

Antimicrobial Activity of Three Solvents Fraction of Three Sudanese Medicinal Plants

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ABSTRACT

The Plants under study were extracted by different solvents with increasing in polarity, petroleum ether, ethyl acetate and methanol extracts of some medicinal plants (*Glinus lotodios* (seeds), *Eclipta alba* (whole plant) and *Ethulia conyzoides* (Aerial parts) commonly used to treat a variety of ailments. And investigated for their antibacterial activity against four standard bacterial strains *Bacillus subtilis* (NCTC 8236), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and two standard fungal strains *Aspergilliu niger* (ATCC 9763) and *Candida albicans* (ATCC7596) in vitro. The plants extracts at a concentration of 100 mg/ml were applied using the agar plate well- diffusion method. the ethyl acetate and methanol extracts obtained from *Ethulia conyzoides* give more crude extract were showed high activity against all tested organisms, while the methanol extract of *Glinus lotodios* showed significant of all tested bacterial only and have no sensitivity towards fungal strains. The least antimicrobially active plant was *Ethulia conyzoides*. *Ethulia conyzoides* have more crude extract of methanol (9.81) and showed broader spectrum towards *Bacillus subtilis* (I Z = 32mm). The methanol extract of *Ethulia conyzoides* suitable candidates for the development of novel antibacterial compounds.

Key words: medicinal plants – *Ethulia conyzoides* - methanol - antimicrobil - *Bacillus subtilis*

INTRODUCTION

The increasing problems of antibiotic drug resistance by pathogenic organisms in the past few decades and recently have led to the continuous exploration of natural plant products for new antibiotic agents^[1-4]. Many of these products are produced in plants as secondary metabolites and often used in plants for defense against microbial attack^[4].

Medicinal plants are a source of great economic value all over the world, and represent a rich source of antimicrobial agents; traditionally, crude plant extracts are used as herbal medicine for the treatment of human infectious diseases^[5,6].

Preparation and administration of plants drugs should be done by experts only, Therefore, an extensive study is required to detect the medical properties of the plant. Several medicinal plants have been tried against pathogenic microorganisms^[7,8].

plants are the main medicinal source to treat infectious diseases. The present study were screened for their antimicrobial activity of different extracts from three plant species against four standard bacteria, two gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and two gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and against two fungi (*Candida albicans* and *Aspergillus niger*), which have been exploited in traditional medicine for the treatment of various ailments^[9,10].

The bacterial agents including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Proteus vulgaris* cause several human infections ^[9,10].

The plant has been used as an anti-inflammatory agent in wound healing, anti-anxiety, anti-stress, anti-mutagenic, and spasmolytic agent and spasmolytic activities ^[11].

Plant products have been part of phytomedicines since time immemorial. These can be derived from any part of the plant like bark, leaves, flowers, seeds ^[12], i.e., any part of the plant may contain active components like, alkaloids, flavanoids, glucosides, tannins, gums, resins, essential oils, fatty oils, carbon compounds, hydrogen, oxygen, nitrogen salts of some chemicals and others few of these chemicals are toxic with residual effects. Hence, preparation and administration of plants drugs should be done by experts only.

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MATERIALS AND METHODS

Plant materials and extraction

Plants screened were listed in Table (1) with their botanical /vernacular names families and chemical constituents. The three different medicinal whole plants namely: (*Glinus lotodios*, *Ethulia conyzoides* and *Eclipta alba*) were collected from West White Nile (Khartoum State). The plants were identified in the Botany Department, Om Drman Islamic University. Each plant was spread and then air dried on sterile blotter under shade.

Table(1)

Botanical and Vernacular Names	Family	Part used	Chemical constituents
<i>Glinuslotoides</i> / Raba	Aizoaceae	Seeds	Triterpenoids, saponinglycosides, Flavonoids . oleanane glycosides, ^[13] .
<i>Eclipta alba</i> / Annual herb	Asteraceae	Whole Plant	apigenin, luteolin, amyrin etc. antifungal, Alkaloid, ^[14] .
<i>Ethuliaconyzoides</i> /Um-riehana	Asteraceae	Aerial parts	Anthelmintic coumarin, anticoagulant, ^[15]

Solvent Extraction

The three different medicinal plants were shade dried and pulverized. 30 g of powdered material was packed in Soxhlet apparatus and subjected to continuous percolation for five hours using 600 cm³ of petroleum ether, ethyl acetate and methanol (80%) as solvents. All extract were filtered through Whatmann filter paper No. 1, and concentrated under vacuum and dried in a dessicator. The extracts obtained and stored in refrigerator and were dissociated in dimethyl sulfoxide for prior to use.

