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Studies on Antimicrobial Effects of Chromatographic Separated Active Components of *Moringa Oleifera* Seed Extract

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Abstract
Petroleum ether was employed to extract Moringa oleifera seeds that had been subjected to oven drying and subsequently reconstituted in n-hexane. The combination of Chloroform and petroleum ether (5:5) was adopted for the separation of the extract from the oven dried seeds as it came out as the best solvent system that aided its separation on column chromatography and thin layer chromatography (TLC) plates. The extract separated to four distinct bands on both the column chromatography packed with silica gel and retained such after exposure to ultra-violet light (254nm and 356nm) and subsequent exposure to iodine vapour. The separated bands were discovered to have antimicrobial activity on Staphylococcus aureus (45%) which was the most susceptible organism to the extract followed by Escherichia coli (31%) and the fungus Aspergillusniger (13%), while Pseudomonas aeruginosa (11%) demonstrated the least susceptibility to the extract. However, upon subjecting the data generated to inferential statistics, the chromatographic separated extracts did not exert significant (P<0.05) antimicrobial effects on the test organisms employed.

Keywords: Antimicrobial, Chromatography, *Moringaoleifera*, Seed extracts.

INTRODUCTION
Moringaoleifera plant which is widely cultivated and known for its multifunctional attributesin Africa and numerous countries in the world including India, Sri Lanka, Ceylon, Thailand, Burna, Mexico, Malaysia and the Philippines(Fahey, 2005). Every part of a typical *Moringaoleifera* tree has been reported by Akanni *et al.* (2014) as having medicinal attributes and thereby having huge potentials for its development into marketable medicinal and industrial by-products. Several authors(Abdulkarim *et al*., 2005;Anwar*et al*., 2007; Oluduro, 2012) reported that ranging from the leaves, fruits, flowers to the immature pods of the tree can be eaten and are employed in India, Pakistan, Hawaii, the Philippines and some African countries as a richly nutritive vegetable. According to Dhakar *et al.* (2011), *Moringaoleifera* has been used over the years as available substitute for imported food supplements with a view to combating malnutrition amongst mothers and infants due to its nutritive chemical components.

Moringaoleifera has been reportedly used for the treatment of many diseases ranging from sexually transmitted diseases, fungal infections, bacterial infections, inflammation, diarrhoea

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and malnutrition (Fahey, 2005; Arabshabi-Delouee *et al*., 2009; Rahman*et al*., 2009; Farooq*et al*., 2012). Foidl*et al.* (2001), ascribed the extensive research studies being conducted on the plant over the last twelve (12) years to the advantageous benefits that it possesses. Owing to *Moringaoleifera* richness in fat content (Anwar and Bhanger, 2003; Anwar and Rashid, 2007; Farooq*et al*., 2012)reportedly indicated that it can be employed as biofuel, vegetable oil, cosmetic,medicine and animal feed. Some authors (Abdulkarim *et al*., 2005; Rahman *et al*., 2009; Compaore *et al*., 2011)have equally reported that *Moringa oleifera* seeds are very rich in protein.

However, Anwar *et al*. (2007) reported that the polypeptides obtainable in the seeds have got the best natural coagulant that aids its ability to bind to several moieties and can thereforebe employed to treat water meant for human consumption. Katayon *et al*. (2005) equally reported that the seeds of *Moringa oleifera* have got coagulating attributes which enable its effective use in water and wastewater treatment. This study however investigated and reported the antimicrobial potency of chromatographic separated extracts of *Moringa oleifera* seeds subjected to oven drying.

MATERIALS AND METHOD

Collection and identification of *Moringa oleifera* seeds

Moringa oleifera seeds were collected at the back of doctors' quarters situated at the Ekiti State Specialist Hospital, Ado-Ekiti, South West Nigeria. The seeds were identified in the Department of Plant Science, Ekiti State University, Ado Ekiti.

