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Evaluation of the antibacterial activities of leaf extracts of *Achyranthus aspera*

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The fresh leaves of *Achyranthes aspera* Linn. soaked with water is traditionally used to treat skin infection in Ethiopia. The leaves are also applied traditionally to stop bleeding during cutoff in local people of Ethiopia. The above activities of the plant might be associated with antimicrobial activities. The leaves collected from their wild habitat were air dried under shade and ground into fine powder. The leaf powder was soaked in solvents (1 g: 10 ml) and shacked for 72 h. After filtering, the mixtures were concentrated by using rotary evaporator and the extract was prepared at 50, 100 and 200 mg/ml in 3% Tween 80 for antibacterial test. The disks were loaded with extract solution and allowed to evaporate. Then, the discs were placed on culture bacteria in the Muller Hinton agar and incubated for 24 h. Finally, the inhibition zones were measured in mm. The antibacterial activities and the minimum inhibitory concentration (MIC) test of the extracts were assessed by disk diffusion and agar dilution methods, respectively. Both 80% methanol and chloroform extract of *A. aspera* leaf showed significantly higher inhibition zone than the negative control, 3% Tween 80, but the extracts inhibition was significantly lower than the standard drugs (chloroamphenicol and ampiciline). Both 80% methanol and chloroform extract of *A. aspera* leaf showed MIC on *Shigella boydii* and *Salmonella typhi* at 6.25 mg/ml. The minimum bactericidal concentration (MBC) of the 80% methanol and chloroform extract of *A. aspera* leaf was measured at 6.25 and 12.5 mg/ml on *S. boydii* and *S. typhi,* respectively. The bacterial inhibition increased with the 80% methanol and chloroform extracts of *A. aspera* leaves concentrations. The 80% methanol *A. aspera* leaf extract which was administered orally with a single dose of 500, 1000 and 2000 mg/kg body weight was not toxic to albino mice.

Key words: *Achyranthes aspera,* inhibition zone, antibacterial activity, acute toxicity, minimum inhibitory concentration, minimum bactericidal concentration.

INTRODUCTION

Medicinal plants are used in spiritual therapies, manual techniques and exercises which can be used to treat, diagnose or prevent illness (WHO, 2003). More than 200,000 natural products of plant origin are known and most of them are being identified from higher plants and microorganisms (Ramawat and Merillon, 2007).

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In Ethiopia, more than 80% of the people use traditional medicines to treat different types of infections in the major health care system (Bekele, 2007).
However, herbalists traditionally use the plant without evaluating and optimizing the doses of biologically active ingredients. Plant extracts from roots, barks, stems, leaves and seeds are used traditionally to treat different types of infections (Ogbulie et al., 2007).

Achyranthes aspera Linn.which belongs to the family Amaranthaceae is distributed throughout tropical and sub tropical regions. *A. aspera* is an important medicinal plant and it is traditionally used in various diseases like odontalogic, rheumatism, bronchitis, skin disease, rabies, fever, dysentery, diabetes and used as antifungal and antibacterial activity (Dey, 2011). Researches in India demonstrated that this plant possesses activities like antiperiodic, diuretic, purgative, laxative, antiasthmatic, hepatoprotective, anti-allergic and various other important medicinal properties (Srivastav et al., 2011).

In Ethiopia, *A. aspera* with local name “Telenje” is used traditionally to treat various illnesses. Previously, it was shown that powdered *A. aspera* or fresh leaves with water are applied on skin to treat parasitic skin diseases in southern Ethiopia (Mesfin et al., 2009). The leaf and root extracts of *A. aspera* is also applied on human skin to stop bleeding in the North West part of Ethiopia (Giday et al., 2006). Traditional healers in Ethiopia soak *A. aspera* leaf in water to treat various diseases. On the other hand, in plant extraction techniques, methanol dissolves most of the secondary plant metabolites. Chloroform is supposed to medium solvent to plant material as a result in this research, 80% methanol and chloroform were used as solvent for the antibacterial activities of *A. aspera* leaf. Thus, the objective of this study is to evaluate the antibacterial properties of the 80% methanol and chloroform extracts of leaves of *A. aspera* on selected pathogenic bacteria.

MATERIALS AND METHODS

Chemicals

Methanol (Reagent chemical Services Ltd., United Kingdom), chloroform (Merck KGaA, 64271, Darmstadt, Germany), nutrient agar (Oxoid LTD., Bsingstoke, Hampshire, England), Müller-Hinton agar (Oxoid LTD., Bsingstoke, Hampshire, England), barium chloride sulfuric acid (SDFCL Fine Chemical Ltd., Mumbai, India), Tween-80 (Uni-Chem Chemical Reagents), sodium chloride (Nike Chemical, India), cotton swab (Nataso, India), tetracycline (Oxoid Ltd., United Kingdom), chloramphenicol (Oxoid Ltd., United Kingdom), barium chloride (BDH Chemicals Ltd. Poole, England) werer used for the study.