Antimicrobial activity

All extracts of four different plants were tested by disc diffusion method ^[13].

A test stock concentration of 10 mg/ml for methanol : H₂O (80:20) extracts were prepared by dissolving 0.1 g of each extract in 10 ml of methanol in separate test tubes. The antimicrobial activities of each extract were tested against standard Gram positive bacteria (*Bacillus subtilis* National Culture Type Collection NCTC 8236) *Staphylococcus aureus* American Type Culture Collection ATCC 25923, Gram negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* (ATCC 27853)) and fungi (*Aspergillus niger* ATCC 9763 and *Candida albicans* ATCC 7596) using agar well diffusion method and the resultant inhibition zones were measured and tabulated as means. The zones were measured with a transparent ruler and the result recorded in millimeters. The screening was done in triplicates. Negative controls involving the addition methanol instead of the extracts were included.

Antimicrobial Activity

The antimicrobial activity was determined by the agar well diffusion method against different strains of bacteria. Each test bacterium was spread onto sterile Muller-Hinton Agar (Hi-Media). A 6 mm diameter well was cut from the agar using a sterile cork-borer; subsequently each well was filled with 0.1 ml of the plant extract. Sterile dimethyl sulfoxide (DMSO) served as negative.

Bacterial organisms:

The standard organisms were obtained from the national collection of type culture (NCTC), Colindale, England and American type culture collection (ATCC), Rock Ville, Land, USA. Table (2).

Table (2)

Bacteria used	Code No.
<i>Bacillus subtilis</i>	NCTC 8236
<i>Staphylococcus aureus</i>	ATCC 25923
<i>Escherichia coli</i>	ATCC 25922
<i>Pseudomonas aeruginosa</i>	ATCC 27853

Antifungal Activity

The fungal suspensions cultures were maintained on sabouraud dextrose agar, incubated at 25°C for 4 days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

Fungal organisms

The source of fungi was the American type culture collection (ATCC), Rockville, Maryland, USA (Table 3).

Table (3)

Fungi used	Code No
<i>Aspergillus niger</i>	ATCC 9763
<i>Candida albicans</i>	ATCC 7596

RESULTS AND DISCUSSION

The antibacterial properties of the petroleum ether, ethyl acetate and methanol extracts of three medicinal plants (*Glinus lotodios*, *Eclipta alba* and *Ethulia conyzoides*) at concentration 100 mg /ml were tested

against four standard bacterial strains (*Staphylococcus aureus*(ATCC 25923), *Bacillus subtilis*(NCTC 8236), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa*(ATCC 27853) , and against two standard fungal strains *Aspergillus niger* (ATCC 9763) and *Candida albicans* (ATCC7596). The results of the antimicrobial activities are presented as per their measurement of zone of inhibition in millimeter (Table 4). The results showed that methanol was the best solvent for extracting antibacterial substances of *Ethulia conyzoides* extract while ethyl acetate was the best solvent for extracting antifungal substances from *Glinus lotodios*.

The results of the antimicrobial activities are presented as per their measurement of zone of inhibition in millimeter. This resulting was depended on the number of pathogenic microorganisms inhibited and the diameter of inhibitory zones produced, it was also observed that the Gram positive bacteria *Bacillus subtilis* was the most sensitive microorganism inhibited by all extracts of plants except *Eclipta alba*. All extracts have very weak or no

growth inhibition was observed against fungal strains except ethyl acetate and methanol extracts of *Ethulia conyzoides* shows high inhibition zone against **Aspergillus niger**(18 mm)and *Candida albicans*(22 mm) Table (4) . Furthermore, the petroleum ether extracts have no inhibitory activity against all tested organism . *Ethulia conyzoides* showed high antibacterial activity against *Bacillus subtilis* (IZ =35mm) with methanol extract , also ethyl acetate extract of *Glinuslotodios*.

was found effective against all tested + ve and - ve , bacteria *B.subtilis*, *S.aureus*,*psaeruginosa* and *E. coli* with inhibition zoens (IZ= 20 , 21 18 , 18 mm) respectively.

