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Antimicrobial Potentials of *Apis Multiflora* Honey in Combination with Coffee and Cinnamon Extracts against Common Human Pathogenic Bacteria

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Abstract

Background: Traditional medicines have been used widely by the people of Ethiopia for treatment of various ailments. However, scientific evidences regarding their antibacterial potential are scarce. The present study aim to assess an *in vitro* antibacterial activity of *Apis multiflora* honey in combination with coffee and cinnamon bark extracts against the standard and clinical isolates of human pathogenic bacteria.

Methods: Antimicrobial activities of honey and the extracts were tested against *Escherichia coli*, *Citrobacter* species, *Staphylococcus epdermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 2923). Agar well diffusion and micro-well broth dilution techniques were used to determine the antibacterial activity and minimum inhibitory concentration.

Result: Honey exerted a maximum bacterial inhibition against *Citrobacter* species and *E. coli* (29 mm). Coffee extracts displayed best antibacterial activity against *S. aureus* (25-26 mm) and cinnamon extract exhibited the maximum inhibitory effect against *S. epidermidis* (31 mm). The combination of honey with cinnamon was most effective against *P. aeruginosa* (27 mm), whereas the combination of honey with coffee and cinnamon extracts was most effective against *S. aureus* ATCC 2923 (35 mm). The antibacterial activity exerted by a reference antibiotic ceftraxone against different test strains ranged from 24-37 mm.

Conclusion: Coffee and cinnamon extracts, and honey have demonstrated a broad spectrum antibacterial effect. Our data indicating that these natural products have potential to be used as alternative antimicrobials for treatment of pathogenic bacteria. Thus, we recommend further investigations of each extract to elucidate bioactive compounds responsible for the observed antibacterial activity.

Keywords: Honey, Cinnamon, Coffee, Antimicrobial activity

Introduction

Infectious diseases represent an important cause of morbidity and mortality worldwide [1,2]. Chemotherapy of infected individuals with antimicrobial drugs is one of the widely used strategies for the control of infectious diseases [3-5]. However, the recent upsurge of antimicrobial drug resistance and its spread has posed a unique challenge to the global infectious diseases control program [3-5]. Further, affordability and accessibility of newer pharmaceutical antimicrobials are issues of major concern in developing countries, especially in rural areas. Therefore, a search for safe, cheaper and effective antimicrobial agents is of urgent necessity to cope with this global challenge. With this regard, traditional medicinal plants are one of the potential sources of alternative antimicrobial agents that can be used for treatment of various ailments [6,7].

Medicinal plants have played crucial roles in traditional health care system since the origin of mankind. There has been a worldwide increase in public interest toward traditional remedies, involving natural products, in the past decades [8]. Presently, it is estimated that up to 80% of population in developing countries rely on herbal medicines to meet their primary health care needs [9] and herbal remedies are being available in drug stores and supermarkets [8].

In Ethiopia, traditional medicines continue to be an important segment of primary health care to the majorities of rural population [10]. This wide usage of traditional remedies among the population of Ethiopia could be in part attributed to their probable efficacies against some diseases, cultural acceptability, and their accessibility and affordability compared to allopathic medicines. Despite the wide usage, information regarding the safety and *in vivo* or *in vitro* efficacies of Ethiopian traditional medicines are very limited. Thus, careful screening of the safety and efficacies of these traditional medicines is of paramount importance.

Honey has been used as a medicine since ancient times and is still being used in many traditions. A number of studies revealed the antimicrobial potential of honey for treatment of ulcers, bed sores, wound infecting methicillin-resistant *Staphylococcus aureus* and bacterial gastroenteritis in infants [11,12]. In Ethiopia, honey has been and being used traditionally for treatments of respiratory infections, coughs and gastrointestinal complaints either singly or in combination with other beverages like coffee. Similarly, coffee is used for treatment of respiratory infections and stomach pain in combination with honey and/or Cinnamon barks. Extracts of coffee have been reported to

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inhibit *Staphylococcus aureus*, *Salmonella typhi*, *Shigella dysenteriae*, *Vibrio cholera*, *parahaemolyticus* and *Yersinia enterocolitica* [13] and cinnamon extracts have been demostrated to exert their antimicrobial effect against a wide range of pathogenic bacteria and fungi [14,15]. However, scientific evidences regarding their effect in combination are missing. The aim of the current study was to investigate the antibacterial activities of a combination of honey with coffee and Cinnamon extracts against common human pathogenic bacteria.