Plant collection and identification

The fresh leaves of *A. aspera* were collected from natural vegetation in Dejen district, Eastern Gojjam Zone, Amhara Regional State, Ethiopia. The leaves were collected in June 2014 and the specimen were identified and authenticated by taxonomist at College of Natural Sciences of Addis Ababa University. It was deposited at the Herbarium with voucher number *A. aspera* (ah001).

Preparation of solvent extraction

The fresh leaves were washed three times with tap water and once with sterile distilled water. After wash, the plant materials were air dried in shade at room temperature (25–30°C). Following the drying process, about 300 g leaves of the plant were powdered by milling, sieved through a fine mesh (Canadian Series sieves with 500 um opening) and stored at room temperature for further use as described by Subbarayan et al. (2010). 50 g of powder of *A. aspera* was soaked into 500 ml of chloroform and 500 ml 80% methanol in separate flasks. The mixtures in the Erlenmeyer flask were placed on a platform shaker of 120 rpm for 72 h at room temperature (Mohana et al., 2009). Then, the solutions were filtered by Whatman no. 1 filter papers and the solvent extracts were concentrated separately using rotary flash evaporator (Buchi Laboratoriums- Tuchnics AG CH-9230 Flawil/Schweiz). After complete evaporation of the solvents each of the extract was weighed and dissolved in 3% Tween-80 for bacterial assay.

Antibacterial susceptibility test determination of leaf extracts of *Achyranthes aspera*

Staphylococcus aureus, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa and Shigella boydii were obtained from Microbiology Department, Ethiopian Public Health Institute (EPHI), Addis Ababa. These bacteria were screened for bacterial susceptibility test at different doses (50, 100 and 200 mg/ml) of *A. aspera* extracts and standard antibiotics ampiciline (30 µg/disk) and chloramphenicol (30 µg/disk). Bacterial broth culture was prepared at a density of 10^8 cells ml^{-1} which approximately equal to 0.5 McFarland standards. The test microorganisms were uniformly swabbed on the Mueller Hinton Agar (MHA) using the cotton swab. The paper disc diffusion technique was applied to determine the antimicrobial activities of the tested plant extracts as described by Bauer (1966). Sterile paper discs (6 mm in diameter) were immersed in stock solutions containing 50, 100 and 200 mg/mL of plant extracts prepared in 3% Tween-80. Then, the discs were allowed to dry for 15 min (Chantana et al., 2005) and paper disks were placed on the agar plate using sterile forceps. The plates were then incubated for 24 h at 37°C and diameters of the inhibition zones were recorded. All tests were carried out in triplicates and the data were calculated using SPSS version 20 statistics.

Minimum inhibitory concentration (MIC) determination by agar dilution method

Minimum inhibitory concentration (MIC) was determined by agar dilution methods as described by European society of clinical microbial and infectious diseases (ESCMID, 2000). Nineteen milliliter of molten Mueller Hinton Agar (MHA) and 1 ml of extracts from each plant at different concentration (50, 25, 12.5, 6.75, 3.125 ml) were mixed thoroughly and poured on Petri dish. It was then allowed to dry to avoid drops of mixture. The bacterial suspensions in 0.85% saline contains about $1.5x10^8$ cells ml^{-1} colony forming unit (CFU) which were standardized with 0.5 MacFarland. The suspensions of organisms were diluted in 0.85% saline to give $10^7 CFU ml^{-1}$. 1 µl of bacterial suspension (approximately $1x10^4$ CFU) was inoculated on plate having MHA and extracts as described by (ESCMID, 2000). After drying the inoculums spot, the plates were inculpated at 37°C 24 h. The MIC was determined by observing the growth bacteria with our naked eyes.

Table 1. Percentage extract yield from the leaves of chloroform *A.aspera* and *C.hirtsuta* extracts

Solvents/ plant extract	Dry powder (g)	Solvent (ml)	Ratio (w/v)	Yield in (g)	Yield in (%)
80% Methanol/ *A.aspera*	50	500	1:10	6.1	12.2
Chloform/ *A.aspera*	50	500	1:10	4.05	8.10

Table 2. The inhibition of *A. aspera* leaves extract on selected bacteria.

