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Evaluation of Antimicrobial Activities of *Sinapis alba and Brassica nigra* Leaves Against Selected Microorganisms

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Abstract

Many traditional practitioners in developing countries use the herbaceous plant to treat a different type of microbial infection. Eritrea is one of the developing countries where most of their communities are dependent on herbal medicines for the treatment of infectious disease. However, this malpractice follows incorrect dosage, administration, formulation, frequency and other non-scientific methods with the inevitable negative effect of the practice which makes it inconvenient for the clients who seek treatment. Therefore the current study was carried out to get a scientific evidence of antimicrobial activity of two selected important herbal plants. Active part from leaves of *Sinapis alba* and *Brassica nigra* were extracted by continuous hot extraction (Soxhlet technique), and different concentrations were obtained by ethanol, N-hexane, aqueous and DMSO solvents. Microorganisms (*Escherichia* Coli and *Staphylococcus* aureus from bacterial strains and *Candida Albicans* from fungal strain) were selected for testing antimicrobial activity of the plants. Then the extracted solutions were diffused to selected standard organisms inoculated in Muller Hinton Agar using well diffusion technique. Ethanol extracts of *S. Alba* of 2500mg/ml dissolved in DMSO concentration against *E. coli* have shown a significant activity with inhibition zones of 30mm. This plant in the same concentration also had a considerable effect against *S. aureus* and *C. Albcaians* with a prompting result of 28mm and 25mm zones of inhibition respectively which is greater than the positive control. Moreover, this plant showed almost an equal activity at 1000mg and 250mg which are 20mm and 13mm respectively for *C. Albicans*, 26mm and 23mm for *S. aureus* and for *E. coli* 25mm and17mm. N-hexane extracts of the same plant also showed a remarkable activity at concentrations of 1000mg, 250mg and 50mg, where the zones of inhibition against *S. aureus* were 18mm, 20mm and 25mm respectively. Ethanol-extract of this plant diluted in ethanol also showed activity at the lowest concentration. Generally, both plants extracted using N-hexane and Ethanol extracts gave a remarkable activity against all the selected micro-organisms.

1 Introduction

Eritrea is blessed with marine and terrestrial biodiversity and its accession to the Convention on Biological Diversity on 21 March 1996 is evidence to its government's desire to preserve and develop its biodiversity. Moreover, according to different researches launch on their scientific evidence, many of plant leaves, seeds, and even roots contain medically important compounds. In developing countries like Eritrea, most of their communities are dependent on traditional healers; consequently, the numbers of traditional practitioners are much more dominant over professional medical doctors[1]. And they do believe that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics[2]. Traditional

healers widely use natural herbs for treatment of bacterial, fungal and parasitic infections, but most of them don't fulfill the scientific requirements[3]. Hence the superstition believes of the community is not completely eradicated in addition to the above, living condition of the communities, shortage of supply of clean water and lack of awareness on hand washing practices, the prevalence of the disease is high in Eritrea. Though there is no research based data available here in Eritrea regarding the antimicrobial resistance, WHO and other different pharmacovigiliance center reports showed, that inappropriate use of antibiotics possess a significant increase in antibiotic resistance and sever adverse effect globally[4].Micro-organisms like *Escherichia coli* are normally inhabitant of the human gastrointestinal tract and are among bacterial species most frequently isolated from stool culture and consist of a diverse group of bacteria. Pathogenic *E. coli* strains are associated with diarrhea are referred to as diarrheagenic, can be transmitted through contaminated water or food, and directly contact with infected one[5]. *Staphylococcus aureus* is a leading cause of community-acquired and healthcare association bacteremia, Methicillin-resistant *S. aureus* (MRSA) is one of the most important antibiotic resistant bacteria in the United States[6].*Candida. albicans* is the most frequently encountered pathogenic human fungal species and commonly colonizes host mucosal and moist skin surfaces this opportunistic microbe can rapidly transition from commensal to pathogen, causing an array of infections ranging from localized mucosal to severe systemic infections with high morbidity and mortality rates[7] .*Sinapis alba* and *B. nigar* family of *Brassicaceae or Cruciferae* grow well in hot and dry environments widely distributed around the Mediterranean region, where it was most likely originated[8]. Many countries, traditionally, have been applying those plants in cases of digestive complaints. An infusion of the seeds is useful in the treatment of chronic bronchitis, rheumatism, muscular and skeletal pains; it stimulates circulation in the pain area[9]. It is also believed that these herbal plants have a role in reduction of the risk of chronic diseases, including cardiovascular diseases and cancer[10]. These two edible vegetables which provide nutrients and health-promoting phytochemicals such as vitamins, fiber, soluble sugars, minerals, glucosinolates and also due to the presence of phenolic compounds, these brassica species are rich in carotenoids, tocopherols, ascorbic acid and anti-oxidative property[11]. According to some recent researches, it was reported that seeds of essential oil of *S. alba* and *B. nigra* (i,e mustard family) has a good result against different clinically significant bacteria[12]. The chief objective of this study was to determine the possible anti-bacterial and anti-fungal activity of *S. alba* and *B. nigra* by applying hot continuous extraction methods at different concentrations.

