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ORIGINAL ARTICLE

Microbiological Studies on the Effect of Medicinal Plant Extracts on Diabetic Foot Ulcer Bacteria

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ABSTRACT

Background: *The emergence of microbial resistance towards antibiotics increased in a terrible rate. Screening of antimicrobial effect of plant extracts represents hope for discovery of new antimicrobial agents.* ***Objectives:*** *This research aimed to study the influence of the extracts of several medicinal plants on diabetic foot ulcer bacteria.* ***Methodology:*** *Swabs from deep tissues were collected from 56 patients attending the Outpatient clinic of diabetic foot Unit, and diagnosed clinically as diabetic foot infections. The specimens were examined to identify the causative bacteria and their antibiotic susceptibility pattern. Antimicrobial activity of ethanol extracts of ten medicinal plant parts (cinnamon, henna, fennel, black cumin, eucalyptus, clove, chamomile, ginger, sloenstemma and basil) were investigated using well diffusion method. Phytochemical screening of effective plants extracts were performed using tests for alkaloids, glycosides, cardiac glycosides, saponins, phenols, sterols, tannins, flavonoids and diterpen.* ***Results:*** *The commonest isolated organisms were S. aureus (33.9%), followed by S. epidermidis (16.9%), P. aeruginosa (15.3%), P. mirabilis (13.6%), K. pneumoniae (10.2%), E. coli (6.8%) and P. vulgaris (3.4%). Most bacteria were resistant to tested antibiotics and 33.9% were multi-drug resistant bacteria. Ethanol extract of solenstemma, clove, black cumin, and basil had effective growth inhibition effect against isolated bacteria. Phytochemical screening clarified that these plant parts contain powerful secondary metabolites and active materials which explained their antimicrobial activity.* ***Conclusions:*** *Some medical plants showed antimicrobial activity against resistant bacteria, thus could be leading and useful therapeutic agents against many bacterial infections.*

INTRODUCTION

Diabetic foot ulcer is a serious complication affecting the majority of patients with diabetes [1]. Diabetic foot infection (DFI) was defined as the existence of a non healing wound with evidences of inflammation, with or without systemic toxicity, and with a definite bacterial culture [2]. Treatment program of antibiotics, wound care, and possibly hospitalization are necessary [3]. Most DFIs are poly-microbial with aerobic Gram-positive cocci especially *staphylococci* the commonest causative organisms. Aerobic Gram-negative bacilli are common associated pathogens in chronic infections or after antibiotic therapy and obligate anaerobes are common pathogens in ischemic or necrotic wounds [4]. Antibiotic resistance is a worldwide challenge related to high morbidity and mortality. Multidrug resistant patterns in Gram-positive and -negative bacteria had resulted in difficult-to-treat or even untreatable infections with conventional antimicrobials. Dramatic increase in expanding resistance occurs and can disseminate to other patients and the community [5]. The emergence of microbial resistance towards antibiotics increased in a terrible rate. The current shortage of effective drugs, lack of effective prevention measures and few new antibiotics underdevelopment will require the evolution of new options for therapy and alternative antibiotic treatments [6]. The plant kingdom appears as huge supply of active compounds with biological activities and antimicrobial properties (phytochemicals). These phytochemicals, are secondary metabolites in higher plants such as: alkaloids, steroids, flavonoids, terpenoids, tannins, etc [7]. The field of active compounds from plant sources was attractive target for scientists working on fighting infections. In recent years, there was evolution of interest in natural products which had antibacterial and antifungal activities. The specific role of some phytochemicals is still unclear. Screening of antimicrobial effect of plant extracts represents hope for discovery of new antimicrobial agents [8]. Large number

of researches had extensively studied the antimicrobial effect of plant extracts worldwide [8-11]. Therefore, the current study aimed to isolate bacteria pathogens from DFI and to study their current trends of antibiotic susceptibility. Also to evaluated the antibacterial properties of ten different extracts of medicinal plant against isolates from DFI.

