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In vitro antimicrobial activity of three medicinal plants of Ethiopia against some selected bacterial isolates

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Medicinal plants constitute natural source of antimicrobial drugs that will provide essential compounds to fight against disease. In this study, the antibacterial activity of ethanol extracts of *Moringa stenopetala*, *Thymus serrulatus*, and *Terminalia brownii* were investigated against selected pathogenic Gram positive and Gram negative bacteria. *In vitro* antibacterial activities of the ethanol extracts were tested at a concentration of 50, 25, and 12.5 mg/ml by using agar disc diffusion method and zone of inhibitions were determined. Furthermore, minimum inhibitory concentrations were determined for plants that showed antibacterial activity (>15 mm zone of inhibition). The results indicated that only *T. serrulatus* and *T. brownii* exhibited antimicrobial activity against one or more test pathogens. Both extracts of these plants showed strong and dose dependent activity when compared with *M. stenopetala* which demonstrated no activity. Interestingly, *T. serrulatus* showed broad spectrum activity against the tested bacteria. Therefore, ethanol extracts of *T. serrulatus* and *T. brownii* showed promising antimicrobial activity justifying their usage in traditional medicine.

Key words: Antibacterial, Ethiopia, *Moringa stenopetala*, *Terminalia brownii*, *Thymus serrulatus*.

INTRODUCTION

Despite tremendous progress in medicine, infections caused by bacteria, fungi, virus and parasites are still major threat to human and animal health. In the last three decades, few antibiotics were produced but clinical efficacy of these antibiotics is being threatened by the emergence of multi drug-resistant pathogens (Khond et al., 2009). Moreover, antibacterial pharmaceuticals are not accessible to majority of the communities in developing countries (Cheruiyot et al., 2009). Therefore, actions must be taken to reduce these problems, such as controlling the use of antibiotics, understanding the genetic mechanisms of resistance and developing new antibiotics and new therapeutic strategies. Advances in identifying new sources of natural products with antimicrobial activities and expanding antibiotic chemical diversity are providing chemical leads for new drugs

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(Walkty et al., 2014).

Traditionally used medicinal plants produce a variety of compounds for the treatment of various ailments. These medicinal herbs constitute indispensable components of the traditional medicine practiced worldwide due to the low cost, easy access, and ancestral experience; and they are considered as candidates for developing new antimicrobial drugs (Abdalla et al., 2013; Madduluri et al., 2013). Over the past few decades, numerous studies have been conducted on plants to explore possible candidates for antibiotics (Frey and Meyers, 2010). Ethnobotanical studies revealed that wider range of Ethiopian plants are being used in treatment of many diseases in the traditional health care system of the country (Giday et al., 2007; Teklehaymanot et al., 2007). Crude extracts of some Ethiopian plants are known to possess strong antimicrobial activity indicating that these plants can serve as sources of effective drugs against certain microbial agents (Mancini et al., 2015; Taye et al., 2011). *Moringa stenopetala* (bak.) Cuf. (Moringaceae), *Thymus serrulatus* (Lamiaceae) and *Terminalia brownii* (Combretaceae) are among plants which are frequently used in traditional medicine of Ethiopia for the treatment of infectious and non-infectious diseases (Asfaw et al., 2000; Mekonnen and Drager, 2003; Mancini et al., 2015; Wilson and Woldo Gebre, 1979).

