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Evaluation of the mosquito Repellency properties and antibacterial effects of lemon grass (*Cymbopogon Citratus*) extracts

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Abstract
The incidence of malaria infection from infected female Anopheles mosquito is a global health challenge. Insect repellency is used to force back insect; preventing insect borne diseases. The antibacterial and repellency property of lemongrass extracts was determined using microbiological methods. The leaf of the plant was air-dried, blended and extracted using methanol and isolates of *Salmonella typhi*, *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus***.** The repellency property was tested on shaved albino rats for a period of 6 hours. The results showed that the methanol extract exerted the highest inhibitory property with diameter of 22.44± 0.51mm, 17.21±0.51mm and 14.28± 1.12mm on *Staphylococcus aureus, Salmonella typhi* and *Escherichia coli* respectively. The chloroform extract exerted the highest zone of inhibition on *Bacillus cereus* having a diameter of 13.66± 0.33mm. Comparatively, the methanol extract was more effective than most of the commercial antibiotics used, which also repelled female anopheles mosquito for 5½ hours before any landing was observed. Observations from this research shows that lemongrass has antibacterial effect against the bacteria used and can be used to design new antibacterial or to fortify the existing ones. The extract can also be used for repelling insects and mixed with creams to repel vectors mosquitoes.

Keywords: antibacterial, mosquito repellency, lemongrass, extracts, antibiotics

Introduction
By definition, medicinal plants are plants which contain bio-active ingredients that support development of cells and consequently support growth of the entire being (Arhoghro *et al.*, 2012) [2]. Nature has been a wellspring of therapeutic herbs for many years and since the start of man (Momoh, 2015). They are various plants that have some medicinal properties with one or more of its organs containing substances that can be used for therapeutical purposes or which are precursors for synthesis of useful drugs (Odugbemi, 2006) [11]. Medicinal plants include; Plants used for extraction of pure substances for direct medicinal use. Plants parts or plants used medicinally in galenical preparation, food and perfumery plants used medicinally. Microscopic and macroscopic plants used for isolation of drugs especially antibiotics, Plants fiber used for preparation of surgical dressings. (Sofowora, 2008) [17].
Cymbopogon citratus (Lemon grass) is an herb known worldwide as lemon grass. It is an aromatic perennial tall grass with rhizomes and densely tufted fibrous root. It has short underground stems with ringed segments, coarse, green slightly leathery leaves in dense clusters (Omotade, 2009) [12]. It is commonly used in teas, soups, and curries. It is also suitable for use as poultry, fish, beef, and seafood (Hayam *et al.*, 2013) [6]. Moreover, its fresh and dried leaves are currently used in traditional cuisine. Lemon grass is also a folk remedy for cough, elephantiasis, flu, gingivitis, headache, leprosy, malaria, ophthalmia, pneumonia and vascular disorders. Lemon grass is principally taken as a tea to remedy digestive problems; diarrhea and stomach ache (Arhoghro *et al.*, 2012) [2]. *C. citratus* is a rich source of citral, which is used in perfumery, pharmaceutical industries, and production of bioactive compounds (flavonoids and vitamin C).
Its applications in Nigeria include cures for upset stomach, malaria, insect repellent and as an antioxidant (tea) according to Opeyemi *et al.*, (2015) [14].
Plant based mosquito repellents are an alternative to the use of insecticide-based repellent. They may be applied to the skin to protect an individual from the bites of mosquitoes. A single bite from an infected mosquito can result in transmission of disease and the fact that people use synthetic chemical-based mosquito repellents to protect themselves from mosquito bite but in exchange get more serious problems (Kranti *et al.*, 2015) [7]. Repellents are substances that act locally or at a distance, deterring an Arthr-opod from flying to, landing on or biting human or animal skin (Kranti *et al.*, 2015) [7].
The aim of this study is to determine the inhibitory or antibacterial and insect repellency properties of *Cymbopogon citratus* leaf extract and the repellency properties in other to establish if it could be used to combat resistance in selected bacteria and prevent possible infections from vectors.