As can be seen from the results, methanol extract of is the most fastidious species against bacteria. All species of plants included in the present study were also found to be active on at least one of the selected microbial strains.

The antibacterial activity profile of *Ethuliaconyzoides* extract against all the tested organisms strains indicated that all organisms were susceptible. with justification, Gram- positive bacteria are frequently reported to have developed multi-drug resistance to many of the antibiotics currently available in the market. extracts.

The reason for the difference in sensitivity between microbial strains might be ascribed to the differences in morphological constitutions between these microorganisms, Therefore, the cell wall of Gram-negative organisms which are more complex than the Gram-positive ones act as a diffusional barrier and making them less susceptible to the antimicrobial agents than are Gram-positive^[16, 17].

The search for substances with high antibacterial properties has been one of the most intensive researches of this time . It is known that plant produce certain chemicals which are naturally toxic to bacteria but not to humans . Extracts of various Sudanese medicinal plants have been reported to possess antibacterial activity^[18 - 21]. It is an established fact that intensive use of antibiotics is often followed by the development of resistant strains . Because of this drug resistance , the search for new antibiotics continues unabated.

The activity of the plant extracts against bacteria is an indication of the presence of broad or narrow spectrum antibiotic compounds or simply metabolic toxins in the plant^[22].

plants used in this study different extracts were different in their antimicrobial efficacy depending on the extractive solvent used.

CONCLUSION

It is concluded that this study would lead to the establishment of some valuable compounds that has to be used to formulate new, different and more potent antimicrobial drugs of natural origin. Presently many countries in Africa have policies directed at developing biotechnology capabilities, through funding of projects, training of researchers and creation of specialized research institutes^[16].

The antibacterial screening insured the importance of these plants and the intensive use by the healers as traditional medicines and pointed a good guiding for further research studies in these plant.

Table (4) The result of diameter of the zones of inhibition (IZ)

Name of plant	Part used	Yield%	Solvent used	Test organism used MDIZ (mm)					
				Bacteria				Fungi	
				B.s	S.a	P.s	E.c	Ca	A.s
<i>Glinus lotoides</i>	Whole plant	0.25	Pt	17	12	-	12	-	-
		1.62	E a	20	21	18	18	-	-
		1.88	Me	26	18	18	26	12	-
<i>Eclipta alba</i>	Whole plant	0.24	Pt	15	-	16	15	14	-
		1.55	E a	16	13	12	10	16	12
		2.52	Me	17	14	14	10	14	13
<i>E thulia conyzoides</i>	Whole plant	0.51	Pt	18	-	-	13	-	-
		3.32	E a	29	31	28	22	22	18
		4.81	Me	32	30	26	30	23	15

M.D.I.Z = Mean Diameter of growth Inhibition Zone in mm. Average of 2 replicates ; 0 = No Inhibition Zone

MDIZ >18 Sensitive; 14-17 Intermediate; <14 = Resistant (-) No activity.

Standard fungal :Ca :*Candida albican* , As: *Aspergillu*

Standard bacteria: S.a :*Staphylococcus aureus* , B.s : *Bacillus subtilis* , E.c : *Escherichia coli* , P.a : *Pseudomonas aeruginosa*

Solevent: pt: petreolumether , E a : Ethyl acetate , Me : methanol

REFERENCES

- 1) G. Ayoola, F. Lawore, T. Adelowotan, I. Aibinu, E. Ade- nipekun, H. Coker and T. Odugbemi, "Chemical Analysis and Antimicrobial Activity of the Essential Oil of *Syzi- gumaromaticum* (clove)," *African Journal of Microbiol- ogy Research*, Vol. 2, 2008, pp. 162-166.

