Anti-Microbial and Phytochemical Screening of Commiphora africana, Cucumis pustulatus and Vernonia schimperi from Eritrea

Samuel Fissehatsion (sameknchеb@gmail.com)
Asmara College of Health Sciences
Berhane Girmai
Asmara College of Health Sciences
Bereket Berhane
Asmara College of Health Sciences
Samuel Berhane
Asmara College of Health Sciences

Research Article

Keywords: Anti-microbial activity, Commiphora africana, Cucumis pustulatus, Vernonia schimperi, Phytochemical screening

Posted Date: November 29th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2173161/v1

Abstract

Background

Traditional medicines have been widely used in treating many microbial, inflammatory and other types of diseases states in Eritrea, where complimentary remedies are required to the modern health care system.

Methodology

The current research project focusses on three Eritrean medicinal plants; *Commiphora africana*, *Cucumis pustulatus* and *Vernonia schimperi*, which are traditionally used in management of different diseases. The hidden potential of the three plants for their anti-microbial activity was assessed with the employment of zone of inhibition. The plants were extracted by cold extraction using three solvents arranged in their increasing polarity starting with Petroleum ether, methanol and distilled water. The in vitro anti-microbial activity was performed by agar well diffusion method.

Result

Percentage yield was calculated for each plant extract. The cold methanol extracts of these plants exhibited the maximum yield while the minimum yield was obtained with petroleum ether. The gram-positive bacteria *Staphylococcus aureus* was the most susceptible, while the gram-negative bacteria, *Escherichia coli* was moderately susceptible. Desirable result has shown against *Candida albicans* with the methanol extracts of both *Commiphora africana* and *Cucumis pustulatus*. The most active antibacterial activity was obtained from the plant *Commiphora africana*. As a standard anti-biotic, Ciprofloxacin and Fluconazole were taken as positive control for the bacterial and fungal medias respectively and distilled water as a negative control.

Conclusion

Commiphora africana and *Cucumis pustulatus* have shown the highest activities. Based on the outcomes these plants have compelling anti-fungal and anti-bacterial property. However further in-vivo studies, advanced phytochemical screenings, toxicity tests are recommended.

Introduction

Nature is creative, it always accompanies solutions for every problem, happiness for sorrow, birth for death, and the same too medicine for disease. Human kind has known the unavoidable and unpredictable upcoming of infections, before their causative agent has discovered dawn history [1]. From the ancient times when civilization started, human beings have identified the use of different herbs in treating infections [2]. Several studies agree the knowledge on plant use is the result of many years of man's interaction and selection on the most desirable, most vigorous and most successful plants present in the immediate environment at a given time [2, 3, 4]. To use your environmental resources for different purpose is the gift from nature. And to apply the knowledge of science to your environment and to use appropriately is second blessing of nature.

The world's poorest countries are most in need of affordable, effective treatments for diseases. According to the WHO report, about 80% of African population depend on traditional medicine for their basic health service needs [5].The WHO emphasizes, the significance and ability of traditional medicineto carry out the theme "Health for All"

in Africa, hence advised and assisted to promote their traditional medicine as a significant component of the healthcare system[6]. Besides, more than 50% of modern clinical drugs origin are the natural products [7]. Hence, many studies have done on plants used as traditional medicines for their antimicrobial activities; to use as remedy for many disease of microbial origin [8,].

In Eritrea the use of herbs to treat different types of ailment is a wide spread practice from ancient times [9]. However, little has been done to study the antimicrobial activity of Eritrean flora. In this research the potential bioactivity of three traditionally used plants, namely; *Commiphora africana* (Local name-Anqua), *Cucumis pustulatus* (Local name-Hafafle), *and Vernonia schimperi* (Local name-Sigemo)are tested for their antibacterial and antifungal activities. The plants were selected based on their ethno-medicinal use against infection and inflammation, such as toothache, disease of the gum, abdominal pain, wound healing, diarrhoea and tonsillitis in the areas where they grow.

Methodology

Materials and chemicals: Distilled water, methanol, petroleum ether, Muller Hinton Agar, Sabouraud Dextrose Agar, normal saline, chlorohexidine, Fluconazole and Ciprofloxacin. And materials were shaker, vacuum pump, butchner funnel with its flask, rotary vapour, Whatman filter paper №.1,petri dishes, beakers, test tubes, air-sensitive balance, grinder, hood, freezer, cork borer and auto clave.

