WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 4, 501-509. Research Article ISSN 2277– 7105

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANTS USED BY ERITREAN TRADITIONAL HEALERS

***[1]Atul Kaushik, [1]Amanuel Aron, [1]Senai Mihreteab, [2]Nuredin Mohamedkassm and [1]Ephraim Michael Kidane**

[1]Medicinal chemistry Unit, School of Pharmacy, Asmara College of Health Sciences, 118, Adi shimaglle, Asmara, Eritrea 8566.

[2]School of Allied Health Profession, Asmara College of Health Sciences, 118, Adishimaglle, Asmara, Eritrea 8566.

Article Received on
26 Jan 2015,

Revised on 17 Feb 2015,
Accepted on 08 Mar 2015

***Correspondence for Author**
Atul Kaushik
Medicinal chemistry Unit,
School of Pharmacy,
Asmara College of Health Sciences, 118,
Adi shimaglle, Asmara,
Eritrea 8566.

ABSTRACT

This experimental based study is aimed to validate the traditionally used plants *viz Meriandra bengalensis, Urtica urens L.* and *Sida schimperiana* for antimicrobial potential against different strains of bacteria and fungi (*Escherichia coli, Staphylococci aureus and Candida albicans*). Among these plants, *Meriandra bengalensis,* and *Sida schimperiana*, showed positive results. *Meriandra bengalensis,* and *Sida schimperiana* showed mild activity against *S. aureus* and *C. albicans* while *Urtica urens L.* was not showing any activity against all the tested microorganisms. TLC analysis was also carried out for all the extracts. R.f. values of the respective spots (major constituents) were determined which may help in further separation and isolation investigations. The results lend scientific credence to justify the use of these plants against bacterial infections.

KEYWORDS: *Traditional medicine, Antimicrobial activity, Crude extracts,*

1.0 INTRODUCTION

The term natural products today is quite commonly understood to refer to herbs, herbal concoctions, dietary supplements and alternative medicine.[1] Most of the discoveries and developments have been based on herbs, folklore, or traditional or alternative medicine. Researches on the bioactivity of tropical medicinal plants have demonstrated that most of them are safe and effective therapies[2, 3]. Tropical rural communities, have a very low access

to modern pharmaceuticals due to their high cost. Therefore, locally available medicinal plants can contribute to health care needs and generate economic benefits for tropical rural communities. The WHO Traditional Medicine Programs have conducted various researches on medicinal plants and proven the safety and efficacy for the treatment of common tropical diseases including malaria and skin, lungs and gastrointestinal tract infections. Therefore, the wise and scientific use of these resources can contribute to the health of large number of the rural community in these areas that have limited or no access to modern health care.[4] By keeping all these points in mind, *Meriandra benghalensis***,** Local (Tigrigna) name: Nhba, *Urtic urens* L. *Local (Tigrigna) name:* Am'e, and *Sida schimperiana* Local (Tigrigna) name: Tefrerya, were used for the present study.

2.0 Experimental work

2.1 Sample collection and Identification

Meriandra benghalensis, Leaf, *Urtica urens L.*, leaf and stem and *Sida schimperiana* whole plant were collected. Plants samples were collected on basis of information gathered from local traditional healers. The authentication was done by Dr Ghebrehiwet Medhanie, Taxonomist, Eritrean Institute of Technology (EIT) Eritrea. Voucher specimens were deposited in the school of pharmacy, ACHS, Asmara, Eritrea.

2.2 Processing of samples

The fresh leaves of *Meriandra benghalensis*, fresh leaves and stems of *Urtica urens*, and fresh whole plant material of *Sida schimperiana* were shade dried (25-35°C) for 10-15 days. The dried plant parts were crushed and weighed before extraction (Table 1).

