

**Research Article****Evaluation of antibacterial activity of *Momordica charantia*, *Ocimum sanctum* and *Prosopis juliflora* against some selected bacteria**Abubakar Dabo Dalhat<sup>1\*</sup>, Dalha Wada Taura<sup>2</sup>, Dambazau Ado Musa<sup>2</sup>, Sadiya Suleiman Ayuba<sup>2</sup>, Musayyiba Shu'aibu<sup>2</sup>, Adam Uba Muhammad<sup>2</sup><sup>1</sup>Department of Biotechnology, Faculty of Life Science Mewar University, Gangrar, Chittorgarh (RAJ) India.<sup>2</sup>Department of Microbiology Faculty of Life Science Bayero University Kano PMB: 3011

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**Abstract**

**Background:** Medicinal plants have been used in traditional medicine practice since ancient time. Hundreds of chemical compounds which offer defence against insects, bacteria, fungi, diseases and herbivorous mammals are synthesised by these plant. **Objectives:** The objective of present study was to determine the antibacterial activity of some plants used by herbalist to treat wound and urinary tract infections. **Material and Methods:** Aqueous and methanol extracts of leaves of three important medicinal plant species, *Momordica charantia*, *Ocimum sanctum* and *Prosopis juliflora* has been tested individually for their antibacterial activity against *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *klebsiella pneumoniae* and *Streptococcus pyogenes* isolated from wound and urine using agar well diffusion method. **Results and conclusion:** On the basis of results obtained from the preliminary antibacterial activity, aqueous and methanolic leaf extracts while tested individually showed various degrees of activity. Methanolic extract was found to be the most active in respect to the wide range of inhibition zones against all test bacteria. The methanolic leaves extracts of *M. charantia* showed comparatively a high degree of activity followed by *P. juliflora* and *O. sanctum*. The diameter of ZI was 21mm for methanolic extract of *M. charantia* extract against *S. pyogenes*. The least activity was observed against *P. aeruginosa* showing 7 mm and 8 mm by methanolic and aqueous leaf extract of *O. sanctum* at 50 mg/ml.

**Keywords:** *Momordica charantia*, *Ocimum sanctum*, *Prosopis juliflora*. Zone of inhibition, antibacterial

**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. Nowadays, microbial infections are major public health problems in the developed and developing countries. Antibiotics are used to treat these infections. As a result of indiscriminate use of commercial antibiotics, the incidence of multiple antibiotic resistance in human pathogens is increasing (Jeyachandran and Mahesh, 2007). The use of plants and herb extract in the treatment of human ailments is a very ancient art, a practice that has been passed on for generations and scientists in Africa and other developing countries and other are conducting

research into local plants abundant in the continent for their possible use in traditional medicine (Nneamaka, 1991). Despite being the resources of promising drugs for many diseases the biological and pharmacological properties of many plants are still unknown (Ayyanar and Ignacimuthu, 2008; Agbafor et al., 2011; Roy et al., 2011; Vinoth et al., 2011; Mishra and Tripathi, 2011).

***Momordica charantia*:** *Momordica charantia* Karela contains an array of biologically active plant chemicals including triterpens, proteins, steroids, alkaloids, saponins, flavonoids and acids due to which plant possesses anti-fungal, anti-bacterial, anti-parasitic, anti-viral, anti-fertility, anti-tumorous, hypoglycemic and anti-carcinogenic properties (Beloin et al., 2005; Grover and Yadav, 2004).

***Prosopis juliflora*:** This herb is well-known in the folkloric system of medicine because of its ethnobotanical importance. The high-potential activity of crude extracts of this plant compared to selective antibiotics lead to evaluate the new antimicrobial agents to fight against the drug-resistant

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pathogens (Navya et al., 2011). Moreover, the leaves were found to be effective in reduction or eradication of the phytopathogens like *Xanthomonas campestris* and *Agrobacterium rhizogenes* in an eco-friendly way (Sheikh et al., 2012).

**Ocimum sanctum:** *Tulsi* "Queen of herbs" is described as sacred and medicinal plant in ancient literature. It is an important symbol of the Hindu religious tradition.