Extract/solvent	Dose (mg/ml)	Mean inhibition ±SEM				
		E. coli	***P. aeruginosa***	***S. aureus***	***S. boydii***	***S. typhi***
A.a/Me	50	6.65±0.50^{*d}	7.54±0.43^{*d}	6.61±0.23^{*d}	6.83±0.12^{*d}	6.60±0.12^{*d}
	100	7.43±0.23^{*d}	7.98±0.58^{*d}	7.00±0.12^{*d}	7.33±0.18^{*d}	7.17±0.44^{*d}
	200	8.2±0.12^{*d}	8.57±0.57^{*d}	8.72±0.18^{*d}	9.50±0.29^{*d}	8.73±0.18^{*d}
A.a/Ch	50	7.74±0.09*	7.92±0.06^{*d}	6.91±0.06^{*d}	7.53±0.06^{*d}	7.31±0.11^{*d}
	100	8.44±0.13^{*d}	8.2±0.15^{*d}	7.80±0.11^{*d}	9.3±0.17^{*d}	8.12±0.06^{*d}
	200	11.12±0.06^{*d}	11.63±0.19^{*d}	10.5±0.5^{*d}	11.83±0.06^{*d}	10.3±0.12^{*d}
Amp	30µg	12.5±0.29^{*d}	15.33±0.33^{*d}	18.00±0.29^{*d}		
Chl	30µg				23.66±0.33	26.83±0.6
	T80	00±00	00±00	00±00	00±00	00±00

A.a=*Achyranthes aspera;* Me=methanol; Ch=chloroform; Amp=ampiciline; Chl=chloroaphinicol; T80=3% Tween 80; C=chloroamphnicol; ^{d}=T80 inhibition, *=is significantly higher than.

Minimum bactericidal concentration (MBC) determination

Some portions of tests were taken from the (MIC) test plate and were sub-cultured on solid nutrient agar by making streaks on the surface of the agar. The plates were incubated at 37°C for 24 h and the MBCs were determined after 24 h. Plates that did not show growth were considered to be the MBC for the extract.

Oral acute and sub-acute toxicity

OECD guideline (2001) was used as a reference for oral acute toxicity on mice. Female Swiss albino mice, 5 in each group were administered orally with 500, 1000 and 2000 mg/kg b.wt from each extract which was dissolved in 3% Tween 80 and 0.2 ml 3% Tween were administered as control. For acute toxicity studies; gross physical changes and body weight were assessed for 14 days.

Data analysis

Data was analyzed by using window software; SPSS, version 20. The results were presented as the Mean±standard error of the mean (Mean±SEM) and statistical significance was considered at 95% confidence interval (P<0.05). For toxicity test and bacterial sensitivity test was compared by using one way ANOVA and followed by Tukey's test.

RESULTS

The highest and minimum yield of the *A. aspera* extract obtained from 80% methanol and achloroform was 12.2% and 8.1%, respectively as shown in Table 1.

Both chloroform and 80% methanol extract of *A. aspera* showed significantly higher inhibitions than negative control, 3% Tween 80, but all extract had significantly lower inhibitions than the corresponding standard discs as it shown in Table 2.

The MIC values of *A. aspera* extracts range from 6.5 to 25 mg/ml on *E.coli, P. aeruginosa, S. aureus, S. boydii* and *S. typhi* as shown Table 3

The MBC values of the two extracts ranges from 12.5 to 25 mg/ml concentrations on five bacteria as described in Table 4.

There were no physical signs such as depression, decrease in feeding activities and hair erection after extract administration at different doses within 24 h. Similarly, there was no mortality in the 14 days follow up after crude extract administration. The weight of the mice increases significantly in each group from day 0 to day 7 and day 14 in both extract and water administered mice as shown in Table 5.

DISCUSSION

The methanol and chloroform extracts of *A. aspera* leaves were active against potentially pathogenic bacteria. Furthermore, the percent inhibition of both extracts of *A. aspera leaves* on bacteria was dependent on extract concentration. However the methanol and

Table 3. Minimum inhibitory concentration (MIC) of leaves of *A. apera.*

Bacterial spp.	Extract	Concentration in mg/ml					
		1.5625	3.125	6.25	12.25	25	50
E.coli	*A.aspera*[a]	+	+	+	+	*	-
	A.aspera[b]	+	+	+	*	-	-
P. aeruginosa	*A.aspera*[a]	+	+	+	*	-	-
	A.aspera[b]	+	+	+	*		-
S. aureus	*A.aspera*[a]	+	+	+	+	*	-
	A.aspera[b]	+	+	+	*	-	-
S. boydii	*A.aspera*[a]	+	+	*	-	-	-
	A.aspera[b]	+	+	*	-	-	-
S. typhi	*A.aspera*[a]	+	+	*	-	-	-
	A.aspera[b]	+	+	+	*	-	-

[a]80% methanol, [b]chloroform, +, growth of bacteria. * Minimum inhibitory concentration; -, for no growth of bacteria.

