

Antibacterial activities of *Microglossa pyrifolia* (Lamk.) Kuntze, *Leucas deflexa* Hook. and *Indigofera spicata* Forssk

Jemal Dilebo

Department of Pharmacy, College of Medicine and Health Sciences, Ambo University,
P.O.Box 19, Ambo, Ethiopia
Email: jdilebo@gmail.com
Mobile: +251913021659

Abstract

Microglossa pyrifolia, *Leucas deflexa*, and *Indigofera spicata*, traditionally used medicinal plants for treatment of diarrhea and other ailments by three ethnicities in South west Ethiopia, i.e., Bench, Meinit, and Sheko were studied for antibacterial activity against some selected species of bacteria using agar - well diffusion method. 80% methanol leaf extract of *M.pyrifolia* showed mean diameter of zone of inhibition of 16.5 and 18.5 mm against *S.aureus* and 11 and 12 mm against *S.sonnei* at 50 and 100 mg/ml concentration, respectively. Whereas, the 80% methanol root extract of *I.spicata* was active against *S.aureus* and *P.aeruginosa* with mean diameter of zone of inhibition of 11 and 14.5 mm against *S.aureus* and 8.5 and 10 mm against *P.aeruginosa* at 50 and 100 mg/ml concentration, respectively. However, 80% methanol leaf extract of *L.deflexa* was active only against *S.aureus* with mean diameter of zone of inhibition of 11 and 17.5 mm at 50 and 100 mg/ml concentration, respectively. On MIC test, the extract of *M.pyrifolia* had 8 mg/ml against both *S.aureus* and *S.sonnei*. The *in vitro* antibacterial activity may be the rationale for the traditional use of these medicinal plants to treat diseases such as diarrhea.

Key words: *Microglossa pyrifolia*, *Leucas deflexa*, *Indigofera spicata*, antibacterial activity, diarrhea

Introduction

Due to relevance in traditional medicine among ethnicities in South west Ethiopia i.e, Bench, Sheko, and Meinit, three medicinal plants such as *Microglossa pyrifolia* (Asteraceae), *Leucas deflexa* (Lamiaceae), and *Indigofera spicata* (Fabaceae) were selected for antibacterial activity screening.

M. pyrifolia is an erect or scandent shrub which grows up to 5 m and mostly grows along forest edges, river forests, grass land, bush land, and waste land and wide spread in tropical Asia and Africa [1]. The leaves, above ground, and root (mixed with *I.spicata*) is used for treatment of meningitis by Bench people [2]. The leaves are also used for treatment of hard swelling on the skin by Meinit people [3] and for treatment of jaundice and herpes by Sheko people [4].

L.deflexa is an erect sparsely branched herb which grows up to 1 m with stems ribbed hairy and leaves opposite, elliptic-oblong to ovate, hairy on both surfaces [5]. The leaves are used to treat diarrhea and ascariasis in adults and the above ground part to treat diarrhea in children by Bench people [2]. The leaves are also used to treat diarrhea, ascariasis, joint dislocation, *Tinea corporis*, abdominal cramp (also root), snakebite, and as snake repellent by Shekos [4] and to treat diarrhea in children, ascariasis, and stomachache by Meinit [3].

I.spicata is prostrate to weakly ascending, spreading, perennial herb which grows vertically up to 50 cm and horizontally up to 1 m. It has strong, deep, woody taproot. It is commonly known in English as creeping indigo, lawn indigo or trailing indigo. It is distributed from Senegal to Ethiopia, south to South Africa; also in Madagascar, tropical Arabia, and tropical Asia [6]. The root of *I. spicata* is used to treat diarrhea, cough, malaria, stomachache, toothache, retained placenta, evil eye, and headache in human and to treat cough in cattle and sheep, and blackleg in cattle by Meinit [3]. The whole or above ground part is used to treat meningitis and the leaves to treat *tinea nigra* and above ground part to treat wound by Bench people [2].

Except study on antibacterial activity of *M.pyrifolia*, literature search has shown no publication to the best of author's knowledge regarding antibacterial activities of *L.deflexa* and *I.spicata*.

Materials and methods

Bacterial species

Staphylococcus aureus ATCC 25923, *Shigella sonnei* ATCC 25931, *Salmonella typhimurium* ATCC 13311, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 were obtained from Mizan Branch Public Laboratory.

Collection of plant materials

Leaves of *L.deflexa* and *M. pyrifolia* as well as the root and aerial part of *I.spicata* were collected from different places around Mizan- Aman town from January to February 2013.