Materials and Methods

Collection of samples

Honey samples were collected from local bee-keepers near Gondar town (15 km), Gondar, Ethiopia during the flowering season of 2013. The samples were collected in sterile screwed cups and kept in a cool and dry place until test. Cinnamon barks and Coffee grains were purchased from the local market of Arada, Gondar, Ethiopia.

Preparation of honey solutions

Honey samples collected from the local farmers were melted in water bath (at 45°C) and filtered with sterile mesh to remove debris. The filtrates were checked for purity by streaking on blood agar plates followed by overnight incubation at 37°C. Samples that had not shown contamination were then stored at 4°C until used. Honey dilutions (12.5, 25, 50, 75%) were made in distilled water and used immediately for the test.

Preparation of cinnamon and coffee extracts

Cinnamon and coffee extracts were prepared in accordance with the Ethiopian traditional way of processing these products for usage. Briefly, cinnamon barks were cleaned with deionized water and dried in the sunlight for two days. The barks were further dried in an oven (at 40°C) for 30 minutes and grinded into fine powders. Cinnamon extracts were prepared by soaking 10 g powders in 50 ml of absolute ethanol or methanol in a 250 ml Erlenmeyer flask. The flasks were sealed with an aluminum foil and placed on a shaker for 48 hr at room temperature. Following the incubation, the mixtures were centrifuged at 3,500 RPM for 20 min and filtered through Whitman filter paper No.1. The filtrates were dried at 40°C in a dry oven until semi-solid substances were obtained and then further dried in a crucible at 45°C. The extracts were stored at -20°C until used. Methanol and ethanol extracts were dissolved in dimethyl sulfoxide (DMSO).

Similarly, for preparation of coffee extracts, coffee beans were washed thoroughly with distilled water and roasted for 18–20min at 217°C. The roasted coffee beans were crushed into fine powders using mortar and pestle. The extracts were prepared by mixing 7.5 g of coffee powders with 31 ml of absolute ethanol or methanol followed by incubation on shaker for 48 h at room temperature. Filtrations, evaporation of the solvents and storage of the resulting extracts were done as indicated for cinnamon extracts.

Preparation of test organisms

A total of six bacteria strains were used for screening the antimicrobial activities of the extracts and honey. Four clinical isolates, *S. epdermidis, S. aureus*, *E.coli, and Citrobacter* were obtained from the University of Gondar Teaching Hospital diagnostic laboratory. The standard bacteria, P. *aeruginosa* (ATCC 27853*) and S. aureus* (ATCC 2923*)* were already available at the laboratory of medical microbiology, the University of Gondar, Ethiopia.

Preparation of inocula

Bacteria inocula were prepared by sub-culturing a loopful of each strain in nutrient agar slopes at 37°C for 24 hr. Colonies from the overnight cultures were picked with sterile loop and inoculated into sterile test tubes with 3 ml nutrient broth. The turbidity of bacterial suspension was adjusted to 0.5 McFarland's standard giving a bacterial load of about 1×10^8 CFU/ ml [16].

Determination of antibacterial activities of test samples

Antibacterial activities of each extract and honey was tested against six strains of bacteria using agar-well diffusion assay. Briefly, 100 µl of each bacterial suspension, equivalent to 0.5 McFarland's standard, was evenly distributed on Muller Hinton agar (MHA). Then, 50 µl of each extracts were applied to the wells prepared by well borer. Ciprofloxacin disc and DMSO were included for the positive and negative controls respectively. The plates were then incubated at 37°C for 24 hr. At the end of incubation period, the antimicrobial activity of each sample was determined by measuring the diameter of inhibition zone around the wells.

