Phytochemical Analysis and Antimicrobial Activity of Neem Leaf, Lemon Grass and Mixture of Neem Leaf & Lemon Grass Essential Oil Extract(S) Against Some Selected Gram Positive and Gram Negative Organisms.

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Abstract- ***In this study, the antimicrobial activity and phytochemical constituents of neem leaf, lemon grass and mixture of neem leaf + lemon grass essential oil extract were evaluated. Oil extracts of neem leaf, lemon grass and mixture of neem leaf + lemon grass were obtained by solvent extraction method using n-hexane. Antimicrobial activity screening of plants' oil extracts was conducted using agar well diffusion method and the oil extracts were tested against two-gram positive bacteria (Staphylococcus aureus and Bacillus subtilis) and two-gram negative bacteria (Salmonella typhi and Escherichia coli). Results: Phytochemical components of the oil extracts were alkaloids, saponins, tannins, flavonoids, glycosides, phenol, flavonoids, steroids and carbohydrates. Flavonoids, alkaloids and carbohydrate were most present in Neem leaf, lemon grass and mixture Neem leaf + lemon grass extracts while saponins, tannins and phenols are in moderate concentration in the three extracts, while steroid was only present in Lemon grass extract, and glycoside was only present in Neem leaf extract. There was significant antimicrobial activity in the test carried out, with the mixed extract at known quantity having the highest zone of inhibition 10.00± 0.17^{a} against B. subtilis at 400mg/ml and lemon grass had the lowest zone 3.00± 0.06^{d} against E. coli at 400mg/ml. Conclusion: The antimicrobial activity of Neem extract, Lemon grass extract and mixed extract may be attributed to the present of phytochemical constituent present in the extracts. The present study showed that the mixture of Neem leaves and lemon grass extract exhibited significant activity compared to the individual extracts and could be exploited as the source of a potent bioactive ingredient for pharmaceutical products for the treatment of bacterial infections and also to improve the quality of health care.***

Indexed Terms- ***Phytochemical Analysis, Essential oil extract, antimicrobial avtivity, lemon grass (Cymbopogon citratus) and neem (Azadirachta indica).***

I. INTRODUCTION

Essential oils are naturally occurring volatile and odoriferous aromatic extracts of plants. These plant essences are mostly located in cells, ducts and glands of leaves, barks, roots, buds, flowers and fruits of most plant matrix [18]. Lemon grass (Cymbopogon citratus), is an odorous tropical grass which yields oil that smells of lemon, used in cooking, perfumery and medicine (Concise Oxford Dictionary Tenth Edition). The genus Cymbopogon belongs to the grass family, Poaceae (syn. Gramineae). The Poaceae family has about 700 genera and 11,000 species widely distributed in all regions of the world [4].

Lemon grass oil is widely used in perfumery, cosmetics, soaps, detergents and confectionary and in the production of vitamin A [11]. The essential oils of the grasses of species of Cymbopogon have an industrial profile; they are used in beverages,

foodstuffs, fragrances, household products, personal care products such as deodorants, herbal tea, skin care products, insect repellents, pharmaceuticals, in tobacco etc. [1]. High antibacterial and remarkable antifungal activities make the lemongrass oil a potential food preservative [10].

Neem (*Azadirachta indica*) belonged to *Meliceae* family and grows rapidly in the tropic and semi-tropic climate. It is also observed that this tree could survive in very dry and arid conditions. Neem oil is widely used as insecticides, lubricant, drugs for variety of diseases such as diabetes and tuberculosis. This oil could also prolong leather goods when it is applied on them. The neem has a wide range of several therapeutic properties based on its characteristics, such as antifungal, antibacterial, antioxidant, antiviral, anti-inflammatory, analgesic, antipyretic, and immune stimulant activity [17,24]. The leaf extract is commonly used as an antibacterial agent. In addition, the neem has several applications, such as antiseptic, healing, anthelmintic; use in medicinal soaps, creams and toothpaste [17,21].

Ronicely [20] evaluated the effect of drying air velocity upon the chemical composition of the essential oil extracted from Brazilian lemon grass leaves. The essential oil components, after drying, were compared with the values obtained from the fresh plant (control). All drying treatments showed no difference for the main components (myrcene, geranial and neral) of the essential oil obtained from lemon grass, when compared to the fresh plant (control).

