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Phytochemical Screening and Antimicrobial Potential of *Lepidium sativium* and *Rumex nervosus* in Eritrea

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Authors' contributions

This study was carried out in collaboration between all authors. Authors YSG, MSD and NAF designed and carried out the study and wrote the manuscript. Author MSD supervised the study and proofread the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Some of the widely used plants for the treatment of eye diseases, especially animals, include *Lepidium sativium* and *Rumex nervosus*. Therefore, the phytochemical screening and antimicrobial activities of the crude extracts of the seeds *L. sativium* and leaves of *R. nervosus* were investigated. Freshly matured seeds of *L. sativium* and leaves of *R. nervosus* were collected, and sequentially extracted by soxhlet extractor using different polarity solvents including n-hexane, methanol, ethanol and water. The extracts were thus screened for the presence of the prominent secondary metabolites which display characteristic bioactivities. Comparatively, the methanol and ethanol extracts displayed most of the metabolites including alkaloids, flavonoids, tannins, phenols, glycosides and steroids. Moreover, the antimicrobial activities of the extracts were tested against *Staphylococcus aureus, Escherichia coli* and *Candida albicans* using agar well diffusion method.

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The ethanolic extracts of *R. nervosus* leaves showed the highest activity (18.00 mm) against *S. aureus* and the water extracts gave the lowest zone of inhibition (6.00 mm) against *E. coli*. In all the extracts, the growths of the two bacterial and fungus strains were inhibited by the methanol and ethanol extracts. The best antifungal activity was observed in the methanol extract of the seeds of *L. sativium* against *Candida albicans* (20.00 mm). The results revealed that, the ethanol and methanol extracts of both plants were the two best extractive solvents with potential inhibitory activity against microbial growth. The observed activities were related to the presence of the noticeable phytochemicals in those plants. Therefore, this finding strongly supports the claim of the local community to use *L. sativium* and *R. nervosus* for the treatment of different pathogenic bacterial infections associated to eye diseases. However, the traditional aqueous extraction practice should be modified by addition of alcohol in order to maximize the extraction efficiency and thus the bioactivity of the plants.

Keywords: Lepidium sativium; Rumex nervosus; phytochemicals; antimicrobial; eye disease.

1. INTRODUCTION

Infectious diseases remain the most common cause of several illness and death worldwide, the causative agents of infectious include bacteria, viruses, fungi and parasites [1]. Medicinal plants have source for the invention of novel drugs and 25 % of modern drugs contain one or more active principles of plant origin [2]. According to WHO, medicinal plants would be the best source to obtain a variety of drugs. Therefore, medicinal plants should be investigated to better understand their properties, safety and efficacy [3]. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, phenolic compounds, which are part of essential oils, tannins, terpenoids, alkaloids, and flavonoids. These metabolites have been found *in vitro* to have antimicrobial properties.

Many plants have been evaluated not only for their inherent antimicrobial activity, but also for their action as a resistance-modifying agent. The enhancement of antibiotic activity or the reversal of antibiotic resistance by natural or synthetic nonconventional antibiotics has led to the classification of these compounds as modifiers of antibiotic activity. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms [4]. Herbal molecules are safe and would overcome resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell [5].

Eye disease is a serious problem in Eritrea especially for animals due to the bacterial infection, therefore there is a need to identify the microbes as well as develop cheap and less hazardous drugs from medicinal plant to minimize this bacterial infection. With novel mechanism of action that is less susceptible to resistance by microbes, eye diseases in remote areas of Eritrea are still traditionally cured by medicinal plants. Based on some ethnobotanical information, the treatment of eye disease in Eritrea, especially in the treatment of animals, includes the use of plants such as *Barleria eranthemoides, Lepidium sativium, Cynoglossum lanceolatum, Zehneria scarab, Ocimum hadiense and Rumex nervosus.*

