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Research Article

Investigation of the antimicrobial potentials of some phytochemical extracts of leaf and stem bark of *Berlinia grandiflora* (Leguminoceae) *Caesalpinioidae* against pathogenic bacteria

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Background: *Berlinia grandiflora* is a tropical pant which grows in most countries of West and Central Africa. Its bark sap, stem bark and leaves are used locally by the Igbos of South Eastern Nigeria to treat various health conditions including microbial infections.

Objective: The objectives of our study was to investigate the antimicrobial potentials of its aqueous, methanol and butanol stem bark and leaves extracts against eight pathogenic bacteria.

Methodology: The effects of various concentrations of the extracts were determined against the pathogenic bacteria and the minimum inhibitory concentrations (MICs) were established using standard methods. The MIC evaluations established the susceptibility of the bacteria to the extracts and the zones of inhibition assays quantified the antibacterial activities of the extracts. Similar studies with ciprofloxacin hydrochloride as reference drug were undertaken.

Results: The results indicate that the phytochemical extracts of the plant parts possess appreciable antibacterial activity against selected species of pathogenic bacteria. *Staphylococcus aureus* showed the highest sensitivity to the extracts with the lowest MIC of 21 mg/ml and the largest zone of inhibition of 28.0 ± 0.01 mm with the methanol stem bark extract and 41.0 ± 0.11 mm for ciprofloxacin hydrochloride. *Serratia marcescens* had the highest MIC (140 mg/ml) with the butanol stem bark extract. The methanol stem bark and leaves extracts showed similar antibacterial activity against the isolates with MIC ranging from 50 mg/ml for most bacteria and 75 mg/ml for a few. The zones of inhibition ranged from 18.0 ± 0.10 mm to 28.0 ± 0.1 mm for the extracts and 12.0 ± 0.02 mm to 41.0 ± 0.11 mm for ciprofloxacin hydrochloride. The extracts exhibited appreciable antibacterial activity in the zones of inhibition assay against the pathogenic bacteria.

Conclusion: The study has shown that the phytochemical extracts of the plant parts have antibacterial activity against the pathogenic bacteria and has partially validated its ethnomedicinal use.

Key words: *Berlinia grandiflora*, phytochemical extracts, antimicrobial activity

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1. Introduction

Berlinia comprises about 20 species which are confined to tropical Africa with almost all the species found in West and Central Africa (Hutchison and Dalziel, 1963). *Berlinia grandiflora* is widespread in Guinea, Mali, Nigeria, Central Africa and Democratic Republic of Congo (Hutchison and Dalziel, 1963). It is a forest tree that is sometimes planted as ornamental tree and shade tree in villages and coffee plantations. It is occasionally browsed by livestock especially sheep and goats. The Igbos of South Eastern Nigeria refers to it in their local languages as abaa, dokar, rafi, and ububa while the Yorubas of the South West Nigeria refer to it as apado. Its bark sap is applied to sores and wounds, and bark decoctions are administered to treat haemorrhoids and liver complaints (Gill, 1992). The bark is used to ease labour during child birth and gastrointestinal disorders. A decoction of the leafy twigs is used as febrifuge and antiemetic while leafy decoctions are taken as tonic (Gill, 1992). Reports of its biological activities are scanty and include analgesic activity of stem bark extract (Asuzu et al, 1993), antihelminthic activity of stem bark (Enwerem et al, 2001), antihelminthic activity of stem bark and its active principle, betulinic acid (Enwerem et al, 2001).

In South Eastern Nigeria, the leaf and the stem bark extracts of the plant are used by traditional herbalists to treat microbial infections. However, this ethnomedicinal use of the plant parts has not been validated scientifically. This study investigated the antimicrobial potential of the aqueous, methanol and butanol extracts of the leaves and stem bark against pathogenic bacteria as part of our evaluation of its antimicrobial activity.