Plant collection and authentication:*Commiphora africana*'sbarks and *Cucumis pustulatus*'s leaves were collected from Southern region of Eritrea specifically Dubarwa (15.0896^0 N, 38.8339^0 E) and Dekemhare (15.0715^0 N, 39.0424^0E) sub-zones (Tsegaro and Adigolgol villages) respectively. Whereas *Vernonia schimperi*'s leaves were collected from Central region in Asmara (15.3229^0 N, 38.9251^0E). We received a formal consent from Ministry of Agriculture to start the sample collection of the wild plants for the purpose of the study. Fresh plant collection was done in the summer season from August to September 2015, when the plants are matured. The plants were authenticated by Taxonomist Professor Ghebrhiwet Medhanie and his team by Herbarium methods against Herbarium specimens. The voucher ID of *Commiphora africana, Cucumis pustulatus and Vernonia schimperi* are 754, 506 and 869 respectively. And has been deposited at Mai-Nefhi College of Science, Herbarium of Department of Biology.

Extraction of crude extract: The plant materials were thoroughly washed, shade dried at room temperature, powdered, and prepared for cold extraction.110gm of *Commiphora africana*'s bark, 130gm of *Cucumis pustulatus*'s leaves and 140gm of *Vernonia schimperi's* leaves were taken and macerated using different volumes of Distilled water, Methanol, and Petroleum ether (illustrated in table No.1) in decreasing polarity for 48 hours with the help of shaker for the sensitivity test. The soaked materials were filtered with help of vacuum pump in order to facilitate the filtration process and then the crude extract were collected in a clean container. The crude extract was then concentrated and solvent recovered by distillation using rotary evaporator, under reduced pressure and temperature of 40°C. The concentrated extract was dried and weighed. The crude extracts were diluted with sterile distilled water and kept in an airtight bottle in the refrigerator until the experiment was carried out. Distilled water was taken as a negative control.

Antibacterial test (ABT): Two standard bacteria, a gram positive *Staphylococcus aureus* (ATCC 10231) and gram negative *Escherichia coli* (ATCC 25922) were obtained from National Health Laboratory (NHL) microbiology unit, Asmara, Eritrea. Mueller Hinton agar plates were seeded with the test organisms and the plates left to dry for five

minutes. After drying, wells were made in the agar using sterile cork borer measured6mm in diameter[10]. Hundred microliters (100 µl) of crude extracts of different concentration (250 mg/ml, 125 mg/ml and 62.5 mg/ml) for each bacterium were dispensed into the labelled wells by well streak methodand standard drug ciprofloxacin, the positive control was impregnated by disk diffusion method. The culture media incubated at 37°C, for 24hrs. After the defined period; inhibition zone was measured with Vernier calliper for the crude extracts and standard drug. Analysis were done in duplicates and mean was recorded. Overall, cultured bacteria with zones of inhibition equal to or greater than 8 mm were considered susceptible to the tested extract[10]. The inhibition zone diameter of each crude extract against each microbe is illustrated in the result tables and graphs.

Antifungal test (AFT): A standard *Candida albicans* (ATCC 10231) was obtained from the same unit as for the standard bacteria. The antifungal assay was also performed in well streak diffusion with Sabauraud dextrose media. The crude extracts were impregnated to the wells of fungal agar Medias at different concentration (250 mg/ml, 125 mg/ml and 62.5 mg/ml). From each extract, hundred micro litres were dropped in the different holes with 6mm in size. And standard drug Fluconazole as positive control also performed as disk diffusion. The cultured media incubated at 37°C, for 72hrs. Sensitivity test of fungus was accomplished. The inhibition zone was measured using a Vernier calliper for the crude extracts. Analysis were duplicated and mean was taken. The inhibition zone diameter of each crude extract against each microbe is illustrated in the result tables and graphs. Inhibition zones greater than or equal 8mm were considered as sensitive to the extracts. [10] Prior to each procedure, all the material used for the antimicrobial test was sterilized properly and media, specifically, were autoclaved at 121^{o}C for 30 minutes.

Phytochemical Screening: determining pharmacologically active constituents from plants remains to be a long and tedious process. Several methods could be used for the identification and characterization of the active components of plant extracts. One such technique is chemical screening that will allow localization and targeted isolation of constituents with potential activities. Phytochemical screening was done using different standards [11].

Alkaloids: 0.2gm of the crude extract was added to five drops of HCl and then it was filtered and finally the filtrate was mixed with Wagner's reagents to form a Brown or precipitate which indicates the presence of an alkaloids.

Saponins: 0.2gm of crude plant extract was mixed with 20 mL distilled water and shaken in a graduate cylinder for 15 minutes to form a foam with a 1 cm layer indicating the presence of saponins.