Table 4. Determination of minimum bactericidal concentration (MBC).

Bacterial spp.	Extract	Concentration in mg/ml				
		3.125	6.25	12.25	25	50
E.coli	*A.aspera*[a]	+	+	+	*	-
	A.aspera[b]	+	+	+	*	-
P. aeruginosa	*A.aspera*[a]	+	+	+	*	-
	A.aspera[b]	+	+	*	-	-
S. aureus	*A.aspera*[a]	+	+	+	+	*
	A.aspera[b]	+	+	+	*	-
S. boydii	*A.aspera*[a]	+	+	*	-	-
	A.aspera[b]	+	+	*	-	-
S. typhi	*A.aspera*[a]	+	+	*		-
	A.aspera[b]	+	+	+	*	-

[a]for 80% methanol, [b] for chloroform, + for growth of bacteria, * for Minimum inhibitory concentration.

chloroform extracts showed different bacterial inhibition at same the concentration. The chloroform extract of *A. aspera* showed the highest inhibition zone (11.83±0.29) at the concentration (200 mg/ml) on *S.boydii* compared to 80% methanol extracts of *A. aspera* inhibition (10.3±0.26) on the same bacteria at the same concentration. The methanol extract showed only 11.63 mm inhibition on *P. aeruginosa* at 200 mg/ml by using paper disk diffusion methods, but other researchers reported that the extracts of *A. aspera* showed up to 14 mm inhibition on *Pseudomonas* species by well plate method (Manjula et al., 2009). This result variation might be due to the application of different antimicrobial methods and variation in the chemo types of the plant. Similarly, *A.*

Table 5. Acute toxicity test of 80% methanol *A. aspera* leaves extract against body weight.

Group	Dose (mg/kg)	Mean body weight ±SEM		
		Pre (D0)	Post (D7)	Post (D14)
a	NC	$^{w2w3\#}$25.97±0 .29	$^{w2\#}$34.77±0.17^{*w1}	36.55±0.19^{*w1w2}
b	500	$^{bcw2w3\#}$25.21±0.52	$^{cw3\#}$34.54±0.18^{*w1a}	35.62±0.51^{*w1w2ab}
c	1000	$^{bcw2w3\#}$25.70±0.24	$^{w3\#}$33.67±0.89^{*w1a}	35.50±0.36^{*w1w2ab}
d	2000	$^{bcw2w3\#}$25.50±0.17	$^{w2w3\#}$33.53±0.26^{*w1a}	$^{w3\#}$34.12±0.27^{*w1a}

D0= day 0; NC= negative control ^{a}= weight of mice before extract administration; ^{b}= weight of mice at 7 days extract administration; ^{c}= weight of mice at 14 days extract administration; ^{w1}= weight of mice before water administration; ^{w2}= weight of mice at 7 days water administration; ^{w3}= weight of mice at 14 days of water administration; *= is significantly higher weight than; $^{\#}$= is significantly lower weight than.

aspera leaves extracts in various solvents showed the antimicrobial effect on *E. coli, E. aerogenes S. aureus* and *P. aeruginosa* in the isolates organisms from patients of diabetes (Kavishankar et al., 2011).

The MIC values of 80% methanol extracts in this research were 12.5, 6.25mg/ml and 6.25 on *S. aureus, S. boydii* and *S. typhi*, respectively, but in the related species, the methanolic extract of the leaves of *Achyranthes coynei* was efficient on *Staphylococcus epidermis, Bacillus subtilis, S. aureus* and *P. aeruginosa* with MIC value of 0.62±0.00 mg/ml (Ankad et al., 2013).

People in southern Ethiopia used powdered *A. aspera* dry or fresh leaf with water to treat skin diseases (Mesfin et al., 2009). This research agreed with traditional healers in that the plant extracts which inhibit the growth of bacteria such as *P. aeruginosa* infecting and affecting the skin. The inhibition zone of the standards drug chloroamphenicol (30 µg) was interpreted in such a way that its bacterial inhibition is resistant (≤12 mm), intermediate (13-17 mm) and sensitive (≥18 mm) as described in Bauer et al. (1966). Based on these criteria, the chloroamphenicol inhibition on *S. boydii* (23.66±0.33 mm) and *S. typhi* (26.83±0.6 mm) in this research was more sensitive.