Materials and Methods
3.1 Location and Environment of Study
The research was carried out at the Microbiology laboratory, Elizade University, Ilara-Mokin Ondo State.

3.2 Materials
The materials used in this work include lemon grass leafs, rotary evaporator, conical flask, Petri dishes, test tubes, bijou bottles, measuring cylinder, thermometer, sterile container for sample collection, foil paper, chloroform, methanol, distilled water, cotton wool, paper tape, prepared slants and slides.

3.3 Collection and Preparation of Plant Sample
Lemon grass (*Cymbopogon citratus)* was obtained from Ilara-Mokin, Ondo State, Nigeria. Its identity was confirmed in Biology Department, Elizade University before air-drying. The leaf was cut into smaller pieces and was air-dried for 14 days and milled into fine powdery form with the use of a blender (Qasa blender, product type: Blender and Grinder model QBL-18L40, power voltage: 230 v 50 HZ, power input: 350 W speed, 10,000-13,000 rpm) according to Momoh (2015) and was kept in a sterile bottle.

3.4 Sterilization of Glass Wares
All the glassware used such as conical flask, glass Petri dishes, measuring cylinder, McCartney bottles and test tubes were properly cleaned and sterilized using oven at a temperature of 180°c for 2 hours (Cheesbrough, 2014).

3.5 Concentration and Preparation of Stock Solution
Two different solvent; methanol and chloroform were used for the extraction of the samples. Each powder (100 g) was measured and placed into 2 separate beakers. Exactly 400 ml of 98% methanol was dispensed into the first beaker containing the extract and 400 ml of 98% chloroform was also dispensed into the second beaker containing the extract (1:4). The beakers were shaken well using a mechanical shaker to obtain a homogenous extract-solvent mixture. The samples were left to soak at room temperature for 3 days (72 hours). The extract was decanted, sieved through muslin bag and filtered using Whatman no 1 filter paper and then lyophilized. The stock solutions of the extract were stored in sterile capped McCartney bottles and kept at room temperature.

3.6 Test Organisms
The test organisms used in this study were 2 Gram positive bacteria *(Staphylococcus aureus, Bacillus cereus*) and 2 Gram negative bacteria (*Escherichia coli* and *Salmonella typhi).* They were obtained from National Institute for Medical Research (NIMR), Yaba Lagos, Nigeria.

3.7 Preservation of Culture
This was done using the standard method described Cheesbrough (2014).

3.8 Confirmatory Tests on Clinical Isolates
Clinical isolates collected were subjected to confirmatory tests such as Gram's staining, biochemical reactions and sugar fermentation test to confirm their identity before used (Cheesbrough, 2014).

3.9 Antibacterial Analysis
The testing of the bacterial cultures for the inhibitory effect for extracts of lemon grass was performed using agar well diffusion method described by Ewansiha *et al*., (2012).

Insect Repellency
Anopheles mosquitoes were bred for four days, this was done by putting clean water in a container and adding little portion of leaves to the water and kept in a dark room. Grown-up anopheles mosquitoes feast upon the leaves and lay their eggs on the surface of the water and the eggs are additionally stuck together in pontoons. The eggs hatch into hatchling (1st instars) in the wake of interacting with water. The container is been kept into an enclosure properly netted (rat cage) and permitted to finish its life cycle there. Four (4) albino rats were purchased from FUTA Akure, Ondo state, Nigeria. For every rat, a portion of their body was shaved utilizing sterile forceps scissors and cutting-edge blade and they were all marked A, B, C and D. Rat A was rubbed with methanol extract concentrate using sterile swab stick, similarly Rat B was rubbed with chloroform extract concentrate. A known repellent was rubbed on Rat C (Drug field repellent cream) and was used as positive control, additionally Rat D was used as negative control and was not rubbed with any repellent. The rats were kept in the cage of 4 compartments each containing 25-30 female anopheles mosquitoes for 6 hours. The time it took the anopheles mosquitoes to land on each rat and the number of mosquitoes per hour was observed and recorded.