Mondall [16] Studied the efficacy of different extracts of neem leaf on seed borne fungi Aspergillus, Rhizopus and chemical characterization of the neem leaf extracts in vitro on the culture medium. The growth of both the fungal species was inhibited significantly ($p<0.01$) and controlled with both alcoholic and water extract of all ages and of the concentrations used. The alcoholic extracts of neem leaf was most effective in comparison to aqueous extract for retarding the growth of Rhizopus and Aspergillus. The crude aqueous and alcoholic leaf extracts of neem was more effective in inhibitions of growth of the fungi Aspergillus in comparison to inhibitory effects on Rhizopus growth in the artificial culture medium. Leaf extracts of neem which are cheap and environmentally safe are promising for protecting crop species against the fungal infestation and leading towards improvement of the crop in terms of yield and productivity. The lemongrass essential oil shows a wide spectrum of biological activities.

Study evidenced that plants fruits, oil, leaves, bark and other parts have important role in diseases prevention due to their rich source of antioxidant [3]. Quercetin and sitosterol, polyphenolic flavonoids, were purified from neem fresh leaves and were known to have antibacterial and antifungal properties [3,13].

Generally, the leaf, flower, root and seed are used in herbal medicine all over the world [7]. Smith [23] reported that the flower and young fruit are used as curries. Phytochemical investigation of plant extracts shows the presence of active principles in the plant parts like flower, bark, leaves, root, fruits, etc. phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produce these chemicals to protect itself but research works demonstrates that many phytochemicals can protect humans against disease [25,15,8]. Knowledge of phytochemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances. Plants are rich in a wide variety of secondary metabolites such as alkaloids, flavonoids, Saponins, and tannins, etc, which have been found to in-vitro have anti-microbial properties [2]. This study focusses on the extraction, phytochemical analysis and determination of the antimicrobial effect of neem leave, lemon grass and the mixed oil extracts (lemon grass and neem leaves essential oil extract) on some selected microbial isolates (gram positive bacteria (*Staphylococcus aureus and Bacillus subtilis) and* gram negative bacteria (*Salmonella typhi* and *Escherichia coli*). The findings of this research work could serve as an important guideline in the area of the use of the mixed extracts (lemongrass (*Cymbopogon citratus)* and neem leaves (*Azadirachta indica*)) as cure for bacterial infections

II. MATERIAL AND METHODS

2.1 Plant material and Essential oil extraction

The leaves of lemon grass (*Cymbopogon citratus)* and neem (*Azadirachta indica*) free from disease were collected from lamingo near JUTH, Jos North, Plateau State. The collected leaves samples of lemongrass (*Cymbopogon citratus)* and neem (*Azadirachta indica*) were washed with ordinary water to remove dirt, dust, and other contaminated agents. Furthermore, the plant leaves were washed with distilled water for more cleaning and were allowed to drip. The plants leave samples were air dried in a shade at room temperature for about three weeks to remove the moisture content. The shade dried leaves were subjected to size reduction into fine powder (grinded using mortar and pestle). About 100g of lemon grass powder, 100g of neem leave powder and a combination of 50g of lemongrass powder and 50g of neem leave powder were soaked with N-hexane in different beakers for 72hrs at room temperature. The liquid phases were decanted and filtered with filter paper thereafter, the aqueous extract were concentrated using Laboratory rotary evaporator.

2.2 Bacterial Strains

The clinical isolates ***Staphylococcus aureus, Bacillus subtilis, Salmonella typhi*** and ***Escherichia coli*** Specie were obtained at the Jos University Teaching Hospital JUTH, Jos, Plateau State for the tests against neem leaves, lemon grass and lemon grass & neem leaves oil extracts.

2.3 Phytochemical analysis

Preliminary phytochemical analysis was carried out for identification of bioactive chemical constituents in the neem leaf and lemon grass extracts using the standard procedure described by Brain and Turner [5] and Evans [9]. For each test, 1 ml of each solvent extract was used for analysis, in exception for the saponin test in which 3 ml solvent extract was used.