METHODOLOGY

- **Sample collection and processing**

Patient attending the Outpatient Clinic of Diabetic Foot Unit, Mansoura Specialized Medical Hospital from January, 2014 to December 2014 and diagnosed clinically as diabetic foot infections. Patients who received antibiotic treatment systemically within the previous 72 hours were excluded from the study. This study was approved from the Medical Research Ethics Committee, Mansoura University. Swabs from deep tissue were collected from patient's wounds. The specimens was collected under aseptic precautions and transported to the Microbiology Department, Microbiology Diagnostics and Infection Control Unit (MDICU),. The samples were inoculated onto blood agar plates and examined after 48 hours incubation at 37 ºC. When no growth appeared plates considered free from aerobic organism.

- **Isolation and identification of bacteria**

Bacterial growth was identified according to the colony characters, hemolytic activity, microscopic examination by Gram's stain and biochemically according to standard microbiological procedures [12].

- **Antibiotic susceptibility testing**

Pathogenic bacteria isolated from DFI were tested against different antibiotic discs by the standard disk diffusion method. The inhibition zones were interpreted based on Clinical and Laboratory Standards Institute (CLSI) guidelines[13]. The following antibiotic discs were used: penicillin(G), 10 ug, amoxicillin/clavulanic acid (AMC), 30 ug, ampicillin/sulbactam (SAM), 20 ug, cefadroxil (CFR), 30 ug, cefoperazone (CEP), 75 ug, cefuroxime (CXM), 30 ug, ceftazidime (CAZ), 30 ug, cefotaxime (CTX), 30 ug, ceftriaxone (CRO), 30 ug, cefepime (FEP), 30 ug, meropenam (MEM), 10 ug, piperacillin (PRL), 100 ug, amikacin (AK), 30 ug, levofloxacin (LEV), 5 ug and clindamycin (DA), 2 ug. Bacteria that were resistant to three or more classes of antibiotics were considered as multi-drug resistant bacteria (MDR) [14].

- **Inocula preparation**

The bacteria subjected to further studying were MDR isolates. They included: *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis (S. epidermidis), Esherichia coli (E. coli), Klebsiella pneumoniae (K. pneumoniae), Proteus vulgaris (P. vulgaris), Proteus mirabilis (P. mirabilis)* and *Pseudomonas aeruginosa (P. aeruginosa).* The size of inoculum of the test strain standardized according to the CLSI guidelines [15].

- **Plant material and preparation of extract**

Green parts of ten tested wild medicinal plants were freshly collected. These were Leaf from *Ocimum basilicum* (basil), *Solenostemma argel* (sloenstemma), *Lawsonia inermis (*henna), *Cinnamomum camphora* (camphor) and *Marticaria chamomilla* (chamomile); seed from *Feniculum vulgare* (fennel) and *Nigell sativa* (black cumin); rhizome from *Zingiber officinale* (ginger); bark from *Cinnamum verrum* (cinnamon) and flower from *Syzgium aromaticum* (clove). These plants were dried at room temperature (20-25 ºC) and ground into a powder using a blender. The dried plants powder was macerated with methanol (80%) with continuous shaking for 48 h at room temperature. The extract was filtered through Whatman filter paper (No.2) and the filtrate was evaporated to dryness using vacuum rotary evaporator. Stock solution of extracts were prepared by diluting the dried extracts with 10% dimethyle sulfoxide (DMSO) solution to obtain a final concentration of 10 mg/ml [16].

- **Antibacterial activity of medicinal plants by well diffusion method**

Well diffusion method was employed for detection of antibacterial activities of medicinal plant extracts. 1 ml bacterial suspension was inoculated on Mueller Hinton agar medium. By using a sterile cork borer, wells of 6 mm diameter were cut from the agar. The wells were filled by adding 20 ul of the different plant extracts, while DMSO used as negative control and vancomycin (1µg/ml) as positive control. The plates were incubated for 24 h at 37 ºC. After incubation, the inhibition zones around each well were measured with caliper, recorded and considered as indication for antibacterial activity [17].

