

## Research Article

# Studies on synergistic effect of *Mormodica charantia*, *Ocimum sanctum* and *Prosopis juliflora* leaves extract against some clinical isolates of bacteria

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## Abstract

**Objective:** Aim of present study synergistic antibacterial effect of *Mormodica charantia*, *Ocimum sanctum* and *Prosopis juliflora*. **Material and methods:** Aqueous and methanol extracts of leaves of three important medicinal plant species, *Mormodica charantia*, *Ocimum sanctum* and *Prosopis juliflora* has been tested individually and in combination for their antibacterial activity against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *klebsiella pneumoniae* and *Streptococcus pyogenes* using agar well diffusion method. **Results and conclusion:** Results showed higher antibacterial activity in combination of extracts of the medicinal plants studied. The methanolic leaf extracts of *P. juliflora* and aqueous leaf extracts of *M. charantia* showed 2.1 and 1.9 cm zone of inhibition (ZI), respectively against *S. aureus* and *S. pyogenes* while tested individually. Whereas, the combination of methanolic leaf extracts of *M. charantia* + *P. juliflora* (1:2) and *M. charantia* + *O. Sanctum* (2:1) showed 2.3 cm ZI against *S. aureus* and *S. Pyogenes* respectively. Similarly, the highest antibacterial activity of 2.5 cm ZI was observed against *S. Pyogenes* in combination of methanolic leaf extracts (2:1:1) of all the three plants. This study clearly demonstrates the synergistic activity of plant extracts against different bacteria.

**Keywords:** *Mormodica charantia*, *Ocimum sanctum*, *Prosopis juliflora*, synergistic, zone of inhibition

## Introduction

Infectious diseases caused by bacteria and fungi affect millions of people worldwide, throughout the history of mankind, infectious diseases have remained a major cause of death and disability. Today infectious diseases account for one-third of all deaths in the world; the World Health Organization estimates that nearly 50,000 people die each day throughout the world from infectious diseases. Medicinal plants are the resources of promising drugs for many diseases. The use of plants and herb extract in the treatment of human ailments is a very ancient art and World-over, the scientists are conducting research,

exploring the possibilities of utilizing medicinal plants for their phytochemicals, antioxidant, anticancer and antimicrobial (Ayyanar and Ignacimuthu, 2008; Agbafor et al., 2011; Roy et al., 2011; Vinoth et al., 2011; Mishra and Tripathi, 2011) or finding out pharmacologically active compounds from medicinal plants for their possible use in traditional medicine (Nneamaka, 1991).

World-over, the scientists are exploring the possibilities of utilizing or finding out pharmacologically active compounds from medicinal plants. For example, screening of medicinal plants for their phytochemicals, antioxidant, anticancer and antimicrobial activities is the prime concern for finding out an effective phytochemically active principle (Ayyanar and Ignacimuthu, 2008; Agbafor et al., 2011; Roy et al., 2011; Vinoth et al., 2011; Mishra and Tripathi, 2011). Majority of these kind of research are concerned with the study of the solvent extracts of plant

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parts and testing them individually for selective pharmacological activities, such as antibacterial (Mishra and Mishra, 2011), hypoglycemic and hypolipidemic activities (Sharma et al., 2007). However their activity in combine form is unavailable. Recent studies show that some plants extract in combination of two or more exhibit effective antimicrobial activity against a wide range of microorganisms including drug resistant bacteria (Prakash et al., 2006a, b; Karmegam et al., 2008). Hence three medicinal plants namely, *Mormodica charantia*, *Ocimum sanctum* and *Prosopis juliflora* were selected in this to test their synergistic effect against some clinical isolate of bacteria. *Momordica charantia* leaves are used in treatment of menstrual troubles, burning sensation, constipation, fever (malaria), colic, infections, worms and parasites, as an emmenagogue, measles, hepatitis and helminthiasis (Kumar et al., 2010). Fresh leaves essential oil had shown more antibacterial properties compared to dried leaves essential oil of *Ocimum sanctum* and in case of fungus the property is just the reverse (Mondal et al., 2007). various solvent extracts of different parts of *P. juliflora* are reported to possess antibacterial activity by many researchers (Sathiya and Muthuchelian, 2008; Seetha Lakshmi et al., 2010; Navya et al., 2011; Singh et al., 2011; Hari Prasad et al., 2011).

