Optimization of antibiotic activity of composites of ethanolic extracts of flower of *Mangifera indica, Gongronema latifolium* leaves and *Citrus sinensis* peel using the mixture experimental design of the response surface methodology

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Abstract— *The generation of pathogenic microorgannisms is overwhelming the potency, safety and cost of synthetic antibiotics. The study south insight for the use of plant materials to fight microbes and optimized antibiotic activity of pure, binary and ternary blends of ethanol extracts of flower of Mangifera india, Gongronema latifolium leaves, Citrus sinensis peel on Streptococcus aureus using the Simplex Lattice {3,3} mixture experimental design of the response surface methodology (RSM). Fourteen (14) blends of the plants' parts were produced and tested on the S. aureus. Inhibition zones inhibited by the extract blends ranged between 11-19 mm. Blends C and BC exhibited the highest value of 19 mm. Other blends equally exhibited some inhibition effects on the growth of the test microorganism, however, decreasing in values as their proportions in the blends carried. ANOVA on the data revealed that the model of the experiment was significant ($p<0.05$; $R^2=0.8350$), the pure; A, B, C, and the ternary blends, ABC, were significant in the model ($p<0.05$). Although other blends were n ot statistically significant ($p>0.05$), the graphic and the equation indicated their positive contributions to the model. The model showed overall mean inhibition zone of 14.11 mm compared to 22.5 mm observed in Levofloxacin on the test organism. The study showed that ethanolic extracts of the plants' parts could provide the basis for engineering and synthesis of potent antibiotics.*

Keywords— Synthetic antibiotics, shynergism, Simplex Lattice design, antibiotics activity, zone of inhibition.

I. INTRODUCTION

Microbial infectious diseases are leading causes of morbidity and mortality in the developing world. It is estimated that about 60% of the earth's biomass comprises of microbes. The genetic, metabolic and physiological diversity of microbes makes the war against them difficult, hence it continued negative effect on health the world over (Radulovic *et al.,* 2013).Synthetic antibiotics are the major antimicrobial drugs used to control and treat health problems in man and farm birds and animals (Cech *et al.,* 2013). Most antibiotics are expensive, generate multi-drug resistant pathogens and parasites, they are toxic to man and the environment. In order to circumvent the negative effect of synthetic antibiotics, herbal medicine is gaining some recognition and prevalence of use (Welz *et al*., 2018). Herbal medicines are eco-friendly and bio-friendly because the plant sources are nutritious and edible. They contain phyto chemicals; vitamins and nutrients which are needed separately in foods for good health. Besides, they have served as foods and health tonics for generations today without reported adverse health effect (Welz et al., 2013).

Antimicrobial activities of many plants have been widely studied as possible alternatives to the synthetic counterparts. For instance, (Rasulovic *et al*., 2013) have reported activities of extracts of parts of *Moringa oleifera* against some pathogenic microorganisms. *Mangiferaindia, Gongronema latifolium* and, *Citrus sinensis* peel are underutilized wastes of edible plants (Ugochukwu and Babady, 2002). *Citrus sinensis* peels are rich in Vitamin C, fibre, and many nutrients, including phenolics and flavonoids which are also good antioxidant agents. *M. indica* contains alkaloids, they are used as a basic medicinal agent for their analgesic, antispasmodic and bactericidal effects. According to Chinedu and Friday (2015), *Gongronema latifolium* leaves contain alkaloids, glycosides, tannin, saponin, and flavonoids all of which are antioxidants and antimicrobial. *Staphylococcus aureus* is a leading cause of food poisoning (Ogston *et al.,* 1984).It is a gram-positive, catalase positive cocci belonging to the staphylococcaceae family. *S. aureeus* is approximately 0.5-7.5μm diameter, non-motile, non-spore forming,

facultative anaerobes. *S. aureus* is part of human flora and are primarily found in the nostrils and other body cavities, often implicated in a variety of food borne diseases (Vameen *et al.*, 2009).

Mixture experimental design of the response surface methodology optimizes the blending of individual components to obtain superior activity over single effect. The design chosen in this work is due to it accuracy, simplicity, robustness, predictability, and reproducibility (Bondari, 1999).