- **Preliminary screening of phytochemical substances**

About 100 g of air-dried plant powder were relaxed with 200 ml 70% methyl alcohol for 6 hours, and then filtered. The filtrates were centrifuged at 2000 rpm for about 10 min. The supernatant was obtained and allowed to evaporate until completely dried [18], then used for the following tests:

- ***Test for alkaloids***

Two ml of plant extract were added to 2 ml of conc. hydrochloric acid. Mayer's reagent drops were added. Development of white precipitate or green color indicates the presence of alkaloids [19].

- ***Test for glycosides***

Two ml plant extract were added to chloroform (3 ml) and 10% ammonia solution. Development of pink color indicated glycosides presence [20].

- ***Test for cardiac glycosides (Legal's test)***

Sodium nitroprusside in pyridine and sodium hydroxide were added to plant extracts. Presence of cardiac glycosides was indicated by formation of pink to red color [20].

- ***Test for saponins (Foam test)***

Plant extract (2 ml) was diluted with distilled water (2 ml) and shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam showed the presence of saponins [21].

- ***Test for phenols (Ferric chloride test)***

Extract were treated with a solution of ferric chloride (3-4 drops). Formation of bluish black color showed the presence of phenols [22].

- ***Test for sterols (Salkwski test)***

Alcoholic plant extract (2 ml) was left to dry. The residue was suspended in chloroform (2 ml) and filtered, then the filtrate was treated with few drops of sulphuric acid (conc.). Steroids were detected by formation of brown ring while phytosteroids detected by bluish brown ring [20].

- ***Test for tannins (Lead acetate test)***

Plant extract (5 ml) was added to few drops of lead acetate solution (10%). The test was positive for tannins by appearance of yellow or red precipitate [20].

- ***Test for flavonoids (NaOH test)***

To 2 ml of Plant extract (2 ml) was treated with few drops of sodium hydroxide solution. Flavonoids were detected by Formation of strong yellow color [23].

- ***Test for diterpenes (Copper acetate test)***

Extract was dissolved in water and added to copper acetate solution (3-4 drops). Positive diterpenes test was detected by formation of green color [24].

- **Investigation of total active materials**
- ***Estimation of total phenol content (TPC)***

The amount of TPC in extract was detected with the Folin Ciocalteu reagent. Gallic acid used as a standard and the amount of phenol expressed as µg/mg gallic acid equivalent to (GAE) [25] .

- ***Estimation of total flavonoid content (TFC)***

The amount of TFC in plant extract was estimated by colorimetric method using aluminum chloride assay. TFC was expressed as mg rutin/g dry weight (mg rutin/g DW), through the calibration curve of Rutin [26].

- ***Estimation of total tannins***

This quantitative method relays on precipitation of tannin using copper acetate solution, converting copper tannate to copper oxide and weighing the residual copper oxide [19].

- ***Estimation of total saponins***

About 2g plant parts were dispersed in 20 % ethanol. The combined extracts were concentrated and purified. The saponins content was calculated in percentage according to Okwu and Ukanwa [27].

- ***Estimation of total alkaloids***

Ethanol extract of plant parts (2 gm) was concentrated, filtered and extracted with chloroform. Then evaporated and weighed to estimate the percent [28].

RESULTS

In this study, 56 patients with diabetic foot ulcer were included, from which 59 bacterial species were isolated. There was higher male prevalence (62.5%) compared with female patient (37.5%). The mean age of the patients was 65.8 ± 13.8 years (mean ± SD; range, 36-75 years).

Microbiologic results

We reported 48 out of 56 samples yielded positive bacterial culture. Thus ulcer infection was detected in 85.7% cases (table 1). The current study detected mono-microbial infection in 66.1% patients while 19.6% had poly-microbial nature.