2. Materials and Methods

2.1 Drugs and chemicals

Ciprofloxacin hydrochloride used as the standard drug in the study was in safe conditions for use while the chemicals and solvents were of analytical grade. Ciprofloxacin hydrochloride, nutrient agar and nutrient broth were products of Sigma Aldrich Laborchemikallien, GmBH, Germany, butanol, Meck, Germany and methanol, (Scharlau Chemie S.A. Spain).

2.2 Clinical bacterial isolates

Eight pathogenic bacteria were used. These included *Staphylococcus aureus*, *Escherichia Coli*, *Alcaligenes faecalis*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Enterobacter aerogenes*, *Proteus vulgaris* and *Klebseilla pneumoniae*. They were obtained from the University of Benin Teaching Hospital Medical Microbiology Laboratory stock Unit. They had been isolated from samples of patients for clinical diagnosis of their infections. They were authenticated using standard morphological and biochemical assays (Cheesbrough, 2004) in the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Benin.

2.3 Plant materials

The fresh stem bark and leaves of the plant were collected in July, 2011 from a mature tree in Uli village, Ihiala Local Government Area, Anambra State, Nigeria. The preliminary identification was in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin. Identification and authentication was done at the Forestry Research Institute of Nigeria (FRIN), where a voucher specimen of the plant is deposited (Voucher Specimen no: FHI 108436).

2.4. Extraction of plant materials

The stem bark was carefully separated from the woody part, cut into small bits, shade dried and pulverized using a grinder (Lab. Mill serial NO. 4745, Christy and Norris Ltd, England) and stored in dry airtight glass jar. The leaves were shade dried, pulverized and stored in dry airtight glass jar and labelled accordingly. Each of the powdered plant materials (500 g) were macerated separately in 98% methanol (2 L) and butanol (2 L) in glass jars for 72 hours and were shaken intermittently throughout the period. The extracts were filtered separately. Another portion of each of the plant materials (500 g) were macerated separately in distilled water (2 L) for 24 hours and were shaken intermittently. The extracts were filtered separately. Each of the methanol filtrates and butanol were evaporated to dryness at reduced pressure using a rotary evaporator to obtain a dark brown residue (stem bark) and dark greenish residue (leaves) until a constant weight was obtained and the yield with reference to the powdered material in each case noted. Each of the aqueous filtrates was evaporated to dryness in an hot air oven set at 40 °C to obtain a dark brown residue (stem bark) and dark greenish residue (leaf) until a constant weight was obtained and the yield with reference to the powdered material in each case noted. The extracts obtained were stored in the refrigerator set at a temperature of –4 °C until when required for experiments reported in our study.

2.5. Antibacterial assay

Determination of the effects of various concentrations and minimum inhibitory concentrations (MICs) of the plant extracts against the pathogenic bacteria

The agar dilution method described by George and Robert (1996) was used to determine the effects of various concentrations of the extracts on the pathogenic bacteria in order to establish the minimum inhibitory concentrations (MICs) of the extracts. The minimum inhibitory concentrations of the extracts of stem bark and leaves were evaluated using different final concentrations in nutrient agar against *Staphy. aureus*, *E. Coli*, *A. faecalis*, *P. aeruginosa*, *S. marcescens*, *E. aerogenes, P. vulgaris* and *K. pneumoniae*. For the aqueous stem bark and leaf extracts the concentrations were as shown in **Table 1** and **2**, respectively (Supporting Information). The concentrations for the butanol stem bark extract and leaves extract concentrations were as shown in **Table 3** and **4**, respectively (Supporting Information). For the methanol stem bark and leaves extracts, the concentrations used were as shown in **Table 5** and **6**, respectively (Supporting Information).

Each concentration of the extracts was in triplicate plates. Each plate was inoculated with 0.1 ml (10^6 CFU/ml) of overnight nutrient broth culture of each of

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the clinical bacterial isolates. The inoculums were spread uniformly on the surface of the agar with the aid of a sterile glass spreading rod. The plates were incubated at 37 °C and observed for growth after 24 hours. The lowest concentration of each extract which inhibited the growth of each pathogenic bacterium was considered as the MIC for the bacterium.