Determination of MIC and MBC

Minimum inhibitory concentration (MIC) was determined using the broth microdilution as described previously [17]. Briefly, the honey was serially diluted to the concentration of 75%, 50%, 25%, 12.5%, 6.25%, and 3.125% in a 96-well microtiter plate. Similarly, the extracts were serially diluted to concentrations ranging from 3.125 mg/ml to 100 mg/ml. Then, 100 µl of test organisms, 5×10^5 CFU, were added to duplicate wells of each dilution and incubated at 37°C for 24 hr. DMSO treated wells and wells with no bacteria (media only) were included for controls. The lowest concentration at which the bacteria did not show visible growth was defined as the MIC of a test sample.

Minimum Bactericidal Concentration (MBC) was determined by inoculating 100 µl of culture medium from the wells with no visible growth in the MIC test and subculturing on a fresh MHA at 37°C for 24 hr. The concentration at which no visible bacterial colonies were seen on MHA was considered as MBC of the test sample [18].

Statistical analysis

Results are presented as arithmetic mean of at least duplicate observations. Data were tested for statistical significance by two ways ANOVA with Bonferroni posttests. P value less than 0.05 was considered statistically significant.

Results

The antibacterial activities of honey (75% v/v) and extracts of coffee and cinnamon were tested against six strains of common human pathogenic bacteria. The bacterial inhibition effects of honey and each extract are depicted in Table 1. The antimicrobial activities exhibited by honey were ranged from 19 mm-29 mm inhibition zone diameter (IZD). Honey showed maximum inhibition against *Citrobacter* species and *E. coli* (29 mm). The methanol extract of coffee was most effective against *S.aureus* (25 mm). Similarly, its ethanolic extract exhibited the highest (26 mm IZD) antimicrobial activity against *S.aureus*, both the standard strain and the clinical isolates. But, it showed the lowest (14 mm IZD) antibacterial activity against *S. epidermidis* and *P.aeroginosa*. The methanol extract of cinnamon showed a strong inhibitory effect (31 mm) against *S. epidermidis* and the lowest inhibition against *E.coli*. The bacterial inhibition effect exerted by methanol extract of cinnamon

Solution /Extracts (100 mg/mL)	Solvent	Antimicrobial activity (mm)					
		S. aureus	*S.aureus ATCC2923*	*S.epdermidis*	Citrobacter Spp	*E.coli*	*P.aeruginosa ATCC27853*
Honey 100%	-	28	24	25	27	25	22
75%	W	24	20	23	29	29	19
50%	W	22	18	20	25	25	12
Coffee	M	25	25	12	13	24	15
	E	26	26	14	17	23	14
Cinnamon	M	24	24	31	19	15	17
	E	19	19	19	17	17	15
HCf	M	25	24	16	14	19	21
	E	21	20	14	24	19	22
HCn	M	20	16	20	22	22	20
	E	24	24	17	21	21	27
HCfCn	M	30	30	0	0	0	13
	E	33	35	0	0	12	0
Ceftraxone (30µg)		36	35	35	30	32	32

W=Water, E=Ethanol, M=Methanol, HCf: Combination of honey and coffee; HCn: combination of honey and Cinnamon; HCfCn: combination of honey, coffee and cinnamon. Honey was use at 75% concentration in the combinations.

Table 1: Antimicrobial activity of honey and its synergetic effect with coffee and cinnamon against different bacteria, 2013.

against *S.epidermidis* was similar to that of the positive control ceftraxone (35 mm). On the other hand, ethanol extract of cinnamon exerted a moderate (17-19 mm IZD) inhibitory effect against all test organisms.

Combination of methanol extract of coffee with honey had improved the antimicrobial activity of the extract against *P.aeroginosa* (Table 1). Similarly, treatment with combination of the ethanol extract of coffee and honey showed better antibacterial activity against *P.aeroginosa* and *Citrobacter* species. The overall bacterial inhibition activity of the coffee extracts in combination with honey was even slightly lower than the individual effects of each sample against most test strains. On the other hand, combination of honey with the methanol extract of cinnamon had improved the bacterial inhibition effect of cinnamon against *E.coli*, *Citrobacter* species and *P.aeroginosa*. Combination of the ethanol extract of cinnamon with honey had improved the antibacterial activity of the extract against all test strains except *S. epidermidis*. The activity displayed by this mixture against *P. aeroginosa* was significantly higher ($p<0.05$) than the antimicrobial activity of each sample. Conversely, treatment with a combination methanol extracts of cinnamon with honey showed a reduced inhibitory effect against Gram positive *S. aureus, S. aureus* ATCC 2923, and *S. epidermidis* compared to the activity of individual samples (Table 1). The antibacterial activity displayed by this combination against *S. epidermidis* was significantly lower than the individual samples ($p<0.05$).