3.11 Antibiotics Sensitivity Test
The antibiotic sensitivity test was carried out in order to know the sensitivity of the microorganism to the different commercially available antibiotics. Disc diffusion method was applied to determine the effect of standard antibiotics on the bacterial isolates as described by Prescott *et al.*, (2013).

3.12 Statistical Analysis of Result
Result obtain was subjected to descriptive one way analyses of variance, SPSS version 21 Microsoft windows 10 and Duncan multiple range test was used as follow up test.

4. Results
Plate 1 shows a picture of lemon grass in a garden. The result of the confirmatory test carried out on all the test isolate showed that the bacteria conform to their identity as *Staphylococcus aureus* was gram positive cocci in bunch form, while *Salmonella typhi* was gram negative rod. *Bacillus cereus* was gram positive rod with terminal spore while *Escherichia coli* were gram negative rod. All other test including catalase and sugar fermentation test confirmed the identity of these organism as they correspond positively. The results of the confirmatory test on the isolate are shown in table 1(A and B).

Figure 1 and plate 2 showed the diameters of zones of inhibition of the extract on test organisms. The methanol extract exerted the highest zone of inhibition on both *Staphylococcus aureus, Salmonella typhi and Escherichia coli* respectively. It has a diameter of 22.44±0.51mm diameter on *Staphylococcus aureus* and 17.21±0.51mm diameter on *Salmonella typhi* and 14.28± 1.12mm diameter on *Escherichia coli*. Chloroform extract had the highest diameter of zone of inhibition on *Bacillus cereus* having a diameter of 13.66± 0.33mm. All the extracts where effective in all the test organisms with the least zone of inhibition recorded for chloroform extract on *Escherichia coli* using Muller Hilton agar. Comparatively, both Muller Hilton agar and nutrient agar tend to have equal support for the test organisms as both did not show any sharp contrast on the effect of the extract on the test organisms. However, it was more clear and easy to measure on Muller Hinton agar than on the nutrient agar.

Table 2 shows the susceptibility of the test isolate to standard antibiotics. Only chloramphenicol and streptomycin showed zones of inhibition on all the test isolates. Chloramphenicol showed the highest zone of inhibition of 12.50±0.77mm diameter on *Bacillus cereus*

while the bacteria were resistant to Augumetin, Erythromycin, Clindamycin, Cotrimoxazole, Chlorhexidine, and Cloxacillin. *Escherichia coli* was resistant to all the antibiotics used with the exception of gentamycin, chloramphenicol and streptomycin. Streptomycin exerted the highest diameter of zone of inhibition of *Escherichia coli* with a diameter of 20.21±0.91mm diameter. Only chloramphenicol and streptomycin inhibited *Staphylococcus aureus* with a diameter of zone of inhibition of 10.21± 0.12mm and 11.10±0.55mm respectively. The same pattern was observed for *Salmonella typhi* which was resistant to all the antibiotics with the exception of chloramphenicol and streptomycin respectively.

Figure 2 shows a comparative evaluation of the effect of the extract with the 2 most effective antibiotics on the Gram negative test bacteria such as *Escherichia coli* and *Bacillus cereus*. Streptomycin was the most effective on *Escherichia coli* while chloramphenicol was least effective. Therefore, while the effect of the extract were not up to the effect of streptomycin, they were however more than the effect of chloramphenicol thereby making the extract more effective than chloramphenicol antibiotic and all other antibiotics to which the organisms was resistant. *Salmonella typhi* on the other hand had the highest or most effective inhibition for methanol extract on Muller Hilton agar and Nutrient agar respectively. Chloramphenicol was more effective than streptomycin as a standard antibiotic.