Table 1. Type of bacterial growth isolated from diabetic foot ulcer

Type of bacterial growth	No. of patient samples	Percentage of growth
Monomicrobial	37	66.1%
Polymicrobial	11	19.6%
No growth	8	14.3%
Total	56	100%

Among the isolated organisms, 50.85% were Gram-positive bacteria and 49.15% Gram- negative bacteria, *S. aureus* was the most common isolate followed by *S. epidermidis*, *P. aeruginosa* and *P. mirabilis*. The number and percentage of pathogenic bacteria isolated from DFI samples are shown in table 2.

Table 2. The Number and percentage of bacterial pathogens isolated from diabetic foot ulcer samples

Pathogenic bacteria	No.	Percentage (%)
Staphylococcus aureus	20	33.9 %
Staphylococcus epidermidis	10	16.95 %
Klebsiella pneumonia	6	10.17 %
Escherichia coli	4	6.78 %
Pseudomonas aeruginosa	9	15.25 %
Proteus vulgaris	3	5.09 %
Proteus mirabilis	7	11.86 %
Total	59	100 %

Antibiotic sensitivity testing results

Antibiotic sensitivity testing demonstrated that the isolated bacteria were resistant to the commonly used antibiotics. Antibiotic susceptibility test of 59 bacteria showed that 20 (33.9%) were MDR. Resistance pattern to different antibiotics among the isolates was; penicillin (100%), amoxicillin/clavulanic acid (93%), ampicillin/sulbactam (90%), cefadroxil (83%), cefoxitin (100%), cefoperazone (38%), cefepime (70%), meropenem (80%), amikacin (93%), cefuroxime (92%),

ceftriaxone (66%), cefotaxime (76%), piperacillin/tazobactam (28%), levofloxacin (64%) and clindamycin (37%). All *S. aureus* were resistant to cefoxitin, hence designated as methicillin resistance *S. aureus* (MRSA). Among the MDR isolates, 7 strains were chosen for further investigations.

Effect of plant parts extract on isolated species.

Investigation of the crude ethanol extracts of the basil, sloenstemma, henna, camphor, chamomile, fennel, black cumin, ginger, cinnamon and clove showed different degree of growth inhibition, by using the well diffusion test. Solenostemma and clove extracts caused growth inhibition of all tested MDR bacteria (*S. aureus*, *S. epidermidis*, *K. pneumoniae*, *E. coli*, *P. mirabilis*, *P. aeruginosa*, and *P. vulgaris*), while black cumin seeds extract was effective against all isolates except *S. epidermidis* and *P. vulgaris*. Basil showed antibacterial activities against all bacterial species except *E. coli* and *K. pneumoniae*. Whereas, others plant extracts henna, camphor, chamomile, fennel, ginger and cinnamon showed no activity against MDR bacteria. This result was clarified in table 3.

Table 3. Effect of plant parts extract on isolated species

Bacterial species	Zone of Inhibition (mm)									
	Basil	Sloenstemma	Ginger	Chamomile	Clove	Camphor	Black cumin	Fennel	Henna	Cinnamon
S. aureus	12 M.S	24 H.S	R	R	12 M.S	R	24 H.S	R	R	R
S. epidermidis	16 M.S	24 H.S	R	R	18 S	R	R	R	R	R
K. pneumoniae	R	22 S	R	R	24 H.S	R	16 M.S	R	R	R
E. coli	R	22 S	R	R	24 H.S	R	24 H.S	R	R	R
P. aeruginosa	12 M.S	22 S	R	R	22 S	R	16 M.S	R	R	R
P. mirabilis	22 S	22 S	R	R	12 M.S	R	28 H.S	R	R	R
P. vulgaris	11 M.S	22 S	R	R	20 S	R	R	R	R	R

R→Resistant; M.S→Mild sensitive (11-16 mm); S→Sensitive (17-23); H.S→High sensitive (24 -28mm).