2 Materials and methods

2.1 Plant material

Leaves of *S. alba* and *B. nigra* were collected during late summer 2018 from highlands of Eritrea and confirmed by botanist.

2.2 Preparation of extracts

Leaves of both *S. alba* and *B. nigra* were changed into powder after carefully washed by tap water and dried in clean conditions, then 25gm powder of each leaf were placed at the thimble, which is used for filtering and located at the chamber of Soxhlet apparatus, and flask which contains the extracting solvent, these were absolute ethanol, N-hexane and distilled water. And they were boiled till they reach their boiling point, at around 78°C, 63°C and 100°C respectively and the vapors produced condensed in to condenser and the crude extract obtained is stored for further solidification process by rotary evaporator under vacuum pressure and heat to produce a thick concentrated extract, the final extracts were also dried by placing in to water bath. The dried extracts produced by both Soxhlet and rota-vapor are then dissolved or changed in to known concentration of 2500mg/ml, 1000mg/ml, 250mg/ml, 50mg/ml by Ethanol, N-hexane, distilled water and Dimethyl Sulfoxide (DMSO). Finally, a total of forty-eight (48) different concentrations were obtained from the extracts and ready for *in vitro antimicrobial test.*

2.3 Micro-organisms and Media

Clinically significant standard organisms were used, *E. Coli* (ATCC 25922), *Candida albicans* (ATCC 10231), and *Staphylococcus aureus* (ATCC 25923) were used and inoculated in their respective media MacConkey for *E. coli*, Saboraud dextrose agar for *C. albicans* and Mannitol salt agar for *S. aureus*. Muller Hinton Agar was used for the well diffusion antimicrobial testing of all the organisms and extracts. Muller Hinton agar with 4mm depth and 6mm diameter wells made by cork borer, the suspension of microorganisms were prepared against McFarland standard turbidity and the discs for the positive control containing known concentration were used for antimicrobial susceptibility test. Therefore using disc diffusion method, the positive control discs were diffused in a Muller Hinton Agar media inoculated with above mentioned organisms. And for extracted plants well diffusion method was used, where the extracts were placed in the wells made by 6mm disc borer on the surface of Muller Hinton Agar and which were inoculated with the above mentioned standard organisms and incubated at 37ºC for 24 hours. The diameter of inhibition zones was measured in millimeter (mm) the next day and the results were recorded.

2.4 Reagents

Absolute ethanol, N-hexane, distilled water and Dimthyl Sulfoxide (DMSO) were used.

2.5 Quality control

To check the quality of the media used in this study, American Type Culture Collection (ATCC) standard organisms were used. And as positive control two antimicrobial discs of Chloramphenicol and Ciprofloxacin were used and tested against *S. aureus* and *E. coli,* Fluconazole against *C. albicans* and the inhibition zones were within accepted zones of inhibition. All solvents used for extraction without the extracts were also used as a negative control.

3 Result

Both in *S. aureus* and *E. coli*, the inhibition zones with diameter less than 12mm were considered as having no antimicrobial activity, diameters between 12 and 16mm were considered moderately active and these with 16 mm were considered highly active. Where as in *C. albicans*, inhibition zones with diameter less than 14mm were considered resistant, diameters between 15 and 18mm were considered moderately active and 19mm and above were considered highly active[13,14]. Results for the antimicrobial activity of the plants are summarized in **tables 1, 2 and 3,** against clinically significant microorganisms of *E. coli*, *S. aureus* and *C. albicans*, respectively in a solvent of N-hexane, absolute ethanol and distilled water.