Treatment with combination of both extracts of coffee and cinnamon with honey exerted higher bacterial inhibition effect against the standard and clinical isolates of *S. aureus* (Table 1). Interestingly, honey with ethanol extracts of coffee and cinnamon showed similar inhibitory effect to the reference antibiotic ceftraxone (35 mm IZD) against the standard *S. aureus*. Apart from this effect, the combination of the three samples did not show antimicrobial activity against the remaining test strains of bacteria.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of honey, methanol and ethanol extracts of coffee and cinnamon against the test pathogens are given in Table.2. The MIC and MBC of honey against test bacterial strains ranged from 12 to 25 mg/ml and 25 to 50 mg/ml, respectively. The MIC and MBC values for ethanol extract of coffee ranged from 12.5 to 25 mg/ml and 25-50 mg/ml, respectively, whereas its methanol extract showed the MIC value of 12.5 mg/ml and MBC value of 25 mg/ml. The ethanol extract of cinnamon showed the MIC value ranged from 12.5 to 50 mg/ml and MBC values ranged from 25 to 50 mg/ml. The methanol extract of cinnamon showed the MIC values ranged from 6.25 to 25 mg/ml and MBC value of 25 mg/ml against all test organisms.

Discussions

The present study investigated the antibacterial potential of honey, and extracts of coffee and cinnamon against six strains of common human pathogenic bacteria. The results clearly indicated the antibacterial activity of honey as well as the extracts against all Gram negative and Gram positive bacteria strains used in this study. This data indicating that all the test samples have a broad spectrum antibacterial activity. As described previously, bacterial sensitivity to antimicrobial drugs classified as resistant, if an induced zone of inhibition by an antimicrobial drug is less than 8 mm; intermediate, if it is between 8-11 mm and sensitive, if it exerted an inhibition zone diameter of 12 mm or more [19]. According to this report, all test strains of bacteria were sensitive to the antibacterial effect of honey, and the ethanolic and methanolic extracts of coffee and cinnamon.

The antimicrobial activity exerted by honey against *Citrobacter species* and *E.coli* were comparable with that of ceftraxone, a reference antibiotic ($p>0.05$). The observed activity of honey could be due to its acidity, osmolarity and hydrogen peroxide contents which have been reported to display antimicrobial activity [20,21]. Despite the slight numerical variation between our observation and others, the overall broad spectrum antibacterial effect of honey is in a good agreement with previously published reports [20-22]. In fact, the antimicrobial effect of honey can be influenced by the type of flowers from which it is made, which in turn influenced by geographic variations and flowering seasons [22]. Thus, the slight difference between this study and others could be attributed to the geographic variation and the type of flowers from which the honey was made.

Both methanol and ethanol extracts of coffee were most effective against *S. aureus*, the standard and clinical isolates, and against enteric pathogen *E. coli*. Further, both coffee extracts showed lower MIC and MBC values against Gram negative as well as Gram positive test strains, indicating a broad spectrum antibacterial activity of the extracts. Of note, there was no significant difference between the antibacterial

Microorganisms		MIC			MBC		
		Honey§	Coffee	Cinnamon	Honey§	Coffee	Cinnamon
S. aureus	E	12	12.5	12.5	25	50	25
	M		12.5	6.25		25	25
S. aureus ATCC2923	E	12	12.5	12.5	25	25	25
	M		12.5	6.25		25	25
S. epidermidis	E	25	25	50	50	50	50
	M		12.5	25		25	50
Citrobacter	E	12	12.5	25	50	25	50
	M		12.5	25		25	50
E. Coli	E	25	12.5	25	25	25	50
	M		12.5	25		25	25
P. aeruginosa	E	12	12.5	12.5	50	25	25
	M		12.5	12.5		25	25

E: Ethanol; M: methanol; §: dilution made in water, Values are in mg/ml.