Tannins: 0.2gm of the crude extract was added to 1% gelatin solution containing NaCl for the formation of a precipitate, which in turn indicates the presence of tannin in the crude extract.

Flavonoid: Using an alkaline reagent test, a few drops of NaOH solution were added to 0.2gm of crude extract for the formation of an intense yellow colour that becomes colourless on the addition of 10 drops of 1% hydrochloric acid showed the presence of flavonoids.

Results And Discussion

Percent Yield of Plant Extracts (w/w):The extracted yields of stem bark of *Commiphora africana* were 10% in water, 6.4% in ethanol and 0.99% in petroleum ether, for the dried leaves of *Cucumis pustulatus* the yields were 20% in water, 11.5% in ethanol and 1.2% in petroleum ether; and in the preparation of dried extract from dried leaves of *Vernonia schimperi*, yields of 15% in water, 12.5% in ethanol and 1.1% in petroleum ether were obtained.

Phytochemical screening:In the phytochemical screening of *Commiphora africana* bark showed flavonoids, tannin, alkaloids, and saponins showed the same result as to these tests as in several studiesin Nigeria [12, 13]. In the screening of *Cucumis pustulatus* flavonoid, tannins, and saponinswere detected, but alkaloids were absent. In phytochemical screening of the leaves of *Vernonia schimperi* was found to reveal for the presence of saponins, tannins, and flavonoids. The same to study done by Sarfaraz et al. [14] this medicinal plant showed negative result to the test for alkaloids.

Flavonoids, tannins, terpenoids, alkaloids have antimicrobial activities. [15]This implies the anti-microbial activities of these plants might be any one or more of these secondary metabolites.

The main aim of this research is to see if the traditionally practiced plants have the suggested medicinal action and make ground for further research. The experimental portion of the research was started by collecting, shade drying for at least two months, grinding to reduce the particle size and extracting of the dried powders with different solvents. It was cold extraction method, since traditionally these plants are kneaded with cold water and some of them with saliva. Lastly to obtain the suspected constituent in the native form as it is used traditionally and protect thermolabile constituents of the plants. So in the extraction of *Commiphora africana*, *Cucumis pustulatus* and*Vernonia schimperi* extraction solvents were petroleum ether, methanol and distilled water in order of increasing polarity. The crude dried extract obtained was higher in methanoland water extracts and lower in petroleum ether extracts. In the case of antimicrobial activity tests, three micro-organisms namely*Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* were selected and obtained from NHL. The method of selection criteria was based on the resistance, cultural usage of plants against disease and gram classification, which *Escherichia coli* is gram negative and with great emergence of resistance[16, 17], *Staphylococcus aureus* from gram positive and with great capability of resistance[18]and *Candida albicans* from fungus with different and new mechanisms of resistance. [19]Ciprofloxacin has a positive control, the selected strains have shown sensitivity, similarly *Candida albicans*has shown sensitivity to Fluconazole, and also the solvent distilled waterwas taken as negative control and didn't show any inhibition zone. Methanol extract was showing predominating inhibition zone, this could be attributed to different polarities and dissolving potential of the solvent for both polar and non-polar chemical constituents. Water extract ranked secondly and petroleum ether showed the lowest activity.

Antibacterial Assay:*Staphylococcus aureus* is susceptible to the three plants at different concentrations except to water and petroleum ether extract of *Vernonia schimperi*. The best inhibition zone was shown by *Vernonia schimperi* of methanol extract from the lower to the higher concentration in sequence, and the highest was at 250mg/ml that is 33mm. All extracts of *Commiphora africana* and methanol extract of *Cucumis pustulatus* were active against *Escherichia coli* consistently but *Vernonia schimperi* didn't show any activity. The highest inhibition was 23mm at 250mg/ml by *Commiphora africana*. Generally,*Commiphora africana* had broadest and better activity than the rest medicinal plants as documented in table No.two (2) in comparison with rest of inhibition zones of *Cucumis pustulatus*in tableNo.3 and *Vernonia Schimperi*in table No.4.

Antifungal Assay:*Candida albicans* was found to be susceptible to those plants except for the lowest and 125mg/ml of both methanol and petroleum ether extracts of *Vernonia schimperi*. The most desirable inhibition zone was 26mm at 250mg/ml of methanol extracts of *Commiphora africana* and *Cucumis pustulatus*. The activities of medicinal plants against their concentration in serial dilution method are presented in Table 2,3, and 4.

In a nutshell *Commiphora africana* showed broad spectrum of antimicrobial activity against the selected microbes (bacteria and fungus) in all extracts and concentrations.

