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Susceptibility pattern of clinical isolates of uropathogens from Southwest Nigeria to antibiotics and extracts of *Dalbergia latifolia* Roxb (Fabaceae)

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Summary

Background: Resistance of uropathogens to conventional antibiotics is increasing, thus creating the need to search for newer and effective antimicrobial agents to treat urinary tract infections (UTI).

Methods: The susceptibility pattern of twenty clinical isolates of uropathogenic bacteria to standard antibiotics and extracts of *Dalbergia latifolia* was investigated using Agar Disc and Agar Well Diffusion methods, respectively. Minimum Inhibitory Concentrations (MICs) of the extracts were determined by Agar Dilution method on some of the uropathogens.

Results: All the test pathogens were resistant to Amoxicillin, Cotrimoxazole and Augmentin. The isolates had 25 % (4), 12.5% (2), 18.75% (3), 68.75% (11) susceptibility to Nitrofurantoin, Gentamicin, Nalidixic acid and Ofloxacin respectively while 87.5% showed resistant to Tetracycline. The *E. coli* and *K. pneumoniae* strains had the highest susceptibility to Ofloxacin while *P. mirabilis* 4 was susceptible to Ofloxacin. The uropathogenic *S. aureus* was highly resistant to the antibiotics, however *S. aureus* 3 and *S. aureus* 4 were susceptible to gentamicin while *S. aureus* 1 and *S. aureus* 5 were susceptible to chloramphenicol. Gentamicin and chloramphenicol were the most active on *S. aureus*. The extracts showed good activity on most of the uropathogens in which 81.81 % (18), 9.0% (2) and 63.63% (14) of the test pathogens were susceptible to the leaf, stem and root (successively) of *D. latifolia*. The MIC values of extracts on test organisms ranged from 0.063 to 2.0 mg/mL.

Conclusion: The results revealed varied patterns of susceptibility of the uropathogens to conventional antibiotics, necessitating rational use of antibiotic in routine treatment of UTI to prevent development of resistance. Further, activity of *D. latifolia* extracts on the uropathogens justified its folkloric use and underlined the potentials of the plant to furnish antimicrobial agents for the treatment of UTIs, including those resistant to conventional antibiotics.

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Résumé

Contexte: La résistance des uropathogènes aux antibiotiques conventionnels augmente, créant ainsi le besoin de rechercher des agents antimicrobiens plus récents et efficaces pour traiter les infections des voies urinaires (IVU).

Méthodes: Le profil de sensibilité de vingt isolats cliniques de bactéries uropathogènes aux antibiotiques standard et aux extraits de Dalbergia latifolia a été étudié en utilisant les méthodes Agar Disc et Agar Well Diffusion, respectivement. Les concentrations minimales inhibitrices (CMI) des extraits ont été déterminées par la méthode de dilution d'agar sur certains des uropathogènes.

Résultats: Tous les pathogènes testés étaient résistants à l'amoxicilline, au cotrimoxazole et à l'augmentation. Les isolats présentaient respectivement une sensibilité de 25% (4), 12,5% (2), 18,75% (3), 68,75% (11) à la nitrofurantoïne, à la gentamicine, à l'acide nalidixique et à l'ofloxacine, tandis que 87,5% étaient résistants à la tétracycline. Les souches d'E. Coli et de K. pneumoniae étaient les plus sensibles à l'ofloxacine, tandis que P. mirabilis 4 était sensible à l'ofloxacine. Le S. aureus uropathogène était très résistant aux antibiotiques, cependant S. aureus 3 et S. aureus 4 étaient sensibles à la gentamicine tandis que S. aureus 1 et S. aureus 5 étaient sensibles au chloramphénicol. La gentamicine et le chloramphénicol étaient les plus actifs sur S. aureus. Les extraits ont montré une bonne activité sur la plupart des uropathogènes dans lesquels 81,81% (18), 9,0% (2) et 63,63% (14) des pathogènes testés étaient sensibles aux feuilles, aux tiges et aux racines (successivement) de D. latifolia. Les valeurs de CMI des extraits sur les organismes d'essai variaient de 0,063 à 2,0 mg / mL.

