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Antimicrobial activity of *Taverniera Abyssinica* A. Rich against human pathogenic bacteria and fungi

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Medicinal plants represent a rich source of antimicrobial agents. Even though hundreds of plant species have been tested for antimicrobial activities, the enormous mass of them have not been adequately evaluated. *Taverniera abyssinica* A. Rich is a widely used Ethiopian endemic medicinal plant commonly known under the local name of "Dingetegna". Medicinal plant preparations are generally very popular in developing countries with a long tradition in the use of them. Root of the medicinal plant was extracted by maceration method using three different extraction solvents. Disc diffusion assay and agar dilution method were used to determine antimicrobial activity against *Staphylococcus aureus, Enterococcus fecalis* and *Escherichia coli* and clinical isolate of *Candida albicans* and *Aspergillus flavus.* To compare extraction solvents and the difference in sensitivity of test microorganisms, one-way analysis of variance was used. *T. abyssinica* A. Rich extracts exhibited remarkable difference in antimicrobial activity between water and alcohol extract. On the other hand there were little differences in antimicrobial activities of extracts obtained using ethanol and methanol as solvents. As a whole, extracts showed better antimicrobial activity against *S. aureus*, *E. faecalis* and *C. albicans* while *E. coli* and *A. flavus* were the most resistant microorganisms to this medicinal plant. Antimicrobial activity of the medicinal plant varies with extraction solvent and tested microorganisms. Even though the local people are using this medicinal plant in treatment of various types of infectious disease, the medicinal plant has little antimicrobial activity. *S. aureus* was the most sensitive microorganism as compared to other tested microorganisms.

Key words: Antibacterial, Antifungal, Crude extract, Ethiopia, *Taverniera abyssinica* A. Rich.

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. In different countries, plants are used medicinally and are the source of several effective and powerful drugs (Rahmoun et al., 2012; Gemechu et al., 2015). Even though hundreds of plant species have been tested for antimicrobial activities, the enormous mass of them have not been adequately evaluated (Das et al., 2010; Rahmoun et al., 2013). The antimicrobial agent contained in plants usually extracted using different solvents and the antimicrobial properties of the extracts may vary

(Sharma et al., 2013;Karmegam et al., 2012; Wang, 2010).

Taverniera abyssinica A. Rich is an Ethiopian endemic medicinal plant commonly known under the local name of "Dingetegna". It belongs to the *Fabaceae* family (Thulin et al., 1989; Abera et al*.,* 2010; Abera, 2014). It is a threatened medicinal plant that usually grows in a bush land limestone areas with an altitudinal range of 1700 to 2300 m above sea level (Abera, 2014). According to ethno-botanical information, *T. abyssinica* A. Rich has been traditionally used for the treatment of various diseases in Ethiopia. A small bundle of the roots are chewed and the juice swallowed for immediate relief of fever, discomfort, stomachache and for many other pains (Kelbessa et al., 1992; Thulin, 1989).

The medicinal importance of *T. abyssinica* A. Rich has been identified by the findings of different chemical compounds isolated from the rootstocks (Noamesi et al., 1990; Abera, 2010). The study done on crude extracts and purified substances of *T. abyssinica* A. Rich plant were tested for their antipyretic and analgesic properties (Dange et al., 1990). In other study carried out, the extracts showed strong nematicidal activities towards *C. elegans.* Medicarpin and 4-hydroxymedicarpin were isolated as nematicidal constituents from the extracts (Stadler et al., 1995). The local people are also using this medicinal plant in treatment of various types of infectious disease

Medicinal plant preparations are generally very popular in developing countries with a long tradition in the use of them (Sharma et al., 2013). On the other hand, scientific evidence carried out to assess the antimicrobial activity of medicinal plant is limited. The aim of this study was to evaluate antibacterial and antifungal activity of root of *T. abyssinica* A. Rich (Dingetegna) which is well known endemic medicinal plant in Ethiopian.

MATERIALS AND METHODS

Study design

In-vitro experimental study of antibacterial and antifungal activity of *T. abyssinica* A. Rich was carried out by disc diffusion method and agar dilution method to determine minimum inhibition concentration, minimum bactericidal and fungicidal concentration, respectively. Positive and negative controls were used to monitor antimicrobial activity in all assays. All measurements were repeated three times and mean ± SD was used to describe the measurements.

