EDITORIAL

IN VITRO ANTIMCROBIAL ACTIVITY OF *MANGIFERA INDICA* L

Salih Osman Mohammed Ahmed ,Mohammed Alfatih Ahmed Omer , Aisha Zoheir Almagboul , Ashraf Nabiel Abdrabo
Medicinal and Aromatic Plants Research Institute, National Centre forResearch, Ministry of Science and Technology. p.o.box 2404, khartoum, Sudan.

Abstract
The chloroform, methanol and aqueous extracts of *Mangifera indica* seeds were subjected to preliminary antimicrobial activity against two Gram- positive bacteria (*Bacillus subtilis, Staphylococcus aureus*) three Gram-negative bacteria *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and two fungi, *Aspergillus niger and Candida albicans.*The seeds chloroform and methanol extracts showed high activity against all organisms tested. The aqueous extract showed high activity against both Gram-positive organisms and one Gram-negative bacteria namely *Proteus vulgaris*, low activity against *Escherichia coli,* and was inactive against *Pseudomonas aeruginosa.* Therefore the active chloroform and methanol extracts were further tested against sixty clinical ; *Staphyllococcus aureus* (n=15), *Escherichia coli* (n=15), *Proteus vulgaris* (n=15) and *Pseudomonas aeruginosa* (n=15) and the aqueous extract was tested against thirty clinical isolates of *Staphyllococcus aureus* (n=15) and *Proteus vulgaris* (n=15) collected randomly from specimens from Sudanese patients. The clinical isolates exhibited low susceptibility compared to the standard organisms. The standard organisms were tested against reference antibiotics (Ampicillin and Gentamicin). It was found that the chloroform extract of *Mangifera indica* seeds at 200 mg/ml was more effective than 20µg/ml Ampicillin and15ug/ml Gentamicin against the majority of the organisms tested.The methanol extract at 200mg/ml was more effective than 40µg/ml Gentamicin against the organisms tested.The aqueous extract was more effective than 40µg/ml Ampicillin against the majority of the organisms tested. All extracts were inactive against *Aspergillus niger.*Both chloroform and methanol extracts were active against *Candida albicans* while the aqueous extract was inactive. Therefore *C.albic*ans is more susceptible than *A.niger*.The chloroform extract inhibited *C.albicans* with inhibitory action between 25-50µg/ml Nystatin and the methanol extract inhibited *C.albicans* with inhibitory action almost similar to 10µg /ml Clotrimazole.Therefore the high activity of the plant might justify its uses in traditional medicine.

EDITORIAL

ملخص:

تم اختبار خلاصـــة الكلوروفورم والميثانول والماء لثمار المانجو ضـــد نوعين من البكتريا موجبة الجرام وهى العصـــوية الرقيقةو العنقودية الذهبية وثلاثة أنواع من البكتريا ســـالبة الجرام)الاشـــريكيه القولونيةو الزائفة الزنجارية و المتقلبة الاعتيادية (أظهرت الخلاصـــة الكلوروفورمية والميثانولية فعالية عالية تجاه كل الكائنات المختبرة .خلاصـــة الماء أظهرت فعالية عالية ضـــد نوعين من البكتريا القياســـية الموجبة الجرام وضـــد نوع واحد من البكتريا الســـالبة الجرام وهي المتقلبة الاعتيادية ,وفعالية متوســـطة ضـــد الاشـــريكيه القولونية وعدم فعالية ضـــد الزائفة الزنجارية الخلاصـــات الفعالة تم اختبارها ضـــد ستين عينة من ســـلالات ســـريريةمعزولةمن مرضـــى ســـودانيين بطريقة عشـــوائية تم اختبار الخلاصـــات تجاه نوعين من الفطريات القياســـية وهي الرشـــاشـــية الســـوداء والمبيضـــة البيضـــاء .ومن ثم تم اختبار الكائنات المعيارية تجاه مضادات .حيوية كأدوية مرجعية وتمت مقارنتها مع فعالية خلاصات النباتا

Introduction

The purpose of the study was to evaluate the antimicrobial activity of *Mangifera indica* seeds extracts against different microbes using the agar plate diffussion method.