The aim of the study was to determine the antimicrobial potency of composites ethanol extract of flower of *Mangifera India, Gongronema latifolium leaves* and, *Citrus sinensis* peel on *Staphylococcus aureus* using the mixture experimental design of the Response Surface Methodology (RSM).

II. MATERIALS AND METHOD

2.1 Procurement of plant material and microbial cultures

Flowers of *Mangifera Indica* tree was collected from a local farm in IkotOsurua, *Gongronema latifolium* leaves and *Citrus sinensis* were obtained from a local market in IkotEkpene Local Government Area. The plants were identified and authenticated by the Botany unit of the Department of Science Technology, Akwa Ibom State Polytechnic, IkotOsurua, IkotEkpene, Akwa Ibom State as the plant parts.

2.2 Test microorganism

Test microorganism was collected and handled according to the method of Cheesbrough (2003). Pure culture of *Staphylococcus aureus* was obtained from the General Hospital, IkotEkpene Local Government Area, Nigeria. The sample was aseptically transferred to and maintained on nutrient agar, and subcultured regularly and preserved on solid media at 4^{o}C for further analysis and certainty.

2.3 Inoculaum preparation

3-4 loopful of isolated colonies was inoculated into 5 ml of suitable broth, incubated at about 37^{o}C. The actively growing bacterial suspensions were adjusted with suitable broth to obtain turbidity visually comparable to that of 0.5 McFarland standard equivalents to approximately $1x10^{8}$cfu/ml.

2.4 Sterilization

The plant materials were surfaced sterilized separately soaks in 1% mercuric chloride ($HgCl_2$) for 5 minutes and rinsing them in 4 to 5 times with distilled water before oven dried at 40^{o}C. The piece was then grounded with a manual grinder separately into powder form.

2.5 Ethanolic extraction of plant parts

1.5 Kg of powdered plant parts were separately extracted with 2 L 95% ethanol by maceration at room temperature for 12 days. The extracts were filtered using Whatman No.42filter paper; the extract was concentrated to dryness with rotary evaporator at reduced pressure. The concentrated extract was weighed and stored in an airtight glass container and kept in a refrigerator.

2.6 Preparation of blends of ethanol extract of the plant parts

The blends of ethanolic extracts of plants' parts were prepared according to the method of Bondari, (1999), in Table 1. The augmented Simplex Lattice mixture experimental design implied1, 0.5, 1/3, 2/3 or referred to as {q, m}Simplex Lattice Design, where q represents the number of factors involved with m+1 equally spaced proportions from0 to 1 for each component. All possible mixtures for {q=3, m=2} and {q=3, m=3} (Table 2). Graded proportions of the ethanolic extracts of the plant parts were mixed together to obtain blends of the extracts as follows; pure A, B, C, binary, AB, AC, BC, and the tenary and A, B, C blends. Antibiotics activity of each blend was tested on *Staphylococcus aureus* comparing the values with Levofloxacin, as a standard antibiotic.

TABLE 1
THREE-COMPONENT (A, B, AND C) SIMPLEX LATTICE
DESIGN FOR 0, 1/3, 2/3, AND 1 DESIGNATED AS {3, 3} DESIGN SITUATIONS

Component	Unit	Lower Proportion	Upper Proportion
A	-	0	1
B	-	0	1
C	-	0	1

$$A + B + C = 1 \text{ or } 100\% \quad (1)$$

2.7 Theory of mixture experimental design

According to Bondari, (1999), statistical modeling in a mixture experiment models the blending surface such that predictions of the response for any mixture component, singly or in combination, can be made empirically. Testing of the model adequacy is also an important part of the statistical procedure. The component proportions in a mixture experiment vary together like in factorial experiments because they are constrained to sum to a constant (1 or 100% for standard designs).

2.8 The Agar disc diffusion technique

The agar disc diffusion was carried out according to the method adopted by. Sterile filter paper disc 6.0 mm in diameter were impregnated with blends of flower of *Mangifera indica leaves, Gongronema latifolium leaves* and *Citrus sinesis* peel (Table 2). The blend impregnated filter papers were carefully placed aseptically on the surface of the nutrient agar that was pre-inoculated with the test organisms using sterile forceps in each plate. The plates were left undisturbed for 15 minutes after which there were incubated at 37^{o}C for 24 hours.