Conclusion: Les résultats ont révélé des schémas variés de sensibilité des uropathogènes aux antibiotiques conventionnels, nécessitant l'utilisation rationnelle de l'antibiotique dans le traitement de

routine des infections urinaires pour empêcher le développement d'une résistance. En outre, l'activité des extraits de D. latifolia sur les uropathogènes justifiait son utilisation folklorique et soulignait le potentiel de la plante à fournir des agents antimicrobiens pour le traitement des infections urinaires, y compris celles résistantes aux antibiotiques conventionnels.

Mots clés: *Uropathogènes, antibiotiques, résistance, extraits de Dalbergia latifolia, CMI*

Introduction

Urinary Tract Infections (UTI) is a global problem with an estimated 130-175 million cases reported annually [1] and microbial resistance to antibiotics is increasing [2,3]. *Escherichia coli* are the most frequent cause of community-acquired UTI, accounting for >80% [4,5] while *Klebsiella* is second, accounting for 6-17% of all nosocomial UTI [6,7]. Gram positive *Staphyloccocus aureus* is also implicated in UTI [8,9]. Various studies have shown that uropathogens (*E. coli, K. pneumoniae, P. mirabilis, S. aureus* and others) have become less susceptible to commonly used antibiotics worldwide but varies according to geographical area [10–12]. The emergence of multidrug resistant uropathogens is responsible for increased morbidity and mortality, outbreaks of UTI in many communities including Southwest Nigeria [9,12]. Therefore, there is urgent need to search for new and effective antimicrobial agents to treat MDR uropathogen-mediated UTI. Medicinal plants have formed the basis for new drug development and approximately 80% of the world population depends on traditional medicine for their primary health care [13,14]. Screening bioactive extracts of medicinal plants used to treat infections locally is a sure way of discovering new antimicrobial agents [15,16].

Dalbergia latifolia Roxb. (Fabaceae) is a pantropical tree called "Indian Rosewood" and was introduced to Nigeria from India [17]. It is a small tree, with characteristic nearly circular leaflets, white flowers in clusters and fruits in bunches at the ends of the branches [17]. Ethnomedicinally, the plant is used to treat cough, bronchitis, internal body pains, piles and urinary tract infections, in a similar recipe to *D. taxatilis* belonging to the same genus [18]. Earlier reports have shown that *D. latifolia* possessed antiamnesic effect [19], antioxidant property [20], hypolipidemic, hypoglycaemic activity [21] and antimicrobial activity [22,23]. This study therefore reports the susceptibility patterns of clinical isolates of uropathogens from Nigeria to extracts of *Dalbergia latifolia* compared with conventional antibiotics.

Materials and methods

Collection of plant materials

Fresh leaves, stem bark and root bark of *Dalbergia latifolia* were collected at the Botanical Garden, University of Ibadan. Identification and authentication of the plant were carried out at the Forestry Research Institute of Nigeria (FRIN) where voucher specimen with herbarium number FHI 109552 was deposited.

Extraction procedure

The leaves, stem bark and root bark of *D. latifolia* were dried at room temperature for about three weeks and grinded to coarse powder. Cold extractions were carried out as follows. Powdered leaves (100g) were soaked in 500mL of methanol, with intermittent shaking for 48hours. The extracts were then filtered using a clean sterile muslin cloth and then Whatman No.1 filter paper, and stored at 4^{o}C for subsequent use.

Collection of clinical isolates

Clinical isolates (5 different strains of each organism) of *E. coli, S. aureus, P. mirabilis and K. pneumoniae* (supposedly obtained from patients diagnosed with UTI showing significant bacteriuria) were collected at the Medical Microbiology Laboratory of Ladoke Akintola University of Technology Teaching Hospital, Osogbo, Southwest Nigeria. Confirmation of identity of the isolates was done by standard bacteriological procedures [24, 25]. The isolates were maintained on Mueller Hinton agar slope and stored at 4^{o}C prior to use.