Table 2: The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of honey and the ethanol and methanol extracts of Coffee and cinnamon against the test human pathogenic bacteria.

activity of the methanol and ethanol extracts of coffee, suggesting that both solvents are equally potent for extraction of antimicrobial agent from coffee samples. Treatment with coffee extracts in combination with honey showed better effect against *P. aeruginosa* compared to the extracts alone. Phytochemical analysis of coffee extracts demonstrated the presence of organic acids, alkaloids, phenols, tannic acids, caffeine and several aromatic compounds [13,23]. Based on these report it is tempting to speculate that the observed antibacterial effect of coffee extracts could be due to the effect of one of or combinations of these chemicals. However, the compounds responsible for the observed effect need to be identified.

The methanol extract of cinnamon was most effective against *S. epidermidis* (31 mm) compared to ceftraxone (35 mm). However, this antibacterial effect was significantly reduced ($p<0.05$) when the extract was used in combination with honey. This suggests that the antibacterial agent in cinnamon extract could be partly inhibited or diluted by the constituents of honey. In contrast, combination of ethanol extract of cinnamon with honey showed better bacterial inhibition effect than the extract alone, albeit the difference didn't reach the statistical significance. The antimicrobial activity of cinnamon extracts could be assigned to compounds like cinnamaldehyde and trans-cinnamaldehyde that have been reported to be the major constituents of the essential oil and extracts of this plant [24-26]. Cinnamaldehyde and trans-cinnamaldehyde have been demonstrated to disrupt bacterial cell membrane that lead to ion leakage and death of bacteria [25]. Further, trans-cinnamaldehyde has been reported to inhibit acetyl-CoA carboxylase [26]. The broad spectrum antibacterial activities of both cinnamon extracts are consistent with a number of reports that demonstrated a wide range antimicrobial potential of crude extracts and essential oil of this plant parts [24-26]. Taken together, these data demonstrated that cinnamon has a potential to be a source of potent antimicrobial agent with a broad spectrum activity.

The overall antibacterial activities exhibited by honey and the extracts were slightly lower than that of the reference antibiotic ceftraxone which is in line with a number of previous reports [27,28]. From these observations, it can be presumed that the antibacterial activities of the products could be enhanced by isolating and concentrating the active ingredients responsible for the aforementioned effects. Combination of the extracts with honey showed improved efficacy against specific strains of test organism. Reduction in the activity of extract up on combination with honey could be attributed to the dilution effect. However, loss of antibacterial activity up on co-application of the coffee and cinnamon extracts with honey suggest the presence of compounds with antagonistic effect. Nevertheless, the observed efficacies of extracts with honey against some test strains justify the traditional use of these combinations.

Conclusions

The present study demonstrated a broad spectrum antibacterial potential of honey and the extracts. The activities of these natural products against the standard and clinical bacterial isolates indicate their potential as sources of potent antibacterial agent that can be used against multidrug resistant microorganisms. Thus, we recommend bioassay-guided fractionation of each extract and identification of bioactive compounds responsible for the antibacterial activity.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

TT, SZ, SM, DB, conceived the study and participated in sample collection. BT, BB, ME and TD designed the study. DB, SZ and SM assisted in data collection and conducted the experiments. TD performed data analysis, interpretation and wrote the manuscript. All authors read and approved the final manuscript.