2.9 Measurement of zones of inhibitions

Inhibitions zones of each blend were measured according to the method of Heatley *et al.,* (2009). The diameters of the zones of inhibition of growth of the test microorganism (the paper disc) were measured using the millimeter rule.

2.10 Data analysis

Experimental design, analysis of variance and optimization analyses on the data obtained from inhibition zones of microbial colonies were carried out using the Design*Expert Ver. 11. (Stat-Ease, Inc. 2021 East Hennepin Ave, Suite 480, Minneapolis, MN 55413).

III. RESULTS AND DISCUSSION

3.1 Result

Table 2 shows response of *S. aureus* in terms of inhibition zone, to the blends of ethanolic extracts of flower of *Magnifera indica leaves, Gongronema latifolium* leaves and *Citrus sinensis* peel compared with Levofloxacin. According to the table, inhibition zones of the pure the blends of extracts of A, B, C on the test organisms ranged from 13.00 to 19.50 mm. Pure blend of *C. sinensis* showed the highest inhibition zone of 19.50 mm, followed by A, 13.0 mm, and C, 12.0 mm. The binary blends, AB, AC, BC exhibited inhibition zones of 13.0, 15.0, 19.0 mm respectively on the test organism. Also, the binary blend, AC exhibited the highest inhibition zone of 19.0 mm on the test organism, followed by AB and AC at 15.0 and 13.0 mm respectively. The centroid blends; A0.333 / B0.333 / C0.333; A0.667 / B0.166 / C0.166; A0.166 / B0.667 / C0.166; A0.166 / B0.166 / C0.667; A0.666 / B0.166 / C0.166 exhibited some growth inhibition on *S. aureus*but not comparable with the pure blends of the extracts. The results shared some comparison with the Levofloxacin, which showed a mean inhibition zones of 22.5 mm in all the trials. Synergism was observed in binary blends AB, BC. The phenomenon could be promoted by molecular interactions of chemical compounds in the plants, part extracts. The observation was in agreement with that reported by Lawal *et al.,* (2013) on ethanol extract of orange peel on *S. aureus*. The model of the design was significant ($p=0.0172$, $R^2= 0.8350$) and mean inhibition zone of 14.11 mm. Figure 1 shows that *C. sinensis* exhibited the highest potency of antibiotic activity against the test microorgnsism, from the figure also, the potency of *C. sinensis* reduced as its proportion in the blends reduced. The contour plot, (figure 2) further explained the variability of antibiotic activity of each plant part extract as the proportion varied. Equation 2 supports the practical significance of the model, showing the contribution of each

blend to the model. The growth inhibition activity of blends of the extract showed promising potential in the utilization of the plants to combat activities of pathogenic microorganisms.

TABLE 2
PROPORTIONS OF BLENDS OF EXTRACTS OF *MANGIFERA INDICA* (A), *GONGRONEMA LATIFOLIUM* (B), AND *CITRUS SINENSIS PEEL* (C) RESPONSES ON *STAPHYLOCOCCUS AUREUS*

	Composites			Coordinates	IZ (mm)	IZL (mm)
Runs	A	B	C			
11	1	0	0	1.00, 0.00, 0.00	13.0	22.5
9	0.167	0.167	0.667	0.16, .167, .667	11.5	22.5
13	0.00	0.00	1.00	0.00, 0.00, 1.00	19.5	22.5
10	0.333	0.333	0.333	0.333, 0.333, 0.333	12.0	22.5
7	0.667	0.167	0.167	0.667, 0.167, 0.167	13.0	22.5
2	0.50	0.50	0.00	0..50, 0.50, 0.00	13.0	22.5
5	0.00	0.50	0.50	0.00, 0.50, 0.50	19.0	22.5
12	0.00	1.00	0.00	0.00, 1.00, 0.00	13.0	22.5
6	1.00	0.00	0.00	1.00, 0.00, 0.00	13.5	22.5
1	0.50	0.50	0.00	0.50, 0.50, 0.00	11.5	22.5
8	0.167	0.667	0.167	0.167, 0.667, 0.167	12.5	22.5
4	0.00	1.00	0.00	0.00, 1.00, 0.00	12.0	22.5
3	0.50	0.00	0.50	0.50, 0.00, 0.50	15.0	22.5
14	0.50	0.50	0.00	0.50, 0.50, 0.00	13.5	22.5

IZ (mm) = inhibition zone in millimeters, IZL (mm) = inhibition zone of Levofloxacin in millimeters, coordinates are points on the edges of experimental space, Run = randomized experimental runs.