Screening of phytochemical materials in medicinal plants extract

Phytochemical screening of the four medicinal plants extract (basil, solenstemma, clove and black cumin) that had antibacterial effect against MDR bacteria, indicated that these plant parts had powerful secondary metabolites and active materials such as: alkaloids, glycosides, cardiac glycosides, saponins, phenol, tannins, flavonoids and diterpene. Results are demonstrated in tables 4 and 5. These secondary metabolites were accountable to their antibacterial effect.

Table 4. Phytochemical screening of plant parts

Secondary metabolities	Phytochemical screening Tests	Plant parts			
		Basil	Solenstemma	Clove	Black cumin
Alkaloids	Mayer's test	-ve	-ve	+ve	+ve
Glycosides	Glycosides test	+ve	+ve	+ve	+ve
Cardiac glycosides	Legal's test	-ve	-ve	+ve	+ve
Saponins	Foam test	+ve	+ve	+ve	+ve
Phenol	Ferric chloride test	+ve	+ve	+ve	+ve
Sterol	Salkawskis test	-ve	-ve	-ve	-ve
Tannins	Lead acetate test	+ve	+ve	+ve	+ve
Flavonoids	NaOH test	+ve	+ve	+ve	+ve
Diterpene	Copper acetate test	-ve	-ve	-ve	+ve

Table 5. Total active materials in different experimental plant parts.

Total active materials	Plant parts			
	Basil	**Solenstemma**	**Clove**	**Black cumin**
Total flavonoid (mg/gm rutin)	265±1	210±3	310±5	332±2
Total phenolic acids (mg/gm gallic acid)	309±2	209±3	352±1	362±3
Total saponins (%)	1.5±0.3	2.4±0.2	2.1±0.5	1.7±0.1
Total alkaloids (%)	-ve	-ve	1.7±0.2	3.5±0.4
Total tannins (%)	2±0.5	1.98±0.4	1.5±0.4	2.1±0.7

DISCUSSION

In the present study, among the 56 patients having DFI, there was a higher male prevalence. This result agreed with that of Patil and Mane[29], who reported that male patients with diabetic foot ulcer were predominant (78.6%) compared with female patient (21.4%). Male predominance might be explained on the basis that the males spend more time working outdoors, exposing their foot to more traumas. We detected ulcer infection in 85.7% of cases, in which mono-microbial infection were detected in 66.1% patients while 19.6% had poly-microbial nature.

This result consistent with Raja[30], who reported that mono-microbial growth was more than poly-microbial growth among DFI. However, this result was in disagreement with most previous studies who recorded the predominant of poly-microbial infection [31,32].

Our results showed that 50.85% were Gram positive bacteria and 49.15% Gram negative bacteria, a result that was close to the Citron et al. [32] in which Gram positive comprised 80.3% of the aerobic organisms. However, Raja[30] showed that 52% were Gram negative bacteria while 45% were Gram positive bacterial infection. In our study, *S. aureus* was commonest isolate followed by *S. epidermidis*, *P. aeruginosa* and *P. mirabilis*.

This was consistent with results reported by Sharma et al.[31] who found that *S. aureus* was the predominant (38.4%) isolate followed by *P. aeruginosa* (17.5%) and *P. mirabilis* (14%).

Citron et al. [32] detected the predominant Gram positive was *S. aureus* (76.6%), followed by *Enterococci* (35.7%) and mong Gram-negative *P. aeruginosa* (19.7%) followed by *P. mirabilis* and *Klebsiella* sp.. Also Raja[30] found *S. aureus* the most common, followed by *Proteus* sp and *P. aeruginosa*.