Extraction of plant materials with 80% methanol

Each plant material was dried under shade and finely powdered using mortar and pestle. About 100 g of the powdered plant material was macerated with sufficient volume of 80% methanol in conical flask for about 48 hr. Then, the filtrate was separated from the marc using filter paper equivalent to Whatman no.1 and the marc was macerated until the filtrate became free of any visible color. The entire filtrate was collected and concentrated in oven (Electric thermostatic heated dry box, India) at 40 °C. Then, the concentrated extract was transferred into wide-mouth glass jar and dried further. Finally, the dried extract was removed from the oven and kept at room temperature in dark place until used for the antibacterial test.

Preparation of samples

Samples of the extracts were prepared at 50 and 100 mg/ml concentration using 1% dimethyl sulphoxide (DMSO). Ciprofloxacin hydrochloride 0.1 mg/ml was prepared in sterile distilled water and used as reference drug.

Preparation of inoculum

Initially, stock culture of each species of bacteria was prepared from deep-frozen culture and maintained on Müller-Hinton agar at a temperature around 4 °C in refrigerator. For the test, inoculum was prepared from fresh culture grown for 24 hr. In this, sufficient quantities of colonies were aseptically transferred into 5 ml of sterile nutrient broth and shaken by hand until it matches 0.5 McFarland standard [7].

Antibacterial activity test

Antibacterial activity test was done by agar-well diffusion method [8]. In this, about 20 ml of molten and sterile Müller-Hinton agar was aseptically poured into sterile petri dishes (9 cm diameter). After congealing of the agar, 0.5 McFarland turbidity adjusted inoculum suspension was dipped with sterile cotton swab, pressed firmly against the inside wall of the test tube just above the fluid level and rotated to remove excess of the liquid. This was then streaked over the entire Müller-Hinton agar surface, rotating the plate approximately 60 degrees after each application to ensure an even distribution of the inoculum and finally swabbed all-round the edge of the agar surface and allowed to dry. Then, four wells each having a diameter of 6 mm and approximately equal distance between them were aseptically formed using a sterile stainless steel borer. Then, two of the wells were aseptically filled with 100 µl of either 50 or 100 mg/ml of the extract and the remaining two wells were filled with the same volume of either 0.1 mg/ml of ciprofloxacin hydrochloride or 1% DMSO as reference drug and negative control, respectively using micropipette. Then, the petri dishes were left for 2 hr at room temperature to allow diffusion into the agar gel and then incubated (model D2S, serial No., 9SF252, GENLAB, England) at 37 °C for 24 hr. The antibacterial activity test was done in duplicate and the diameter of zone of inhibition was measured using ruler (Eber hard Faber®) and the mean and standard error of the mean were recorded.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration was determined by broth dilution method [9].

Preparation of samples

Firstly, stock dispersion of each extract was prepared using 1% DMSO and the stock dispersion was then serially diluted in vials containing sterile nutrient broth to make the final concentration of the extract 4, 8, 16, 32, and 64 mg/ml in final 5 ml of dispersions.

MIC determination

Two pairs of seven sterile test tubes each containing 5 ml of sterile nutrient broth were placed in rack. From each pair one test tube was used for sterility confirmation (test tube which did not contain test bacteria and extract) and another test tube was used for suitability confirmation (test tube which contained test bacteria but not extract). Then, 2.5 ml of turbidity adjusted suspension of test bacteria was poured into each test tube using sterile syringe, except test tube kept for sterility confirmation. Then, immediately to this, 2.5 ml of previously serially diluted extract was aseptically poured into the corresponding test tubes except into the sterility and suitability confirmation test tubes. Finally, the concentration of the extract in each test tube became 1, 2, 4, 8, and 16 mg/ml. Then, the rack containing the test tubes was incubated at 37 °C for 24 hr. The minimum inhibitory concentration of the extract which inhibited growth was taken as MIC of the extract against the test bacteria.

Result and discussion

Antibacterial activity

In this study, the extract of *M.pyrifolia* showed growth inhibitory activity against *S.aureus* and *S.sonnei*. However, it did not inhibit the growth of other species of bacteria (Table 1). It had mean diameter of zone of inhibition of 16.5 and 18.5 mm against *S.aureus* and 11 and 12 mm against *S.sonnei* at 50 and 100 mg/ml concentration, respectively. On the other hand, the extract of *L.deflexa* showed activity only against *S.aureus*. Whereas, that of the root extract of *I.spicata* showed activity against *S.aureus* and *P.aeruginosa*. However, the extract of the aerial part of *I.spicata* did not show activity against any bacteria. Unlike the extracts, ciprofloxacin hydrochloride inhibited the growth of all tested species of bacteria with mean diameter of zone of inhibition of 36.5 to 54 mm (Table 1).