Table 1: Dry weight of samples

Plant name	***Meriandra bengalensis***	***Urtica urens L.***	***Sida schimperiana***
Total dry weight (gm)	100.563	62.904	100.074

2.3 Preparation of various extractives

100 g of plant materials of *M. benghalensis* and *S. schimperiana*, and 62 g of plant materials of *Urtica urens* were extracted by 70% alcohol using cold extraction method. The method involves dried powdered plant in conical flasks with subsequent addition of 70% alcoholic solution and kept on shaking using electric shaker (Stuart scientific) for 24 hrs. After filtration, crude extracts were dried at controlled temperature using Rotavapour (BUCHI R-114). Crude extracts were mixed with water and fractionated by chloroform by liquid -liquid

extraction method. Aqueous and chloroform extracts were separated, dried and yield were calculated. All the extracts were labeled, stored in clean glass bottles and kept in a refrigerator at 4 ^{0}C for further use.

2.4 Phytochemical screening

Phytochemical analysis of the crude extracts were performed according to standard method by Edeoga et al. (2005).[5] The qualitative analysis tests were performed for various constituents viz flavonoids, saponins, tannins, terpenoids, steroids, cardiac glycosides and glycosides.

2.5 *TLC Analysis*

Samples were deposited on TLC plates with micro capillary tubes eluted using optimized solvent systems allowing the extract to be separated. TLC plates were sprayed by visualizing agents and the R.f. value measured.[6] Chloroform extracts, TLC plate was viewed using UV light and revealed 9 spots of *M. bengalensis*, 12 spots for *Urtica urens L.*13 *spots for S. schimperiana.* For the aqueous extracts, TLC plates scanned using UV light and revealed 4 spots of *M. bengalensis*, 4 spots of *U. urens L.* and 3 spots of *S. schimperiana* (Table 4 and 5).

2.6 *Antimicrobial susceptibility test*

2.6.1 Test organisms

The standard strains of pathogenic bacteria and fungi were used. They includes gram positive bacteria, *Staphylococcus aureus* (ATCC 25923); gram negative bacteria, *Esherichia coli* (ATCC 25922); and fungal strain, *Candida albicans* (ATCC 10231). Biochemical analysis was carried on each of the test organisms for confirmation as described by MacFaddin[7], Fobres *et al*.[8] and Leboffe and Pierce[9]. The Bergey's Manual of Systematic Bacteriology[10] was used for species authentication.

2.6.2 Assay

The agar disk diffusion method was used to assess the antimicrobial activities of all the extract. Bacterial and fungal stock organisms were inoculated on Muller Hinton agar media and incubated for 24-48 hrs. Grown cultures were used to take 3-5 colony forming units (CFU) for preparation of suspension which was further checked for turbidity equilibration against 0.5 McFarland turbidity standards. Optimally, within 15 minutes after adjusting the turbidity of the inoculums suspension, a sterile cotton swab was dipped into the adjusted

suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum from the swab. The dried surface of a Müeller-Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed.

Highly concentrated semisolid extracts were serially diluted using their respective solvents to get concentrations of 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml. Filter paper sterile discs (6 mm in diameter) were socked in liquefied crude extracts and were subjected to air drying, placed on inoculated culture media. Control disks of extractant solvents were also used. Vancomycin (10µg/disk), Gentamycin (10µg/disk), Ampicillin (10 µg/disk) and Amikacin (30 µg/disk) were used as standard.[11]

2.7.3 Determination of minimum inhibitory concentration (MIC)

MIC was determined for aqueous and chloroform extracts of *M. bengalensis* and *S. schimperiana*. Extracts was diluted to 500mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml and 31.25 mg/ml and were added to different test tubes containing the nutrient broth. This was prepared for each organism and done in triplicate. A colony of 24h cultured organism was inoculated into test tube containing 1ml of normal saline to form a turbidity of 0.5 McFarland standard and was thereafter dispense into the test tube containing the suspension of nutrient broth, aqueous and non-aqueous extract. This was done for all the organisms at the varying concentrations. All test tubes were properly corked and incubated at 37^{o}C for 24h and at 25^{o}C for 96 h for bacteria and fungi respectively. After which they were observed for absence or presence of visible growth. The lowest concentration of each extracts showing no visible growth was recorded as the minimum inhibitory concentration (MIC). It was further standardized in terms of absorbance at 600 nm in a visible spectrophotometer.