2.4 Antibacterial Activity Test of Crude Extracts

Antibacterial activity of Neem leaf and lemon grass extract was evaluated using agar well diffusion assay. A sterilize molten Muller Hinton Agar (Hi Media) was prepared according to manufacturers' instruction and allowed to cool at 45 °C in a water bath. Subsequently, 0.1ml of 18h old bacterial suspensions adjusted to turbidity of 0.5 McFarland Standard was dispensed into empty petri dish. Sterile agar medium was dispensed into the plates containing the bacterial suspension, the agar plates were then left to solidify under Bunsen burner flame for 60 min. After 30 min, equidistant wells were cut in the medium using a 6mm diameter flame sterilized cork borer and labeled. A 100 µg of the test extracts of the different concentrations (400, 200, 100 and 50 mg/ml) and controls were then dispensed into the wells and labeled. The plates were incubated at 37 °C for 24 h and diameters of growth inhibition zones around the wells formed were measured. Antibiotic gentamicin (10 µg ml^{-1}) was used as positive control. They extracts were dissolved using tween 20.

2.6 Determination of Minimum inhibitory concentrations (MIC). The minimum inhibitory concentration (MIC) of plant extracts was determined by broth dilution method according to the method adopted by Gizachew [12]. Sample concentration was prepared from the stock solutions by two-fold dilutions in sterile nutrient broth. four dilutions of the samples ranging from 50,100,200 and 400mg ml^{-1} were tested. The inoculums of test strains prepared from fresh overnight cultures were adjusted to 0.5 McFarland standards, which equals to 1×10^8 CFU ml^{-1} for bacteria. The inoculums then were diluted in 1:100 ratios in order to get 1-5× 10^5 CFU ml^{-1} concentrations. The bacterial suspension of 1 ml was added to each of the tubes and incubated at 37°C for 24 h. The control tubes contained only the bacteria inoculum and tween 20 used to dissolve the extracts with no test extract. After incubation, the visual turbidity was observed and recorded. The lowest concentration without turbidity observed was measured as a MIC of the extracts.

2.5 Minimum Bactericidal Concentrations (MBC). Minimum bactericidal concentrations were evaluated by taking loop full of tubes that did not show visible signs of growth during MIC determination on agar medium. The inoculums were streak plated onto sterile Mueller Hinton agar (Accumix – Verna, India) and then incubated at 37°C for 24 h. The lowest concentration of crude extracts with the absence of growth after incubation at 37 ^{0}C for 24 h was considered as MBC.

III. RESULTS

TABLE 1. Phytochemical screening of Neem leaf, lemon grass and mixture of neem and lemon grass extract

Compound	Neem leaf Extract	Lemon grass Extract	Lemon grass + neem leaf (mixed extract)
Flavonoid	++	++	++
Saponins	+	+	+
Steroids	-	+	-
Tannins	+	+	+
Alkaloid	++	+	++
Phenol	+	+	+
Carbohydrate	++	+	++
Glycosides	+	-	-

Key: ++ = highly present, + = present, - = absent

The result obtained showed the presence of phytochemicals in lemon grass oil, Neem leaf extract and mixture of Lemon grass oil + Neem leaf extract such as flavonoid, tannin, phenol, saponins, alkaloid and carbohydrates. However, steroid was only found in Lemon grass oil and glycosides were found Neem leaf.

3.1 ANTIMICROBIAL ACTIVITY

Sensitivity Test

Table 2: Sensitivity test of the *neem leaf extract*.

Organisms	50 mg/ml	100 mg/ml	200 mg/ml	400 mg/ml	Control
Salmonella typhi	0.00 ± 0.00^{a}	1.10 ± 0.10^{b}	4.33 ± 0.33^{a}	8.10 ± 0.01^{b}	14.43 ± 0.29^{b}
E. coli	0.00 ± 0.00^{a}	0.00 ± 0.00^{c}	3.07 ± 0.07^{c}	9.03 ± 0.09^{a}	20.17 ± 0.12^{a}
S. aureus	0.00 ± 0.00^{a}	0.00 ± 0.00^{c}	1.07 ± 0.07^{d}	5.10 ± 0.10^{d}	14.73 ± 0.37^{b}
B. subtilis	0.00 ± 0.00^{a}	2.20 ± 0.15^{a}	4.27 ± 0.18^{a}	7.03 ± 0.03^{c}	13.13 ± 0.19^{c}
L.S.D	0.44				
P- value	<0.0001 ****				

At P≤0.05 there was a significant difference in the sensitivity test of the oils from *neem leaf* on the selected bacteria isolates. Values are presented as mean standard error of means. Ranking was done across the oil and values with the same super script are not significant.