Processing of seeds

The collected *Moringa oleifera* seeds were subjected to oven drying at a temperature of 80°C for 15 minutes with a view to reducing the moisture content. Subsequently, the seeds were grounded into fine powder with the aid of electric blender. 50 grammes of the powdered seed were dispensed in 250ml size conical flask containing 200ml petroleum ether. The cold extractions of the seeds were derived through filtration with the aid of Whatman filter paper number 1. The collected filtrate was later concentrated to semi-solid residue through evaporation.

Reconstitution of the extracts in diluents

Petroleum ether extracts were reconstituted in n-hexane solvent so as to extract the seed active components and it was subsequently concentrated to semi-solid residue through evaporation. It was then kept at4°C before spotting the extracts on thin layer chromatography (TLC) plates.

Preparation of the TLC plates

The TLC plates were prepared by dipping it into slurry of commercial TLC adsorbents in organic solvents. For the slurry, 35g of silica gel was dissolved in 100ml chloroform-methanol (2:1 v/v). The slurry was stirred properly before it was used. Two clean and dry plates were held back to back together and dipped into the prepared slurry. The slides were slowly expunged and allowed to drain on the edge of a sterile container. The two plates were then separated and allowed to dry for 5 minutes.

Determination of solvent system for the separation of extracts.

Four solvents ranging from petroleum ether, hexane, chloroform and benzene were interchangeably paired with varying ratio with a view to determining the most suitable pair and ratio that had the best performance in terms of separating the extracts.The chromatographic plates were oven dried at 100°C for 15 minutes to remove moisture. The

extracts were then spotted right at the base of the TLC plates and subsequently immersed into a sealed chromatographic tank having petroleum ether: chloroform (5:5). The plates were dried and examined under UV light (254nm and 365nm) before exposure to iodine vapour.

Column chromatography for testing the organisms

The column was packed with silica gel using petroleum ether: chloroform (5:5). The oven dried seed extract was poured through the separation funnel which was followed by pouring the solvent system intermittently to prevent drying and cracking of the packed silica gel. 25ml of each fraction was obtained and concentrated before spotting on the TLC plates. The separated compounds from each spot of the fractions were then detected under UV light.

All the test organisms of different strains of bacteria and a fungus (*Aspergillus niger*) were collected from stock culture in the Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun state. The bacteria were maintained on nutrient agar (oxoid) slants and the fungus was maintained on malt extract (oxoid) slants under sterile conditions and kept in the refrigerator at 4°C prior to bioassay**.**

Antimicrobial assay

The antibacterial and antifungal activities of the seed extracts were examined by spraying strains of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Aspergilusniger* on the chromatographic separated fractions. The plates were then incubated in a sealed humid chamber at 37°C for 24 hours and 25°C for four (4) days for the detection of antibacterial and antifungal zones of inhibition of each fraction respectively.

Statistical analysis

Descriptive statistics was employed to analyze the data generated whiledata were equally subjected to one way analysis of variance using SPSS and means were separated through multiple mean comparison tools.

RESULTS AND DISCUSSION

The results related to the determination of the most efficient solvent system employed for the separation of the oven dried seed extract into district components are shown in Table 1.

Table 1: Determination of the best solvent system for the separation of extracts

Solvent systems (v/v)	Ratio	Effectiveness
Petroleum ether: Hexane	7:3	Not effective
Petroleum ether: Hexane	9:1	Not effective
Petroleum ether: Hexane	6:4	Not effective
Petroleum ether: Hexane	5:5	Not effective
Chloroform: Benzene	9:1	Not effective
Chloroform: Benzene	7:3	Not effective
Chloroform: Benzene	6:4	Not effective
Chloroform: Benzene	1:9	Not effective
Petroleum ether: Hexane	1:9	Not effective
Petroleum ether: Hexane	4:6	Not effective
Chloroform: petroleum ether	9:1	Not effective
Chloroform: petroleum ether	7:1	Not effective
Chloroform: petroleum ether	5:5	Very effective
Chloroform: petroleum ether	6:4	Partially effective

It is evident that out of the various solvent system tested, petroleum ether: chloroform (5:5) came out as very effective and was as a result employed in this study, chloroform: petroleum ether (6:4) was partially effective while the remaining paired solvents and their respective ratios were not effective at all (Table 1).