In this report, the seeds of *Lepidium sativium* and leaves of *Rumex nervosus* were selected based on their wider applications and thus the phytochemicals present and antimicrobial potential of the plants were investigated. *Lepidium sativium* belongs to the family Brassicaceae. The common names of the plant are cress, pepper cress, Garden cress and pepper grass and commonly known as Shinfae in Eritrea. The plant is glabrous erect annual, the leaves and seeds contain volatile oils known as cress-oil. The seeds are small, oval-shaped, smooth surfaced, about 2-3 mm long and 1-1.5 mm wide with reddish brown colour. When soaked in water the seed coat swells and gets covered with transparent, colorless, mucilage [6]. Similarly, *Rumex nervosus* is locally known as Hihot and belongs to Polygonaceae family. It is a 3 m high shrub with few or many straight spines, branch lets with soft or stiff hairs. In Eritrea, it is found in central and northern highlands as well as Eastern escarpments. Its barks serve as a catalyst in wine making [7]. The genus *Rumex* is found to be distributed worldwide but is mostly Asian. This genus includes more than 250 species and contains a

large number of chemically complex and biologically active compounds [8]. Photos of the flowering plants are shown in Fig. 1.

The seed of *Lepidium sativium* is used to treat backache, tympanitis pain and nervous disorders [6]. The various parts of the plant have been used for the treatment of jaundice, liver problems, diarrhoea, dysentery spleen diseases, gastrointestinal disorders, menstrual problems, fracture, arthritis, and other inflammatory conditions [9,10]. The seeds boiled in milk are used for nursing mothers to increase the secretion of milk and relieving the flatulence [11]. *Lepidium sativum* is largely recommended by traditional herbal healers for diabetes control, hypertension, renal disease and physiotherapy [12,13]. The roots and aerial parts of *Rumex nervosus* have been used traditionally for a variety of therapeutic uses, such as antioxidant, cytotoxic, antifertility, anti-inflammatory, antimicrobial, antidiarrheal and antiviral activities [8]. The juice of *Rumex nervosus* is used in Ethiopia to seizure bleeding during male circumcision [14]. Traditionally in Eritrea, the leaves, stems and sometimes roots of *Rumex nervosus* are used as traditional medicines, for the eye disease, taeniacapitis, haemorrhoids, infected wounds, arthritis, eczema, abscess and gynaecological disorders.

2. MATERIALS AND METHODS

2.1 Plant Collection and Sampling

Seeds of *Lepidium sativium* were purchased from a market in Asmara and the fresh matured leaves of *Rumex nervosus* were collected from Adi-hawisha, Zoba Maekel, Eritrea during the month of February of 2018. The plant species were identified and authenticated by a Botanist in the Department of Biology of EIT, Eritrea. The plants were thoroughly washed in running tap water to remove debris and dust particles and then rinsed using distilled water and then finally air dried for 14 days under shade. The shade dried plant materials were powdered using pestle and mortar and thus kept in polythene bags until further use.

2.2 Preparation of Plant Extracts

The powdered plants (200 g) were extracted sequentially using a soxhlet apparatus with n-hexane (98%), ethanol (90%), methanol (99%) for 2 hrs each at various boiling temperatures and the final residue was extracted with distilled water for 6 hrs. All the extracts were concentrated using Buchi Rota evaporator under reduced pressure and the water bath was fixed at 40°C. The crude extracts obtained were stored in sterile capped reagent bottles and refrigerated at 4°C until further use. From the extracts, 0.5 g sample was reconstituted in the respective solvents (in 20 mL) to get a final concentration 25 mg/mL and thus was used for the phytochemical screening. Moreover, each crude extract (0.25 g) was dissolved in 1 mL DMSO to give 250 mg/mL solution for antimicrobial assay [15].

2.3 Phytochemical Screening

The crude extracts of the two plants were tested for the presence different phytochemicals based on previously reported methods [16,17]. The common types of reactions including Salkowski test for terpenoids and steroids, alkaline reagent test for flavonoids, ferric chloride test for phenols, Wagner's reagent for alkaloids, foam test for saponins, Keller-Killian's test for glycosides and lead acetate test for tannins were employed.