The activities of the test substances could be similar if the concentration of the plant extracts blends were assured, the plant blends would be expected to be more potent because they contain many more chemical compounds than the single component of the standard.

Inhibition zone =

13.28*flower of *M. indica* + 12.07**G. latifolium* leaves + 18.72**C. sinensis peel* + 2.71**M. indica* leaves**G. latifolium* - 5.96**M. indica* leaves**C. sinensis* peel + 10.74**G. latifolium* leaves**C. sinensis* peel -123.38**M. indica* leaves**G. latifolium* leaves**C. sinensis* peel (2)

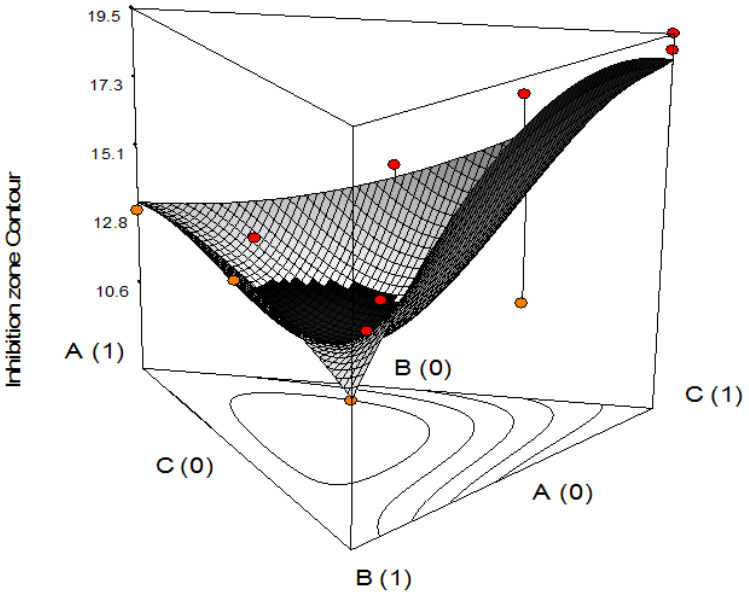

FIGURE 1: Response surface plot of inhibition zones (mm) against concentration of composites plant materials

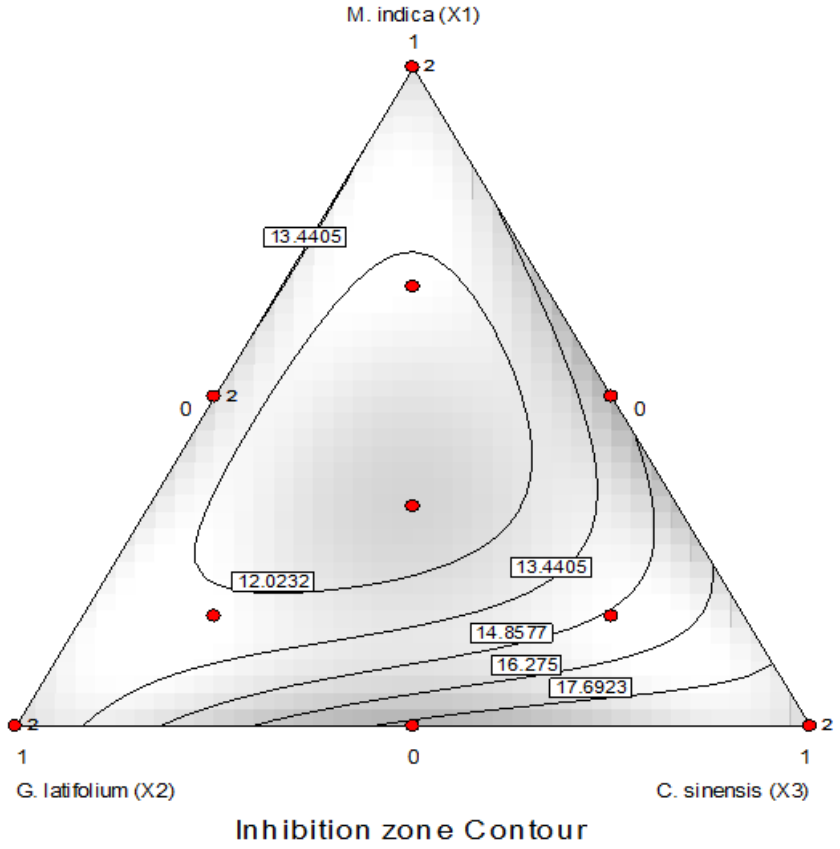

FIGURE 2: Contour plot of inhibition zones of ethanol extracts of flower *Mangifera indica, Gongronema latifolium* leaves and *Citrus sinensis* peel blends on *Staphylococcus aureus*