Collection and extraction of Plant Materials

Root of *T. abyssinica* A. Rich was purchased from local market and authenticated by taxonomist and specimen was deposited at the National Herbarium, Department of Biology, Addis Ababa University Herbarium. The plant materials were washed three times under running tap water followed by rinsing twice with sterile distilled water and then air-dried in an oven at 40°C. Then ground into fine powder with electric grinder (Figure 1). About 25 g of fine powder of the medicinal plant was dissolved in 250 mL of solvents (ethanol, methanol and distilled water) separately in sterilized screw capped 500 mL glass bottles. Then the mixtures were kept in orbital shaker for 12 h at room temperature. Then the extracts were filtered by Whatman No. 1 filter paper. After having filtered extracts, they were evaporated to remove the solvent under vacuum in Rotary Evaporator kept at 40°C. Then the residues from rotary evaporator were allowed to dry in room temperature. The powdered extracts were weighed and dissolved in distilled water to gate stock solution of 200 mg/mL by labeling for each extraction solvents and stored in deep freezer at -20ºC for further use (Parekh et al., 2005; Handa et al., 2008).

Determination of disc diffusion assay

To determine disc diffusion assay, *S. aureus* (ATCC-25923), *E. feacalis* (ATCC-29212) and *E. coli* (ATCC-25922), and clinical isolate of *C. albicans* and *A. flavus* were used to screen antimicrobial activity of the medicinal plant. Mueller Hinton agar medium and Sabouraud's dextrose agar (SDA) were used to carry out disc diffusion assay antibacterial and antifungal activity respectively.

Diffusion discs of approximately 6 mm diameter were prepared from Whatman No. 1 filter paper by puncher and sterilized by autoclave then oven dried in sterile way and each solvent extracts were prepared into a series of concentrations: 10, 20, 40 and 80 mg/mL to determine disc diffusion assay. A 10 µl of each concentration of crude medicinal plant extracts was impregnated in separate sterile disc using sterile micropipette tips and stored at 4°C in separate sterile containers according to their extraction solvents and concentrations. Then disc diffusion assay was carried out using Kirby- Baur disk diffusion method (CLSI, 2009). Gentamycin (10 µg/mL) and Ketoconazole (10 µg/mL) disc were used as positive control for bacteria and fungi respectively. A blank disc impregnated with each solvent was used as negative control. All the tests were conducted in triplicate and the average of the three measurements was used.

Determination of minimum inhibitory concentration

Agar dilutions method was used to determine minimum inhibitory concentration (MIC) of the medicinal plant extracts. For each plant extract, a stock solution of 200 mg/mL was added into a sterilized molten Mueller Hinton agar and SDA after cooled to 45^{o}C in water bath. Then two fold serial dilutions was used to obtain 100, 50, 25, 12.50, 6.25, 3.125 and 1.56 mg/mL concentration of medicinal plant extracts in agar medium. Then the mixture of plant extract and molten agar medium were poured to 90 mm Petri dish and solidified.

Then the plates were inoculated with a loopful of 0.5 McFarland standards diluted suspension of each test microorganisms in small spot. The plates were incubated at 37°C for 24 h and at 27°C for 48

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Figure 1. Root of *Taverniera abyssinica* before grinding, after grinding to fine powder and filtered extract (From left to right).

h for bacteria and fungi, respectively. Then the minimum dilution of the plant extracts completely inhibiting the growth of each organism was taken as the MIC. A control comprising the test organismgrown on fresh agar medium and agar medium with each solvent were used as control. All the tests were done in triplicates (CLSI, 2009).

Determination of minimum bactericidal and fungicidal concentration

Concentrations of the plant extract determined as MIC, the preceding one and one more concentration between the two concentrations were used to determine of MBC and MFC. Then these concentrations of the plant extracts were adjusted in nutrient broth and a Sabouraud's dextrose broth and inoculated with test microorganisms then incubated at 37ºC for 24 and 48 h at 27 ºC for bacteria and fungi, respectively. Then after, a loopful of all broth media was sub-cultured on Mueller-Hinton agar and SDA plates. The inoculated plates were incubated at 37ºC for 48 h and at 27ºC for 72 h for bacteria and fungi, respectively. A control comprising the test organism grown on fresh agar medium and agar medium containing each solvent were used as control. All the tests were done in triplicate.

Statistical analysis

For each assay, all the measurements were replicated three times and the results were presented as mean ± SD. One way ANOVA followed by Tukey's test was used to compare extraction solvents and the difference in the sensitivity of the test microorganisms using the statistical package for social sciences (SPSS) version 16 and P-value ≤ 0.05 were considered as statistically significant.