M. stenopetala is endemic to East Africa and is mainly present in Southern Ethiopia and Northern Kenya (Padayachee and Baijnath, 2012). This plant is known as Shiferaw in Amharic (Makonnen et al., 1997). Various parts of the plant are used by traditional healers to treat cold, anemia, epilepsy (Demeulenaere, 2001), digestive problems, dysentery, malaria, hypertension, stomach pain, visceral leishmaniasis, asthma, diabetes (Mekonnen and Drager, 2003; Padayachee and Baijnath, 2012) and hyperglycemia (Tesemma et al., 2013). Whereas, *T. serrulatus* is endemic to Ethiopia and it is locally known as Tosign (Asfaw et al., 2000). The leaves and flowering parts of *Thymus* species are widely used as tonic, herbal tea, antiseptic, carminative as well as treating cold (Javadi et al., 2013). *Thymus* spp. are also used as antispasmodic, anti-inflammatory, as expectorants and to treat digestive problems in Iran (Nickavar et al., 2005). On the other hand, *T. brownii* is found in many parts of Africa, such as Democratic Republic of Congo, Ethiopia, Kenya and Tanzania (Fyhrquist et al., 2002). In Ethiopia, it is locally known as Weba. Traditionally, it is used to treat bacterial, fungal and viral infections (Mariod et al., 2014), diarrhea, cut wounds, gonorrhea, cough (Abdalla et al., 2013), jaundice, hepatitis, liver cirrhosis, and yellow fever (Kokwaro, 1976; Wilson and Woldo Gebre, 1979).

Despite of the wide spread uses of these plants as treatment against animal and human infectious diseases, only few report exists on the activity of these plants against micro organisms, such as *Salmonella* species, *Escherichia coli* o15:H7, *Bacillus cereus* and *Staphylococcus aureus.* Therefore, the objective of this study was to evaluate the potential antibacterial activity of these medicinal plants against selected gram positive and gram negative bacteria isolated from samples of animal origin.

MATERIALS AND METHODS

Plant collection and extraction

The leaves of *M. stenopetala*, *T. serrulatus* and *T. brownii* were collected from different parts of Ethiopia. The plants were identified by a botanist and voucher specimens were deposited at the Herbarium of the Addis Ababa University. The leaves of each plant were washed with distilled water and allowed to dry under shade. The dried leaves were ground and extracted with 70% ethanol by maceration in such a way that 150 g of each powdered plant material was soaked in 500 ml of 70% ethanol for 24 h with shaking. The solvent was filtered through Whatman filter paper No. 1 (Whatman, UK), while the residues were used for a second extraction with 300 ml of 70% ethanol. After the second extraction, the filtrates were concentrated under reduced pressure using a rotary evaporator at 40°C and crude extracts thus obtained were stored in refrigerator at 4°C until use.

Preparation of test organisms

S. aureus (from bovine milk), *B. cereus* (from poultry), *Salmonella* spp. (from bovine meat) and *E. coli* o15:H7 (from bovine meat) were isolated and confirmed at the laboratory of Veterinary Microbiology, Hawassa University, Ethiopia according to the standard protocol described in Quinn et al. (1999). Liquid cultures were prepared by placing a loopful of bacteria into 10 ml of nutrient broth grown at 37°C. The turbidity of each liquid culture for use in the assays was then adjusted to 0.5 McFarland standard units using sterile nutrient broth.

Antimicrobial activity assay

The disc-diffusion assay (Bauer et al., 1966) was used to determine the antimicrobial potential of investigated extracts. Methanol was used to dissolve *T. serrulatus* whereas sterile water was used for *M. stenopetala* and *T. brownii*. Extract impregnated discs were prepared in such a way that 100 µl of the extracts prepared at three different concentrations (50, 25 and 12.5 mg/ml) was pipetted onto a 6 mm sterile filter paper disc and allowed to dry overnight at 37°C. For sensitivity test, Muller Hinton agar medium was prepared, poured to each sterile petriplates and allowed to solidify at room temperature. 100 µl of liquid bacterial culture was spread onto the plates. Standard antibiotic discs and extract impregnated sterile discs were then placed on the plates. Each plate contained four paper discs; two discs contained extracts at two different concentrations (either of 50, 25 and 12.5 mg/ml), one disc served as a negative control (100 µl sterile water or methanol impregnated disc), and the other standard antibiotic disc served as a positive control (ceftriaxone (30 µg) for *E. coli* o15:H7 and *Salmonella* spp; tetracycline (30 µg) for *B. cereus* and *S. aureus*). Each extract was tested in triplicate, and the plates were inverted and incubated at 37°C for 24 h. At the end of the incubation period, the antimicrobial activity was evaluated by measuring the diameter of inhibition zones. An inhibition zone of 15 mm or more was considered as high antibacterial activity (Mothana et al., 2009).