Mangifera indica, locally known as mango belongs to the family Anacardiaceae. The plant is native of India and has been dispersed in Africa during the last century .In Sudan , it is a cultivated fruit, free with short stocky bole and dense ovate crown . Leaves alternate. Lancelets 20-30 cm long and 3-6 cm broad with long petioles inflorescences in terminal panicles with numorous little flowers yellowish or pink.
Phellandrene , Alpha pinene , Ambolic acid , Arginine Ascorbic– acid , Beta carotene , Gallic acid , Gallo tannic acid , Mangifelic acid , mangifrol peroxidase , phenylalanin , proline – were isolated from the ethanolic extract of the seeds (Kabuki *et al* 2000).Two antifungal compounds known as di-2-ethyl hexyle phthalate and a mixture of various alkyl-phthalate compounds were isolated from the fruit peel of unripe mango (Supyen *et al* 1998)
Traditionally the leaves are used to treat pertussis and diarrhoea.
The roots are used for diarrhoea and dysentery. The fruit is used for diarrhoea.

Plant Material

The plant used in this study was *Mangifera indica* belonging to the family Anacardiaceae. The seeds were collected from a herbalist from Omdurman. (December 2002).
The plant was identified and authenticated by Waeel Alsadig Abdalla (Medicinal Aromatic Plants Research Institute, National Center for Research). A voucher specimen was prepared and deposited at the herbarium of the Institute.

Method of extraction

Fifty grams of the air-dried and coarsely powdered plant material of Mango was exhaustively extracted for 20 hours with chloroform in a Soxhlet apparatus .The chloroform extract was filtered and evaporated under reduced pressure. The extracted plant material was then air dried and repacked in the Soxhlet and exhaustively extracted with methanol.
The residue of the chloroform extract was redissolved in a mixture of petroleum ether and methanol (1:2v /v) and the methanolic extract was filtered and evaporated under reduced pressure .The residue was redissolved or suspended in methanol and the final volume was adjusted to give the specific concentration and kept in the refrigerator till used.
Simultaneously, water extract was prepared by adding 10 ml of boiled distilled water to the coarsely powdered plant material in a beaker on a water bath with occasional shaking for 4 hours.
The aqueous extract was then filtered and the marc was washed with a small volume of boiled distilled water and added to the filtrate, which was then adjusted to 5ml volume and used immediately.

Tested organisms

The plant extracts were tested against two Gram- positive *(Bacillus subtilis* NCTC 8236, *Staphylococcus*

EDITORIAL

aureus ATCC 25923), three Gram- negative (*Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 6380, *Pseudomonas aeruginosa* ATCC 27853), and two fungi (*Aspergillus niger* ATCC 9763, *Candida albicans* ATCC 7596)

- NCTC is the National Collection of Type Culture, Colindale England.
- ATCC is the American Type Culture Collection. Rockville, Maryland, USA.

Sixty clinical isolates of *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, and *Pseudomonas aeruginosa* were collected randomly from Sudanese patients attending Khartoum North Hospital, Khartoum Teaching Hospital Laboratory and the National Health Laboratory.
The bacterial cultures were maintained on nutrient agar slopes and incubated at 37ºC for 18 hours and then kept in a refrigerator at 4ºC till used..

Testing of extracts for antibacterial activity

The cup-plate agar diffusion method was adopted with some minor modifications to assess the antibacterial activity of the prepared extracts. (Kavanagh 1972). Two ml of the standardized bacterial stock suspension (10^8-10^9 colony forming units per ml)were thoroughly mixed with 200 ml of sterile molten nutrient agar, which was maintained at 45ºC.
Twenty ml aliquots of the inoculated agar were distributed into sterile petri dishes. The agar was left to set and in each of these plates ,four cups (10mm in diameter) were cut using a sterile cork borer (No 4) and the agar discs were removed. Alternate cups were filled with 0.1 ml sample of each of the extracts using adjustable pipette, and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37 ºC for 18 hours; two replicates were carried out for each extract against each of the tested organisms.
After incubation, the diameters of the resultant growth inhibition zones were measured, averaged and the mean values were tabulated.

Results and discussion

The seeds extracts of *Mangifera indica* belonging to the family Anacardiacae were screened for antimicrobial activity against five standard organisms two Gram- positive (*Bacillus subtilis*, *Staphylococcus aureus*) three Gram negative (*Escherichia coli*, *Proteus vulgaris*, P*seudomonas aeruginosa*) and against two fungi namely *Aspergillus niger and Candida albicans.*
It will be seen from table 1 that the chlorofrm, methanol and the aqueous extracts of *Mangifera indica* (fam.Anacardiaceae) possed high antibacterial activity against one or more of the tested bacteria.