Figure 3 shows the comparative evaluation of the effect of the extracts and the 2 most effective antibiotics on Gram positive test bacteria used. Streptomycin competes favorably with methanol extract used on Muller Hilton agar while chloramphenicol competes favorably at the same level of zone of inhibition with the methanol extract on Nutrient agar for *Bacillus cereus*. The most effective for *Staphylococcus aureus* was the methanol extract on nutrient agar and chloroform extract on Muller Hilton agar. Streptomycin and chloramphenicol had approximately the same level of zone of inhibition on *Staphylococcus aureus.*

Table 3 shows the results of the repellency test carried out on the extracts using albino rat. The chloroform extract exerted a stronger repellency power against the mosquitoes than the methanol extract. It also competes favorably with the positive control (drug field repellency cream). This is because only one mosquito was able to land on it 5 and half hours after the experiment began while one mosquito landed on the methanol extract and positive control 5hours after. At the end of the experiment (after 6hours), 2 mosquitoes landed on the group that was rubbed with chloroform extract while 2 mosquitoes also landed on the positive control. And three mosquitoes landed on the methanol extract. The negative control had mosquito landing on it 30 minutes after the commencement of the experiment and 12 mosquitoes were sucking from its back 6hours after. Plate 3 and 4 show the pictures of the shaving of the rats used for the repellency test experiment.

Plate 1: Photograph of lemon grass (*Cymbopogon citratus)*

Table 1a: confirmatory test results of the clinical isolates used

isolate	gram's reaction	spore test	oxidase test	methyl red test	molity test	Lactose	sucrose	glucose
1	+ve cocci	_	+	+	+	+_	+_	++
2	_ve rod	_	+	_	+	+_	+_	+_
3	+ve rod	+	+	_	_	++	_ _	++
4	_ve rod	_	_	+	+	++	++	+_

Resistance = -, Sensitive = +, Intermediate = +_

Table 1b: Confirmatory test results of the clinical isolates used

Xylose	Salicin	Arabinose	Indole	Catalase	Confirmed Organism
_ _	++	_ _	+-	+	*Staphylococcus aureus*
_ _	+-	_ _	+-	_	*Salmonella typhi*
+-	+-	_ _	_ _	+	*Bacillus cereus*
+-	_ _	+-	+-	+	*Escherichia coli*

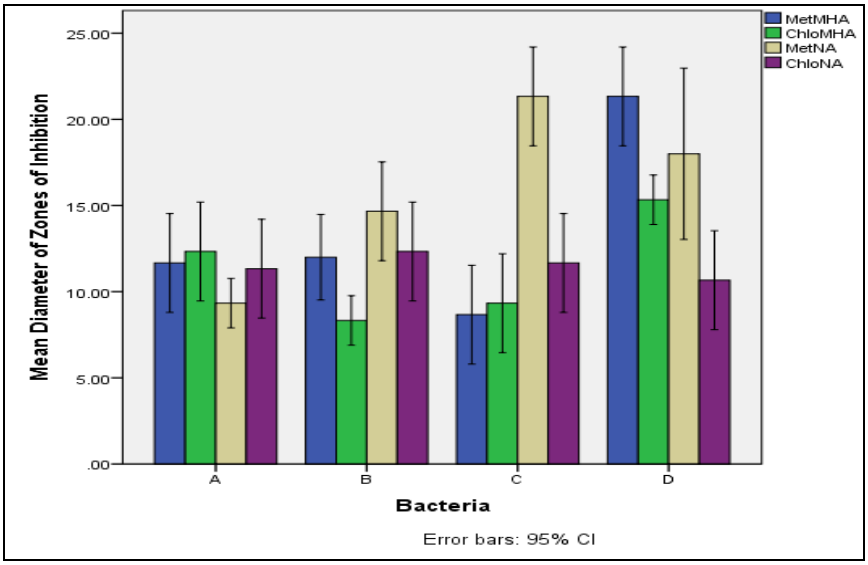

Fig 1: The diameter of zones of inhibition of the extracts on test bacteria.