Susceptibility of isolates to standard antibiotics

The agar-disc diffusion method of Kirby-Bauer was employed following procedure recommended by Clinical and Laboratory Standard Institutes, CLSI [26]. The following antibiotic discs were used; Augmentin (30µg), Amoxicillin (25µg), Erythromicin (5µg), Tetracycline (10µg), Cloxacillin (5µg), Cotrimoxazole (25µg), Gentamicin (10µg), Chloramphenicol (30µg), Nitrofurantoin (300µg), Nalidixic acid (30µg) and Ofloxacin (30µg). The agar plates were observed for susceptibility (zones of inhibition measured in mm) or resistance.

Susceptibility of isolates to plant extracts

This was done using the agar well diffusion method of Perez *et al*. [27] as follows: 0.2ml of overnight broth culture of each of the clinical isolates

standardized by McFarland procedure to contain 1.0 x 10^8 cfu/ml was transferred to and spread on solidified Mueller Hinton agar. A sterile cork borer of 6 mm diameter was used to cut uniform wells in the agar and each well was filled with 3 drops of each extracts at different concentrations. Control experiment was setup with methanol (40%) and gentamicin (10ìg). Plates were incubated at 37°C for 24hours and zones of inhibition were measured in millimeter.

Determination of Minimum Inhibitory Concentrations (MICs)

The MIC of each extract was determined using agar dilution method of Andrews [28]. Each extract was serially diluted to obtain concentrations of 20.0, 10.0, 5.0, 2.5, 1.25, 0.625, 0.3, 0.15, 0.075mg/mL and 2mL of each concentration of the extract was incorporated into 18ml of Mueller Hinton agar, then each of the test isolates was inoculated on the plates by streaking. Plates were incubated at 37°C for 24hours, after which they were observed for growth. The least concentration in which there was no growth was noted as the MIC. All the antimicrobial susceptibility test were carried out in triplicates.

Results

Percentage yield on extraction (Table 1) was highest in the leaves (6.87%) followed by root bark (1.65%) and stem bark (1.25%) with each showing different macroscopic characteristics indicating the presence of different type or quantity of phytoconstituents. A total of twenty (20) isolates which include five isolates each of *S. aureus, E. coli K. pneumoniae* and *P. mirabilis* with reference strains of *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were investigated for susceptibility to conventional antimicrobials used to treat UTI. The isolates of *S. aureus* showed zero% susceptibility to augmentin, amoxicillin, erythromycin and cloxacillin, and 16.7% susceptibility to tetracycline and cotrimoxazole. All the isolates of *E. coli, K. pneumoniae* and *P. mirabilis* showed zero% susceptibility to amoxicillin, cotrimoxazole and augmentin. Ofloxacin was the most active of the antibiotics used against Gram-negative organisms in this study. Also, *P. mirabilis* 2, 3 and 5 were found to be non-susceptible to all the antibiotics used in this study (Table 2).

Staphylococcus aureus 1 showed the highest susceptibility to the root extract of *D. latifolia* with 24mm diameter zone of growth inhibition (Table 3) while *E. coli* 2 has the highest susceptibility to the leaf extract of *D. latifolia*. Most of the test pathogens were not susceptible to the stem bark extract of *D. latifolia* except *S. aureus* 2, *E. coli* 4 and *P. mirabilis,* representing less than 10% susceptibility (Table 4).

Table 5 shows the MIC value of the extracts of *D. latifolia* against the selected uropathogens. Root extracts of *D. latifolia* was the most active with MIC of 0.06mg/mL against *E. coli* ATCC 25922*, S. aureus* 4 and *K. pneumoniae* 5 followed by leaf extracts with MIC of 0.125mg/ml against *E. coli* 2, *S. aureus* 1, *P. mirabilis* 4, and *K. pneumonia* 5.