3.0 Results and discussion

3.1 Yield percentage

Different parts of three plants belonging to three different genus were extracted with 70% ethanol solvent (cold extraction technique) and then fractioned to aqueous and chloroform fractions. The percentage yields of these crude extracts were calculated and given in Table 2.

Table 2: Percentage yields of medicinal plant extracts using two different solvents.

Plant name	Aqueous Extract % (W/W)	Chloroform Extract % (W/W)
Meriandra bengalensis	2.00	2.06
Urtica urens L.	5.17	1.83
Sida schimperiana	5.96	1.24

3.2 Phytochemical analysis

The qualitative analysis was carried out for different phytoconstituents and the results are shown in table 3. The qualitative analysis revealed the presence of alkaloids, tannins, terpenoids, flavonoids, cardiac glycosides and saponins for *Meriandra bengalensis*. For *Sida schimperiana* the tests revealed the presence of alkaloids, tannins, terpenoids, flavonoids and cardiac glycosides. While *Uritca urens L.* possessed alkaloids, tannins, cardiac glycosides and saponins.

Table 3: Qualitative Chemical Analysis of extracts of *M. benghalensis, S. schimperiana &U. urens*

S. No	Name of Test	Test Performed	Observation	Inference		
				M. benghalensis	S. schimperiana	U. urens
1.	TEST FOR ALKALOIDS	Wagner's Test	Red-Brown Precipitate	(+)	(+)	(+)
2.	TEST FOR CARDIAC GLYCOSIDES	Keller killiani test	Reddish brown color at junction	(+)	(+)	(+)
3.	DETECTION OF STEROIDS	Acetic Anhydride Test	Color change from Violet to Blue or Green	-	-	-
4.	TEST FOR SAPONINS	Foam Test	1cm layer of foam	(+)	-	(+)
5.	TEST FOR TANINS	$FeCl_3$ Sol. Test	Deep Blue-Black Color	(+)	(+)	(+)
6.	TEST FOR FLAVONOIDS	Ammonia Sol. Test	Yellow color	(+)	(+)	-
7.	TEST FOR TERPENOIDS	Salkowaski Test	Reddish –brown at the interface	(+)	(+)	-

(+)= Presence; (–) =Absence.

3.3 TLC Results

Further investigation is needed to isolate the separated compounds to elucidate the chemical structure and its bio efficacies. Thin layer chromatography analysis data will support and facilitate the further work on these medicinal plants. Various spots were identified and Rf values were determined for each spot. (Table 4, 5)

Table 4: Chloroform extracts eluted with chloroform: acetone (24:1)

No of spots	*Meriandra bengalensis*			*Urtica urens L.*			*Sida schimperiana*		
	X	Xo	Rf	X	Xo	Rf	X	Xo	Rf
A	1	15.5	0.065±0.053	0.7	15.4	0.045±0.037	0.7	15.4	0.045±0.047
B	8	15.4	0.519±0.073	1.8	15.4	0.117±0.015	8	15.4	0.519±0.025
C	8.9	15.4	0.578±0.024	8.3	15.4	0.539±0.031	10.7	15.4	0.695±0.055
D	10	15.4	0.650±0.052	10.5	15.4	0.681±0.047			

X: distance of the solute **Xo**: distance of the solvent Rf: Retardation factor

Table 5: Aqueous extracts eluted with Acetone: Methanol (3:1)