In this study the extracts of the three medicinal plants were active against *S.aureus*, the only gram – positive pathogen used in this study. An antibacterial acetylenic glucoside has been isolated from the leaves of *M.pyrifolia* [10].

MIC

The MIC of the extract of *M.pyrifolia* was 8 mg/ml against both *S.aureus* and *S.sonnei* (Table 2). Whereas, the root extract of *I.spicata* had MIC of 8 mg/ml and >16 mg/ml against *S. aureus* and *P.aeruginosa*, respectively.

Conclusion

Even though the *in vitro* antibacterial activities of *M.pyrifolia*, *L.deflexa*, and *I.spicata* are low, the traditional use of these plants by Bench, Sheko, and Meinit ethnicities to treat diarrhea has basis.

Acknowledgments

The author acknowledges Mizan – Tepi University for funding the research, Mizan Branch Public Laboratory for providing standard strains of bacteria and allowing to use laboratory facilities, and Ethiopian Food, Medicine and Health care Administration and Control Authority for kindly providing ciprofloxacin hydrochloride.

References

- [1] O. B. Dokosi. Herbs of Ghana, Ghana Universities Press, Accra. 1998, 313-314.
- [2] M. Giday, Z. Asfaw, Z. Woldu, et al. Medicinal plant knowledge of the Bench ethnic group of Ethiopia: an ethnobotanical investigation. Journal of Ethnobiology and Ethnomedicine, 2009, 5:34.
- [3] M. Giday, Z. Asfaw and Z. Woldu. Medicinal plants of the Meinit ethnic group of Ethiopia: An ethnobotanical study. J. Ethnopharmacol., 2009, 124: 513–521.
- [4] M. Giday, Z. Asfaw and Z. Woldu. Ethnomedicinal study of plants used by Sheko ethnic group of Ethiopia. J. Ethnopharmacol., 2010, 132:75–85.
- [5] http://www.zimbabweflora.co.zw/speciesdata/species.php?species_id=149270 (accessed on 20-08-2011)
- [6] http://www.tropicalforages.info/key/Forages/Media/Html/Indigofera_spicata.htm (accessed on 20-08-2011)
- [7] J. M. Andrews. BSAC standardized disc susceptibility testing method (version 6). J. Antimicrob. Chemother., 2007, 60 (1): 20–41.
- [8] L. Boyanova, R. Kolarov, G. Gergova, et al. In vitro activity of Bulgarian propolis against 94 clinical isolates of anaerobic bacteria. Anaerobe, 2006, 12 (4): 173–177.
- [9] I. Wiegand, K. Hilpert and R. E. W. Hancock. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nat. Protoc., 2008, 3 (2): 163–175.
- [10] G. Ruecker, S. Kehrbaum, H. Sakulas, et al. Acetylenic glucosides from *Microglossa pyrifolia*. Planta Medica, 1992, 58(3):266-269.

Table 1: Antibacterial activity of extracts (mean±SEM^a)

Test sample (mg/ml)	Diameter of zone of inhibition (mm ^b)				
	<i>S.aureus</i>	<i>S.sonnei</i>	<i>S.typhimurium</i>	<i>E.coli</i>	<i>P.aeruginosa</i>
Mp-50	16.5±1.5	11±0	-	-	-
Mp-100	18.5±2.5	12±0	-	-	-
Ld-50	11±1	-	-	-	-
Ld-100	17.5±0.5	-	-	-	-
Is root-50	11±0	-	-	-	8.5±0.5
Is root-100	14.5±3.5	-	-	-	10±0
Is aerial-50	-	-	-	-	-
Is aerial-100	-	-	-	-	-
Ciprofloxacin Hcl-0.1	36.5±0.15	44.5±0.5	54±0	54±1	40±0
1% DMSO	-	-	-	-	-

^a SEM = standard error of the mean, ^b diameter of zone of inhibition includes borer diameter, Mp = *M.pyrifolia* extract, Ld = *L.deflexa* extract, Is root = *I.spicata* root extract, Is aerial = *I.spicata* aerial part extract, “-”: indicates no inhibition

Table 2: Minimum inhibitory concentration of the extracts

Test extract	MIC (mg/ml)		
	<i>S.aureus</i>	<i>S.sonnei</i>	<i>P.aeruginosa</i>
Mp	8	8	nt
Ld	>16	nt	nt
Is root	8	nt	>16

Mp=*M.pyrifolia*, Ld= *L.deflexa*, Is root = *I.spicata* root, “nt”= not tested