RESULTS

T. abyssinica A. Rich extracts exhibited remarkable difference in antimicrobial activity between water extract and alcohol extract. On the other hand there were little differences in antimicrobial activities of extracts obtained using ethanol and methanol as solvents. In disc diffusion assay, extracts of *T. abyssinica* A. Rich in water exhibited nearly the same amount of antimicrobial activities against *S. aureus* and *E. faecalis*. From tested fungal species, *T. abyssinica* A. Rich showed antifungal activity against *C. albicans* only at 80 mg/mL in water extract. Furthermore, the extracts in water did not exhibit any activity against *E. coli* and *A. flavus* up to 80 mg/mL (Table 1).

T. abyssinica A. Rich root extract in ethanol showed weak antimicrobial activity against *E. coli* and *A. flavus* at 80 mg/mL whereas the plant extract against *S. aureus* and *E. faecalis* were showed antimicrobial activity starting from 20 mg/mL. Likewise, extracts in methanol also exhibited antimicrobial activities at 80mg/mL against all tested microorganism. In methanol extract, the maximum antimicrobial activity was observed against *S. aureus.* There was significant difference ($P < 0.5$) between antimicrobial activity of water based and alcohol based extracts of the medicinal plant (Table 1).

The minimum inhibition concentration assay also showed water based extracts had weak antimicrobial activity than alcohol based extracts. In this assay, *T. abyssinica* A. Rich showed better antimicrobial activity against *S. aureus* and *C. albicans* when compared with *E. coli*. On the other hand, *E. faecalis* and *A. flavus* were inhibited at 100 mg/mL of water based extract of the medicinal plant. In methanol based extract, the least MIC (6.25mg/mL) was observed against *C. albicans* whereas the highest MIC of 25mg/mL was observed against *E. coli*. Generally, there was no significant difference ($P > 0.05$) in antimicrobial activity of ethanol based and methanol based extracts of *T. abyssinica* A. Rich against selected pathogenic bacterial and fungal species. The plant extracts also showed less antimicrobial activity against *E. coli* and *A. flavus* than other tested micro-organisms (Table 2).

In bactericidal and fungicidal determination assay, the extracts of *T. abyssinica* A. Rich, exhibited varying degrees of antimicrobial activities against the tested organisms. The water based extracts exhibited bactericidal and

Table 1. Antimicrobial activity of *T. abyssinica* root extract obtained using three different extraction solvents with four different concentrations.

Extraction solvents	Extract concentration (mg/ml)	Inhibition zone (mm)				
		Bacterial species			Fungal Species	
		S. aureus	*E. coli*	*E. faecalis*	*A. flavus*	*C. albicans*
Water	10	-	-	-	-	-
	20	-	-	-	-	-
	40	-	-	-	-	-
	80	8.66 ± 0.57	-	8.33 ± 0.57	-	8.33 ± 0.57
Ethanol	10	-	-	-	-	-
	20	8.33 ± 0.57	-	8.00 ± 1.00	-	-
	40	9.33 ± 0.57	-	9.33 ± 0.57	-	8.66 ± 1.15
	80	11.00 ± 1.00	8.33 ± 0.57	12.66 ± 0.57	9.00 ± 1.00	12.00 ± 1.00
Methanol	10	-	-	-	-	-
	20	8.00 ± 0.00	-	-	-	-
	40	9.66 ± 1.15	-	9.00 ± 1.00	-	8.00 ± 1.00
	80	13.00 ± 1.00	8.66 ± 1.154	12.33 ± 0.57	8.33 ± 0.57	11.66 ± 0.57
Positive control		22.33 ± 0.57	20 ±0.00	17.00 ± 1.00	22.33±0.57	23.66±0.57
Negative control		-	-	-	-	-

(-) = No activity; Values are mean of inhibition zone (mm) ± S.D of three replicates.

