L. extract. Accepted in International Journal of Applied biology and Pharmaceutical Technology-IJABPT. Oct-Dec-2013- Vol-4 Issue 4PP.

[21] Jamil, A., M. Shahid, M. M. Khan and M. Ashraf. 2007. Screening of some medicinal plants for isolation of antifungal proteins and peptides. Pak. J. Bot., 39 (1): 211-221.

[22] Kebreab, A.G., K.R. Gunaratna, H. Henriksson, H. Brumer and G. Dalhammar. 2005. A simple purification and activity assay ofthe coagulant protein from *Moringa oleifera* seed. Water Res., 39: 2338-2344.

[23] Lalrotluanga, N. Senthil Kumar and G. Gurusubramanian 2011. Evaluation of the random amplified polymorphic DNA (RAPD) assay for the detection of DNA damage in mosquito larvae treated with plant extracts. Sci Vis 11 (3):155-158.

[24] Liu W., Li P., Qi X., Zhou Q., Zheng L., Sun T. H and Yang, Y. S. 2005. DNA changes in barely (Hordeum vulgare) seedlings induced by cadmium pollution using RAPD. Chemosphere 61: 158-167.

[25] Liu W., Yang Y. S., Li P. J., Zhou Q. X., Xie L. J.and Han Y. P. 2009. Risk assessment of cadmium contaminated soil on plant DNA damage using RAPD and physiological indices. Journal of Hazardous Materials 161: 878-883.

[26] Lockett, C.T., C.C. Calvet and L.E. Grivetti. 2000. Energy and micronutrient composition of dietary and medicinal wild plants consumed during drought. Study of rural Fulani, northeastern Nigeria. Int. J. Food Sci. Nutr., 51(3):195-208.

[27] Maryam S, Sasan M and Hasan M 2010. Cadmium-induced genotoxicity detected by the random amplification of polymorphism DNA in the maize seedling roots. J. Vet. Sci.7 (2), 181–187.

[28] Miki Tamura, Kayo W, Yuzurn M, Katsukiyom Y, Kazuko N. 2001. Molecular characterization of new clinical isolates of Candida albicans and C.dubliniesis in Japan: Analysis Reveals a New Genotype of C. albicans with Group Iintron. J. Clin. Microbiol. 39(12): 4309-4315.

[29] Mohan M. and Srivastava G. 1981. Studies on the extractability and the chemical composition of leaf proteins from certain trees». Journal of Food Science and Technology, India, 18(2) 48-50.

[30] Morita H, Awang K, Hadi AH, Takeya K, Itokawa H, Kobayashi J 2005. Conformational analysis of rhazinilam and three-dimensional quantitative structure-activity relationships of rhazinilam analogues. Bioorg. Med. Chem. Lett. 15 (4):1045-50.

[31] Morton. J. F. 1991. The horse radish tree, *Moringa Rengosperma* (Moringaceae). A boon to arid lands? Economic Botany». 45(3): 318-333.

[32] Napoleon, J. F.,Rosmary,C.A,Udeh, S,Ilo,PC,Ugwu,UB 2009. Antibacterial evalution of *Moringa oleifera* leaf extract on selected bacterial pathogens *Echerichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*, Word journal of pharmaceutical research.2(4):1065-1077.

[33] Nelson J. R., Lawrence C. W. and Hinkle D. C. 1996. Thymine-thymine dimmer bypass by yeast DNA-polymerase-zeta. Science 272: 1646-1649.

[34] Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning.A laboratory Manual (2nd Edn), Cold Spring Harbor Laboratory Press. NY. PP. 6.3–6.59.
[35] Shital S 2010. Characterization of Some Antimicrobial Substances from Seed Coat of Tamarindus indica L. Br. J. Pharmacol.Toxicol. 1(1):29-32.
[36] Silvestro, L., Weiser, J.N. and Axelsen, P.H. 2000. Antibacterial and anti membrane activities of cepropin A in Eschericchia coli. Antimicrob.Agents Chem other. 44:602 – 607.
[37] Somali, M. A. Bajneid and Fhaimani S. S. Al. 1984 .Chemical composition and characteristics of Moringa peregrine seeds and seeds oil». Journal of the American Oil Chemists' Society. 61(1): 85-86.
[38] Sreelatha, S., and Padma, P. R. 2009. Antioxidant activity and total phenolic content in Moringa oleifera leaves in two stages of maturity. Plant Food for Human Nutrition, 64:303-311.
[39] Suarez, M., Entenza, J.M. and Dorries, C. 2003. Expression of a plant – derived peptide harbouring water – cleaning and antimicrobial activities. Biotechnol. Bioeng. 81:13–20.
[40] Vukovic N, Milosevic T, Sukdolak S, Solujic S 2007. Antimicrobial Activities of Essential Oil and Methanol Extract of Teucrium montanum. eCAM ;4(S1)17-20.
[41] World Health Organization, Media Center 2008.Traditional medicine. Fact sheet N°134. http://www.who.int/mediacentre/factsheets/fs134/en/.
[42] Williams, J. G . K , Kubelik, A. R., Livak, K J , Rafalski, J A., Tingey, S. V 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers Nucl Acids Res. 18: 6531-6535.