Key: A – *Bacillus cereus;* MetMHA – Methanol extract on Muller Hilton agar; B – Escherichia coli-ChloMHA – Chloroform extract on Muller Hilton agar; C – *Stphylococcus aureus*-MetNA – Methanol extract on Nutrient agar;
D – *Salmonella typhi*-ChloNA – Chloroform extract on Nutrient agar

Table 2: Susceptibility of Test Isolates (Mm) to Standard Antibiotics

S/N	TET	GEN	AUG	ERY	COT	CLN	CHL	STR	CMC	CXC
B.cereus	4.61±	6.80±	0.00	0.00	0.00	0.00	12.50±0.77	8.67±0.33	0.00	0.00
E.coli	0.00	6.10±0.22	0.00	0.00	0.00	0.00	8.58±0.25	20.21±0.91	0.00	0.00
S.aureus	0.00	0.00	0.00	0.00	0.00	0.00	10.21±0.12	11.10±0.55	0.00	0.00
S.typhi	0.00	0.00	0.00	0.00	0.00	0.00	8.06±0.03	10.40±0.28	0.00	0.00

KEY: TET – Tetracycline, GEN – Gentamycin, AUG – Augumetin, ERY – Erythromycin; COT – Cotrimoxazole, CLN – Clindamycin, STR – Streptomycin, CMC – Chlorhexidine; CXC – Cloxacillin

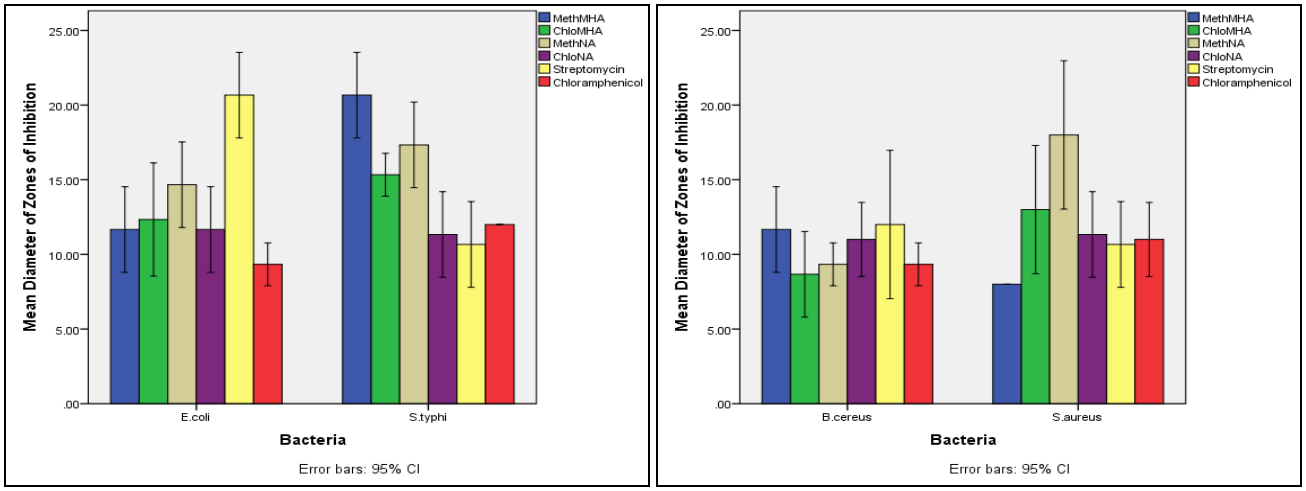

Fig 2 and 3: Comparative evaluation of the effects of the extracts on the two most effective antibiotics on the Gram negative test bacteria and Gram positive test bacteria.