Table 1: Preliminary screening for antimicrobial activity of *Mangifera indica* against the standard organisms.

Family name	Part extracted	Yield %	Solvent system	Inhibition Zone Diameter(mm)						
				B.s	*S.a*	*E.c*	*Pr.v*	*Ps.a*	*A.niger*	*C.albicans*
Anacardiaceae	Seeds	8.7	Chloroform	15	17	15	21	17	0	27
		7.5	Methanol	25	30	26	28	24	0	30
		8.7	Water	19	17	13	33	0	0	0

B.s=Bacillus subtilis; S.a=Staphyllococcus aureus;E.c=Escherichia coli; Pr.v=Proteus vulgaris; Ps.a=Pseudomonas aeruginosa; A.niger=Aspergillus niger; C.albicans=Candida albicans.
Inhibition zone diameter in mm>15mm=Sensitive ; <15=Resistant.
Test concentration used=200mg/ml,0.1ml/cup
Inhibition zones are the mean of four replicates

The chloroform extract of *M.indica* seeds showed high activity (15-21mm) against all bacteria tested.The methanol extract showed very produced activity (24-30mm) against the bacteria tested . The aqueous extract showed variable activity (13-33mm) i.e high activity (17-19mm) against both Gram positive bacteria

EDITORIAL

, low activity (13mm) against the Gram negative bacteria *Escherichia coli*, pronounced activity (33mm) against *Proteus vulgaris* while no activity against *Pseudomonas aeruginosa.*

The invitro antibacterial activty of the methanol and the aqueous extracts showed variable results. This was in ageement with that obtained by Sariam *et a*l 2003.On the controry, while our methanol extract of *M.indica* seeds showed high activity (26mm) against *Escherichia coli* and its aqueous extract showed low activity (13mm) against the same organism,both extracts tested by Sariam *etal* 2003 did not show any significant effect on the growth of *Escherichia coli* .Most pronounced activities of the extracts were observed against *Proteus vulgaris*.

The methanol extract of *M.indica* seeds had a broad antibacterial activity and was more active against Gram positive than Gram negative bacteria with a few exception.This result was in agreement with that produced by Kabuki *etal* 2000.

Table 2 :Screening for antibacterial activity of Ampicillin and Gentamicin against standard organisms.

Drug	**Drug concentration µg /ml**	*M.DI.Z (mm)				
		B.s	***S.a***	***E.c***	***Pr.v***	***Ps.a***
Ampicillin	40	15	30	0	25	0
	20	14	26	0	20	0
	10	13	22	0	17	0
	15	12	19	0	0	0
Gentamicin	40	24	18	24	26	22
	20	22	17	19	22	15
	15	17	14	16	20	12
	5	15	13	12	15	0

Key:
***M.D.I.Z = Mean diameter of growth inhibition zone (mm).**
Average of four replicates.
B.s = Bacillus subtilis., *S.a = Staphylococcus aureus.*
E.c = Escherichia coli., *Pr.v = Proteus vulgaris.*
Ps.a = Pseudomonas aeruginosa., 0 =No activity

The comparison of observation given in tables 1 and2 showed that the chloroform extract of *M.indica* seeds at 200mg/ml was similar to 40µg/ml Ampicillin against *Bacillus subtilis*, almost similar to 15µg/ml against *Staphyllococcus aureus* and 20µg/ml against *Proteus vulgaris*.With Gentamicin, the effect of chloroform extract of *M.indica* seeds was similar to 5µg/ml against *Bacillus subtilis* and 20µg/ml against *Staphyllococcus aureus*, almost similar to 15µg/ml against *Escherichia coli* and *Proteus vulgaris* and 20µg/ml against *Pseudomonas aeruginosa*

The methanol extract of the seeds of *M.indica* at 200mg/ml was more effective than 40µg/ml Gentamicin and Ampicillin against the bacteria tested.With Ampicillin at the same concentration, the methanol extract was also more effective than 40µg/ml against *Bacillus subtilis*, *Proteus vulgaris* and similar to *Staphyllococcus aureu*s.