In our research, antibiotic sensitivity testing showed that the isolated bacteria were resistant to most tested antibiotics and among which, 23.9% were MDR. The high percentage of antibiotic resistance observed may be caused by the widespread usage of broad-spectrum antibiotics in our hospital which led to selective pressure advantage for resistant pathogens. Our research revealed the presence of antimicrobial properties of four medicinal plants; solenostemma, clove, black cumin and basil, with solenostemma and clove showing highest antibacterial effect against all tested bacteria. This observation was in agreement with Emmanuel et al. [10] who studied antimicrobial activity of cloves extract in different concentrations and showed their inhibitory action against *S. epidermidis*, *E. coli*, *P. mirabilis*, *K. pnuemoniae*, *Aspergillus niger*, *Candida albicans*, *Rhizorpus spp* and *Aspergillus flavus.*

Also Simiat et al. [33] reported that clove had a high inhibitory effect on bacterial isolates (*S. aureus*, *E. coli* and *P. aeruginosa*) and fungal isolates (*C. albicans*, *A. flavus* and *Penicillium* species).

On the contrary, Shailesh et al. [11] found that clove failed to show any antibacterial action against *S. aureus* and *Bacillus* in their study. Our result was consistent with another study which detected positive antibacterial effect of ethanol extract of solenostemma against *E. coli*, *P. aeruginosa*, *S. aureus* and *Bacillus subtilis* [34].

Also cumin was reported to have an inhibitory action against *S. aureus*, *Bacillus subtiles*, *E. coli* and *P. aeruginosa* [35]. In the present study, basil was effective against all tested bacteria except *K. pnuemoniae* and *E. coli*. While previous studies reported that basil had antibacterial action against *E. coli*, *P. aeruginosa*, *S. aureus*, *P. mirabilis*, *K. pneumoniae* and *Enterococcus faecalis* at all concentrations [9,35].

In our study, other plants extract e.g. henna, camphor, chamomile, fennel, ginger and cinnamon were inactive against all isolates. However, Mita et al. [8] reported that cinnamon antibacterial had effect against test organisms (*S. aureus*, *K. pneumonia*, *P. aeruginosa* and *P. vulgaris*).

In contrast to our result, others demonstrated that ginger was effective against *E. coli*, *Bacillus*, *S. aureus* and *Aspergillus Niger* [36]. Our screening for phytochemical materials of the 4 effective plant parts (basil, solenstemma, clove and black cumin) indicated that they had secondary metabolites such as alkaloids, glycosides, cardiac glycosides, saponins, phenol, tannins, flavonoids and diterpene, which were accountable to the curative properties of medicinal plants [37]. These secondary metabolites of plants serve as a defense mechanism against attack by micro-organisms, insects and herbivores. The existence of alkaloids and saponins in leaf extract is very essential

and used in bactericidal activities. Saponins have properties of foam formation in water solutions and hemolytic activity and extensively used as detergents, pesticides and bactericidal [38]. Flavonoids revealed a wide range of actions such as anti-inflammatory, antioxidant, antiallergic, and antimicrobial [39]. Tannins combine with proline-rich protein causing block of cell wall synthesis, and have antibacterial action against *S. aureus*, *Streptococci*, *Bacillus subtilis*, *E. coli* and *Salmonella* spp [40].

CONCLUSION

The results reported by the current study are encouraging as some medical plants showed antibacterial effect against most MDR pathogens, but the antibacterial effect varies widely, depending on the kind of medical plant and microorganisms. This study opens up the possibility for the search of new antimicrobials as alternatives to the antibiotics and positively participate in solving the trouble of diabetic foot ulcers and resistance of bacterial strains to antibiotic.