- 2) N. Belmekki, N. Bendimerad, C. Bekhechi and X. Fernandez, "Chemical Analysis and Antimicrobial Activity of *Teucrium polium* L. Essential Oil from Western Algeria," *Journal of Medicinal Plants Research*, Vol. 7, No. 14, 2013, pp. 897-902.
- 3) A. A. Dahpour, P. Rahdari and Z. Sobati, "Chemical Composition of Essential Oil, Antibacterial Activity and Brine Shrimp Lethality of Ethanol Extracts from *Sedum pallidum*," *Journal of Medicinal Plants Research*, Vol. 6, No. 16, 2012, pp. 3105-3109.
- 4) L. Shittu, M. Bankole, T. Ahmed, M. Bankole, R. Shittu, C. Saalu and O. Ashiru, "Antibacterial and Antifungal Activities of Essential Oils of Crude Extracts of Sesame Radiatum against Some Common Pathogenic Micro-Organisms" *Iranian Journal of Pharmacology & Therapeutics (IJPT)*, Vol. 6, No. 2, 2008, pp. 165-170.
- 5) DS Alviano, CS Alviano. Plant extracts: search for new alternatives to treat microbial diseases. *Curr Pharm Biotechnol.* 2009 Jan;10(1):106–121.
- 6) R Zhang, K Eggleston, V Rotimi, RJ Zeckhauser. Antibiotic resistance as a global threat: Evidence from China, Kuwait and the United States. *Global Health.* 2006 Apr 7;2:6.
- 7) H. Haraguchi, S. Kataoka, S. Okamoto, M. Hanafi and K. Shibata, 1999. Antimicrobial triterpenes from *Ilex integra* and the mechanism of antifungal action. *Phytotherapia Res.*, 13: 151-156.
- 8) K.J. Sashi, M. Ramya and K. Janardhan, 2003. Antimicrobial activity of ethnomedicinal plants of Nilgiri Biosphere reserve and Western Ghats. *Asian J. Microbiol. Biotechnol.*, 5: 183-185.
- 9) G. Peirano, Multi resistant enterobacteriaceae new threat to an old prob; expect review of anti infective therapy. *Expert Rev Anti Infect Ther.* 2008;6:657–669.
- 10) Rastogi and Mehrotra (1993). *Index of Indian medicinal plants.* CDR(Luknow). 2:496.
- 11) M. Cheesbrough, *Medical Laboratory Manual for Tropical Countries.* Oxford, United Kingdom: Oxford, Publishers; 1984. Microscopic examination of specimens; pp. 32–33.
- 12) G.M. Cragg, and D.J. Newman, 2001. Natural product drug discovery in the next millennium. *Pharm. Biol.*, 39: 8-17.
- 13) A Endale, B Kammerer, T Gebre-Mariam, PC Schmidt (2005). *J Chromatogr A.* 12; 1083(1-2):32-41
- 14) LC Diogo, Fernandes RS, Marcussi S, Menaldo DL, Roberto PG, Matrangulo PV, Pereira PS, França SC, Juliatti S, Soares AM, Lourenço MV (2009). *Basic Clin Pharmacol Toxicol.* Apr; 104(4):293-9.
- 15) Kadkady, Brimer L, Furu P, Lemmich E, Nielsen HM, Thilborg ST, Thastrup O, Christensen SB (1992). *Planta Med.* Aug;58(4):334-7
- 16) Rastogi and Mehrotra (1993). *Index of Indian medicinal plants.* CDR(Luknow). 2:496.
- 17) Grayer, R.J., Eckert, M.R., Veitch, N.C., Kite G.C., Marin, P.D., Kokubun, T., Simmonds, M.S.I and Poton, A.J. (2003). The chemotaxonomic significance of two bioactive caffeic acid esters, nepetoidins A and B, in the lamiaceae. *Photochemistry* 64 519-52.
- 18) A., Al-magboul A.Z. Omer, M.E.A and Eltohami M.S. (2001) *Sudanese Plants Used in Folkloric Medicine : Screening for Antibacterial Activity. Part X .Fitoterapia.* 72: 810- 817.
- 19) El-egami, A.A.; Almagboul, A.Z.; Omer, M.E.A., ElTohami, M.S. (1998). *Sudanese Plants used in Folkloric Medicine : Screening for Antibacterial Activity .Part X .Fitoterapia.* 69:4,369-373

- 20) Al-kamali , H.H., Ahmed , A.H.,Mohammed, A.S., YahiaA.A.M., Eltayeb I.H. and Ali A.A. (1998). Antibacterial Properties of essential oils from *Nigella sativa* Seeds, *Cymbopogon citrates* leaves and *Pulicariaundulata* aerial parts. *Fitoterapia*.69 : 77-7
- 21) Eisa, M.M. , El Egami , A ., Omer ,M.E.A. and Elegami , A.(2000) . Antimicrobial Activity of *Dichostachyscinerea*. *Fitoterapia* 71: 324-327.
- 22) Parekh, J. and S. Chanda, 2007. *In vitro* antimicrobial activity of *Trapanatans* L. fruit rind extracted in different solvents. *Afr. J. Biotechnol.*, 6: 766-770.