Conclusion

In conclusion, the present study showed that almost all solvents extract of *Commiphora africana*, *Cucumis pustulatus*and *Vernonia schimperi* have great activity against the selected pathogenic microorganisms. This suggest identifying the right biologically active components which may be worth further investigation and structural elucidation for their active principle. As in the result this study reinforces the traditional claim of these plants in treatment of infection especially *Escherichia coli* or*Staphylococcus aureus* origin as its described in the beginning.

Declarations

Ethics approval and consent to participate

All the experimental research and field studies on plants where taken based on the institutional guidelines and legislation.

Availability of data and materials

The datasets used and analysed during the current study available from the corresponding author on reasonable request.

Consent for publication

Not applicable

Competing interests

No competing interests.

Funding

Self-funded project, but the acknowledge institutions help us in respective chemicals and equipments to accomplish the research.

Authors' contributions

Samuel Fissehatsion, Bereket Berhane, Samuel Berhane: Were responsible for study plan, plant collection, laboratory analysis and result interpretation.

Prof. Berhane Girmai: was responsible for study plan and guiding laboratory procedures.

All authors read and approved the final manuscript.

ACKNOWLEDGEMENT

Authors acknowledge to Asmara college of Health Sciences, especially to the school of pharmacyfor providing all the necessary materials used in this scientific work. Eritrean National Health Laboratory (NHL) for their contribution and technical guidance in the sensitivity tests. Last but not least, authors acknowledge to the

Taxonomist Prof. Ghebrhiwet Medhanie and his team from Mai-Nefhi College of Sciences, Biology Department for the authentication process of the study plants.

Author details

1. Department of Pharmacy, Asmara College of Health Science, Asmara- Eritrea.
2. Dean of College, Asmara College of Health Sciences, Asmara-Eritrea.

References

1. Morens DM, Fauci AS. Emerging Infectious Diseases: Threats to Human Health and Global Stability. PLoSPathog. 2013; 9(7):1-3. DOI:10.1371/journal.ppat.1003467.
2. Joshi B, Lekhak S, Sharma A. Antibacterial Property of Different Medicinal Plants:*Ocimum sanctum, CinnamomumZeylanicum, Xanthoxylumarmatum and Origanummajorana*. KUSET. 2009 01; 5 (1): 143-50.
3. Matasyoh JC, Maiyo ZC, Ngure RM, Chepkorir R. Chemical composition and antimicrobial activity of the essential oil*Coriandrumsativum.* J Food Chem. 2008 07 30; 113 (2009): 526-29.
4. Magassouba FB, Diallo A, Kouyat´e M, Mara F and Mara O, et al. Ethnobotanical survey and antibacterial activity of some plants used in Guinean traditional medicine. Journal of ethnopharmacology. 2007 07 09; 114 (2007):44-53. DOI:10.1016/j.jep.2007.07.009
5. World Health Organization. African Health Monitor: Traditional Medicine our culture, our future. WHO,Brazzaville, Congo. 2003:4(1).2.
6. World Health Organization. Traditional Medicine Strategy 2002-2005. WHO, Geneva, Switzerland. 2001:1.
7. Stuffness M, Douros J. Current status of the NCI plant and animal product program. J. Nat. Prod.1982; 45: 1-14. Cited by: Moorthy K, Srinivasan K, Subramanian C, Mohanasundari C, Palaniswamy M. Phytochemical screening and antibacterial evaluation of stem bark of *Mallotusphilippinensis var. Tomentosus.* Afr. J. Biotechnol. 2007 07 04; 6(13): 1521-23. Available from URL:http://www.academicjournals.org/AJB
8. Subramani SP, Goraya GS. Some Folklore medicinal plants of Kolli hills: Record of a WattivaidyasSammelan. J. Econ. Taxon. Bot. 2003; 27 (3): 665-78.Cited by: Moorthy K, Srinivasan K, Subramanian C, Mohanasundari C, Palaniswamy M. Phytochemical screening and antibacterial evaluation of stem bark of *Mallotusphilippinensis var. Tomentosus.* Afr. J. Biotechnol. 2007 07 04; 6(13): 1521-23. Available from URL:http://www.academicjournals.org/AJB
9. Senai AW. Legislative Regulation of Traditional Medicinal Knowledge in Eritrea vis-à-vis Eritrea's Commitments under the Convention on Biological Diversity: Issues and Alternatives. LEAD j.2010; p. 130. Available from URL: http://www.lead-journal.org/content/10130.pdf
10. Nayan R. Bhalodia, V. J. Shukla. Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal. J. Adv. Pharm. Tech. Res. 2011 Apr-Jun; 2(2): 104-9. DOI:10.4103/2231-4040.82956
11. Pandey A, Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. J PharmacognPhytochem. 2014;2(5): 115-9.
12. Goji ADT, Dikko AAU, Bakari AG, Mohammed A, Ezekiel I, Tanko Y. Effect of Aqueous-ethanolic Stem Bark Extract of Commiphora Africana on Blood Glucose Levels on Normoglycemic Wistar Rats. Int. J. Anim. Veter. Adv.2009; 1(1): 22-4.