The aqueous extract of *M.indica* at 200mg/ml was more effective than 40µg/ml

Ampicillin and Gentamicin against *Proteus vulgaris*, similar to 20µg/ml Gentamicin against *Staphyllococcus aureus* ,15µg/ml Gentamicin against *Bacillus subtilis* and 5µg/ml Gentamicin against *Escherichia coli*.With Gentamicin, the aqueous extract of *M.indica* at 200mg/ml was effective as 15µg/ml

EDITORIAL

against *Bacillus subtilis, Escherichia coli* .The 20µg/ml against *Staphyllococcus aureus*, 5µg/ml against aqueous extract was also more effective than 40µg/ml Ampicillin and Gentamicin against the bacterial strain of *Proteus vulgaris*.

The data given in table 1 showed that the methanol and the aqueous extracts were inactive against *Aspergillus niger* while highly active (27-30mm) against *Candida albicans* .The aqueous extract was inactive against both *Aspergillus niger* and *Candida albicans.*

Therefore 200mg/ml of the chloroform of the seeds of *M.indica* was nearly as effective as 25-50µg/ml of Nystatin against the ATCC fungal strain of *Candida albicans* while the methanol extract of the seeds at the same concentration was as effective as10µg/ml Clotrimazole against *Candida albicans.*

It is further seen from table 4 that the chloroform and methanol extracts of the seeds of *M.indica* were active against a large number of clinical isolates of *Escherichia coli. Staphyllococcus aureus, Proteus vulgaris* and *Pseudomonas aeruginosa.*

All the above results obtained with standard chemotherapeutic agents against the same standard tested organisms (tables 2,3), and the plant extracts resulting in 15mm or more growth inhibition zones were considered to be active and those resulting in less than 15mm were inactive.(Barry *et.al* 1970 Cruickshank *et.al* 1975) .

Table (3) Screening for antifungal activity of reference drugs against standard organisms

Drug	Concentration µg/ml	*M.D.I.Z (mm)	
		Asper.niger	*C.albicans*
Nystatin	50	17	28
	25	14	26
	12.5	0	23
Clotrimazole	20	24	43
	10	19	30
	5	17	33

Key : *M.D.I.Z = Mean diameter of growth inhibition zone (mm). Average of four replicates
Asper.niger= Aspergillus niger.
C.albicans=Candida albicans ; 0 = No activity

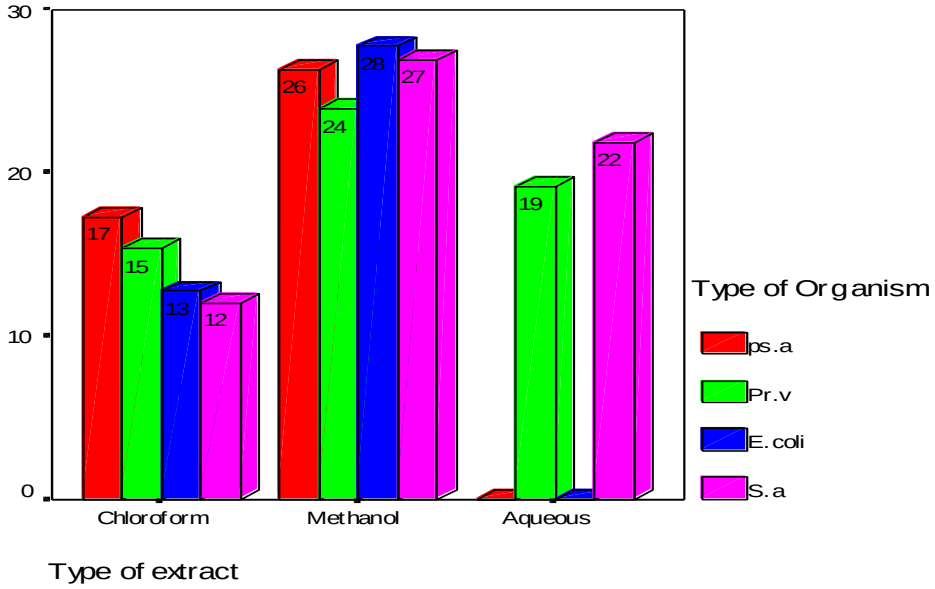
Table 4: Sensitivity of clinical isolates towards *Mangifera indica* L seeds extracts*:*