Growth inhibition action of blends of ethanolic extracts of flower of *Mangifera indica*, *Gongronema latifolium* and *Citrus sinensis* peel on *Staphylococcus aureus* showed good potential of antibiotic activity of the plants and good comparability of the blends with that of the Levofloxacin antibiotic. The lower inhibition zone exhibited by the blend could be attributed to lack of standardization of the concentration to meet the specificity of the test organism. Levofloxacin is a pure substance and organism specific plant extracts are broad spectrum against a wide range of microorganism.

IV. CONCLUSION

The constituent of the plants extracts could be a source of lead compounds for the development of potent broad spectrum antibiotics. The study revealed synergy and antagonism of activity as the proportion of the ethanolic extracts of plants plant varied, this could guide the combination of the component extract for optimal activity. More work should be done on the microbial activity of other parts of the plants in addition to effect of solvents and extraction time.

REFERENCES

[1] Bondari, K. (1999). Interaction in entomolong: multiple comparisons and statistical interactions in entomological experimentation. *Journal of Entomological Science.* **34:** 57-71.

[2] Cech, N. B., Junio, H. A., Ackermann, L. W., Kavanaugh, J. S. and Horswill, A. R.. (2013) Quorum quenching and antimicrobial activity of Goldenseal (*Hydrastiscanadensis*) against Methicillin-Resistant Staphylococcus aureus (MRSA). Planta Medica, 78(14):1556-1561.

[3] Cheesbrough, M.(2003). Medical laboratory Manual on tropical health technology.(low priced). England: Orington Cambridgeshire. Pp.20-23.

[4] Chinedu, I. and Friday, O. U. (2015). Phytochemical Analysis of *Gongronema latifolium* Benth Leaf Using Gas Chromatographic Flame Ionization Detector. International Journal of Chemical and Biomolecular Science, 1(2): 60-68.

[5] Heatley,N. G., Florey,H.W., Chain,E. ,Jennings,. A and Sanders, A.G. and Abraham E. P. (2009). Method for measuring the sensitivity of micro-organisms to antibiotics. Oxford University Press London **10** (1): 200-204

[6] Lawal, D. I., Bala, J. A., Aliyu, S. Y. and Huguma, M. A. (2013). Phytochemical Screening and In Vitro Antibacterial Studies of the Ethanolic Extract of *Citrus sinensis* (Linn.) Peel against Some Clinical Bacterial Isolates. *International Journal of Innovation and Applied Studies*. 2 (2): 138-145.

[7] Ogston, A.On Abscesses. Rev Infect Dis. 1984; 6:122–128. [PubMed: 6369479]

[8] Radulović, N. S. 1., Blagojević, P. D., Stojanović-Radić, Z. Z., Stojanović, N. M.. (2013). Antimicrobial plant metabolites: structural diversity and mechanism of action. Curr Med Chem. 2013; 20(7):932-52.

[9] Ugochukwu, N. H and Babady, N. E., (2002).Antioxidant effects of *Gongronema latifolium*in hepatocytes insulin dependent diabetes Filoterapia, 73 (7-8), 612 -618.

[10] Vameem,M.A., Nadim,H., Akhtar,N. Iram,Jared, I. and Hameed,A.(2009). Antibiotics Susceptibility profile of Methicillin-Resistant *Staphylococcus* isolated from nasal sample of hospitalized patient. *Journal of Clinical microbiology*. **25**(5): 225-334.

[11] Welz, A. N., Agnes E. and Klaus M. (2018).Why people use herbal medicine: insights from a focus-group study in Germany. BMC Complementary and Alternative Medicine. 18:92. https://doi.org/10.1186/s12906-018-2160-6.