Determination of the effect of various concentrations of ciprofloxacin hydrochloride and minimum inhibitory concentration (MIC) against the pathogenic bacteria

The agar dilution method described by George and Robert, (1996) was used to determine the effects of various concentrations of ciprofloxacin hydrochloride on the pathogenic bacteria in order to establish the minimum inhibitory concentrations (MICs) of ciprofloxacin hydrochloride.

The MICs of ciprofloxacin hydrochloride against *Staph. aureus*, *E. Coli*, *A. faecalis*, *P. aeruginosa*, *S. marcescens*, *E. aerogenes, P. vulgaris* and *K. pneumoniae* were determined in triplicates using final concentrations of 5, 7.5, 10, 15, 20 and 30 mg/ml of ciprofloxacin hydrochloride in nutrient agar. Each plate was inoculated with 0.1 ml of 10^6 CFU/ml of overnight nutrient broth culture of each of the clinical bacterial isolates. The inoculums were spread uniformly on the surface of the agar with the aid of a sterile glass spreading rod. The plates were incubated at 37 °C and observed for growth after 24 hours. The lowest concentration of ciprofloxacin which inhibited the growth of each pathogenic bacterium was considered as the MIC for the bacterium.

Evaluation of antibacterial activity of aqueous, butanol and methanol extracts of stem bark and leaves of *Berlinia grandiflora* using zones of inhibition

The agar well diffusion method as described by Perez et al (1990) was used. The antibacterial activity of the aqueous, butanol and methanol extracts of *Berlinia grandiflora* was evaluated using *Staph. aureus*, *E. Coli*, *A. faecalis*, *P. aeruginosa*, *S. marcescens*, *E. aerogenes, P. vulgaris* and *K. pneumoniae.* The MIC against each bacterium exhibited by each extract was used in the zone of inhibition experiments. Petri dishes in triplicates were labelled with each of the eight clinical bacterial isolates and 25 ml of nutrient agar pipetted into them aseptically. The nutrient agar was allowed to set and was inoculated with 0.1 ml (10^6 CFU/ml) of an overnight culture of each of the clinical isolates, in separate petri dishes. The inoculums were spread uniformly on the surface of the agar with the aid of a sterile glass spreading rod. Using a sterile cork borer, wells of 6 mm in diameter were made in the nutrient agar of each plate and labelled accordingly with the extract type and ciprofloxacin hydrochloride, to which the bacterial isolates were sensitive. The standard drug, ciprofloxacin hydrochloride (0.2 ml equivalent to 20 µg) of each of its MIC against each bacterium was aseptically pipetted into the well at the center while 0.2 ml of each of the minimum inhibitory concentrations of the extracts tested was aseptically pipetted into the appropriately labelled well. The plates were left for 10 minutes on the bench top for the drug and extracts to diffuse into the agar. The plates were incubated at 37 °C for 24 hours and examined for zones of inhibition around the wells. The zones of inhibition were measured and recorded. The mean and standard error of mean (Mean ± SEM) was determined for the triplicate plates in each case.

3. Results

The maceration of 500 g of the powdered leaves in distilled water, butanol and methanol yielded 35 g (7.0%), 20 g (4.0%) and 25 g (5.0%) respectively, while the powdered stem bark yield 40 g (8.0%), 22 g (4.4%) and 25 g (5.0%) respectively. The results of the effects of various concentrations of the aqueous, methanol and butanol extracts of leaves and stem bark of *Berlinia grandiflora* are presented in **Table 1 – 6** (Supporting Information). The results indicate that the extracts inhibited the growth of the pathogenic bacteria at different concentrations suggesting that the clinical bacterial isolates have different sensitivity patterns to the extracts. **Table 7** (Supporting Information) shows the effects of various concentrations of ciprofloxacin hydrochloride against the pathogenic bacteria. The results indicate that the clinical bacterial isolates showed different sensitivity patterns to ciprofloxacin hydrochloride with *Proteus vulgaris* being the most susceptible to ciprofloxacin.