Clinical isolates (n)		Sensitive isolates(n) Seeds extracts		
Microorganisms	Source	$CHCL_3$	MeOH	H_2O
E.coli *15*	Urinary tract (15)	11	15	0
S. *aureus*. 15	Abscess (2)	0	2	2
	Ear swab (11)	8	11	11
	Sputum (2)	1	2	2
Pr.vulgaris. 15	Ear swab (8)	8	8	8
	Urinary tract (7)	4	6	7
Ps aeruginosa 15	Ear swab (6)	5	6	0
	Urinary tract (9)	9	9	0

The sensitive organisms are those exhibiting 15mm inhibition zone and more extracts tested at200mg/ml.
E.coli = Escherichia coli .a = Staphylococcus aureus Pr.v = Proteus vulgaris.
Ps.a = Pseudomonas aeruginosa., 0 =No activity

Graph1: Showing the average of inhibition zone of *Mangifera indica* extracts against clinical Isolates

EDITORIAL



Ps.a = Pseudomonas aeruginosa , *E.c = Escherichia coli.,*

Pr.v= Proteus vulgaris, *S.a= Staphylococcus aureus*

Univariate Analysis of Variance(SPSS)

Tests of Between-Subjects Effects

Dependent Variable: Mean Inhibition zone Diameter (mm)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	15024.861[a]	11	1365.896	30.442	.000
Intercept	51850.139	1	51850.139	1155.588	.000
Organisms	1580.061	3	526.687	11.738	.000
Extract	8259.478	2	4129.739	92.040	.000
Organism * Extract	5185.322	6	864.220	19.261	.000
Error	7538.000	168	44.869		
Total	74413.000	180			
Corrected Total	22562.861	179			

a. R Squared = .666 (Adjusted R Squared = .644)

Post Hoc Tests

Type of extract

EDITORIAL

Multiple Comparisons

Dependent Variable: Mean Inhibition zone Diameter (mm)
LSD

(I) Type of extract	(J) Type of extract	Mean Difference (I-J)	Std. Error	Sig.
Chloroform	Methanol	-11.8500*	1.2230	.000
	Aqueous	4.1333*	1.2230	.001
Methanol	Chloroform	11.8500*	1.2230	.000
	Aqueous	15.9833*	1.2230	.000
Aqueous	Chloroform	-4.1333*	1.2230	.001
	Methanol	-15.9833*	1.2230	.000

Based on observed means.
*. The mean difference is significant at the .05 level.

Means

Repor

Inhibition zone Diameter (mm)

Type of extract	Mean	N	Std. Deviation
Chloroform	14.4000	60	8.9844
Methanol	26.2500	60	5.5192
Aqueous	10.2667	60	11.4564
Total	16.9722	180	11.2272

Conclusion

The methanol extract of the seeds of *M.indica* possessed more pronounced and diverse antibacterial activity than its chloroform and aqueous extracts.This suggests that the active principles may be relatively polar compound (s) .These results support the uses of the seeds of *Mangifera indica* as antimicrobial therapy in folkloric medicine in Sudan and the neighbouring countries.

Refrences

1. 1-Barry, Al; Garcia,F.;Thrupp,LD(1970). Interpretation of sensitivity test results .Am.J.Clin. Path .53:149.
2. Cruickshank, R, Duguid, J.P. Marmaion B.p and Swain.R.H.; (1975Medical Microbiology; R.Cruickshank; J.P.,Duguid;BP. Marmion and R.H Swain (Eds.)Churchill Livingstone (Pub.), Edinburgh, p.120, 12 th. Ed.
3. Kabuki – T, Nakajima-H, Arai-M, Ueda-S, Kuwabara-Y, Dosako-S (2000). Characterization of novel antimicrobial compounds from mango (*Mangifera indica L.*) kernel seeds. Food Chemistry .71:1,61-66
4. 4-Sariam K, Hemalatha S, Kumar A, Srinivasan T, Ganesh J, Shankar M, Venkatarman (2003).Evaluation of antidiarrhoeal activity in seeds extracts of Mangifera indica. J.Ethnopharmacol 84 (1):11-15.
5. Supyen – D**,** Chairangai –N, Sardsud-V, Johnson-GI (Ed), Hayley-E (ED), Joyce-DC. (1998) Non-

resorcinol antifungal compounds in mango peel (*Mangifera indica* L.). ACIAR-Proceeding- series. No 80, 115-120.

6. 6-SPSS (Statistic Package for social sciences) program, version 10
7. Kavanagh F (1972**)** Analytical Microbiology. F.Kavanagh (Ed.) Vol 11,Academic Press, New York and London p1