The methanol extract of *A. aspera* leaf in this research (2000 mg/kg) on mice did not reveal any sign symptom and mortality which was agreeable to Bhosale et al. (2010) with no clinical signs of toxicity and mortality on mice at 2000 mg/kg at 80 methanol extract of *A. aspera*. The weight of mice before treatment significantly increased in both extracts and water administered mice at all concentrations on day 7 and day 14. This data showed that the extracts at or lower 2000mg/kg are not toxic to mice.

Conclusion

A. aspera which is used as medicinal plant in different parts of Ethiopia showed antibacterial activities on some pathogenic bacteria (*E. coli, P. aeruginosa, S. aureus, S. boydii* and *S. typhi*) but it is not toxic to albino mice at 2000 mg/kg. This research indicates further studies on the plants to isolate and identify the responsible biological active compounds in this plant.

CONFLICTING OF INTERESTS

The authors declare that they have no conflict of interest.

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REFERENCES

Ankad G, Upadhya V, Pai SR, HV, Roy S (2013). *In vitro* antimicrobial activity of Achyranthes coynei Sant. Asian. Pac. J. Trop. Dis. 3(3):192-195.

Bauer AW, Kirby WM, Sherris JC, Turck M (1966). Antibiotic Susceptibility Testing by a Standardized Single Disk Method. Am. J. Clin. Pathol. 45:493-496.

Bekele E (2007). Study on Actual Situation of Medicinal Plants in Ethiopia. Prepared for Japan Association for International Collaboration of Agriculture and Forestry, Addis Ababa. p 1-47.

Bhosale U, Radha Y, Popale P, Zambore M, Somani RS (2010). Antinociceptive evaluation of an ethanol extraction of *Achyranthes aspera* (Agadha) in animal model for nociception. Int.J. Phytomedicine. 2:240-245.

Dey A (2011). *A. aspera* L: Phytochemical and Pharmacological Aspects: A review. Int. J. Pharm. Sci. Rev. Res. 9(2):72-82.

European Society of Clinical Microbiology and Infectious Diseases (ESCMID) (2000). Clinical Microbiology and Infection 6(9): 509-515.

Giday M, Teklehaymanot T, Animut A, Mekonnen Y (2006). Medicinal plants of the Shinasha, Agew-awi and Amhara peoples in northwest. Ethiopia. J. Ethnopharmacol. 110:516-525.

Kavishankar GB, Lakshmidevi N, Mahadeva Murthy S (2011). Phytochemicalanalysis and antimicrobial properties of selected medicinal plants against bacteria associated with diabetic patients. Int. J. Pharm Bio. Sci. 2(4): 509-518.

Manjula M, Indira V, Dhasarathan P (2009). Phytochemical characterization and antibacterial activity screening of Achyranthes aspera. Asian J. Microbiol. Biotech. Environ. Sci. 11(2): 365-368.

Mesfin F, Demissew S, Teklehaymanot T (2009). An ethnobotanical

study of medicinal plants in Wonago Woreda, SNNPR, Ethiopia. J. Ethnobiol. Ethnomed. 5:1-28.
Mohana DC, Satish S, Raveesh KA (2009). Antimicrobial evaluation of some plant extracts against some human pathogenic bacteria. Adv. Biol. Res. 2:49-55.
Ogbulie JN, Ogueke CC, Nwanebu FC (2007). Antibacterial properties of Uvaria chamae, Congronema latifolium, Garcinia kola, Vemonia amygdalina and Aframomium melegueta. African J. Biotechnol. 6(13):1549-1553.
Organization for Economic Co-operation and Development (OECD) (2001). OECD guideline for testing of chemicals. Acute Oral Toxicity – Fixed Dose Procedure p 1-14.
Ramawat SK, Merillon JM (2007). Bioactive Molecules and Medicinal Plants. Springer-Verlag, Berlin, Berlin Heidelberg. P 323.
Srivastav S, Singh P, Mishra G, Jha KK, Khosa RL (2011). *Achyranthes aspera-n,* an important medicinal plant: A review. J. Nat. Prod. Plant Resour. 1(1):1-14.
Subbarayan PR, Sarkar M, Impellizzeri S, Raymo F, Lokeshwar BL, Kumar P, Agarwal RP, Ardalan B (2010). Anti-proliferative and anti-cancer properties of Achyranthes aspera: specific inhibitory activity against pancreatic cancer cells. J. Ethnopharmacol. 131: 78–82.
World Health Organization (2003). Factsheet 134: traditional medicine. Geneva P 1.