Discussion

Generally, in this study *P. mirabilis* was the most resistant uropathogen. The most active of all the antibiotics used was ofloxacin followed by nitrofurantoin and nalidixic acid (Table 2), however *P. mirabilis* was resistant to all the antibiotics used. Our results are comparable with various similar studies reported. Kibret and Abera, 2011 [29] reported high resistance rates of *E. coli* to erythromycin (89.4%), amoxicillin (86.0%) and tetracycline (72.6%), while we recorded 100% resistance in this study. Mohsen *et al.,* 2010 [30] reported the resistance rates of *E. coli* from urine culture to ampicillin and amoxicillin to be 98.4% and 83.7% respectively which is also lower than 100% resistance recorded in this work. Also, Mohsen *et al.,* 2010 [30] reported that uropathogens were most susceptible to amikacin (93.3%), ciprofloxacin (91.5%), nitrofurantoin (89.8%) and nalidixic acid (78.7%) while we recorded a lower susceptibility for

Table 1: Extraction Yields and Visual Characteristics of Extracts of *Dalbergia latifolia*

Extracts	% Yield	Observed Characteristics
Leaf	6.87	A dark brown and sticky substance
Stem bark	1.25	A brown crystalline powder
Root bark	1.63	A brown and sticky substance

Table 2: Susceptibility of the test pathogens to selected antibiotics

Test organisms	Antibiotic susceptibility							
	Amx	Cot	Nit	Gen	Nal	Ofl	Aug	Tet
E. coli 1	R	R	S	I	S	S	R	R
E. coli 2	R	R	S	I	I	S	R	R
E. coli 3	R	R	S	R	R	R	R	R
E. coli 4	R	R	R	I	I	S	R	R
E. coli 5	R	R	R	R	S	S	R	R
E. coli ATCC 25922	R	R	I	R	I	S	R	R
K. pneumoniae 1	R	R	R	R	R	S	R	R
K. pneumoniae 2	R	R	R	R	R	S	R	R
K. pneumoniae 3	R	R	R	R	S	S	R	R
K. pneumoniae 4	R	R	R	S	I	S	R	I
K. pneumoniae 5	R	R	S	S	I	S	R	R
P. mirabilis 1	R	R	R	I	I	I	R	R
P. mirabilis 2	R	R	R	R	R	R	R	R
P. mirabilis 3	R	R	R	R	R	R	R	R
P. mirabilis 4	R	R	R	R	R	S	R	I
P. mirabilis 5	R	R	R	R	R	R	R	R

R: Resistant, I: Intermediate, S: Susceptible, Aug: Augmentin (30µg), Amx: Amoxicillin (25µg), Cot: Cotrimoxazole (25µg), Gen: Gentamicin (10µg), Nit: Nitrofurantoin (300µg), Nal: Nalidixic acid (30µg), Ofl: Ofloxacin (30µg), Tet: Tetracycline (30µg)

Table 3: Susceptibility of uropathogenic *S. aureus* to selected Gram positive antibiotics

Test Organisms	Antibiotic susceptibility							
	Amx	Cot	Nit	Gen	Nal	Ofl	Aug	Tet
S. aureus 1	R	R	R	R	R	R	R	S
S. aureus 2	R	R	R	R	R	I	R	I
S. aureus 3	R	R	R	I	R	S	R	I
S. aureus 4	R	R	R	R	R	S	R	R
S. aureus 5	R	R	R	R	R	R	R	S
S. aureus ATCC 25923	R	R	R	R	R	R	I	R

R: Resistant, I: Intermediate, S: Susceptible, Aug: Augmentin (30µg), Amx: Amoxicillin (25µg), Ery: Erythromicin (5µg), Tet: Tetracycline (10µg), Cxc: Cloxacillin (5µg), Cot: Cotrimoxazole (25µg), Gen: Gentamicin (10µg), Chl: Chlroramphenicol (30µg).