Minimum inhibitory concentration (MIC) assay

Based on the screening test, MIC of *T. serrulatus* and *T. brownii* were determined. The agar dilution method recommended by the National Committee for Clinical Laboratory Standards (Prudent et al., 1995) was used with minor modification. A series of two fold dilution of each extract, ranging from 0.125 to 64 mg/ml was prepared in Muellur Hinton agar at 40°C. Plates were dried at room temperature for 30 min prior to spot inoculation with 10 µl aliquots of culture. Inoculated plates were incubated at 37°C for 18 h and the MICs were determined. Experiments were carried out in triplicate. Inhibition of bacterial growth in plates containing test extracts was assessed by comparison with growth in the positive control plates (20 mg/ml of ceftriaxone for *E. coli* o15:H7 and *Salmonella* spp.; 20 mg/ml of tetracycline for *B. cereus* and *S. aureus*). The MICs were determined as the lowest concentration of extract inhibiting visible growth of each organism on the agar plate (Delaquis et al., 2002).

RESULTS

The disk diffusion method for antimicrobial susceptibility testing was initially performed to determine the antibacterial activities of crude ethanol extracts of the leaves of *M. stenoptala*, *T. serrulatus* and *T. brownii* against *B. cereus*, *E. coli* o15:H7, *Salmonella* spp. and *S. aureus. T. serrulates* exhibited concentration dependent antibacterial activity against *E. coli* o15:H7, *B. cereus* and *S. aureus*, whereas *T. brownii* was active only against *Salmonella* spp. At the three concentrations, the minimum zone of inhibition of both plant extracts was 15 mm (Table 1). However, *M. stenopetala* was found to be inactive against all tested bacteria.

The MIC of *T. serrulatus* and *T. brownii* against all tested bacteria are summarized in Table 2. Only those extracts which inhibited the growth of bacterial strains in disc diffusion method were subjected to MIC evaluation. It was found out that *E. coli* o15:H7 was relatively the most susceptible bacteria with the lowest MIC values of crude extracts of *T. serrulatus* (2 mg/ml). On the other hand, MIC of *T. serrulatus* (against *B. cereus* and *S. aureus*) and *T. brownii* (against *Salmonella* spp.) was 4 mg/ml.

DISCUSSION

Plants contain various types of bioactive molecules which are under the targets of extensive research worldwide (Walkty et al., 2014). In the present work, 70% ethanol extract of *M. stenopetala*, *T. serrulatus* and *T. brownii* were subjected to antimicrobial study against *B. cereus, E. coli* 015:H7, *Salmonella* spp. and *S. aureus*. The result of this study shows that the crude extracts of *T. serrulatus* showed concentration dependent inhibition against *B. cereus*, *S. aureus* and *E. coli o15:H7*. At the lowest test concentration (12.5 mg/ml), its potency was comparable to that of standard antibiotics ceftriaxone and tetracycline; and even better than the standard antibiotics at relatively higher concentrations (25 and 50 mg/ml). The antimicrobial activity of *T. serrulatus* found during the current investigation were in agreement with the findings of earlier researchers who determined the antimicrobial activity of aerial parts of related *Thymus* spp. against a wide range of microorganisms (Akrayi and Abdulrahman, 2013; Pirbalouti et al., 2011). In addition, our study results show that *T. serrulatus* did not have activity against *Salmonella* spp. However, some other *Thymus* spp. (*Thymus lanceolatus*) showed inhibitory effect on *Salmonella* spp. (Benbelaïd et al., 2013). The discrepancy may be due to variation in plant parts used, effect of climate, extraction method, and composition of extracted products.