Table 1: Antimicrobial activity of *Sinapis alba* and *Brassica nigra* leaves against *E. coli*

Extracted plant	Concentration of extraction in (mg/ml)			
	2500	1000	250	50
S. Alba-N-Hexane DMSO	R	R	R	R
B. Nigra Ethanol DMSO	R	R	R	R
B. Nigra, N-Hexane-DMSO	R	R	R	R
S. Alba Ethanol, DMSO	30	25	17	R
B. Nigra Ethanol, Ethanol	26	20	18	12
B. Nigra Hexane Hexane	R	R	R	R
S. Alba Ethanol, Ethanol	R	R	R	R
S. Alba, N-Hexane, N-Hexane	R	R	R	R
S. Alba, H_2O, DMSO	R	R	R	R
B. Nigra, H_2O DMSO	R	R	R	R
S. Alba, H_2O, H_2O	R	R	R	R
Nigra, H_2O, H_2O	R	R	R	R

(R); indicated for Resistance; which is less than 12 mm zone of inhibition against *E. coli*

As the above tables stated, Ethanol extracts of *S. alba* in 2500mg/ml of DMSO concentration against *E. coli* has shown significant activity with inhibition zones of 30mm.

Table 2: Antimicrobial activities of *Sinapis alba* and *Brassica nigra* leaves against *S. aureus.*

Extracted plant	Concentration of extraction in (mg/ml)			
	2500	1000	250	50
S. Alba-N-Hexane DMSO	R	18	20	25
B. Nigra Ethanol DMSO	R	R	R	R
B. Nigra, N-Hexane-DMSO	R	R	R	R
S. Alba Ethanol, DMSO	28	26	23	17
B. Nigra Ethanol, Ethanol	R	R	R	R
B. Nigra Hexane Hexane	R	R	R	R
S. Alba Ethanol, Ethanol	R	R	R	R
S. Alba, N-Hexane, N-Hexane	R	R	R	R
S. Alba, H_2O, DMSO	R	R	R	R
B. Nigra, H_2O DMSO	R	R	R	R
S. Alba, H_2O, H_2O	R	R	R	R
Nigra, H_2O, H_2O	R	R	R	R

(R); indicated for Resistance; which is less than 12 mm zone of inhibition against *S. aureus*

Table 3: Antimicrobial activities of *Sinapis alba* and *Brassica nigra* leaves against *C. Albicans*.

Extracted plant	Concentration of extraction in (mg/ml)			
	2500	1000	250	50
S. Alba-N-Hexane DMSO	R	R	R	R
B. Nigra Ethanol DMSO	R	R	R	R
B. Nigra, N-Hexane-DMSO	R	R	R	R
S. Alba Ethanol, DMSO	25	20	13	6
B. Nigra Ethanol, Ethanol	12	12	20	18
B. Nigra Hexane Hexane	17	16	6	6
S. Alba Ethanol, Ethanol	R	R	16	18
S. Alba, N-Hexane, N-Hexane	R	R	R	R
S. Alba, H_2O, DMSO	R	R	R	R
B. Nigra, H_2O DMSO	R	R	R	R
S. Alba, H_2O, H_2O	R	R	R	R
Nigra, H_2O, H_2O	R	R	R	R

(R); indicated for Resistance; which is less than 14 mm zone of inhibition against *C. Albicans*

This plant in the same concentration also had a remarkable effect against *S. aureus* and *C. albicans* with a prompting result of 28mm and 25mm zones of inhibition respectively which is greater than the positive controls (Fig.1). And it showed similar activity at 1000mg and 250mg which are 20mm and 13mm respectively for *C. albicans*, 26mm and 23mm for *S. aureus* and for *E. coli* 25mm and17mm. N-hexane extracts of the same plant also showed a considerable activities at concentrations of 1000mg, 250mg and 50mg, where the zones of inhibition against *S. aureus* were 18mm, 20mm and 25mm respectively. Ethanol-extract of this plant, *S. alba* diluted in ethanol also showed activity at its lowest concentration against *C. albicans* (Table 3 and Fig. 2).