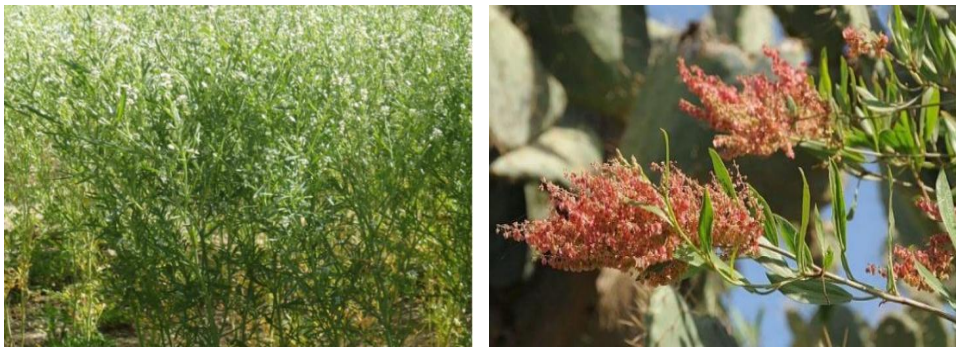


Fig. 1. *Lepidium sativium* (left) and *Rumex nervosus* (right)

2.4 Antimicrobial Screening

2.4.1 Chemicals and materials

The materials and chemicals used include McFarland solution, saline water, incubator, safety cabinet, an autoclave for sterilizing, Mueller-Hinton agar, Saboradi agar, 6 mm diameter well borer, sterile micro syringe, and DMSO.

2.4.2 Media preparation

The mixture of Mueller-Hinton agar powder and sterile distilled water were stirred with a sterilized glass rod and covered with a cotton wool, over which an aluminium foil was tightly wrapped and then autoclaved for 15 min at 121^{o}C. Soon after autoclaving, the agar was allowed to cool and placed inside a water bath at about 50^{o}C to maintain the media in a molten stage in order to minimize the amount of condensation that forms. Then, the agar medium was allowed to cool to room temperature in the laminar flow hood prior to pouring it into the Petri plate. Plates were dried faster in lower humidity by keeping them in a laminar flow hood. The freshly prepared and cooled medium was poured into flat-bottomed petri-dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm [18].

2.4.3 Preparation of bacterial suspension

Using a sterile inoculating loop, one or two colonies of the organism to be tested were taken from the subculture plate. The organism was suspended in 3 mL of physiological saline. The test tube containing the saline was then vortexed to create an overall smooth suspension. McFarland standards are used as reference to adjust the turbidity of any given bacterial suspension. McFarland solution is an essential material needed before testing the microorganisms for their sensitivity. This was done to make sure that the number of bacteria is within a given range to standardize the microbial testing. This would also help avoid any error in result, because if the suspension is too heavy or too diluted, an error might occur for any given antimicrobial agent [19].

2.4.4 Well diffusion method

Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a small volume about 0.1 ml of the bacterial suspension was inoculated onto the dried surface of Mueller-Hinton agar plate and Saboradi agar of the fungal suspension and streaked by the sterile cotton swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60°C each time to ensure an even distribution of inoculum and finally the rim of the agar was swabbed. The lid was left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the crude extracts on the respective well [18].

2.4.5 Test bacterial organisms

Two species of bacteria, *Staphylococcus aureus* (gram positive, ATCC 25923) and *Escherichia coli* (gram negative, ATCC 25922) and *Candida albicans* (fungus, ATCC 10231) were used as indicator microorganisms to detect the anti-microbial activities. The bacterial strains were selected due to their effect associated with eye infections. The analysis was done in the microbiology laboratory, National Health Laboratory, Asmara, Eritrea. All the strains were obtained as actively growing cultures from the microbial culture collection of the microbiology laboratory.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

The phytochemicals including triterpenoids, steroids, flavonoids, phenols, alkaloids, saponins, glycosides, tannins were investigated. As presented in Table 1, most of the phytochemicals were present in large or small amount in most of the extracting solvents. Most of the phytochemicals were present in the ethanol and methanol extracts of both plants. Steroids and glycosides were not present in the hexane extracts of both plants and this could be due to the non-polar nature of the solvent. Alkaloids, flavonoids and phenols which are polar compounds were found in almost the solvents but not appreciably much in the n-hexane extracts. Moreover, tannins and phenols were found in the water extracts of both plants. These results revealed that these plants have quite a number of chemical constituents, which may be responsible for the different pharmacological actions related to those plants and the existing secondary metabolites promote characteristic antimicrobial activities. The results infer that the two plants are rich in potentially bioactive metabolite and thus their use as herbal medicine

by the public can be demonstrated. Moreover, the phytochemicals present in the plants can serve as a valuable source of information and provide appropriate standards to establish a base for identification and elucidation of the different types of bioactive chemicals using advanced spectroscopic methods.