gentamicin (37.7%), ofloxacin (75%), nalidixic acid (55.0%). Our results have indicated that, within a decade, uropathogenic bacteria have undergone significant reduced susceptibility to the tested antibiotics. *Proteus mirabilis* has been implicated in meningitis, empyema, osteomyelitis and gastroenteritis. Also, it frequently causes nosocomial infections of the urinary tract (46%), surgical wounds (24%) and lower respiratory tract (30%). Less frequently, *Proteus* species cause bacteraemia (17%), most often in elderly patients [31]. Therefore, the high-level antimicrobial resistance (> 95%) recorded in this study is a cause for concern.

Both Gram positive and Gram negative uropathogens used in this study were multidrug resistant (Table 3). The least susceptible was *P. mirabilis* while the most susceptible was *E. coli*. The most active antibiotics on Gram positive organism (*S. aureus*) was chloramphenicol and gentamicin while ofloxacin was the most active antibiotic on the

Table4: Susceptibility of uropathogens to extracts of *Dalbergia latifolia*

Test Pathogens	Zones of Inhibition (mm) of Extracts at 20mg/mL			
	Leaves	Stem bark	Root bark	Gentamicin 10µg/mL
S. aureus 1	15	—	24	12
S. aureus 2	18	11	15	25
S. aureus 3	13	—	—	15
S. aureus 4	12	—	13	—
S. aureus 5	15	—	10	22
S. aureus ATCC 25923	11	—	12	16
E. coli 1	12	—	11	22
E. coli 2	23	—	15	14
E. coli 3	12	—	11	22
E. coli 4	—	—	16	30
E. coli 5	—	—	—	15
E. coli ATCC 25922	12	—	—	20
K. pneumoniae 1	12	—	14	25
K. pneumoniae 2	13	—	16	30
K. pneumoniae 3	13	—	—	—
K. pneumoniae 4	10	—	—	—
K. pneumoniae 5	18	—	15	20
P. mirabilis 1	—	—	—	—
P. mirabilis 2	11	—	—	—
P. mirabilis 3	—	—	—	—
P. mirabilis 4	13	—	15	30
P. mirabilis 5	12	14	12	—

Keys: — = No zone of inhibition

Table 5: MIC of extracts of *Dalbergia latifolia* on selected test pathogens

Organisms	MIC mg/mL		
	DLL	DLS	DLR
S. aureus 4	ND	1.0	0.06
E. coli ATCC 25922	ND	2.0	0.06
E. coli 2	0.125	ND	ND
P. mirabilis 4	0.125	ND	ND
S. aureus 1	0.125	ND	ND
K. pneumoniae 5	0.125	2.0	0.06

Keys: DLL= *Dalbergia latifolia* leaf; DLS= *Dalbergia latifolia* stem bark; DLR= *Dalbergia latifolia* root bark*;* ND= Not determined

Gram negative organisms. The sensitivity pattern of clinical isolates of *S. aureus* to chloramphenicol and gentamicin in this study was 66.6% and 50% respectively which were found lower than the findings of Nwankwo and Nasiru, 2011 [32] who reported the susceptibility pattern of uropathogenic *S. aureus* to gentamicin and chloramphenicol to be 92.4% and 61.9% respectively. In this study, *S. aureus* isolates were totally resistant to augmentin, amoxicillin, cloxacillin (all penicillin class) and erythromycin. This is similar to the study conducted by Saravanan *et al*., (2013) where all clinical MRSA strains (100%) were resistant to penicillin, rifampicin, ampicillin, gentamicin and amoxicillin [33]. This is another strong indication that resistant uropathogens are fast rendering antimicrobial drugs obsolete thereby making drug treatment of UTI more difficult and expensive. This is why it is imperative to intensify the search for newer and effective antimicrobial agents.