The retardation factor (Rf) values of bands that developed and obtained from the TLC plates placed in the solvent system petroleum ether: chloroform (5:5) are depicted in Table 2.

Table 2: Rf values of bands developed on TLC plated in solvent system of Chloroform: petroleum ether (5:5)

Bands	**ULTRAVIOLET LIGHT**		**IODINE VAPOUR**
	Rf at 254nm	Rf at 365nm	Rf values
1	0.05	0.05	0.05
2	0.11	0.11	0.11
3	0.19	0.19	0.19
4	0.41	0.41	0.41

The seed extracts separated into four distinctive bands leading to the discovery of four Rf values. The Rf values obtained from the TLC plates ranged from 0.05 in the first band and went up to 0.41 in the fourth band. It was discovered that the same Rf values were obtained using the short wavelength (254nm) and the long wavelength (365nm) across the first band, second band, third band and fourth band respectively (Table 2).

On exposure of the TLC plates to iodine vapour in a tightly closed container, it was observed that the Rf values also ranged from 0.05 to 0.41 (Table 2).

Antimicrobial activities of the generated four extract fractions on the test organisms and their respective mean values are shown in Table 3. The results obtained and depicted in Figure 1 indicate that the higher the zone of inhibition, the higher the antimicrobial potency of the fractions of the extracts on the test organisms employed. *Staphylocuccusaureus* (45%) was the most susceptible organism to the extract followed by *Escherichia coli* (31%) and the fungus *Aspergillusniger* (13%) while *Pseudomonas aeruginosa* (11%) demonstrated the least susceptibility to the effect of the extract. Thesedescriptive results confirmed the antimicrobial effect of each fraction obtained from the column chromatographic separation of the oven dried seeds.

Table 3. Antimicrobial activities of the extract on the test organisms

	Zone of inhibition (mm)					
Test Organisms	First fraction	Second fraction	Third fraction	Fourth fraction	Mean values	Control
Escherichia coli	2.0	6.0	8.0	1.0	4.25	0.0
Staphylococcus aureus	3.0	12.0	10.0	0.0	6.25	0.0
Pseudomonas aeruginosa	1.0	4.0	1.0	0.0	1.5	0.0
Aspergillusniger	2.0	2.0	2.0	1.0	1.75	0.0

