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Antagonistic activity of *Eleutherine palmifolia* Linn

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ABSTRACT

Objective: The aim of the present study was to investigate the phytochemical constituent and the *in vitro* Antimicrobial activity of the wonderful medicinal herb *Eleutherine palmifolia*. **Methods:** *Eleutherine palmifolia* bulb was extracted with ethanol. The extract was evaluated for their phytochemical constituent's and their antimicrobial activity against the multidrug resistant pathogens following the standard protocols. The active drug principle present in the extract was analysed by GC-MS. **Results:** The qualitative phytochemical evaluation of the *Eleutherine palmifolia* bulb revealed the presence of Phenols, Sterols, Phlobatannins, Proteins, Steroids, Tannins and Reducing sugar. The extract showed higher antimicrobial activity in comparison to the standard antibiotics. A promising control over methicillin resistant *Staphylococcus aureus* and *Acinetobacter baumannii* was found. GC-MS analysis of the plant extract showed the presence of Decane, Cyclohexane, (1, 2-dimethylbutyl) and Cyclohexanecarbonyl Chloride etc. **Conclusions:** The present investigation reveals that the plant *Eleutherine palmifolia* bulb may act as potent source of antimicrobial agent against methicillin resistant *Staphylococcus aureus* and *Acinetobacter baumannii*.

1. Introduction

Eleutherine palmifolia is a herb from Iridaceae, a botanical family that comprises of 90 genera and about 1200 species. It is a native of tropical America. It was introduced into the Philippines, and is now naturalized in some parts of the Islands, occurring in abaca plantations and occasionally in waste places. In the Traditional system of Medicine, the macerated bulbs are applied on the stomachs of children to relieve gas pain. A decoction of the bulbs is diuretic. This plant is used by some populations as a vermifuge, for painful and irregular menstruation, as an abortive and antifertility agent [1].

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a bacterium responsible for several difficult-to-treat infections in humans. It is also called multidrug-resistant *Staphylococcus aureus*. MRSA is any strain of *Staphylococcus aureus* that has evolved resistance to beta-lactam antibiotics, which include the penicillins (methicillin, dicloxacillin, nafcillin, oxacillin etc.) and the cephalosporins. Strains unable to resist these antibiotics are classified as methicillin sensitive *Staphylococcus aureus*. The development of such resistance does not cause the organism to be more intrinsically virulent than strains of *Staphylococcus aureus* that have no antibiotic resistance, but resistance does make MRSA infection more difficult to treat with standard types of antibiotics and thus more dangerous. Certain groups of people are at a higher risk of infection with MRSA [2].

Acinetobacter baumannii is a pleomorphic aerobic gram-negative bacillus (similar in appearance to Haemophilus influenzae on Gram stain) commonly isolated from the hospital environment and hospitalized patients. *Acinetobacter baumannii* is a water organism and preferentially colonizes aquatic environments. *Acinetobacter* species have low virulence but are capable of causing infection. *Acinetobacter* infections are uncommon but, when they occur, usually involve organ systems that have a high fluid content (eg, respiratory tract, CSF, peritoneal fluid, urinary tract), manifesting as nosocomial pneumonia, infections associated with continuous ambulatory peritoneal dialysis (CAPD) or catheter-associated bacteria. *Acinetobacter baumannii* is a multiresistant aerobic gram-negative bacillus sensitive to relatively few antibiotics. Multidrug-resistant *Acinetobacter baumannii* is not a new or emerging phenomenon, but *Acinetobacter baumannii* has always been an organism inherently resistant to multiple antibiotics [3].

With the continued emergence of antibiotic resistance and drug resistance pathogenic microbial strains, it is necessary to search for further powerful antimicrobial compounds.

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Perusal of literature reveals that information on the antimicrobial activity and chemical analysis of *Eleutherine palmifolia* is totally lacking. Hence in the present study we report the First hand information on *Eleutherine palmifolia* (L) antimicrobial active, phytoconstituents and the volatile organic matter.

2. Materials and Methods

The fresh *Eleutherine palmifolia* bulbs were collected from Keeripaarai mountain range, Kanyakumari District, Tamil Nadu and were identified by Dr. J.V. Sudakar, Botanical Survey of India, Southern region centre, Tamilnadu Agriculture University Campus, Coimbatore.

Outer dry scales of *Eleutherine palmifolia* bulb of about 50 gm were taken and crushed well, macerated with mortar and pestle with 200 ml of ethanol. The solvent was filtered using filter paper funnel to remove debris. The clear soup of the solvent extract is stored. The qualitative preliminary phytochemical analysis of the solvent extract was analysed for phenols, sterols, proteins, resins, steroids, tannins, glycosides, terpenoids, reducing sugars, Pholabatannins using the standard protocols [4].

The test microorganisms used for screening were 9 bacteria (4 Gram positive and 3 Gram negative) *Streptococcus* sp (MTCC code- 890), *Lactobacillus acidophilus* (MTCC code- 447), *Shigella sonnei* (MTCC code-2957), *Klebsiella pneumonia* (MTCC code-3384), methicillin resistant *Staphylococcus aureus*, *Acinetobacter baumannii*, *Salmonella paratyphi* (MTCC code-3220), *Bacillus subtilis* (MTCC code 121),*Pseudomonas aeruginosa* (MTCC code 4676) and two fungi *Candida albicans* (MTCC code-183) and *Aspergillus flavus* (MTCC code-277). These organisms were identified and procured from Institute of Microbial Technology (IMTECH-CSIR) Chandigarh, India.