The minimum inhibitory concentrations (MICs) values of ciprofloxacin hydrochloride and the aqueous, methanol and butanol extracts of *Berlinia grandiflora* are presented in **Table 8**.

The result of the zones of inhibition of the extracts and ciprofloxacin against each pathogenic bacterium is presented in **Table 9**. The zones of inhibition ranged from 18.0 ± 0.10 mm to 28.0 ± 0.01mm for the extracts and that of the reference drug, ciprofloxacin ranged from 12.0 ± 0.02 mm to 37.0 ± 0.14 mm. The methanol stem bark extract produced the largest zone of inhibition (28.0 ± 0.01 mm) against *Staph. aureus*. Generally the extracts exhibited appreciable antibacterial activity in the zones of inhibition assay against the pathogenic bacteria. *Staph. aureus* showed the largest zones of inhibition both for the extracts and ciprofloxacin while *A. faecalis* showed the smallest zone of inhibition for most of the extracts and ciprofloxacin.

4. Discussion

The use of medicinal plants remains the mainstay in health care delivery system in most developing countries. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. The use of medicinal plants to treat infectious diseases is on the increase now orchestrated by the unavailability, unaffordability of the existing antimicrobial agents, coupled with emergence of multidrug resistant pathogens that hamper clinical efficacy of existing antibiotics (Davis, 1994; Lamikanra and Ndep, 1993). Many rural dwellers are now reinforcing their use for medicinal plants to treat microbial infections. This documents the need for scientific research to validate the ethnomedicinal uses of these plants and provide leads for new antimicrobial agents.

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Table 8: Minimum inhibition concentrations (MICs) values of aqueous, methanol and butanol extracts of *Berlinia grandiflora* and ciprofloxacin hydrochloride against the pathogenic bacteria

Pathogenic Bacteria	Minimum inhibitory concentration values (mg/ml)						
	Aqueous Extracts		Butanol Extracts		Methanol Extracts		Ciprofloxacin (µg/ml)
	Bark	Leaves	Bark	Leaves	Bark	Leaves	
Staphylococcus aureus	21.0	21.0	22.0	55.0	55.0	55.0	10.0
Escherichia coli	24.0	23.0	100.0	50.0	50.0	50.0	25.0
Alcaligenes faecalis	50.0	47.5	100.0	75.0	75.0	75.0	30.0
Serratia marcescens	75.0	35.0	140.0	55.0	55.0	55.0	30.0
Enterobacter aerogenes	90.0	90.0	95.0	55.0	55.0	55.0	30.0
Klebsiella pneumoniae	75.0	75.0	75.0	50.0	50.0	50.0	30.0
Pseudomonas aeruginosa	70.0	23.0	23.0	50.0	50.0	50.0	25.0
Proteus vulgaris	47.5	23.0	23.0	50.0	50.0	50.0	7.5

Table 9: Zones of inhibition of various MICs of extracts of *Berlinia grandiflora* and ciprofloxacin hydrochloride against the pathogenic bacteria