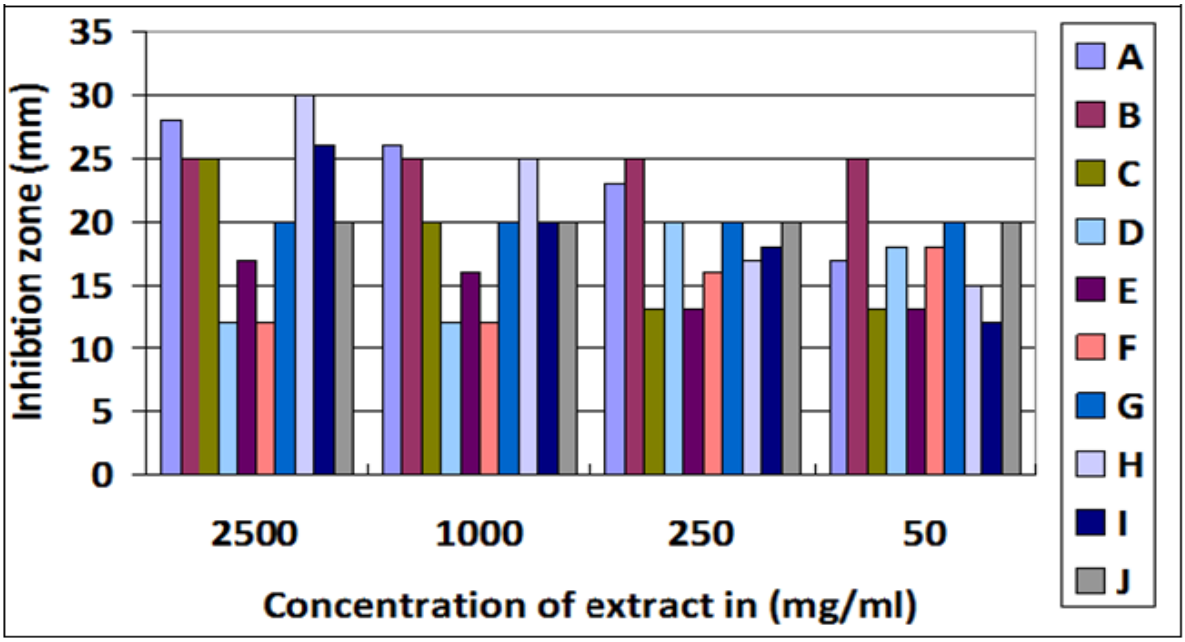

Fig 1: Antimicrobial activity of S. alba and B.nigra against microorganisms and control; *A - S.alb-Eth-DMSO-S.aureus;B-ChloramphenicalS.aures;C-S.alb-Eth-DMSO-C.albicans;D-B.nigra-Eth-Eth C.albicans; E- B.nigra-Hex-HexC.albicans; F-S.alba-Eth-Eth-C.albicans;G - Fluconazol C.albicans;H- S.alba-Eth-DMSO E.Coli;I - B.nigra-Eth-Eth E.coli;J - Chloramphenical E. coli*

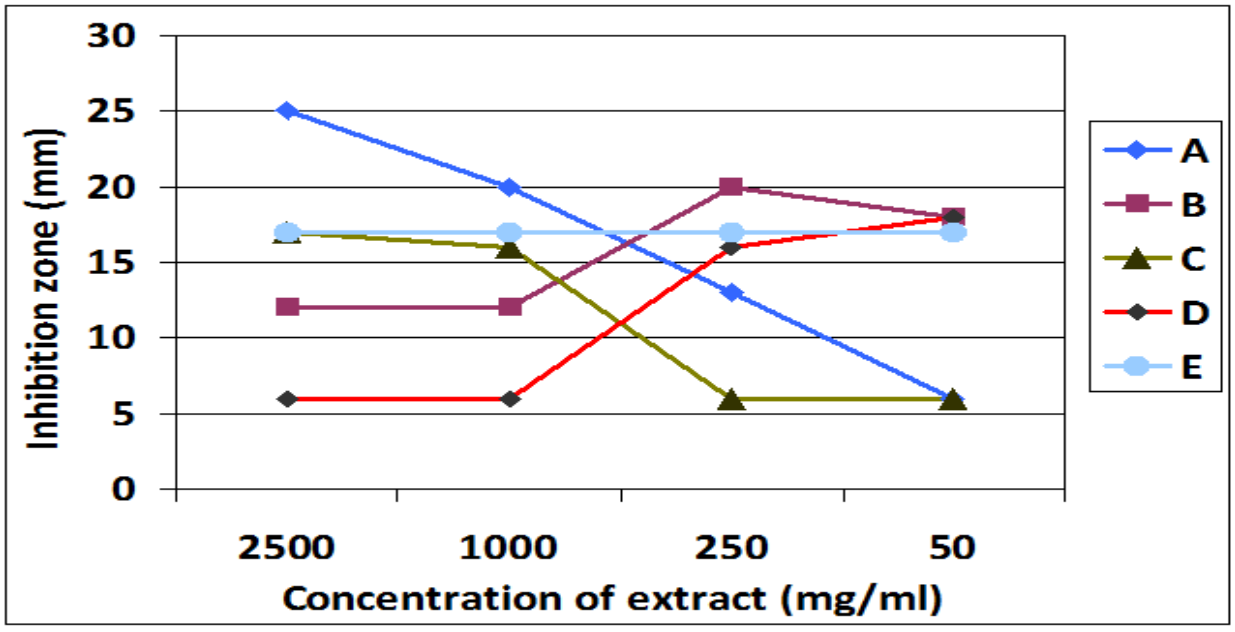

Fig 2: Antimicrobial activity of S.alba and B.nigra against C. albicans; A-S.alba-Eth-DMSO-C.albicans; B-B.nigra-Eth-Eth C.albicans; C-B.nigra-Hex-Hex C.albicans; D-S.alba-Eth- Eth C.albicans; E-Control

Ethanol-extract of *B. nigra* dissolved in ethanol showed an activity against *E. coli* at high concentration and *C. albicans* at low concentration whereas against *S. aureus*, it didn't show any activity at all concentrations (Tables 1 and 3). The same plant extracted by N-hexane showed activity only against *C. albicans* in the first two highest concentrations, whereas aqueous solvent didn't show any activity against all organisms at any concentration.