## Materials and methods

### Preparation of crude leaf extracts

**Solvent extraction:** The leaves of the plants were washed with tap water, blotted with filter paper and spread over newspaper for air drying under shade. After complete dryness, the leaves of each plant were powdered using a mixer grinder. A known quantity of leaf powder (100g) of each plant was taken in a 500 ml conical flask and added with 300 ml of methanol (95%). The methanol-leaf powder mixtures were kept at room temperature for 48 h and rapidly stirred using glass rod every 8 h. After 48 h, the extract of each plant was filtered through Whatman No. 1 filter paper to exclude the leaf powder. Then each filtrate was concentrated using vacuum evaporator. A greasy final material (crude methanolic-leaf extract) obtained for each plant was transferred to screw cap bottles, labelled and stored under refrigerated (4°C) condition till use (Shihabudeen et al., 2010).

**Aqueous extraction:** For aqueous extraction, 50 g of air-dried powder of each plant leaves was placed in 300 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, labelled and stored under refrigerated (4°C) condition until use (Shihabudeen et al., 2010).

### Screening of the phytochemical constituents

All the plants extracts were subjected to phytochemical analysis to determine some of its secondary metabolites such as saponins, tannins, alkaloids, terpenoid, flavonoid, phenol and cardiac glycosides in the plants extract. The presence of alkaloids was detected with Dragendorff's reagent (Firdouse and Alam, 2011) and Wagner's reagent (Lalitha and Jayanthi, 2012), whereas Flavonoids by shinoda test and ferric chloride test (Subash et al., 2013), and phenol with phoshomolybdic acid reagent (Kumar et al., 2007). Terpenoids was identified with chloroform and sulphuric acid (Edeoga et al., 2005) and saponins by froth test (Parekh and Chanda, 2007). Tannins were spotted with ferric chloride test (Kumar et al., 2007; Parekh and Chanda, 2007) and lead acetate test whereas anthraquinones by Borntrager's test and cardiac glycosides by Keller-Kiliani test and Legal test (Singh, 2012; De et al., 2010).

## Bacterial susceptibility testing

### Preparation of various concentrations of extracts

One gram (1000mg) of each plant extracts were reconstituted individually in 20ml dimethyl sulphoxide (DMSO) to obtain 20ml of a 100mg/ml solution. A portion of the 100mg/ml solution was diluted with an equal volume of DMSO to obtain a 50mg/ml solution. The double dilution procedure was continued to obtain lower concentrations of the extracts (25mg/ml, 12.5mg/ml and 6.25mg/ml).

### Antibacterial assay

The *in vitro* antibacterial activity of aqueous and methanolic leaves extracts of these plants were performed using agar well diffusion assay on Mueller Hinton Agar (MHA) according to the Obeidat et al. (2012).

### Agar well diffusion method

After solidification of MHA, 0.1ml of the standardized inoculums suspension of the bacteria were 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. 0.1ml of the dilution series of the plant extracts were added onto the wells on the seeded medium. 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. One hundred micro litre (100µl) of Gentamicin at concentration of 500 µg/ml was used as an antibiotics reference standard and blank Dimethyl Sulfoxide (DMSO) were used in separate plate as positive and negative control respectively. The experiment was performed in triplicate for each bacterial strain and the antibacterial activity of each extract was expressed in terms of the mean of the diameter of zone of growth inhibition in mm (Thakur et al., 2012).

### Minimum Inhibitory Concentration (MIC)

**Broth dilution method:** The MIC was defined as lowest concentration able to kill any microbe. Dilutions of the plant extract were prepared in sterile nutrient broth to get a final concentration of 3.125mg, 6.25mg, 12.5mg, 25mg, 50mg and 100mg/ml respectively (Mahfuzul Hoque et al., 2007). To each of these dilutions, a loop full of culture adjusted to 0.5 McFarland standard, was inoculated and all the tubes were incubated at 37°C for 24 hrs. The tube with no extract was served as the growth/negative control (no agent added), while the tube containing the only extract and broth was served as broth/positive control (no bacteria). The turbidity of the tubs indicates the amount of bacterial growth. The tube with no antibacterial agent was the most turbid. As the concentration increases, the turbidity

decreases until the MIC (minimum concentration showing no detectable bacterial growth) is reached (Akinyemi et al., 2005).