The result of this study also shows that *T. brownii* extracts showed antibacterial activity only against *Salmonella*. This was contrary to an earlier study result where *T. brownii* did not show any activity against salmonella species (Abdalla et al., 2013); however, it has well exhibited activity against *S. aureus*. In addition, *T. brownii* has antibacterial activity against *S. aureus*, *E. coli, Salmonella* and *B. cereus* as reported by Mbwambo et al. (2007). The observed variation might be attributed to the variation in the plant parts and extraction solvent used. Nevertheless, 70% ethanol extracts of leaves of *T. brownii* was used in the present study instead of methanol extracts of barks, wood and whole roots.

In an earlier study, *M. stenopetala* showed antimicrobial activities against *S. auerus*, *E. coli* and *Salmonella* spp. (Tesemma et al., 2013). However, in the present study, the result clearly demonstrated that this plant was devoid of any antimicrobial potential against tested organisms. The reason might be due to the variation in the plant parts and extraction solvents used; where in this study, leaves of ethanol extracts of plant was used instead of acetone extracts of root wood. Other studies on methanol and n-hexane extracts of *M. stenoptala* (Eilert et al., 1981; Walter et al., 2011) and methanol and aqueous extracts of bark and leaf extracts of *M. stenoptala* (Biffa, 2005) revealed that the plant was effective in inhibiting the growth of *E. coli* and *S. aureus*; but only *S. aureus*. Earlier, the compound, namely 4(α-L-Rhamnosyloxy)benzylisothiocyanate isolated from the seeds of *M. stenoptala* showed profound antimicrobial activity against *Mycobacterium phlei* and *B. subtilis* (Eilert et al., 1981).

The discrepancies of these findings with other previous studies are expected as phyto-constituents and are known to vary with ecological factors and seasonal variation (Rafique and Chaudry, 1999). Furthermore, the type of solvent and different phytoconstituents might have played a role (Thaker and Anjaria, 1986). Such factors are known to cause negative or positive effects on the treatment of test microorganisms. It is worth to mention that the antimicrobial components of a plant might have changed in concentration with the age of the plants (Mangla and Kamal, 1989). Environmental factors

Table 1. Mean zone of inhibitions (mm) of three plant extracts against test bacteria.

Bacteria	Mean zone of inhibitions										
	T. serrulatus (mg/ml)			*T. brownii* (mg/ml)			*M. stenopetala* (mg/ml)			Control	
	50	25	12.5	50	25	12.5	50	25	12.5	±ve	-ve
Salmonella species	-	-	-		21.5±0.5	16.5±0.5	15.5±0.5	-	-	18.0±0	-
E. coli 015:H7	19±0	17.5±0.5	15.5±0.5	-	-	-	-	-	-	18.0±1.0	-
B. cereus	20±0	17.5±0.5	15±1.0	-	-	-	-	-	-	16.0±1.0	-
S. aureus	19.5±0.5	18.5±0.5	15.5±0.5	-	-	-	-	-	-	17.0±1.0	-

Values are mean inhibition zone (mm) ± standard deviation (SD) of three replicates, P<0.05. (± ve): Positive control (Ceftriaxone disc for *Salmonella* species and *E. coli 015:H7*; Tetracycline disc for *B. cereus* and S*. aureus*). (-ve): Negative control: sterile water for *T. brownii* and *M. stenopetala*, methanol for *T. serrulates.* (-): no zone of inhibition.

Table 2. Minimum inhibitory concentration (MIC) values (mg/ml) of ethanol extract of *T. serrulatus* and *T. brownii* against tested bacteria.

Plant species	*B. cereus*	*E. coli* o15:H7	*Salmonella* spp.	*S. aureus*
T. serrulatus	4	2	-	4
T. brownii	-	-	4	-

(-): no zone of inhibition.

like excessive rain fall and drought are also reported to enhance the quality of active compounds or diminish it (Vlachos et al., 1997).

It is concluded that the findings of this study justify the claimed traditional uses of *T. serrulatus* and *T. brownii* to treat various infectious diseases in Ethiopia. The results of the present study warranted to initiate in-depthinvestigation on the antimicrobial potential of these plants especially after fractionation, isolation and characterization of active phytoconstituents.

Conflict of interests

The authors declare that the study was carried out purely with the academic interest and there are no competing interests involved.

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