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REFERENCES

1. Shanmugam P, Jeya M, Susan SL. The bacteriology of diabetic foot ulcers, with a special reference to multidrug resistant strains. J. Clin. Diagn. Res. 2013; 7: 441-445.
2. Yerat RC, Rangasamy VR. Clinicomicrobial study of diabetic foot ulcer. Int. J. Med. Pub. Health. 2015; 5(3): 236-241.
3. Mathangi T, Prabhakaran P. Prevalence of Bacteria Isolated from Type 2 Diabetic Foot Ulcers and the Antibiotic Susceptibility Pattern. Int.J.Curr.Microbiol.App.Sci. 2013; 2(10): 329-337.
4. Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJG, Armstrong DG, et al. 2012 infectious diseases society of america clinical practice guideline for the diagnosis and treatment of diabetic foot infections. J. Americ. Pod. Med. Ass. 2013;103(1): 2-7.
5. Akova M. Epidemiology of antimicrobial resistance in bloodstream infections. Virulence. 2016;7(3):252–266.
6. Mühlen S, Dersch P. Anti-virulence strategies to target bacterial infections. Curr. Top. Microbiol. Immunol. 2016; 398: 147-183.
7. Peteros NP, Uy MM. Antioxidant and cytotoxic activities and phytochemical screening of four Philippine medicinal plants. J. Med. Plants. Res. 2010; 4(5): 407-414.
8. Mita V, Krupali M, Ayushi T. Phytochemical Analysis and Antimicrobial Activity of Cinnamomum verum. IJRSI. 2017; 4(4): 2321–2705.
9. Amjad k. Antimicrobial activity of ethanolic extracts of Ocimum basilicum leaf from Saudi Arabia, Asian Network for Scientific Information. Biotechnology. 2013; 12(1): 61-64.
10. Emmanuel OO, Macdonald I, Osayamen CU. Phytochemical Screening and Antimicrobial Sensitivity of Clove Flower (Syzygium aromaticum, L. Merrill and Perry) Bud on Dental Pathogens. Ijppr. Human 2015; 3(2): 1-13.
11. Shailesh. Preliminary phytochemical and antimicrobial screening of Syzygium aromaticum, Elettaria cardamomum and Piper nigrum extracts. J. Pharm. Photochem. 2015; 4(3): 85-89.
12. Holt JG, Krieg NR, Sneath PH, Staley JT, Williams T, Hensy WR. Bergy's manual of determination bacteriological. 9th ed. Williams and Wilkins, Baltimore, Meryland, 1994.
13. CLSI. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Tenth informational supplement. Wayne, Pennsylvania: CLSI, 2010.
14. Shales DM, Gerding DN, John JF, Craing WA, Bomstein DL, Dunean RA. Infect. Central Hosp Epidermal. 1997; 18: 275-291.
15. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 15th informational supplement. CLSI document M100-S15. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 25, 2005.
16. Mehraban F, Nasim OT, Fereshteh J. Antidermatophyte activities of Ecalyptus camaldulensis in comparison with griseofulvin. IJPT. 2005; 4: 80-83.
17. Chattopadhyay RR, Bhattacharyya SK, Medda C, Chanda S,Datta S, Pal NK. Antibacterial activity of black myrobalan (Fruit of Terminalia chebula Retz.) against uropathogen *Escherichia coli*. Pharmacognosy Magazine. 2007; 11: 212-215.
18. Lakshmi MS, Kumar VS, Deepika J, Begum FI. Evaluation of antibacterial activity of methanol extract of leaves of Adhatodavasica on mastitis pathogens. Hygeia. J. D. Med. 2013; 5: 1–4.
19. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global J. Pure Appl. Sci. 2001; 8: 203–

208.