Pathogenic Bacteria	Zones of Inhibition (mm)						
	Aqueous Extracts		Butanol Extracts		Methanol Extracts		Ciprofloxacin
	Bark	Leaves	Bark	Leaves	Bark	Leaves	
Staphylococcus aureus	21.0 ±0.03	27.0 ±0.0.12	27,0 ±0.04	18.0 ±0.02	28.0 ±0.01	27.0 ±0.08	27.0 ±0.04
Escherichia coli	18.0 ±0.10	19.0 ±0.22	23.0 ±0.21	23.0 ±0.0.13	19.5 + 0.08	27.0 + 0.20	18.0 + 0.20
Alcaligenes faecalis	19.0 ±0.30	19.0 ±0.24	19.0 ±0.18	19.0 ±0.11	19.5 ±0.19	19.0 ±0.30	22.0 ±0.21
Serratia marcescens	23.0 ±0.11	20.0 ±0.10	18.0 ±0.0.11	19.0 ±0.03	19.5 ±0.13	18.0 ±0.34	41.0 ±0.11
Enterobacter aerogenes	24.0 ±0.02	19.0 ±0.23	23.0 ±0.0.14	18.0 ±0.22	25.0 ±0.04	25.0 ±0.26	19.0 ±0.30
Klebsiella pneumoniae	19.0 ±0.07	19.0 ±0.03	19.0 ±0.60	23.0 ±0.40	19.0 ±0.20	19.0 ±0.18	16.5 ±0.22
Pseudomonas aeruginosa	22.0 ±0.14	21.0 ±0.21	18.0 ±0.33	20.0 ±0.44	23.0 ±0.11	21.0 ±0.09	12.0 ±0.02
Proteus vulgaris	23.0 ±0.02	25.0 ±0.22	22.0 ±00.15	18.0 ±0.50	24.0 ±0.09	24.0 ±0.11	37.0 ±0.14

Our study investigated the antibacterial potentials of the aqueous, methanol and butanol extracts of stem bark and leaves of *Berlinia grandiflora* against eight pathogenic bacteria. The crude extracts inhibited the growth of *Staph. aureus, E. coli, P. aeruginosa* and *P. vulgaris* which have been reported to have multidrug resistance at MIC ranging from 21 mg/l to 23 mg/ml (Onanuga et al, 2005). Our study revealed that *Staph. aureus* was the most sensitive pathogenic bacteria to the extracts. *E. aerogenes* and *K. pneumoniae* were the least susceptible to the extracts. *Berlinia grandiflora* extracts inhibited them at MICS of 90 mg/ml and 70 mg/ml. The methanol stem bark extract produced the largest zone of inhibition against *Staph. aureus* indicating its high sensitivity.

Betulinic acid is a triterpene of natural origin and has been isolated from the methanol, hexane and ethyl acetate extracts of stem bark of *Berlinia grandiflora* (Enwerem et al, 2001). Betulinic acid and its derivatives have been reported to exhibit antimicrobial activity (Perumal and Sriram, 2005). The antimicrobial activity exhibited by the stem bark and leaves extracts of *Berlinia grandiflora* in this study could be ascribed to betulinic acid. Generally, the extracts exhibited appreciable antibacterial activity in the zones of inhibition assay against the pathogenic bacteria.

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5. Conclusion

The study has shown that aqueous, methanol and butanol extracts of the stem bark and leaves of *Berlinia grandiflora* have antibacterial activity against the clinical pathogenic bacteria and could be beneficial in the treatment of various bacterial infections caused by the organisms used in our study.

Conflict of Interest declaration

The authors declare no conflict of interest

References

Asuzu JU, Nwele OC and Anaga AO (1993). The pharmacological activities of the stem bark methanolic extract of *Berlinia grandiflora*. *Fitoterapia*. **64(6):** 529 – 534.

Cheesbrough M (2004). District Laboratory practice in Tropical Countries, Low price edition, Cambridge University Press Cambridge, pp. 63 – 70.

Davis J (1994). Inactivation of antibiotics and the dissemination of resistance genes. *Science*. **246:** 375 - 382.

Enwerem NM, Wambebe CO, Okogun JI, Akah PA and Gamaniel KS (2001). Antihelminthic screening of stem bark of *Berlinia grandiflora. J. Nat. Remed*. **1:** 17 – 20.

Enwerem NM, Okogun JI, Wambebe CO, Okorie DA and Akah PA (2001). Antihelminthic activity of the stem bark extracts of Berlinia grandiflora and one of its principles betulinic acid. *Phytomed*. **8:** 112 – 114.