Spot	*Meriandra bengalensis*			*Urtica urens L.*			*Sida schimperiana*		
	X	Xo	Rf	X	Xo	Rf	X	Xo	Rf
A	2.1	16.7	0.126±0.04	1.8	16.7	0.108±0.036	2.2	16.7	0.132±0.026
B	4.3	16.7	0.257±0.060	2.3	16.7	0.138±0.061	2.7	16.7	0.162±0.045
C	6.7	16.7	0.401±0.043	4.8	16.7	0.287±0.041	3.0	16.7	0.180±0.032
D	8.3	16.7	0.497±0.015	6.5	16.7	0.390±0.025	5.0	16.7	0.299±0.065
E	9.3	16.7	0.557±0.013	7.1	16.7	0.425±0.063	5.6	16.7	0.335±0.024
F	12	16.7	0.719±0.032	7.7	16.7	0.461±0.025	7.9	16.7	0.473±0.036
G	14	16.7	0.838±0.043	8.9	16.7	0.533±0.060	8.7	16.7	0.521±0.045
H	15.2	16.7	0.910±0.015	10.5	16.7	0.629±0.062	9.5	16.7	0.569±0.047
I	15.7	16.7	0.940±0.02	12.8	16.7	0.766±0.025	11.8	16.7	0.707±0.036
J				13.7	16.7	0.820±0.076	13.7	16.7	0.820±0.012
K				14.7	16.7	0.880±0.015	14.3	16.7	0.856±0.055
L				15.5	16.7	0.928±0.026	14.8	16.7	0.886±0.064
M							15.5	16.7	0.928±0.012

X: distance of the solute **Xo**: distance of the solvent Rf: Retardation factor

3.4 Antimicrobial activity

The results demonstrated that both aqueous and non aqueous extracts of *U. urens L.* did not show any activity against the three tested microorganisms. On the other hand the aqueous and chloroform extracts of *M. bengalensis* showed small zones of inhibition against *S. aureus* and *C. albicans* respectively as compared to standard antibiotic discs. The aqueous and chloroform extracts of *S. schimperiana* also showed activity against *S. aureus.*

The plants were active against the gram-positive bacteria (*S. aureus*) as compared to the gram-negative bacteria (*E. coli*). The reason for the difference in sensitivity between gram-positive and gram-negative bacteria might be ascribed to the differences in morphological constitutions between these microorganisms, gram-negative bacteria having an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to antimicrobial chemical substances. On the other hand, the gram-

positive bacteria are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier. Therefore, the cell walls of gram negative organisms are more complex in lay out than the gram positive ones acting as a diffusional barrier and making them less susceptible to the antimicrobial agents than are gram positive bacteria. The results of antimicrobial study are tabulated below.

Table 6: Zones of inhibition of the extracts: NZ; No zone of growth inhibition, NT; Not tested

Plant name	Solvent	Concentrations	Zone of inhibition (mm)		
			E.coli	*S.aures*	*C. albicans*
Meriandra bengalensis	**Aqueous**	*500 mg/ml*	NZ	**15**	NZ
		250 mg/ml	NZ	**11**	NZ
		125 mg/ml	NZ	**10**	NZ
		62.5 mg/ml	NZ	**8**	NZ
		31.5 mg/ml	NZ	NZ	NZ
	Chloroform	*500 mg/ml*	NZ	NZ	**12**
		250 mg/ml	NZ	NZ	**9**
		125 mg/ml	NZ	NZ	**8**
		62.5 mg/ml	NZ	NZ	NZ
		31.5 mg/ml	NZ	NZ	NZ
Urtica urens L.	**Aqueous**	*500 mg/ml*	NZ	NZ	NZ
		250 mg/ml	NZ	NZ	NZ
		125 mg/ml	NZ	NZ	NZ
		62.5 mg/ml	NZ	NZ	NZ
		31.5 mg/ml	NZ	NZ	NZ
	Chloroform	*500 mg/ml*	NZ	NZ	NZ
		250 mg/ml	NZ	NZ	NZ
		125 mg/ml	NZ	NZ	NZ
		62.5 mg/ml	NZ	NZ	NZ
		31.5 mg/ml	NZ	NZ	NZ
Sida schimperiana	**Aqueous**	*500 mg/ml*	NZ	**13**	NZ
		250 mg/ml	NZ	**9**	NZ
		125 mg/ml	NZ	NZ	NZ
		62.5 mg/ml	NZ	NZ	NZ
		31.5 mg/ml	NZ	NZ	NZ
	Chloroform	*500 mg/ml*	NZ	**13**	NZ
		250 mg/ml	NZ	**8**	NZ
		125 mg/ml	NZ	NZ	NZ
		62.5 mg/ml	NZ	NZ	NZ
		31.5 mg/ml	NZ	NZ	NZ
Standard antibiotic disk (Control)	**Amikacin**	*30 µg/disk*	20	23	NT
	Ampicillin	*10 µg/disk*	17	27	NT
	Gentamycin	*10 µg/disk*	19	22	NT