### Minimum bactericidal concentration (MBC)

Sterile Mueller – Hinton agar plates were inoculated with the sample from the MIC tubes that show no visible bacterial growth, the lowest concentration in which no growth occurred on the medium was recorded as the MBC.

### Results and Discussion

The percentage yields of the crude extracts after extraction method were calculated (table 4). The percentage yield of methanol extract was the highest with *P. juliflora* (22.1%) and *M. charantia* (17.46%), while for *O. sanctum* the water extract gives the highest yield (15.6%) (Table 1). This result was fairly in coherence with result of Abalaka et al. (2009) in which aqueous extract of *M. charantia* yielded 20% extract and 25.25%, *P. juliflora*, 12.76%, *M. charantia* by methanolic extract (Thakur et al., 2012).

Phytochemical analysis of all the methanolic and aqueous crude extracts of the plants were analysed for the presence of secondary metabolites by specific reactions and identified by observing the intensity of colour developed and/or the

appearance of precipitation in the reactions (Table 2). The glycosides were detected in all the extract tested. Saponins are only present in methanolic extract of *M. charantia*. Steroids were not detected in *P. juliflora* whereas flavonoids reported its presence only in *O. sanctum* and *P. juliflora*. Anthraquinones and saponins were not detected in all *P. juliflora* extracts as confirmed by the absence of pink-violet/ red colour and frothing, respectively. Phytochemical analysis studies of these plants have been reported earlier and different bioactive compounds have been identified. In a recent study, phytochemical analysis of different parts of *P. juliflora* extracts revealed distinct classes of secondary metabolites namely, tannins, phenolics, flavonoids, alkaloids, triterpenes and steroids (Singh, 2012). *M. charantia* has been reported to contain alkaloids, tannins, glycosides and steroids (Abalaka et al., 2009).

On the basis of results obtained from the preliminary antibacterial activity it was found that the methanolic extract gave a higher percentage yield of crude extracts and it was considered to be the most active in respect to the wide range of inhibition zones against all test bacteria and presence of bioactive phytochemical compounds (Table 3). Additionally,

**Table 1.** Percentage yields of leaf extracts of *M. charantia*, *O. sanctum* and *P. juliflora*

Extracts	Weight of Starting Material (g)	Weight of Dry Extract (g)	Percentage yield (%)
MC <sub>MX</sub>	100	17.46	17.46
MC <sub>AX</sub>	50	8.12	16.24
OS <sub>MX</sub>	100	15.00	15.0
OS <sub>AX</sub>	50	7.80	15.6
PJ <sub>MX</sub>	100	22.10	22.1
PJ <sub>AX</sub>	50	9.40	18.8

MC<sub>MX</sub> = *M. charantia* methanolic extract, MC<sub>AX</sub> = *M. charantia* aqueous extract, OS<sub>MX</sub> = *O. sanctum* methanolic extract, OS<sub>AX</sub> = *O. sanctum* aqueous extract, PJ<sub>MX</sub> = *P. juliflora* methanolic extract and PJ<sub>AX</sub> = *P. juliflora* aqueous extract

**Table 2.** Results of phytochemical screening of the extracts

Plant chemical constituents	<i>M. charantia</i>		<i>O. sanctum</i>		<i>P. juliflora</i>	
	Methanolic	Aqueous	Methanolic	Aqueous	Methanolic	Aqueous
Saponins	-	-	+	-	-	-
Tannins	+	+	-	+	+	+
Alkaloids	+	+	-	+	+	-
Flavonoids	-	-	+	+	+	+
Terpenoid	+	+	+	+	-	+
Steroids	+	+	+	+	-	-
Anthraquinone	+	-	+	-	-	-
Glycosides	+	+	+	+	+	+
Phenol	+	-	+	+	+	+

+ = Presence, - = Absence

among all the crude extracts used for phytochemical screening, most of the chemical phytoconstituents were found to be present in an appreciable amount in the methanolic extract (Table 1). Hence, extraction using methanol as a suitable solvent was selected for the mass extraction of crude phytoconstituents from these plants.

