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COMPARATIVE EFFECTS OF NEEM AND LEMONGRASS LEAF EXTRACTS ON *SALMONELLA SPP*

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ABSTRACT

Comparative effects of neem and lemongrass were investigated against isolated organism from the stool samples collected from patient suffering from typhoid fever. The isolated organism *Salmonella shigella Agar* (SSA) was used in carrying out sensitivity test using disc diffusion method and after the test the result was compared. Ethanol extracts of neem and lemongrass had minimum inhibitory concentrations (MIC) of 3.0µm/ml (3.0mm) and 6.0µm/ml (2.0mm) on neem and lemongrass respectively. Cold water extracts of neem and lemongrass had (MIC) minimum inhibitory concentrations of 12µm/ml (1.5mm) and 1.92µm/ml (0.9mm) respectively while hot water extracts of neem and lemongrass had MIC of 6.0µm/ml (1.2mm) and 12µm/ml (1.2mm) respectively. This research work revealed that neem could be more therapeutic than lemongrass on *Salmonella shigella*.

Keywords: Comparative effects, *Salmonella shigella Agar,* minimum inhibitory concentration, Neem and Lemongrass.

INTRODUCTION

Neem (*Azadirachta indica*) is used in traditional medicine as a source of many therapeutic agents. It is also used in Indian culture for the treatment of diseases and grows well in tropical countries. Its twigs provide a chewing stick and are widely used in the Indian subcontinent (Amaskane *et al*., 2010). Earlier studies on neem show that it contains active substances with multiple medicinal properties.

Azadirachta indica is used in folklore medicine for the treatment of diabetes (Shravan *et al*., 2011). Aqueous extract of neem leaf had a good therapeutic potential as antihyperglycemic agent in IDDM (insulin dependent diabetes mellitus) and NIDDM (non insulin dependent diabetes mellitus). Abusyed *et al*. (2008) suggests that anti inflammatory effects of neem extract is less than that produced by dexamethasome. Neem leaves have antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premises. Saseed *et al*, (2008) support the use of neem seeds in traditional treatment of infectious conditions especially those involving the eye and ear.

Lemongrass (*Cymbopogen citrates*) belongs to the section of *Andropogen* called *Cymbopogen* of the family *Germinae.* Medicinal use of lemongrass is known to mankind from time immemorial. Its oil has been used to cure various ailments. Like cough, cold, spitting of blood, rheumatism, lumbago, digestive problem, bladder problems, leprosy and as mouth wash for the toothache and swollen gums.

The oil has been found to posses bactericidal and antifungal properties, which is comparable to penicillin in its effectiveness (Lutterodt *et al*., 2010).

There is high risk of typhoid fever which is a health problem among children, adults and pregnant women. As a result of the emergence of drug resistance micro-organism making it most incurable due to too much intake of antibiotics, hence, the need to try unorthodox therapy, which would be effective in reduction of drug resistance necessitated the research.

The purpose of this research work was to investigate and compare the antimicrobial activity of neem and lemongrass leaf extracts on *Salmonella spp*.

MATERIALS AND METHODS

MATERIALS

HARD WARES

Autoclave, Microscope, Incubator, Inoculating Needle, Oven (Hot Air Oven), Bunsen burner, Spatula, Weighing balance, Measuring Cylinder, Wire Loops, Rank, Cotton Wool, Aluminium Foil and Filter Paper.

GLASS WARES

Beakers, Durham Tubes, Petri-dishes, Universal Bottle, Conical Flask

REAGENTS

Gram's stain, Crystal Violet, Lugol's Iodine, Acetone, Alcohol and Safranin

STERILIZATION OF MATERIALS

Petri-dish, conical flask containing Nutrient agar other glassware were thoroughly washed and autoclaved at 121^{o}C for 15 minutes and dried in hot air oven. Wire loop was also sterilized by direct flaming through the Bunsen burner.

COLLECTION OF SAMPLES

Matured leaves of Neem (*Azadirachta indica*) and Lemongrass (*Cymbopogen citrates*) were collected from Obeleagu Umana in Ezeagu L.G.A of Enugu State, Nigeria on September 2012. The leaves were washed and air dried for 2 weeks. The leaves were equally sliced and ground into small sizes using a hand blender. The fine particles were then kept for extraction. The isolated organism was collected from a patient suffering typhoid fever from parklane hospital Enugu. The stool samples were collected with sterile containers, immediately after it was taken to the laboratory for the analysis.

PREPARATION OF CULTURED MEDIA

The media for culturing were aseptically prepared according to the manufacturer's instruction.

PREPARATION OF *SALMONELLA SHIGELLA AGAR* MEDIA (SSA)

Salmonella shigella Agar Media (SSA) was weighed and dissolved in distilled water. The solution was poured into a conical flask and the mouth of the flask was plugged with cotton wool that was covered with aluminium foil and allowed to cool to about 45^{o}C. This was poured into a sterile petri-dish, that was rotated anti-clockwise and clockwise in order to solidify.