20. Treare GE, Evans WC. Pharmacognosy 17th edition, Bahiv Tinal, London, 149, 1985.
21. Kokate CK, Purohit AP, Gokhale SB. Carbohydrate and derived Products, drugs containing glycosides, drugs containing tannins, lipids and protein alkaloids. Text book of Pharmacognosy, 7, edition: 133 -166, 167- 254, 2001.
22. Ahmad B, Naeem AK, Ghufran A, Innamudin. Pharmacological Investigation of Cassia sophera, Linn. Var. purpurea, Roxb. Med. J. Islamic World Academy Sci. 2005; 15(3): 105-109.
23. Khandeal KR. Practical Pharmacognocy. Nirali Prakashan, Pune, edition: 19, 2008.
24. Nikhal SB, Dambe PA, Ghongade DB, Goupale DC. Hydroalcoholic extraction of Mangifera indica (leaves) by Soxhletion. Int. J. Pharm. Sci. 2010; 2 (1): 30-32.
25. Chun K, Kim D, Lee CY. Superoxide radical scavenging activity of the major polyphenols in fresh plums. J. Agric. Food Chem. 2003; 51: 8067-8072.
26. Malla MY, Sharma M, Saxena RC, Mir MI, Bhat SH. Phytochemical screening and spectroscopic determination of total phenolic and flavonoid contents of Eclipta alba Linn, J. Nat. Prod. Plant Resour. 2013; 3(2): 86-91.
27. Okwu DE, Ukanwa NS. Nutritive value and phytochemical contents of fluted pumpkin (Telfaria Occidentalis Hook f.) vegetable grown with different levels of Turkey droppings. African Crop Science Conference Proceedings. 2007; 8: 1759-1964.
28. Woo WS, Chi HJ, Yun, Hye S. Alkaloid screening of some Saudi Arabian plants. Saengyak Hakhoe Chi (Hanguk SaengyaK Hakhoe). 1977; 8(3): 109-113.
29. Patil SV, Mane RR. Bacterial and clinical profile of diabetic foot ulcer using optimal culture techniques. Int. J. Res. Med. Sci. 2017; 5: 496-502.
30. Raja NS. Microbiology of diabetic foot infections in a teaching hospital in Malaysia: a retrospective study of 194 cases. J. Microbiol. Immuno. Infect. 2007; 40(1): 39-44.
31. Sharma VK, Khadka PB, Joshi A, Sharma R. Common pathogens isolated in diabetic foot infection in Bir Hospital. Kathmandu Univ. Med. J. 2006; 3(15): 295-301.
32. Citron DM, Goldstein EJC, Merriam CV, Lipsky BA, Abtamson MA. Bacteriology of moderate-to-severe Diabetic foot infections and in vitro activity of antimicrobial agents. J. Clin. Microbio. 2006; 45(9): 2819-2829.
33. Simiat OJ, Lateefah AA, Kazeem AA. Phytochemical Screening and Antimicrobial Evaluation of Syzygium aromaticum Extract and Essential oil. Int. J. Curr. Microbiol. App. Sci. 2017; 6(7): 4557-4567.
34. Adam AF, Elassam HA. Beneficial antibacterial, antifungal and anti-insecticidal effects of ethanolic extract of Solenostemma argel leaves. Mediterranean J. Biosci. 2016; 1(4): 184-191.
35. Adam ZA, Omer AA. Antibacterial Activity of Ocimum basilicum (Rehan) Leaf Extract against Bacterial Pathogens in Sudan. Am. J. Res. Comm. 2015; 3(8): 94-99.
36. Riaz H, Almas B, Syed AR, Zia MK, Hamad Y, Ayesha T. Antimicrobial property and phytochemical study of ginger found in local area of Punjab, Pakistan. Int. Current Pharm. J. 2015; 4(7): 405-409.
37. Britto JD, Sebastian SR. Biosynthesis of silver nano particles and its antibacterial activity against human pathogens. Int. J. Pharm. Sci. 2011; 5: 257-259.
38. Shi J, Kakuda Y, Yeung D. Antioxidative properties of lycopene and other carotenoids from tomatoes: Synergistic effects. Biofactors 2004; 21(1-4): 203-210.
39. Hossain MA, Muhammad MD, Charles G, Muhammad I. In vitro total phenolics, flavonoids contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant Tetrastigma from Sabah. Asian Pac. J. Trop. Med. 2011; 4(9): 717-721.
40. Abdulhamid A, Fakai IM, Sani I, Argungu AU, Bello F. Preliminary phytochemical and antibacterial activity of ethanolic and aqueous stem bark extracts of Psidium guajava. Am. J. Drug Discovery Dev. 2014; 4: 85-89.