## Material and methods

### Collection of plant material

The medicinal plants used in this study were *Mormodica charantia*, *Ocimum sanctum* and *Prosopis juliflora*. The leaves from these plants were collected from Utai environ and rinsed twice with distilled water and dried under shade

### Extraction of plant materials

**Methanolic extraction:** The dried plant material were powdered and extracted with methanol using soxhlet apparatus method. All the extracts were poured into sterile dry petriplates and the solvent was evaporated. The sediments were scrapped off, weighed and dissolved in DMSO (Shihabudeen et al., 2010).

**Aqueous extraction:** For aqueous extraction, 150 g of air-dried powder of each plant leaves was placed in 600 ml distilled water and boiled for 6 h. At 2 h intervals, it was filtered through eight layers of muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected and concentrated to make the final volume one-fourth of the original volume. A greasy final material (crude aqueous-leaf extract) obtained for each plant was transferred to screw cap bottles, labeled and stored under refrigerated (4°C) condition until use (Shihabudeen et al., 2010).

### Preparation of various concentrations of the extracts

Four grams (4000 mg) of each plants extracts were reconstituted individually in 20ml dimethyl sulphoxide (DMSO) to obtain 20

ml of a 200mg/ml stock solution. A portion of the 200 mg/ml solution was diluted with an equal volume of DMSO to obtain a 100 mg/ml solution. The double dilution procedure was continued to obtain lower concentrations of the extracts (50 mg/ml)

### Preparation of inoculum

Three loopful of the 24-hour-old bacterial cultures was transferred into 10 ml of freshly prepared nutrient broth, incubated at 37°C for 24 hours and standardize to 0.5 McFarland turbidity standards to obtain the desired cell density of  $1.5 \times 10^8$  colony forming unit (cfu)/ml. The 0.5 McFarland turbidity standard was prepared by adding 0.05 ml of 1% barium chloride dihydrate ( $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ ), with 9.95 ml of 1% sulfuric acid ( $\text{H}_2\text{SO}_4$ ).

### Bacterial susceptibility testing

Six bacterial species, *Staphylococcus aureus*, *klebsielle pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Stretococcus pyogens* and *Stretococcus pneumoniae* were isolated and used for the antibacterial activity test. The organisms were maintained on agar slope at 4°C and sub-cultured for 24 hours before use. These organisms were originally obtained from urine, stool and wound swab sample of the patient attended Muhammad Abdullahi Wasse Specialist Hospital, Kano. The *in vitro* synergistic activity of aqueous and methanolic leaves extracts of these plant were performed using agar well diffusion assay on Mueller Hinton Agar (MHA) according to the Obeidat et al. (2012).

### Agar well diffusion method

Standardized inoculums suspension of the bacteria was swabbed uniformly on Mueller-Hinton Agar (MHA) and the inoculums were allowed to dry for 5 minutes. A sterile cork borer (6mm in diameter) was used to punch four well along the sides of the Petri dish seeded. Zero point one (0.1) ml of the dilution series of the plant extracts were added onto the first and second well respectively while one hundred (100) µl of Gentamicin at concentration of 200 µg/ml and blank Dimethyl Sulfoxide (DMSO) were added in third and fourth well as positive and negative control respectively. The inoculated Petri dishes was allowed to stand on the bench for few minutes for the extract to diffuse into the agar and thereafter incubated at 37°C for 24 hours (Thakur et al., 2012).

### Synergistic effect of the plant extract

To identify the synergistic effect between the plants extract, the crude leaf extracts was mixed in equal (1:1) and different (2:1 and 1:2) ratio in combination of two extracts. For the combined effect of the three plant the ratio were (1:1:1,

1:1:2, 1:2:1 and 2:1:1). These ratios were tested against the test organism. For comparison, individual extracts (aqueous and methanol) were tested for antibacterial activity as described above.

### Results and discussion

In this study, three commonly available medicinal plants used by traditional users in Nigeria were tested against six different bacteria. The result of antibacterial susceptibility testing showed that all the bacteria, *S. aureus*, *S. pyogens*, *P. aeruginosa*, *E. coli*, *K. pneumoniae* and *S. pneumoniae* were highly susceptible to

gentamicin with average diameter zone of inhibitions (ZI) of 17, 15, 16, 15, 19 and 17 mm, respectively (Table 1). Aqueous and methanolic leaf extracts when tested individually for their antibacterial activity, showed various degrees of activity (Table 1 and Table 2). The methanolic leaf extracts of *M. charantia* showed comparatively a high degree of activity followed by *P. juliflora* and *O. sanctum*. The diameter of ZI was 21 mm for *M. charantia* methanolic extract against *S. pyogens*. The least activity was observed against *P. aeruginosa* showing 7 mm and 8 mm by