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References

1. Burke JP (2003) Infection control. A problem for patient safety. N Engl J Med, 348: 651-656.
2. Fauci AS (2001) Infectious diseases: considerations for the 21st century. J Clin Infect Dis 32: 675–685.
3. Rossolini GM, D'Andrea MM, Mugnaioli C (2008) The spread of CTX-M–type extended-spectrum beta-lactamases. Clin Microbiol Infect. 14: 33–41.
4. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, et al. (2010) Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis. 10: 597–602.
5. Mulvey MR, Grant JM, Plewes K, Roscoe D, Boyd DA (2011) New Delhi metallo-β-lactamase in Klebsiella pneumoniae and Escherichia coli, Canada. Emerg Infect Dis. 17:103-106.
6. Cordell GA (2000) Biodiversity and Drug discovery: a symbiotic relationship. Phytochemistry 55: 463-480.
7. Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev. 12: 564-582.
8. Abbot NC, Ernst E (1997) Patients' opinions about complimentary medicine. Forsch. Komplementar med. 4: 164–168.
9. WHO/traditional medicine programme. Policy and activities of the field of traditional medicine. TRM96.2, Geneva, 1996.
10. CSO (1985) Report on rural health survey (1982/83). Stat Bull 1: 118-119.
11. Peter CM, Ray TC (2011) The controlled in vitro susceptibility of gastrointestinal pathogens to the antibacterial effect of manuka honey. Europ J clin microbiol and infect diseases. 30: 569-574.
12. Katrina B, Kamal A, Laurent St-Martin, Alan C (2011) Re-Examining the Role of Hydrogen Peroxide in Bacteriostatic and Bactericidal Activities of Honey. Front Microbiol 2: 213.
13. Daglia M, Tarsi R, Papetti A, Grisoli P, Dacarro C, et al. (2002) Antiadhesive effect of green and roasted coffee on Streptococcus mutans' adhesive

properties on saliva-coated hydroxyapatite beads. J Agric Food Chem. 50: 1225-1229.

14. Kalemba D, Kunicka A (2003) Antibacterial and antifungal properties of essential oils. Curr. Med Chem 10: 813-829.
15. Dorman HJ, Deans SG (2000) Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol. 88: 308-316.
16. Andrews JM (2006) BSAC standard disc susceptibility testing method 12. (version 5). J Antimicrob Chemother 58: 511-529.
17. Shahidi GA (2004) Evaluation of antimicrobial properties of Iranian medicinal plants against Micrococcus luteus, Serratia marceceans, Klebsiella pneumonia and Bordetella branchoseptica. Asian J Plant Sci 3: 82-86.
18. Patel RV, Thaker VT, Patel VK (2011) Anitmicrobial activity of ginger and honey on isolates of extracted carious teeth during orthodontic treatment. Asian Pac J Trop Biomed 1:S58-S61.
19. Baur AW, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standard single disc method. Am J Clin Pathol 45: 493-496.
20. Mavric E, Wittmann S, Barth G, Henle T (2008) Identification and quantification of methylglyoxal as the dominant antibacterial constituent of manuka (Leptospermum scoparium) honeys from New Zealand. Mol Nutr Food Res 52: 483-489.
21. Weston RJ (2000) The contribution of catalase and other natural products to the antibacterial activity of honey: A Review Food Chem 71: 235-239.
22. Taghizadeh M, Saffari M, Pourbabaei M, Mahboubi M (2011) Antimicrobial activity of different honey samples against Pseudomonas aeruginosa in vitro. Biharean Biol 5: 113-115.
23. Oladosu IA, OgundajoAL, Aiyelaagbe OO, Emenyonu N (2011) Phytochemical and Antituberculosis Activity of Coffea brivipes HIERN Extracts. Research Journal of Phytochemistry 5: 130-135.
24. Mishra AK, Mishra A, Bhargava A, Pandey AK (2008) Antimicrobial activity of essential oils from the leaves of cinnamomum spp. Natl Acad Sci Lett 31: 341–345.
25. Rana IS, Singh A, Gwal R (2011) In vitro study of antibacterial activity of aromatic and medicinal plants essential oils with special reference to cinnamon oil. Int J Pharm Sci 3: 376–380.
26. Meades G Jr, Henken RL, Waldrop GL, Rahman MM, Gilman SD, et.al. (2010) Constituents of cinnamon inhibit bacterial acetyl CoA carboxylase. Planta Med 76: 1570–1575.
27. Ebi GC and Ofoefule SI (1997) Investigations into folkloric antimicrobial Activities of Landolphia owerrience. Phytother Res. 11: 149-151.
28. Eloff JN (2004) Quantifying the bioactivity of plant extracts during screening and bioassay-guided fractionation. Phytomedicine, 11: 370-370.