13. Nuhu A, Danmalam UH, Ilyas N, Zakariya AM, Shehu S, Jajere UM. Evaluation of Antiulcer Activity of Commiphora africana (A. Rich) Engl. (Burseraceae) Stem-bark Extracts Using Ethanol Induced Ulcer Model in Albino Rats. Int.J.Curr.Microbiol.App.Sci. 2016; 5(3): 15-25. http://dx.doi.org/10.20546/ijcmas.2016.503.003
14. Ahmed S, Al-Rehaily A, Yousaf M. Preliminary phytochemical screening of Vernonia schimperi. IJMCA. 2014; 4(4): 226-30.
15. Prashant T, Bimlesh K, Mandeep K, Gurpreet K, Harleen K. Phytochemical screening and extraction. Internationale PharmaceuticaSciencia. 2011; 1(1):101-2.
16. Olorunmola1 FO, Kolawole DO, Lamikanra A. Antibiotic resistance and virulence properties in *Escherichia coli* strains from cases of urinary tract infections. Afr. J. Infect. Dis. 2013; 7(1): 1-7. Available from http://dx.doi.org/10.4314/ajid.v7i1.1
17. Niranjan V, Malini A. Antimicrobial resistance pattern in Escherichia coli causing urinary tract infection among inpatients. Indian J Med Res. 2014 06; 139: 945-48.
18. Henry C, Frank D. Waves of Resistance: Staphylococcus aureus in the Antibiotic Era. Nat Rev Microbiol. 2009 09; 7(9): 629–41. DOI:10.1038/nrmicro2200
19. Ford C, Funt M, Abbey D, Issi L, Guiducci C, Martinez D et al. The evolution of drug resistance in clinical isolates of Candida albicans. eLife. 2015;4: eLife.00662. DOI: 10.7554/eLife.00662

Tables

Table 1: Amount of dried plants (gm) with different amount of extraction solvents(ml) and yield gained (gm)

Plants		*Commiphora africana*				*Cucumis pustulatus*				*Vernonia schimperi*			
Variables		C.P	S.U	Y.E	%	C.P	S.U	Y.E	%	C.P	S.U	Y.E	%
Solvents	Water	110	750	11	10	95	1200	19	20	140	1000	21	15
	Methanol	110	500	7	6.4	130	2000	15	11.5	140	900	17.5	12.5
	Petroleum ether	110	500	1	0.9	130	2000	1.5	1.2	140	900	1.56	1.1

C.P=Crude Powder Used (in gm) S**.U**=Solvent Used (in ml) **Y.E**=Yield extract (in gm)

Table 2: Inhibition zone diameter of *Commiphora africana* extracts in mm

		Distilled Water			Methanol Extract			Petroleum ether Extract		
Concentration in mg/ml		250	125	62.5	250	125	62.5	250	125	62.5
Test microbes	*E.coli*	14	12	11	23	19	17	16	15	14
	S.aures	16	14	10	26	25	23	18	12	11
	C.albicans	16	15	12	26	24	23	16	15	14

Table 3: Inhibition zone diameter of *Cucumis pustulatus* extracts in mm

		Distilled Water			Methanol Extract			Petroleum ether Extract		
Concentration in mg/ml		250	125	62.5	250	125	62.5	250	125	62.5
Test microbes	*E.coli*	_ _	_ _	_ _	15	14	11	_ _	_ _	_ _
	S.aures	15	14	13	16	15	13	12	11	10
	C.albicans	20	18	15	26	21	16	19	16	15

_ _: NO Zone of Inhibition

Table 4: Inhibition zone diameter of *Vernonia schimperi* extracts in mm

		Distilled Water			Methanol Extract			Petroleum ether Extract		
Concentration in mg/ml		250	125	62.5	250	125	62.5	250	125	62.5
Test microbes	*E.coli*	_ _	_ _	_ _	_ _	_ _	_ _	_ _	_ _	_ _
	S.aures	18	16	_ _	33	29	23	_ _	_ _	_ _
	C.albicans	15	11	_ _	14	_ _	_ _	15	_ _	_ _

_ _: NO Zone of Inhibition