PREPARATION OF NUTRIENT AGAR (NA)

Nutrient Agar was weighed and dissolved in distilled water. The solution was poured into a conical flask and the mouth was plugged with cotton wool that was covered with aluminium foil and autoclave at 121^{o}C for 15 minutes. This was allowed to cool to about 45^{o}C and then poured into sterile Petri dish and allowed to solidify.

INOCULATION AND INCUBATION

A loopful of the bacterial suspension got from a patient suffering typhoid (stool samples) was inoculated on the surface of the solid *Salmonella shigella agar* medium in streak pattern. It was covered and incubated at 35^{o}C for 24h in an inverted position.

IDENTIFICATION OF THE ORGANISM

After incubation of the bacterial suspension on *Salmonella shigella agar*. *Salmonella spp.* showed brown ring rod.

GRAM STAINING AND MICROSCOPE

Thin smear of the isolates were prepared on a clean non-grease slide, heat fixed by passing it through the flame from Bunsen burner and flooded with crystal violet for 1minute, the excess dye was washed off gently in tap water and the slide drained against a paper towel. After which gram iodine solution was applied on the smear for 1 minute, it was then rinsed again with tap water and decolorized with 95% alcohol for 30seconds, washed with tap water at the end of 30seconds to stop the decolorization and drain. After it was counter stained with safranin for 30 minutes, after 30 minutes, it was washed, drained, blotted and dried with filter paper. A drop of oil immersion was added on the slide and it was then examined with microscope, under (x100) objective lens.

Gram positive organism appeared dark purple to brown while Gram negative organism appeared bright pink to red.

BIOCHEMICAL TEST FOR IDENTIFICATION OF ISOLATE

CATALASE TEST

2 drops of hydrogen peroxide (H_2O_2) was place on a clean slide and the isolate was removed with a sterile wire loop and immersed in the hydrogen peroxide solution. Immediate bubble or effervescence indicates positive result.

INDOLE TEST

5ml of peptone water was introduced into sterile test tube with the aid of a sterile pipette then isolates were inoculated into the peptone water, covered with foil paper it was allowed to stay in the incubator at 37^{o}C for 28h (2days) for accumulation of indole, after 48h, 2drops of kovac's reagent was added. Then, the result was read after 15minutes of observation. Red ring on the surface of the peptone water indicated indole positive while yellow ring indicated negative result.

METHYL RED TEST

The sterile glucose – Phosphate peptone water medium was slightly inoculated from a young agar slope culture and incubated at 37^0C for 48h. Then 5 drops of methyl red reagent was added into each tube. The contents were mixed and read immediately positive results were bright red and negative were yellow.

SUGAR FERMENTATION

Fermentation tests were carried out using the following sugar. Glucose, Mannitol, lactose, sucrose, maltose and arabinose. To each 10ml of peptone water in a test tubes, 1.5g of each sugar was separately dissolved into and labelled, 3 drops of 0.01% phenol red was also added. Durham tubes were inserted in an inverted position into the tubes for detection of gas production; the tubes were plugged with non absorbent cotton wool and sealed with aluminium foil. Then put into the autoclave at 110^oC for 15minutes. After sterilization, any trace of air in the test tube was removed by inverting the test tube. The tubes were then aseptically inoculated with small bacterial culture using sterile wire loop. The tubes were incubated for 24h at 37^oC and un-incubated tubes serves as controls. Acid production was indicated by a change in colour from orange (alkaline) to yellow (acid) in the fluid. And gas production was indicated by the presence of air space at the bottom or sealed end of the inverted Durham tubes.

MOTILITY TEST

A loopful of 24h culture of the isolates in peptone water was placed on the sterile slide and emulsified, Vaseline was used at the edge of cover slide to cover the emulsified isolate. The slide was carefully inverted and observed with microscope under (x40) objective lens for motility. Those organisms that are swimming show the motility of organism.

EXTRACTION OF NATURAL CONSTITUENTS

ETHANOL EXTRACTION OF NEEM AND LEMONGRASS

2g each of the dried powdered leaves of *Azadirachta indica* and *Cymbopogen citrates* were weighed and poured into a separate container, 20ml of ethanol was added to each of the container containing the powdered leaves and kept for 48h with periodic shaking. After 48h, it was filtered and the filtrates collected into a separate container.

HOT WATER EXTRACTION OF NEEM AND LEMONGRASS

2g of each dried powdered leaves of *Azadirachta indica* and *Cymbopogen citrates* were weighed and poured into a separate container, 20ml of hot water was added to each of the container containing the powdered leaves and kept for 48h with periodic shaking. After 48h, it was filtered and the filtrates collected into a separate container.