**Table 1.** Antibacterial activity of methanol extracts of different plant extracts

S. No	Organisms	Zone of inhibition in mm					
		<i>Mormodica charantia</i>		<i>Ocimum sanctum</i>		<i>Prosopis juliflora</i>	
		100 mg/ml	50 mg/ml	100 mg/m	50 mg/ml	100 mg/ml	50 mg/ml
1	<i>S. aureus</i>	17	16	15	14	21	19
2	<i>K. pneumoniae</i>	19	18	15	13	20	19
3	<i>E. coli.</i>	20	18	12	11	18	17
4	<i>P. aeruginosa</i>	18	17	10	7	17	16
5	<i>S. pneumoniae</i>	19	17	11	10	18	16
6	<i>S. pyogens</i>	21	21	10	8	19	16

**Table 2.** Antibacterial activity of aqueous extracts of different plant extracts

S. No	Organisms	Zone of inhibition in mm					
		<i>Mormodica charantia</i>		<i>Ocimum sanctum</i>		<i>Prosopis juliflora</i>	
		100 mg/ml	50 mg/ml	100 mg/m	50 mg/ml	100mg/ml	50 mg/ml
1	<i>S. aureus</i>	17	15	15	14	17	15
2	<i>K. pneumoniae</i>	19	16	14	12	16	15
3	<i>E. coli.</i>	18	17	13	11	18	16
4	<i>P. aeruginosa</i>	15	14	10	8	14	11
5	<i>S. pneumoniae</i>	18	16	11	10	15	14
6	<i>S. pyogens</i>	19	17	10	9	17	15

Key: A=*M. charantia*, B=*O. sanctum* C=*P. juliflora*.

**Table 3.** Synergistic activity of methanolic extract of selected plant leaves in combination of two against bacteria

S. No.	Organisms	Zone of inhibition in mm								
		A+B			A+C			B+C		
		1:1	2:1	1:2	1:1	2:1	1:2	1:1	2:1	1:2
1	<i>S. aureus</i>	17	18	16	21	20	23	20	18	22
2	<i>K. pneumoniae</i>	19	20	18	20	20	21	18	16	20
3	<i>E. coli.</i>	20	22	18	20	20	18	17	16	19
4	<i>P. aeruginosa</i>	18	19	17	18	18	17	14	12	18
5	<i>S. pneumoniae</i>	19	21	17	19	20	18	15	13	19
6	<i>S. pyogens</i>	20	23	17	21	22	19	19	17	20

methanolic and aqueous leaf extract of *O. sanctum* at 50 mg/ml (Table 1 and Table 2).

The present investigation fall in line with the studies on screening the activity of the extracts from these plants parts alone obtained by many workers. Evaluation of antibacterial activity of *Prosopis juliflora* (SW.) DC. leaves (Thakur et al., 20014), antifungal and phytochemical analysis of selected medicinal plants (Sukirtha and Lali Growther, 2012), In vitro anti-bacterial activity of *Prosopis juliflora* leaf extract against pathogenic bacteria (Ali et al., 2017), extraction of active components of *Mormodica charantia* (cucurbitaceae) for medicinal use (Abalaka et al., 2009) and in vitro antimicrobial activity of fruit and leaf crude extracts of *Momordica charantia* (Mwambete, 2009) were reported.

The antibacterial activities of extracts in combination of two plants showed different degrees of ZI as shown in table 3 and 4. The diameter 22mm ZI was observed in the following methanolic leaf extract combinations: *A+B*, *A+C* and *B+C*. at 100mg/ml followed by other aqueous combinations. A ZI of 21mm was observed in aqueous leaf extract combination of *A+B* and *A+C* against *S. pyogenes* (Table 4). The highest ZI of 23mm against *S. aureus* and *S.*