3.5 MIC determination

The preliminary and primary screening assays for antimicrobial activity can largely be considered as qualitative assays and are used for identifying the presence or absence of bioactive constituents in the extracts. Minimum inhibitory concentration (MIC) is a quantitative assay and provides more information on the potency of the compounds present in the extracts.

MIC was done on the plants which showed activity, namely, M*eriandra bengalensis and Sida schimperiana* and the results are shown in table 7.

Table 7: MIC values of the extracts

Plant name	Solvent	MIC (mg/ml)		
		E.coli	***S. aures***	***C. albicans***
M*eriandra bengalensis*	**Aqueous**	NT	250	NT
	Chloroform	NT	NT	125
Urtica urens	**Aqueous**	NT	NT	NT
	Chloroform	NT	NT	NT
Sida schimperiana	**Aqueous**	NT	250	NT
	Chloroform	NT	31.25	NT

NT: Not tested.

4.0 CONCLUSION

The phytochemical investigation in this study revealed the presence of various bioactive plant constituents in *Meriandra bengalensis* and *Sida schimperiana* which is believed to be the cause of their antimicrobial properties. The antimicrobial activities shown by *Meriandra bengalensis* and *Sida schimperiana*, though at high concentrations, still holds true to give clue to the local traditional healers making use of it.

With the aforementioned affirmative results from *in vitro* susceptibility test with microorganisms, there still remains jeopardy in our hearts concerning the possible toxicity potentials of those plants. The TLC analysis in the study invites further investigations to isolate and identify the separated compounds for elucidation of the chemical structure and bio efficacies of the active constituents.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Holt, G.A., Chandra, A., (2002). Herbs in the modern healthcare environment- An overview of uses, legalities, and the role of healthcare professional. *Clin. Res. Regulatory Affairs* (USA), 19, p 83-107.
2. Lake, J., (2000). Psychotropic medications from natural products: A review of promising research and recommendations. *Alternative Ther. Health Med*., 6(3), 36, p 39-45, 47-52.
3. Spainhour, C.B., (2001). OTC drugs and nutraceutical. *In*: S.C. Gad (Ed.), Regulatory Toxicology, 2nd ed. Taylor & Francis, London, p 192-204.
4. Hailu T., *Pharmaceutical Studies of Some Selected Medicinal Plants Locally Used in the Treatment of Skin Disorders*, 2004, Addis Ababa University.(M.sc, Thesis)
5. Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical Constituents of Some Nigerian Medicinal Plants. Afr. J. Biotech., 4 (7): 685-688
6. Francis R. and Annick R, Chemical Analysis: Modern Instrumentation Methods and Techniques, 2nd Edition, 1994, Jhon Wiley and Sons Ltd, p 117-123
7. Mac Faddin JF. Biochemical Tests for Identification of Medical Bacteria. 3rd Edition. Williams and Wilkins, Lippincott, New York, 2000. ISBN: 0-683-05318-3
8. Fobres B, Sham D, Weissfield A. *Diagnostic Microbiology*. 11th Edition, Andrew Allen Press, Canada; 2002.
9. Loboffe MJ, Pierce BE. *Microbiology Laboratory Theory and Application*. 1st Edition, Morton Publishing, USA. 2002.
10. Sneath PHA, Mair NS, Sharpe ME, Holt JG. Bergey's Manual of Systematic Bacteriology. Vol. 2, Williams and Wilkins, Philadelphia; 1986.
11. Bauer, A.W., Kirby, W.M.M., Sherris, J.C., and Turk, M. (1966); Antibiotic susceptibility testing by a standardized single disk method: American Journal of Clinical Pathology, 45: p 493-496.