Previously reported phytochemicals found in *L. sativum* revealed that the plant contains triterpenes, alkaloids, flavonoids, tannins and coumarins but notsaponins [9]. The seeds show the presence of volatile essential aromatic oils, carbohydrates, phenolic compounds, flavonoids, alkaloids, proteins, saponins and lipids [4]. It also contains good amount of lignans and antioxidants. The plant is known to contain imidazole, lepidine, β-carotenes, ascorbic acid, linoleic acid, oleic acid, palmitic acid and stearic acid. *L. sativum* contains significant amount of iron, calcium, folic acid, beside vitamins A and C [20]. Moreover, recent studies showed that the leaves of the *R. nervosus* contain palmitoleic acid and palmitic acid [21].

3.2 Antimicrobial Potential of the Crude Extracts

The antimicrobial activity of the extracts was determined by growth inhibition activities of the extracts and thus the diameter of the zone of inhibition exhibited by the extracts against the different microbes was measured. The extracts were reconstituted using DMSO because it doesn't exhibit any activity against the used pathogens. As shown in Table 2, the methanol and ethanol extracts of *L. sativium* exhibited good inhibition activities against all the pathogens. The methanol extract displayed the highest zone of inhibition (20.00 mm) followed by the ethanol extract (16.00 mm) against *C. albicans*. However, the ethanol extract displayed better activity against *S. aureus* and *E. coli,* with zones of inhibition of 15.20 and 13.10 mm respectively, compared to the other extracts. Although the n-hexane extracts displayed a low zone of inhibition (8.20 mm) against both *S. aureus* and *C. albicans* but there was no measurable activity against *E. coli*. Similarly, the aqueous extracts indicated some activities against *E. coli* (10.30 mm) and *S. aureus* (8.20 mm) but no characteristic activity was observed against *C. albicans*. The antibacterial activities of the different extracts were below the activity of the standard (chloramphenicol; 50 µg/mL). As previously reported the petroleum ether extract of seeds of *L. sativium* showed antimicrobial activity against some standard human pathogens. The antimicrobial activity of the water extract was low against *Bacillus subtilis* and *Escherchia coli*. The extract had no effect against *S. aureus* and *C. albicans* [22]. All the extracts had lower activities relative to the standard drug. The antifungal activity of the plant against *Fusarium equisetum, Aspergillus flavus*, and *Alternaria alternate* at 8 mg/ml concentrations in Potato Dextrose Agar showed good results [23]. The phytochemicals, shown in Table 1, including terpenoids, flavonoids, cardiac glycosides and steroids, which were present in different proportions, may have been responsible for the plants antimicrobial potential. Moreover, though there are other reported bioactivities related to *L. sativum*, the type of solvents used, the extraction process and the stains employed were different [13,24,25].

Similarly, as shown in Table 3, the ethanol extract of *Rumex nervosus* was found to have the highest activity against *S. aureus* and *C. albicans* with zones of inhibition of 18.00 mm each. The methanol extract also displayed characteristic activities against all pathogens and its activity against *E. coli* was the highest (15.00 mm) compared to the other extracts. The n-hexane and aqueous extracts showed lower activities against *S. aureus* and *E. coli*; there was no observed activity by both extracts against the fungal strain. The different extracts displayed lower activities compared to the standard drug. Comparing the effect of solvent on the bioactivity of both plants showed similar results against all pathogens. In both cases, the ethanol extract showed a better inhibition on bacterial and fungal growth. The extraction was initially done using ethanol followed by methanol and thus some of the active polar metabolites could have been extracted via ethanol and thus the better activity observed can be attributed to the order of extraction. The effect of extracting solvent on the bioactivity of plant extracts was reported by Beata et al. [26]. The results obtained revealed that the tested polar extracts possess more potential antimicrobial activities against *E. coli, S. aureus* and *C. albicans* when tested using Agar well plate method. As indicated in Table 1, the different active metabolites present in the methanol and ethanol extracts were responsible for the observed activities. Fig. 2 displays some representative pictures that show the characteristic zones of inhibitions for the bacterial and fungal strains.