Table 3: Sensitivity test of the lemon grass extract

Organisms	50 mg/ml	100 mg/ml	200 mg/ml	400 mg/ml	Control
Salmonella typhi	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	2.00 ± 0.00^{b}	5.90 ± 0.07^{b}	14.43 ± 0.29^{b}
E. coli	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{d}	3.00 ± 0.06^{d}	20.17 ± 0.12^{a}
S. aureus	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	1.00 ± 0.00^{c}	4.10 ± 0.10^{c}	14.73 ± 0.37^{b}
B. subtilis	0.00 ± 0.00^{a}	2.00 ± 0.00^{a}	3.13 ± 0.13^{a}	5.90 ± 0.21^{a}	13.13 ± 0.19^{c}
L.S.D	0.38				
P- value	<0.0001 ****				

At P≤0.05 there was a significant difference in the sensitivity test of the oils from *lemon grass extract* on the selected bacteria isolates. Values are presented as mean ± standard error of means. Ranking was done across the oil and values with the same super script are not significant.

Table 4: Sensitivity test of the mixed extract

Organisms	50 mg/ml	100 mg/ml	200 mg/ml	400 mg/ml	control

Salmonella typhi	0.00± 0.00^{b}	1.00 ± 0.00^{c}	4.17 ± 0.09^{c}	8.17 ± 0.12^{c}	14.43 ± 0.29^{b}
E. coli	0.00± 0.00^{b}	0.00 ± 0.00^{d}	5.17 ± 0.17^{b}	9.13 ± 0.13^{b}	20.17 ± 0.12^{a}
S. aureus	1.07± 0.07^{a}	2.13 ± 0.09^{b}	6.00 ± 0.12^{a}	7.03 ± 0.15^{d}	14.73 ± 0.37^{b}
B. subtilis	1.10± 0.10^{a}	3.07 ± 0.07^{a}	6.00 ± 0.17^{a}	10.00 ± 0.17^{a}	13.13 ± 0.19^{c}
L.S.D	0.42				
P- value	<0.0001 ****				

At P≤0.05 there was a significant difference in the sensitivity test of the mixed extract on the selected bacteria isolates. Values are presented as mean ± standard error of means. Ranking was done across the oil and values with the same super script are not significant.

3.2 Minimum inhibitory concentration (MIC)

Table 5 Neem leaf extract

Organism	400 mg/ml	200 mg/ml	100 mg/ml	50mg/ml	MIC	Control
Salmonella typhi	+	-	-	-	400	+
E. coli	+	+	-	-	200	+
S. aureus	-	-	-	-	-	+
B. subtilis	+	+	-	-	200	+

Table 6 Lemon grass extract

Organism	400 mg/ml	200 mg/ml	100 mg/ml	50mg/ml	MIC	Control
Salmonella typhi	-	-	-	-	-	+
E. coli	+	-	-	-	400	+
S. aureus	-	-	-	-	-	+
B. subtilis	+	-	-	-	400	+

Table 7 Neem leaf + Lemon grass extract

Organism	400 mg/ml	200 mg/ml	100 mg/ml	50mg/ml	MIC	Control
Salmonella typhi	+	+	-	-	200	+
E. coli	+	+	-	-	200	+
S. aureus	+	-	-	-	400	+
B. subtilis	+	+	+	-	100	+

3.3 Minimum bactericidal concentration (MBC)

Table 8 Neem leaf extract

Organism	400 mg/ml	200 mg/ml	100 mg/ml	50mg/ml	MBC	Control
Salmonella typhi	-	-	-	-	-	+
E. coli	+	-	-	-	400	+
S. aureus	-	-	-	-	-	+
B. subtilis	+	-	-	-	400	+

Table 9 Lemon grass extract

Organism	400 mg/ml	200 mg/ml	100 mg/ml	50mg/ml	MBC	Control
Salmonella typhi	-	-	-	-	-	+
E. coli	-	-	-	-	-	+
S. aureus	-	-	-	-	-	+
B. subtilis	-	-	-	-	-	+

Table 10 Neem leaf + Lemon grass extract

Organism	400 mg/ml	200 mg/ml	100 mg/ml	50mg/ml	MBC	Control
Salmonella typhi	+	-	-	-	400	+
E. coli	-	-	-	-	-	+
S. aureus	-	-	-	-	-	+
B. subtilis	+	+	-	-	200	+

IV. DISCUSSIONS

The preliminary phytochemical constituents of neem leaf extracts and lemon grass indicates the presents of alkaloids, saponins, tannins, flavonoids, glycosides, phenol, flavonoids, steroids and carbohydrates. Table 1 reveals that flavonoids, alkaloids and carbohydrate are most present in neem leaf, lemon grass and mixture of neem leaf + lemon grass extracts while saponins, tannins and phenols are in moderate concentration in the three extracts, while steroids was only present in Lemon grass extract, and glycoside was only present in neem leaf extract. These results bear similarities to ones obtained by Dash [6].