George ME and Robert CM (1996). Antimicrobial combinations *In*: Antibiotics in medicine, 4th ed., Victor L. (Ed), Williams and Wilkens, London, p. 334.

Gill LS (1992). Ethnomedicinal uses of plants in Nigeria. University of Benin press, Nigeria, pp. 65.

Hutchison J and Dalziel JM (1963). Flora of West Africa. London University press, London, Vol. II, p. 74.

Lamikanra A and Ndep R (1993). The incidence of multiple antibiotics resistance in urinary tract pathogens isolated from Nigerian patients. *Niger. J. Pharm*. **25:** 46 – 49.

Onanuga A, Oyi AR and Olayinka BO (2005). Prevalence of community-associated multiresistant *Staph. aureus* among healthy women in Abuja, Nigeria. *Afr. J. Biotech*. **4:** 942 – 945.

Perez C, Paul M and Bazerque P (1996). Antibiotic assay by agar-well diffusion method *Acta Biol. Med. Exp*. **15:** 113 – 115.

Perumal Y and Sriram D (2005). Betulunic acid and its derivatives: A review on their biological properties. *Curr. Med. Chem*. **12:** 657 – 666.

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Supporting Information

Investigation of the antimicrobial potentials of some phytochemical extracts of leaf and stem bark of *Berlinia grandiflora* (Leguminoceae) *Caesalpinioidae* against pathogenic bacteria

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Table 1: The effects of various concentrations of aqueous leaves extract of B*erlinia grandiflora* against the pathogenic bacteria

Pathogenic bacteria	**Final concentrations of aqueous leaf extract in nutrient agar (mg/ml)**																						
	15	**20**	**21**	**22**	**23**	**24**	**25**	**30**	**35**	**40**	**45**	**47.5**	**50**	**55**	**60**	**65**	**70**	**75**	**80**	**85**	**90**	**95**	**100**
Staphylococcus aureus	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alcaligenes faecalis	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Serratia marcescens	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
**Enterobacter aerogenes*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
**Klebsiella pneumoniae*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Pseudomonas aeruginosa	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Proteus vulgaris	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ *growth of bacteria,*

- *no growth of bacteria*

* *least sensitive bacteria*

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Table 2: Effects of various concentrations of aqueous stem bark extract of B*erlinia grandiflora* against the pathogenic bacteria

Pathogenic bacteria	Final concentrations of aqueous stem bark extract in nutrient agar (mg/ml)																				
	20	**21**	**22**	**23**	**24**	**25**	**30**	**35**	**40**	**45**	**47.5**	**50**	**55**	**60**	**65**	**70**	**75**	**80**	**85**	**90**	**95**
Staphylococcus aureus	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Alcaligenes faecalis	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
**Serratia marcescens*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
**Enterobacter aerogenes*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
**Klebsiella pneumoniae*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
**Pseudomonas aeruginosa*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Proteus vulgaris	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-

+ *growth of bacteria,*

- *no growth of bacteria*

* *least sensitive bacteria*

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Table 3: Effects of various concentrations of butanol stem bark extract of *Berlinia grandiflora* against the pathogenic bacteria

Pathogenic bacteria	Final concentrations of butanol stem bark extract in nutrient agar (mg/ml)																								
	21	**22**	**23**	**24**	**25**	**30**	**35**	**40**	**45**	**47.5**	**50**	**55**	**60**	**65**	**70**	**75**	**80**	**85**	**90**	**95**	**100**	**140**	**150**	**160**	**200**
Staphylococcus aureus	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
**Escherichia coli*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
**Alcaligenes faecalis*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
**Serratia marcescens*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
**Enterobacter aerogenes*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
**Klebsiella pneumoniae*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Proteus vulgaris	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+ *growth of bacteria,*

- *no growth of bacteria*

***** *least sensitive bacteria*

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Table 4: Effects of various concentrations of butanol leaves extract of *Berlinia grandiflora* against the pathogenic bacteria