Table 2. Average minimum inhibitory concentrations, minimum bactericidal and fungicidal concentrations of *T. abyssinica* in three different extraction solvents

Assay methods	Extract solvent	MIC, MBC and MFC (mg/ ml)				
		Bacterial species			Fungal species	
		S. aureus	*E. coli*	*E. faecalis*	*A. flavus*	*C. albicans*
MIC	Water	50.00	*	100.00	100.00	50.00
	Ethanol	12.50	25.00	12.50	25.00	12.50
	Methanol	12.50	25.00	12.50	12.50	6.25
MBC/MFC	Water	100.00	*	100.00	100.00	100.00
	Ethanol	25.00	50.00	25.00	37.50	25.0
	Methanol	12.50	25.00	25.00	12.50	12.50

(*) = No inhibitory or bactericidal/fungicidal activity at 100 mg/ml; MIC=minimum inhibition concentration; MBC= minimum bactericidal concentration; MFC= Minimum fungicidal concentration.

fungicidal effects at 100mg/mL against *S. aureus* and *C. albicans,* respectively. The strongest fungicidal activities were observed in alcohols extracts against *C. albicans, S. aureus* and *E. faecalis* while the weakest bactericidal effects were recorded against *E. coli* (Table 2).

DISCUSSION

Antimicrobial activity of *T. abyssinica* A. Rich varied with extraction solvent used. The successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure (Muhsin and Hussein, 2014). Antimicrobial activity of water extract of *T. abyssinica* A. Rich obtained in the current study was relatively low as compared to positive controls and alcohol extracts. However, this extract showed antimicrobial activity on *S. aureus*, *E. faecalis* and *C. albicans* at high concentration. This indicates that water extract of this medicinal plant at higher concentration can be effective antimicrobial agent against these microorganisms.

Though traditional healers use primarily water but plant extracts from alcoholic solvents have been found to give

more consistent antimicrobial activity compared to water extract. Water soluble flavonoids have no antimicrobial significance and water soluble phenolics are only important as antimicrobial compound in water extract (Lapornik et al., 2005; Das et al., 2010). In contrast, ethanol and methanol extract of *T. abyssinica* A. Rich showed better antimicrobial activity against tested microorganisms that generally increased with the increase in the concentration of the extract. It means that they are more efficient in cell walls degradation which has non-polar character and cause polyphenols to be released from cells (Wang, 2010). In addition to this enzyme polyphenol oxidase are inactivated in methanol and ethanol extract (Karmegam et al., 2012). This may be the reason why the antimicrobial activities of selected medicinal plant showed lower in water extract in our study.

In general, this medicinal plant showed a low antimicrobial activity against *E. coli* and *A. flavus* through all the three extraction solvent used in the current study as compared to other microorganisms. Antimicrobial studies also showed as Gram-negative bacteria show a higher resistance to plant extracts than Gram-positive bacteria. This may be due to the variation in the cell wall structures of Gram-positive and Gram-negative bacteria. Gram-negative bacteria have an outer membrane that is composed of high density lipopolysaccharides that serves as a barrier to many environmental substances including antibiotics (Palombo and Semple, 2001; Robinson et al., 2009).

In Ethiopia, most of people have been using *T. abyssinica* A. Rich for toothbrush and on the other hand *E. faecalis* has been frequently found in root canal-treated teeth in prevalence values ranging from 30% to 90% of the cases (Kunin, 1993). Therefore, without having this information, this people have been benefiting from this medicinal plant. Plant materials remain an important resource to combat serious diseases in the world (Vlietinck *et al.,* 1995; Regassa, 2013). People living in rural areas from their personal experience also know that this medicinal plant is valuable source of natural products of health care. However, they may not understand the scientific facts behind this medicinal plants and their effective way of extraction (Mohammed and Berhanu, 2011; Regassa, 2013). There is no study done on antibacterial and antifungal ctivity of this medicinal plant so far but study showed that it has strong nematicidal activity (Stadler et al., 1995).

Conclusion

Even though the local people are using this medicinal plant in treatment of various types of infectious disease, the medicinal plant has little antimicrobial activity Antibacterial and antifungal activity of *T. abyssinica* A. Rich varies in extraction solvent used. Water was the weakest extraction solvent whereas ethanol and methanol were good solvents to obtain antimicrobial phytochemical from this medicinal plant. Ethanol and methanol extracts showed better antimicrobial activity against *S. aureus*, *E. faecalis* and *C. albicans* while *E. coli* and *A. flavus* were the most resistant microorganisms to this medicinal plant.

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Conflict of interests

The authors did not declare any conflict of interest.

Authors' contributions

GA participated in the design of the study, coordinated and was involved in data collection, experimental work, and also analyzed the data, and drafted the paper. AG and ED participated in the analysis and revised subsequent drafts of the paper. All authors read and approved the final manuscript.

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