Table 1. Qualitative phytochemical analysis of the crude plant extracts

Medicinal plant	Solvent	Phytochemicals							
		Alkaloids	Terpenoids	Steroids	Flavonoids	Tannins	Saponins	Phenols	Glycosides
Lepidium sativium	n-hexane	+	-	-	+	-	+	-	-
	Methanol	+	+	+	+	+	+	+	+
	Ethanol	+	+	+	+	+	+	+	+
	Water	+	+	+	+	-	+	+	-
Rumex nervosus	n-hexane	+	+	-	-	-	-	+	-
	Methanol	+	+	+	+	+	+	+	+
	Ethanol	+	-	+	+	+	+	+	+
	Water	-	+	-	+	+	-	+	+

Key: '+'- Present, '-' Absent of the particular phytochemical

Table 2. Zone of inhibition of the solvent and aqueous extracts (250 mg/mL) of *L. sativium*

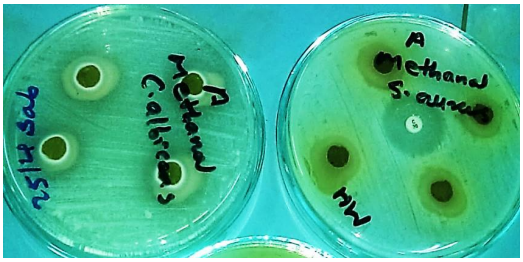
Microorganisms	Zone of inhibition (in mm)				
	Stand.	n-Hexane	Ethanol	Methanol	Water
E. coli	24.00 ± 0.50	-	13.10 ± 0.21	8.00 ± 0.10	10.30 ± 0.00
S. aureus	19.90 ± 0.21	8.20 ± 0.12	15.20 ± 0.32	10.20 ± 0.05	8.20 ± 0.05
C. albicans	*	8.00 ± 0.15	16.00 ± 0.24	20.00 ± 0.43	-

NB: Stand. = Chloramphenicol '' The Standard was not available '-' No observed inhibition*
Results presented as mean ± SD (triplicate)

Table 3. Zone of Inhibition of the solvent and aqueous extracts (250 mg/mL) of *R. nervosus*

Microorganisms	Zone of inhibition (in mm)				
	Stand.	Methanol	n-Hexane	Ethanol	Water
E. coli	24.20 ± 0.60	15.00 ± 0.33	8.00 ± 0.10	10.10 ± 0.13	6.00 ± 0.15
S. aureus	20.40 ± 0.32	16.30 ± 0.25	10.20 ± 0.22	18.00 ± 0.41	10.30 ± 0.00
C. albicans	*	15.10 ± 0.22	-	18.00 ± 0.50	-

NB: Stand. = Chloramphenicol '' The Standard was not available '-' No observed inhibition*
Results presented as mean ± SD (triplicate)



(A) **(B)**

Fig. 2. Representative antimicrobial results of the methanol extract of *R. nervosus* against *C. albicans* (A) and *S. aureus* (B)

4. CONCLUSION

The phytochemical analysis showed the presence of alkaloids, flavonoids, tannins, saponins and others in most of the extracts; this indicates that the extracts contain some of the prominent secondary metabolites. The cognisance of the chemical constituents in a plant helps in understanding the value of folkloric medications and discloses the wrapped areas of therapeutics. The different solvents exhibited a wide range of antimicrobial activities and based on the results observed the ethanol and methanol extracts were the two best extractive solvents for antimicrobial activities of the seeds of *L. sativium* and leaves of *R. nervosus*. Further antimicrobial studies with additional organisms might be relevant in order to give a better picture on the activity of the plants. Therefore, the results establish a good support of the use of *R. nervosus* and *L. sativium* in traditional medicines especially for the people living in remote areas as an alternative treatment for eye diseases.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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