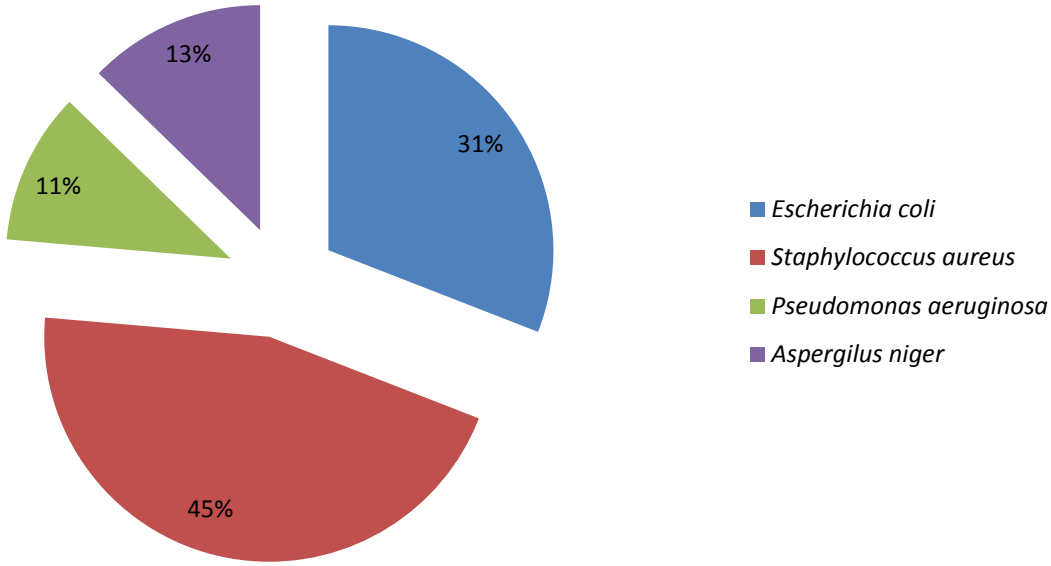

Fig 1. Inhibitory effect of the extracts on the test organisms employed

These results corroborated the findings of Jamil *et al.* (2007)as well as that of Kareab *et al.* (2005) indicating that the seed extracts of *Moringaoleifera* have got antimicrobial properties. Jabeen *et al.* (2008),equally reported that the seed extracts exhibited extremely strong activity against a set of microorganisms of which *Staphylococcus aureus* was among while the extracts had moderate activity against *Escherichia coli, Aspergillusniger* and *Metarhisiumaniscoplae*.

In line with the antimicrobial activity demonstrated by the chromatographic separated extracts of oven dried *Moringaoleifera,* on the test organisms employed in this study, several authors (Madsen et al. 1987; Olsen, 1987; Kawo, 2007) have equally reported such. Interestingly, Guevara *et al.* (1999), did attribute the antimicrobial activity of *Moringaoleifera* seed to the presence of many phytochemicals most significantly to the activity of a short polypeptide named as 4 (ά– L – rhamnosyloxy) benzyl-isothiocyanate in their study.

However, the chromatographic separated extract did not exert significant antimicrobial effects on the test organisms employed as depicted in Table 4.

Table 4: Multiple mean comparisons of the of effect *Moringaoleifera* seed extract on test organisms

Mean Comparison Tools	Comparison of Means of Test Organisms		Mean Difference Between Test Organisms	Std. Error	Sig.	95% Confidence Interval Lower Bound	Upper Bound
Tukey HSD	A	B	-2.00000	2.40875	.839	-9.1513	5.1513
		C	2.75000	2.40875	.672	-4.4013	9.9013
		D	2.50000	2.40875	.731	-4.6513	9.6513
	B	A	2.00000	2.40875	.839	-5.1513	9.1513
		C	4.75000	2.40875	.251	-2.4013	11.9013
		D	4.50000	2.40875	.291	-2.6513	11.6513
	C	A	-2.75000	2.40875	.672	-9.9013	4.4013
		B	-4.75000	2.40875	.251	-11.9013	2.4013
		D	-.25000	2.40875	1.000	-7.4013	6.9013
	D	A	-2.50000	2.40875	.731	-9.6513	4.6513
		B	-4.50000	2.40875	.291	-11.6513	2.6513
		C	.25000	2.40875	1.000	-6.9013	7.4013
LSD	A	B	-2.00000	2.40875	.423	-7.2482	3.2482
		C	2.75000	2.40875	.276	-2.4982	7.9982
		D	2.50000	2.40875	.320	-2.7482	7.7482
	B	A	2.00000	2.40875	.423	-3.2482	7.2482
		C	4.75000	2.40875	.072	-.4982	9.9982
		D	4.50000	2.40875	.086	-.7482	9.7482
	C	A	-2.75000	2.40875	.276	-7.9982	2.4982
		B	-4.75000	2.40875	.072	-9.9982	.4982
		D	-.25000	2.40875	.919	-5.4982	4.9982
	D	A	-2.50000	2.40875	.320	-7.7482	2.7482
		B	-4.50000	2.40875	.086	-9.7482	.7482
		C	.25000	2.40875	.919	-4.9982	5.4982

A= *Staphylococcus aureus* B=*Pseudomonas aeruginosa* C= *Escherichia coli* D=*Aspergilusniger*

CONCLUSION

The results of the present study have shown that chromatographic separated components of oven dried *Moringaoleifera* seed extracts contain considerable anti-microbial activity as revealed by the descriptive statistics employed. However, the results obtained from the inferential statistics revealed that the chromatographic separated extracts had no significant effect on the test organisms employed in this study.

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