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. pyogenes*, *P. aeruginosa*, *E. coli*, *K. pneumoniae* and *S. pneumoniae* were susceptible to gentamicin with average diameter zone of inhibitions (ZI) of 17, 15, 16, 15, 19 and 17 mm, respectively (Table 5). Aqueous and methanolic leaf extracts when tested showed various degrees of activity (Table 3 and table 4). 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 21mm for methanolic of *M. charantia* extract against *S. pyogenes*. The least activity was observed against *P. aeruginosa* showing 7 mm and 8 mm by methanolic and aqueous leaf extract

of *O. sanctum* at 50mg/ml (Table 3 and table 4).

The minimum inhibitory concentration of all the six crude plants extract (three methanolic, three aqueous) ranged from 3.125 to 50 mg/ml, thus showing the effectiveness of the plants against the tested bacteria. The minimum bactericidal concentration of *Momordica charantia* ranged from 6.25mg to 25mg/ml. The Minimum bactericidal concentration is low, thus showing good antibacterial activity against the tested organisms. The MIC and MBC of all the extracts were shown in table 6 and 7.

*Prosopis juliflora* is well-known in the folkloric system of medicine because of its ethnobotanical importance. The plant has been reported to treat oral ailments like toothache (Hebbar et al., 2004). The leaves were used against asthma, bronchitis, conjunctivitis (Agra et al., 2008) as well as against skin diseases, blood and venereal diseases and act as an insecticide (Senthilkumar et al., 2009). The crude extracts of various parts and purified chemical components have been found to possess antimicrobial, insecticidal and

**Table 3.** Antibacterial activity of methanol extracts of different plant extracts

Organisms	Zone of inhibition in mm											
	<i>Momordica charantia</i>				<i>Ocimum sanctum</i>				<i>Prosopis juliflora</i>			
	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5
	mg/ml	mg/ml	mg/ml	mg/ml	mg/m	mg/ml	mg/ml	mg/ml	mg/m	mg/ml	mg/ml	mg/ml
<i>S. aureus</i>	17	16	14	12	15	14	12	9	21	19	17	15
<i>K. pneumoniae</i>	19	18	16	15	15	13	11	9	20	19	17	16
<i>E. coli</i>	20	18	17	16	12	11	9	8	18	17	15	14
<i>P. aeruginosa</i>	18	17	15	12	10	7	-	-	17	16	14	11
<i>S. pneumoniae</i>	19	17	13	13	11	10	8	-	18	16	13	11
<i>S. pyogenes</i>	21	21	20	19	10	8	-	-	19	16	15	13

- = Resistance

**Table 4.** Antibacterial activity of aqueous extracts of different plant extracts

Organisms	Zone of inhibition in mm											
	<i>Momordica charantia</i>				<i>Ocimum sanctum</i>				<i>Prosopis juliflora</i>			
	100	50	25	12.5	100	50	25	12.5	100	50	25	12.5
	mg/ml	mg/ml	mg/ml	mg/ml	mg/m	mg/ml	mg/ml	mg/ml	mg/m	mg/ml	mg/ml	mg/ml
<i>S. aureus</i>	17	15	14	12	15	14	12	9	17	15	13	12
<i>K. pneumoniae</i>	19	16	12	11	14	12	10	9	16	15	12	11
<i>E. coli</i>	18	17	16	14	13	11	9	-	18	16	13	12
<i>P. aeruginosa</i>	15	14	12	10	10	8	-	-	14	11	9	8
<i>S. pneumoniae</i>	18	16	14	11	11	10	8	-	15	14	12	10
<i>S. pyogenes</i>	19	17	14	12	10	9	-	-	17	15	15	12

different pharmacological activities (Malik and Kalidhar, 2005). Moreover *Prosopis juliflora* was also reported to have antibacterial activity on *S. aureus*, *K. pneumoniae*, *E. coli* and *P. aeruginosa* (Sukirtha and Growther, 2012).