Key: MetMHA – Methanol extract on Muller Hilton agar, ChloMHA – Chloroform extract on Muller Hilton agar, MetNA – Methanol extract on Nutrient agar, ChloNA – Chloroform extract on Nutrient agar

Table 3: shows the result of insect repellency using the extracts

Time	Chloroform extract	Methanol extract	Positive control	Negative control
30 min	--	--	--	--
1hr	--	--	--	1
1 ½hrs	--	--	--	1
2hrs	--	--	--	3
2 ½hrs	--	--	--	5

3hrs	--	--	--	6
3 ½hrs	--	--	--	7
4hrs	--	--	--	7
4 ½hrs	--	--	--	9
5hrs	--	1	1	11
5 ½hrs	1	3	1	11
6hrs	2	3	2	12

Plate 2: Photograph of bred female *Anopheles* Mosquito.

Plate 3: Shaving of albino rats for repellency test.

Discussion

The result obtained from the confirmatory test carried out on test isolates shows that the organisms conformed to their identity in agreement with the laid down procedure by Prescott *et al.,* (2013). According to Brooks *et al.,* (2011), *B.cereus* is the only *Bacillus* species that has terminal spore and it's associated with food poisoning. This was also in agreement with the spores viewed for this organism which were all terminal spores.

Of the 2 solvent used, the methanol and chloroform extract. Methanol extract exerted the highest zone of inhibition, it may have extracted more of the active ingredient of the plant due to its polar nature. Previous work done on other plant using methanol as solvent of extraction showed that it has the ability to extract more of the active ingredient of most plants than some other solvents. For instance the extraction of the active ingredient of Beniseed using methanol and N-hexane by Momoh *et al.,* (2012) [8] revealed that the methanol extract was more effective than N-hexane.

The effect of the media used which showed that there was higher zone of inhibition on the Muller Hilton agar than on the Nutrient agar agreed with the result obtained by Aakanksha *et al*., (2013). Equally Nesta *et al*., (2013) stressed that Muller Hilton agar is specially designed for the susceptibility testing for extract and that the clear nature of the agar allows proper view and measurement of the zone of inhibition. This is true as it was easier to measure the zone of inhibition of the extract on Muller Hilton agar than on the nutrient agar used for all the isolate in this work.

On susceptibility of the isolate to commercial antibiotics, chloramphenicol and streptomycin has always been the drug of choice for most of the bacteria according to Momoh *et al.,* (2012) [9]. However, the fact that the methanol extract exerted a higher zone of inhibition on all the test organisms used. It is a pointer to the fact that better antibiotics could be made using this plant. According to Singh *et al.,* (2011) [16], lemon grass oil was more effective against the test organisms used on clinical isolate than commercial antibiotics. These results show that lemon grass has always posse's antibacterial property that could be harnessed for the development of more potent antibiotics to cub menace of resistance. Sofowora, (2008) [17] listed lemon grass as one of the medicinal plant and traditional medicinal plant used in Africa. The result obtained therefore in this research has justified the use of the plant in Africa traditional medicine for curing stomach ache and skin diseases as stated by Odugbemi, (2006) [11]

The repellency effect exerted by the plant against anopheles mosquito may be attributed to its strong scent that the extract possess especially the chloroform extract. Although the chloroform was not used, it was hard to tell if the smell was from the chloroform or from the extract. However, it is reasonable to conclude that the smell may have come from the plant itself, since the whole chloroform solvent was evaporated before using the extract. According to Omoya *et al.,* (2015) [15], plant that possess strong odor may have the ability to repel insect. However the ability to repel female anopheles mosquito by other plant extract has not been thoroughly investigated.

Conclusion

The lemon grass possesses antibacterial property that could be harnessed for the development of more potent antibiotics to cub menace of resistance. It has proven to have a greater effect when used as repellency cream against female anopheles mosquitoes than commercial repellent cream used as control. This therefore offers a solution to the epidemic malaria fever that is common in Nigeria and in Africa at large.

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