DETERMINATION OF SENSITIVITY

The antimicrobial activity (potency) of neem and lemongrass on the test organism was determined by the paper disc diffusion method using whattman filter paper and already prepared, nutrient agar plate. Sterile paper discs were cut into a circular form of about 8mm in diameter and impregnated with the extracts by dipping it into a beaker containing each extracts, the solution was left for about 30minutes, the paper discs were removed from the solution and allowed to air dried. The isolated organism was collected with a sterile wire loop and spread on the surface of solidified nutrient agar plate and the small pieces of the sterile filter paper disc impregnated with each extract were placed on the surface of the culture plates. The plates were incubated at 37^{o}C for 24h. After 24h incubation, the zone of inhibition was observed, measured and recorded accordingly.

RESULTS

TABLE I: MORPHOLOGICAL CHARACTERISTICS OF BACTERIAL ISOLATE.

Morphological Characteristics	**Gram Reaction**	**Presumptive Organism**
Brown round Coloured colonies on SS Agar	Gram Negative Rod	*Salmonella*

TABLE 2: BIOCHEMICAL TESTS ON THE TEST ORGANISM

Identified Organism	Catalase	Indole	Methyl	Motility	Glucose	Lactose
Salmonella	-	+	+	+	AG	G

Key

+ = Positive

- = Negative

A = Acid

G = Gas

AG = Acid and Gas

TABLE 3: THE EFFECTS OF ETHANOL EXTRACT OF NEEM AND LEMONGRASS ON *SALMONELLA SPP.*

CONCENTRATION OF THE EXTRACT AND INHIBITION ZONE							
Concentration of extract	50µm/ml	25µm/ml	12µm/ml	6.0µm/ml	3.0µm/ml	1.92 µm/ml	MIC
Neem Extract	10mm	9.5mm	7.0mm	5.0mm	3.0mm	6.9mm	3.0µm/ml
Lemon Grass Extract	8.0.mm	6.0mm	4.0mm	2.0mm	5.0mm	4.1mm	6.0µm/ml

TABLE 4: THE EFFECTS OF HOT WATER EXTRACTS OF NEEM AND LEMONGRASS ON *SALMONELLA SPP.*

CONCENTRATION OF THE EXTRACT AND INHIBITION ZONE							
Concentration of extract	50µm/ml	25µm/ml	12µm/ml	6.0µm/ml	3.0µm/ml	1.92 µm/ml	MIC
Neem Extract	8mm	6.0mm	3.5mm	1.2mm	0.0mm	3.74mm	6.0µm/ml
Lemon Grass Extract	4.0mm	2.8mm	1.2mm	0.0mm	0.0mm	1.6mm	12µm/ml

TABLE 5: THE EFFECT OF COLD WATER EXTRACTS OF NEEM AND LEMON GRASS ON *SALMONELLA SPP.*

CONCENTRATION OF THE EXTRACT AND INHIBITION ZONE							
Concentration of extract	50µm/ml	25µm/ml	12µm/ml	6.0µm/ml	3.0µm/ml	1.92 µm/ml	MIC
Neem Extract	5mm	3.0mm	1.5mm	0.0mm	0.0mm	1.9mm	12µm/ml
Lemon Grass Extract	3.0mm	1.5mm	0.0mm	0.0mm	0.0mm	0.9mm	1.92µm/ml

DISCUSSION, CONCLUSION AND RECOMMENDATION

DISCUSSION

Comparative effect of neem and lemongrass were investigated and the result obtained shows that all the extracts demonstrated antimicrobial activity against *Salmonella spp*. Ethanol extracts of neem and lemongrass had minimum inhibitory concentrations (MIC) of 3.0µm/ml (3.0mm) and 6.0µm/ml (2.0mm) on neem and lemongrass respectively. Cold water extracts of neem and lemongrass had (MIC) minimum inhibitory concentrations of 12µm/ml (1.5mm) and 1.92µm/ml (0.9mm) respectively while hot water extracts of neem and lemongrass had MIC of 6.0µm/ml (1.2mm) and 12µm/ml (1.2mm) respectively. Table 3, ethanol extracts of neem and lemongrass, showed that at highest concentrations of ethanol extracts of neem and lemongrass (50 µm/ml) the inhibitory zone for neem (10mm) was slightly higher than that of lemongrass (8.0mm). This was also the same in the cold and hot water extracts as shown in tables 4 and 5 above. Neem extracts of ethanol, cold and hot water demonstrated higher effect on *Salmonella spp*. than lemongrass corresponding extracts.

CONCLUSION

In conclusion, the results revealed that neem was more effective than lemongrass on *Salmonella Spp*.

RECOMMENDATION

Based on the results obtained from this research, it is hereby recommended firstly, that government at all levels should encourage and regulate sound and safe practice of unorthodox medicine secondly, there should be public awareness campaign on the need for unorthodox medicine. Such medicinal plant such as *A. indica, C. citrates*, should be planted and tended. Finally, efforts should be made to institute quality control methods in unorthodox medicine dispensation so as, to improve people understanding of it.

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