Much of the protective effects of herbal plants have been attributed to their phytochemical constituents, alkaloids, flavonoids, glycosides, saponins for examples exert multiple biological effects like anti-inflammatory, anti-allergic, antioxidant, anti-diabetic, anti-viral and anti-cancer activities, anti-leprosy activities, antimicrobial activity [22]. Compare to synthetic drugs, use of bio-actives compounds of medicinal plants have several advantages which include fewer side effects, better patient tolerance, relatively less expensive and renewable in nature [26]. In this study the antibacterial activity of Neem leaf, Lemon grass and Neem leaf + lemon grass extracts were determined against four bacteria (two gram positive and two gram negative) as evidenced by their zones of inhibition. According to the tests, the bacterial strains were sensitive to the three extracts which show differences in the zone of inhibition with respect to the concentration.

On the basis of this finding, the widest zone of inhibition in Neem leaf extract was obtained with *E coli*. 9.03 ± 0.09^{a} at 400mg/ml, while *S. aureus* had the lowest with 5.10 ± 0.10^{d} at 400mg/ml. For Lemon grass extract salmonella typhi and B. subtilis showed the highest zone with 5.90 ± 0.21^{a} at 400mg/ml with E. coli having the lowest with 3.00 ± 0.06^{d} at 400mg/ml. B. subtilis had the highest zone measuring 10.00 ± 0.17^{a} at 400mg/ml for mixture of Neem leaf and Lemon grass extract and S. aureus had the lowest, 7.03 ± 0.15^{d} at 400mg/ml.

The results of antimicrobial activity of neem leaf, lemon grass and mixture neem and lemon grass mixture extract against bacterial pathogens were tabulated in the table 2, 3 and 4. The results revealed that mixed extracts (Neem and lemon grass) exhibited significant activity compared to the individual extracts, with its zone ranging from 10.00 ± 0.17^{a} to 7.03 ± 0.15^{d}. neem extract had a moderate activity ranging from 9.03 ± 0.09^{a}mm – 5.10 ± 0.10^{d}mm and the least activity was observed in lemon grass extract ranging from 5.90 ± 0.21^{a} – 3.00 ± 0.06^{d}mm.

The variation in MIC observed could be attributed to the **s**usceptibility and resistance**s** status of the bacteria isolated [14]. The minimum inhibitory concentration of 100 mg/ml against *B. subtilis* suggest that the extract can be a potential antibacterial agent if the

active compound responsible is isolated. This can be deduced from table 7 above. Thus, the results corroborate with studies [19] which reports that the neem leaf and lemon grass extract is a powerful inhibitor agent against the increase and establishment of micro-organisms that cause infectious diseases. However, the mixture of neem leaf extract and lemon grass extract at a known concentration has proven to be more effective as shown in table 10.

CONCLUSION

The study revealed presence of several phytochemicals present in the extract (flavonoids, alkaloids, saponin, phenol, glycoside, tannin and carbohydrate). The positive antimicrobial activity of neem leaf, lemon grass and mixed extracts against the bacteria strain tested could be attributed to the presence of phytochemical constituents present, which justifies this species has biological activities as anti-inflammatory, antimicrobial, antioxidant, antiviral, among others. Considering the relevant MIC values of neem leaf, and mixture of neem and lemon grass extract, it was possible to observe that these samples have some active compounds that caused inhibition of their growth.

The synergistic actions of the phytochemical are very like to be responsible for the various anti -bactericidal, properties exhibited by the oils. These results encourage the continuation of further studies on the isolation of these active constituent for a future development of herbal medicine. It also suggested that more research efforts should be geared towards understanding the mechanisms of antimicrobial susceptibility of the microorganism as this will improve the chances of coming up with novel products which can effectively combat seemingly drug resistance microbes.

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