Pathogenic bacteria	Final concentrations of butanol leaves extract in nutrient agar (mg/ml)											
	45	**50**	**55**	**60**	**65**	**70**	**75**	**80**	**85**	**90**	**95**	**100**
Staphylococcus aureus	+	+	-	-	-	-	-	-	-	-	-	-
Escherichia coli	+	-	-	-	-	-	-	-	-	-	-	-
**Alcaligenes faecalis*	+	+	+	+	+	+	-	-	-	-	-	-
Serratia marcescens	+	+	-	-	-	-	-	-	-	-	-	-
Enterobacter aerogenes	+	+	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	+	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	+	-	-	-	-	-	-	-	-	-	-	-
Proteus vulgaris	+	-	-	-	-	-	-	-	-	-	-	-

+ *growth of bacteria,*

- *no growth of bacteria*

* *least sensitive bacteria*

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Table 5: Effects of various concentrations of methanol stem bark extract of *Berlinia grandiflora* against the pathogenic bacteria

Pathogenic bacteria	Final concentrations of butanol leaves extract in nutrient agar (mg/ml)											
	45	**50**	**55**	**60**	**65**	**70**	**75**	**80**	**85**	**90**	**95**	**100**
Staphylococcus aureus	+	+	-	-	-	-	-	-	-	-	-	-
Escherichia coli	+	-	-	-	-	-	-	-	-	-	-	-
**Alcaligenes faecalis*	+	+	+	+	+	+	-	-	-	-	-	-
Serratia marcescens	+	+	-	-	-	-	-	-	-	-	-	-
Enterobacter aerogenes	+	+	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	+	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	+	-	-	-	-	-	-	-	-	-	-	-
Proteus vulgaris	+	-	-	-	-	-	-	-	-	-	-	-

+ *growth of bacteria,*

- *no growth of bacteria*

* *least sensitive bacteria*

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Table 6: Effects of various concentrations of methanol leaves extract of *Berlinia grandiflora* against the pathogenic bacteria

Pathogenic bacteria	Final concentrations of methanol leaves extract in nutrient agar (mg/ml)											
	45	**50**	**55**	**60**	**65**	**70**	**75**	**80**	**85**	**90**	**95**	**100**
Staphylococcus aureus	+	+	-	-	-	-	-	-	-	-	-	-
Escherichia coli	+	-	-	-	-	-	-	-	-	-	-	-
**Alcaligenes faecalis*	+	+	+	+	+	+	-	-	-	-	-	-
Serratia marcescens	+	+	-	-	-	-	-	-	-	-	-	-
Enterobacter aerogenes	+	+	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	+	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa	+	-	-	-	-	-	-	-	-	-	-	-
Proteus vulgaris	+	-	-	-	-	-	-	-	-	-	-	-

+ *growth of bacteria,*

- *no growth of bacteria*

* *least sensitive bacteria*

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Table 7: Effects of various concentrations of ciprofloxacin hydrochloride against the pathogenic bacteria

Pathogenic bacteria	Final concentrations of ciprofloxacin in nutrient agar (µg/ml)						
	5.0	**7.5**	**10.0**	**15.0**	**20.0**	**25.0**	**30.0**
Staphylococcus aureus	++	+	-	-	-	-	-
Escherichia coli	+++	++	++	+	+	-	-
**Alcaligenes faecalis*	+++	+++	+++	++	++	+	-
**Serratia marcescens*	+++	+++	++	++	++	+	-
**Enterobacter aerogenes*	+++	++	++	++	+	+	-
**Klebsiella pneumoniae*	+++	+++	++	++	++	+	-
Pseudomonas aeruginosa	+++	++	++	+	+	-	-
Proteus vulgaris	+	-	-	-	-	-	-

+++ *heavy growth of bacteria*
++ *moderate growth of bacteria*
+ *minimal growth of bacteria*
- *no growth of bacteria*
* *least sensitive bacteria*

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