4 Discussion

This study is the first antimicrobial activity assessment done in Eritrea for the plants *S.alba* and *B.nigra*. The 25gms of each plant extracted by hot extraction method and the yields obtained as stated in the results, their antimicrobial activities against *E. coli* (Table 1), both plants showed activities in the same extraction solvent, ethanol, but dissolved in different solvents. Ethanol extracts of *S.alba* dissolved in DMSO at 2500mg/ml showed greater inhibition zone of 30mm than ethanol extracts of *B.nigra* dissolved in ethanol at the same concentration. However, in a study done by Valentina B. et al. *S. nigra* extracts showed stronger antimicrobial activity than *S. alba*, except against *E. coli*[15] . These ethanol extracts were the only ones that showed activity against *E.coli*, DMSO dissolved ethanol extracts of *S. alba* exhibited activities at the first three concentrations used while the fourth least concentrated (50mg/ml) didn't show any activity. Ethanol dissolved ethanol extracts of *B.nigra* exhibited activity at all the concentrations used.

For activities against *S.aureus* (Table 2), only *S.alba* showed activity. N-hexane extracts of *S.alba* dissolved in DMSO exhibited stronger inhibition at lower concentration than in the higher concentrations while ethanol extracts of the same plant dissolved in DMSO showed stronger activity in its highest concentrations (28mm inhibition zone at 2500mg/mL) than lowest concentration (17mm inhibition zone at 50 mg/mL). On a study done in Italy, disc diffusion tests on mustard plant has shown antimicrobial properties against *S.aureus* (~19 mm inhibition zone at 10 mg/mL), while they were lower against *E. coli* (12 versus 20 mm)[15]. For activities against *C.albicans* (Table 3), both plants extracted by ethanol dissolved in ethanol had similar activities, where stronger activities were exhibited with decreasing of the concentration. While *S.alba* extracted by ethanol dissolved in DMSO showed activity at the highest concentration and it decreased activity at lower concentrations with no activity at the lowest concentration. Whereas N-hexane extracts of *B.nigra* dissolved in N-hexane exhibited strong inhibition at the two high concentrations (2500mg/ml=17mm and 1000mg/ml=16mm) and no activity at the two low concentrations. Similarly a study done in Jordan by Reema Al Hussaini, flower and leaf extracts of the plants *P. alba* and *P.nigra* against *C. albicans* showed good activity[16,17]. In Fig 1, all the major activities observed are summarized and compared with control antimicrobial drugs. And we can see that *S.alba-eth-DMSO-S.aureus* showed more activity than control used for *S.aureus* which was chloramphenicole. It was also observed that *S.alba-eth-DMSO-C.albicans* in its first three concentrations showed stronger inhibition zones than the control used against *C.albicans*, which was fluconazole. The yeast *Candida albicans* was one of the microorganisms employed in this study and as we can see from Fig 2, the plants' activity against this microorganism was not uniform for *S.alba-eth-DMSO* as concentration decreased the activity also decreased while for *S.alba-ETH-ETH* as concentration of the extract decreased the zone of inhibition increased.

5 Conclusion

Ethanol extracts of the tested medicinal plants exhibited varying degrees of antibacterial activities against all used microorganisms. Strongest activity was observed in ethanol extracts of the plant *S. alba* dissolved in DMSO. Comparing the two plants, *S.alba* showed more antimicrobial activity than *B.nigra* in different concentrations and different solvents. The results obtained in this study validate the use of these plants in the medicinal and pharmaceutical activities in Eritrea.

6 Recommendation

The authors recommend those plants to be tested for anti-fungal activity, particularly against *Tinea versicolor or Pityriasis versicolor.*

7 Acknowledgments

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8 Conflicts of Interest

The authors declare that no conflicts of interest regarding this paper exist.

9 Authors' Contributions

All authors contributed equally on collecting plant, entire extraction procedure, media preparation and reading the results, literature reviewing and drafting the manuscript. All authors read and approved the final manuscript.

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