On comparing the susceptibility of the clinical isolates to the tested antibiotics and crude extracts of *D. latifolia,* it showed that the uropathogens,

including many resistant strains were found more susceptible to the plant's extracts than to standard antibiotics. For instance, *P. mirabilis* 5 which was found to be resistant to all the antibiotics used in this study was sensitive to the leaf, stem bark and root extracts of *D. latifolia* with 12mm, 14mm and 12mm diameter zone of inhibition respectively. This implies that *D. latifolia* contain antimicrobial compounds that are active against multidrug resistant microorganisms. Prasad *et al*. [22] reported the presence of phenolic compounds in *D. latifolia* and concluded that the extracts exhibited enough potential to be used as good source of antibiotics against various microbial pathogens. Mishra and Padhy (2013) also reported the antimicrobial activity of leaves of 21 Indian timber-yielding plants (with *D. latifolia* mentioned), on multidrug resistant *E. coli*, *A. baumannii, C. freundii, E. aerogenes, P. mirabilis* and *P. aeruginosa* [34]. These are evidences that extracts from certain medicinal plants can overcome antimicrobial resistant uropathogens and can be useful in the treatment of UTIs.

Conclusion

This study has clearly demonstrated that uropathogens have decreased susceptibility to antimicrobial drugs, calling for judicious and rational use of antibiotics in the community and clinical practices. Also, the recorded antibacterial activity of *Dalbergia latifolia* has justified its folkloric use to treat infections and revealed the possession of antibacterial activity for potential therapeutic use or produce agents which can be isolated and developed for the treatment of UTIs including those caused by MDR uropathogens.

References

1. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity and economic costs. Am J Med, 2002; 113(1A): 5S - 13S.
2. Davis J. Inactivation of antibiotics and the dissemination of resistance genes. Science, 1994; 264: 375 - 382.
3. Stamm WE and Norrby SR. Urinary tract infections: disease panorama and challenges. Infect Dis J, 2001; 183: s1 - s4.
4. Russo TA and Johnson JR. Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasingly important endemic problem. Microbes Infect, 2003; 5: 449 - 456.
5. Rodríguez-Baño J, Navarro MD, Romero L *et al*. Epidemiology and clinical features of infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli* in non-hospitalized patients. *J Clin Microbiol,* 2002; 42(3): 1089 - 1094.
6. Duggan JM, Oldfield GS and Ghosh HK. Septicaemia as a hospital hazard. J Hosp Infect, 1985; 6: 406 - 412.
7. Bennett CJ, Young MN and Darrington H. Differences in urinary tract infection in male and female spinal cord injury patients on intermittent catheterization. Paraplegia, 1995; 33: 69 - 72.
8. Kunin C. Urinary tract infections. 5th ed. Baltimore: Williams & Wilkins; 1997; pp 3014.
9. Akortha EE and Ibadin OK. Incidence and antibiotic susceptibility pattern of *Staphylococcus aureus* amongst patients with urinary tract infection (UTI) in UBTH Benin City, Nigeria. Afr J Biotech, 2008; 7(11): 1637 - 1640.
10. Winstenley TG, Limb DI, Egginton R *et al.* A 10-year survey of the antimicrobial susceptibility of urinary tract isolates in the United Kingdom: the Microbe Base Project. J Antimicrob Chemother, 1997; 40: 591 - 594.
11. Gupta K. Emerging antibiotics resistance in urinary tract pathogens. Infec Dis Clin N Am, 2003; 17: 243.
12. Okesola AO and Aroundegbe TI. Antibiotic resistance pattern of uropathogenic *Escherichia coli* in South West Nigeria. Afr J Med Sci, 2011; 40: 235 - 238.
13. Cragg GM, Boyd MR, Khanna R *et al.* International Collaboration in drug discovery and development, the NCT experience. Pure Appl Chem, 1999; 71: 1619 - 1633.
14. Newman DJ, Cragg GM and Snader KM. The influence of natural products upon drug discovery. Natural Product Res, 2000; 17: 215 - 234.
15. Idowu PA, Moody JO and Odelola HA. Phytochemical screening of three medicinal plants used to treat infection locally. J Pharm and Bio Resources J, 2005; 2(2): 116 - 119.
16. Kinghorn, AD, Chai H, Sung CK *et al.* The classical drug discovery approach to defining bioactive constituents of botanicals (Review). Fitoterapia, 2011; 82: 71 - 79.
17. Keay RWJ. Trees of Nigeria. New York: Oxford University Press, 1989; 212 - 213.
18. Odugbemi T. Outline of Medicinal Plants in Nigeria. 1st Edition, University of Lagos Press, Nigeria, 2006; p. 77.
19. Prasad DP, Ravi-chandiran V, Kumar KP *et al*. Pharmacological evidences for the antiamnesic effect of *Dalbergia latifolia* Roxb in mice. International J Pharm Chem Biol Sci, 2013a; 3(3): 955 - 961.