*pyogenes* were observed in methanolic leaf extract combination of *A+C* and *A+B* respectively, (Table 3). The combination of methanolic extracts of all the three plants in (2:1:1 proportion) showed a maximum of 25mm ZI against *S. pyogenes* followed by 24mm ZI against *S. aureus* (Table 5). These findings are in coherence with the study reported earlier on synergistic activity of six different plants against pathogenic bacteria by Karmegam et al. (2008). Synergistic activity of aqueous and ethanolic extracts of selected plant leaves, in combination of two, three, four, five and six against test organisms ranged from 0-28 mm zone of inhibition. The highest ZI of 28 mm was observed against *S. aureus* in ethanolic leaf extract combinations of *Balanites aegyptiaca* +*Lobelia nicotianaefolia* (Karmegam et al., 2008). Similarly, Prakash et al. (2006b) reported that the ethanolic leaf extracts of *Catharanthus roseus*, *Lawsonia inermis* and *Chrysanthemum odoratum* showed least activity against methicillin resistant *Staphylococcus aureus* (MRSA) when used individually. Whereas, the combination of these three plant-extracts exerted a higher activity of 26 mm zone of inhibition followed by *C.*

**Table 4.** Synergistic activity of water extract of selected plant leaves in combination of two against bacteria

S. No	Organisms	Zone of inhibition in mm								
		A+B			A+C			B+C		
		1:1	2:1	1:2	1:1	2:1	1:2	1:1	2:1	1:2
1	<i>S. aureus</i>	17	18	16	17	17	17	16	15	18
2	<i>K. pneumonia</i>	18	20	17	19	20	18	15	14	17
3	<i>E. coli.</i>	17	19	15	18	19	18	17	15	19
4	<i>P. aeruginosa</i>	14	16	13	15	16	14	12	11	15
5	<i>S. pneumonia</i>	17	19	14	18	19	17	12	11	16
6	<i>S. pyogenes</i>	18	21	13	19	21	20	13	12	18

Key: A=*M. charantia*, B=*O. danctum* C=*P. juliflora*.

**Table 5.** Synergistic activity of methanolic extract of selected plant leaves in combination of three against bacteria

S. No.	Organisms	Zone of inhibition in mm			
		A+B+C			
		1:1:1	1:1:2	1:2:1	2:1:1
1	<i>Staphylococcus aureus</i>	21	24	17	20
2	<i>Klebsiella pneumonia</i>	20	22	17	20
3	<i>Escherichia coli.</i>	20	19	15	22
4	<i>Pseudomonas aeruginosa</i>	18	18	14	20
5	<i>Streptococcus pneumonia</i>	19	20	15	21
6	<i>Streptococcus pyogenes</i>	21	22	18	25

Key: A=*M. charantia*, B=*O. danctum* C=*P. juliflora*.

**Table 6.** Synergistic activity of water extract of selected plant leaves in combination of three against bacteria

S. No	Organisms	Zone of inhibition in mm			
		A+B+C			
		1:1:1	1:1:2	1:2:1	2:1:1
1	<i>Staphylococcus aureus</i>	17	18	16	18
2	<i>Klebsiella pneumonia</i>	19	19	15	20
3	<i>Escherichia coli.</i>	18	20	14	20
4	<i>Pseudomonas aeruginosa</i>	15	17	12	16
5	<i>Streptococcus pneumonia</i>	18	17	13	20
6	<i>Streptococcus pyogens</i>	18	19	16	21

Key: A= *M. charantia*, B= *O. danctum* C= *P. juliflora*

**Table 7.** Antibacterial activity of the reference antibiotic against the isolated bacteria

S. No	Organisms	Zone of inhibition in mm	
		Gentamicin (200µg/ml)	DMSO
1	<i>Staphylococcus aureus</i>	17	-
2	<i>Klebsiella pneumonia</i>	19	-
3	<i>Escherichia coli.</i>	15	-
4	<i>Pseudomonas aeruginosa</i>	16	-
5	<i>Streptococcus pneumonia</i>	17	-
6	<i>Streptococcus pyogens</i>	15	-

*roseus* +*L. inermis* (23 mm) and *L. inermis* +*C. odoratum* (20 mm) extract combinations against MRSA. Natchimuthu et al. (2012) reported 30 mm ZI against *B. cereus* in combination of aqueous leaf extracts of four plant.

### Conclusion

The antibacterial activity of aqueous and methanolic leaf extracts of *Mormodica charantia*, *Ocimum sanctum* and *Prosopis juliflora* on the selected organisms showed higher inhibition zones when used in combination than that of the single plant extract alone. A ZI of 25mm was obtained against *S. pyogens* by the combination of methanolic extracts of all the three plants used in the study, indicating the high potential of combined use of plant extracts against pathogenic microorganisms. Thus, extract from different plant can be used in combinations against pathogenic bacteria as has been observed from our results and other works.

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### Conflicts of interest

We declared that we have no conflict interests

### Ethical Clearance

An approval of the study was obtained from the research ethics committee of Kano State Ministry of Health, Nigeria.

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