Antibacterial activity of the aqueous, alcoholic, chloroform extract and oil obtained from leaves of *Ocimum sanctum* were studied against *E. coli*, *P. aeruginosa*, *S. typhimurium* and *S. aureus*. Extract obtained from *O. sanctum* were observed equally effective against pathogenic gram-positive and gram-negative bacteria (Mishra and Mishra, 2011). Fresh leaves essential oil had shown more antibacterial properties compared

to dried leaves essential oil of *Tulsi* and in case of fungus the property is just the reverse (Mondal et al., 2007).

*Momordica charantia* extracts also appear to inhibit the growth of numerous gram-negative and gram-positive bacteria, including *Salmonella*, *E. coli*, *Shigella*, *Staphylococcus*, *Pseudomonas*, *Streptococcus*, *Streptobacillus*, and *H. pylori*, and parasitic organisms *E. histolytica* and *Plasmodium falciparum* (Khan and Omoloso, 1998). Abalka et al., (2009) also report the aqueous extract to have good activity against *S. aureus*, *E. coli* and *S. pyogenes*. He reported a zone of inhibition of 15mm, 18mm and 21mm against these organisms

**Table 5.** Antibacterial activity of the reference antibiotic against the isolated bacteria

Organisms	Zone of inhibition in mm	
	Gentamicin (200µg/ml)	DMSO
<i>Staphylococcus aureus</i>	17	-
<i>Klebsiella pneumoniae</i>	19	-
<i>Escherichia coli</i>	15	-
<i>Pseudomonas aeruginosa</i>	16	-
<i>Streptococcus pneumoniae</i>	17	-
<i>Streptococcus pyogenes</i>	15	-

**Table 6.** Minimum Inhibitory Concentration of the selected plant extracts

Organisms	Minimum Inhibitory Concentration(mg/ml)					
	<i>Momordica charantia</i>		<i>Ocimum sanctum</i>		<i>Prosopis juliflora</i>	
	Methanolic	Aqueous	Methanolic	Aqueous	Methanolic	Aqueous
<i>S. aureus</i>	12.5	12.5	25	25	6.25	12.5
<i>K. pneumoniae</i>	6.25	6.25	25	25	6.25	12.5
<i>E. coli</i>	6.25	12.5	50	25	12.5	12.5
<i>P. aeruginosa</i>	12.5	25	50	50	12.5	25
<i>S. pneumoniae</i>	12.5	12.5	50	50	12.5	25
<i>S. pyogenes</i>	3.125	6.25	50	50	6.25	12.5

**Table 7.** Minimum Bactericidal Concentration of the selected plant extracts

Organisms	Minimum Bactericidal Concentration(mg/ml)					
	<i>Momordica charantia</i>		<i>Ocimum sanctum</i>		<i>Prosopis juliflora</i>	
	Methanolic	Aqueous	Methanolic	Aqueous	Methanolic	Aqueous
<i>S. aureus</i>	12.5	12.5	50	50	6.25	25
<i>K. pneumoniae</i>	12.5	12.5	25	50	12.5	12.5
<i>E. coli</i>	12.5	25	50	25	25	25
<i>P. aeruginosa</i>	25	25	100	50	25	50
<i>S. pneumoniae</i>	25	25	50	100	25	50
<i>S. pyogenes</i>	6.25	12.5	100	50	12.5	25

respectively at a concentration of 1100mg/ml which is nearly very similar to our result.

### Conclusion

The presence the presence study an many others prove that these plant are good source of some many secondary metabolites which may be responsible for the antibacterial activity. The activity was found to be concentration and time dependent. The results of cytotoxicity showed that, the plant was weakly toxic. These findings therefore support the local use of these plants for the treatment of bacterial infections such as gastrointestinal, urinary tract and wound infection in Northern Nigeria.

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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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