20. Mohammad K, Siddiqui HH and Sheeba F. *In-vitro* Assessment of antioxidant activity of *Dalbergia latifolia* barks extract against free radicals. Am-Euras J Sci Res, 2011; 6(3): 172 - 177.
21. Mohammad K and Siddiqui, HH. Lipid lowering and hypoglycaemic potential of dried *Dalbergia latifolia* Roxb - Bark extract in Sprague-Dawley rats induced with high fat diets. Int J Nat Prod Res, 2011; 1(4): 49 - 54.
22. Prasad DP, Pranavi A, Kavimani S *et al*. Antimicrobial activity of the root extracts of *Dalbergia latifolia* (Roxb). Global Trends. Pharm Sci J, 2013b; 4(1): 1039 - 1043.
23. Koma OS and Sani IM. Betulinic Acid from antimicrobial root wood extracts of *Dalbergia saxatilis* Hook f. (Fabaceae). Eur J Med Plants, 2014; 4(6): 686 - 694.
24. Cellimore DR. Practical Atlas for Bacterial Identification. Lewis Publishers UK/USA. 2000; p. 37.
25. Cheesbrough M. Medical Microbiology Manual for Tropical Countries. Vol II Microbiology. London: Butterworth, 2003; p 238.
26. Clinical and Laboratory Standards Institute (CLSI). Performance standard for antibiotic susceptibility testing: Document M100-S16. Wayne, P A, 2007.
27. Perez C, Pauli M and Barzerque P. An antibiotic assay by agar well diffusion method. Acta Biol Med Exp, 1990; 15: 113 - 115.
28. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother, 2001; 48: Suppl, SI, 5 - 10.
29. Kibret M and Abera B. Antimicrobial susceptibility patterns of *Escherichia coli* from clinical sources in Northeast Ethiopia. Afr Health Sci, 2011; 11(Suppl 1): S40 - S45.
30. Mohsen M, Ehsan G, Hamid M *et al*. Antimicrobial resistance patterns of *Escherichia coli* detected from hospitalized urine culture samples. Asian J Biol Sci, 2010; 3: 195 - 201.
31. Mansy MSM. Genomic fingerprinting using random amplified polymorphic DNA for discrimination between *Proteus mirabilis* strains. Egypt J Biotech, 2001; (9): 67 - 79.
32. Nwankwo EO and Nasiru MS. Antibiotic sensitivity pattern of *Staphylococcus aureus* from clinical isolates in a tertiary health institution in Kano, Northwestern Nigeria. The Pan African Med J, 2011; 8: 4.
33. Saravanan M, Nanda A and Tesfaye T. Antibiotic Susceptibility pattern of methicillin resistant *Staphylococcus aureus* from septicemia suspected children in tertiary hospital in Hosur, South India. Am J Microb Res, 2013; 1(2): 21 - 24.
34. Mishra MP and Padhy RN. In vitro antibacterial efficacy of 21 Indian timber-yielding plants against multidrug-resistant bacteria causing urinary tract infection. Osong Public Health Res Perspect, 2013; 4(6): 347 - 357.

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