The antimicrobial activity of the reference standard antibiotics (100 μg/ml) was examined by the agar well diffusion method. Inocula of log phase bacterial cultures cultivated in nutrient broth adjusted to 10^5 CFU/ml, fungal cultures cultivated on YPS broth, *Candida albicans* starting inoculam size 10^6 CFU/ml and *Aspergillus niger* starting inoculam size 10^4 CFU/ml were prepared by the spectrophotometric method recommended by NCCLS. These cultures were uniformly spread-plated on Mueller-Hinton agar separately and four wells of uniform sizes were made with a cork-borer in each organism's plate and 50 μl, 75 μl and 100 μl of the bulb extract were pipetted directly into the 3 wells and one well was filled with 100 μl of vehicle solvent ethanol. The bacterial culture plates were incubated at 37 ℃ for 18 to 24 hours and the fungal cultures were incubated at room temperature for 48 hours. After the incubation periods, zones of inhibition were measured.

3. Results

Preliminary phytochemical screening of the *Eleutherine palmifolia* bulb ethanolic extract showed the presence of Phenols, Sterols, Phlobatannins, Proteins, Steroids, Tannins, Reducing sugar and Terpenoids while Resins, Glycosides, Acidic compounds were absent (Table 1).

Table 1

Phytochemical Analysis for *Eleutherine palmifolia* Linn bulb ethanolic crude extract

S. No	Phytochemical Test	Presence or Absence
1	Phenols	+
2	Sterols	+
3	Phlobatannins	+
4	Proteins	+
5	Resins	-
6	Steroids	+
7	Glycosides	-
8	Tannins	+
9	Reducing Sugars	+
10	Terpenoids	+
11	Acidic compounds	-

Agar well diffusion was used to determine the inhibition zones of *Eleutherine palmifolia* bulb ethanolic extract. The bulb showed significant antibacterial and antifungal activity against almost all the organisms (Table 2)

Results of the GC/MS analysis showed that at least 17 compounds were present in ethanol extract of *Eleutherine palmifolia*. These compounds were identified through mass spectrometry attached with GC. The mass spectra of these compounds were matched with those found in the NIST/NBS spectral database and the data are given in Table 3, Figure 1.

Table 2

Antimicrobial activity of *Eleutherine palmifolia* Linn bulb ethanolic crude extract

BACTERIAL PATHOGEN	Solvent	50 μl	75 μl	100 μl	Gentamycin15mcg/disc	Fusidic acid 15mcg/disc	Ketoconazole15mcg/disc
	Zone of Inhibition in mm.						
Shigella sonnei	0	16	18	30	28	NT	NA
Klebsiella pneumonia	21	23	25	28	26	NT	NA
Lactobacillus acidophilus	0	13	15	17	32	NT	NA
Streptococcus sp.	0	20	25	27	29	NT	NA
Salmonella paratyphi	13	15	17	18	30	NT	NA
Pseudomonas aeruginosa	0	10	12	13	24	NT	NA
Bacillus subtilis	0	19	25	27	35	NT	NA
MRSA	0	14	17	33	NT	27	NA
Acinetobacter baumannii	0	15	19	31	17	NT	NA
Aspergillus flavus	0	19	23	25	NA	NA	28
Candida albicans	13	16	19	21	NA	NA	24

Table 3

The Chemical Composition of bulb of *Eleutherine palmifolia* Linn.

S.No	R.T	Name of the Compound	Peak Area (%)
1	10.74	Benzeneethanamine,2-fluro-beta	0.16
2	11.01	Benzeneethanamine,2-fluro-beta	0.25
3	11.60	2,4(1H,3H)-pyrimidinedione,dihydro	0.23
4	12.34	Hydrazinecarboxamide	0.48
5	12.88	Glycyl-di-alanine	0.19
6	14.33	2,4(1H,3H)-pyrimidinedione,dihydro	0.18
7	14.53	Hydroxyurea	0.48
8	14.66	Ethanol,2-(2-Aminoethoxy)	0.74
9	15.50	1-Heptadecanamine	0.97
10	16.75	Cyclohexanecarbonyl Chloride	17.14
11	17.39	Cyclohexane,(1,2-dimethylbutyl)	11.19
12	17.84	Decane	30.22
13	19.14	Cyclohexane, hexyl	33.02
14	19.88	9-Octadecene,1,1-dimethoxy	0.45
15	19.99	Benzyl alcohol,alpha,-(1-amino	1.69
16	20.46	Cyclododecanol,1-aminomethyl-	0.25
17	21.40	Propane	0.38

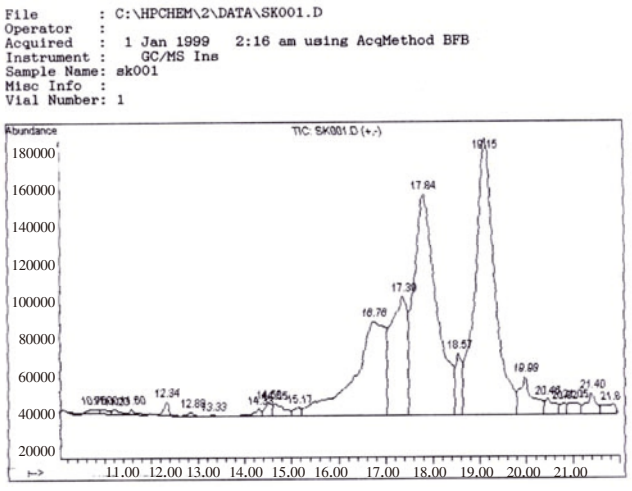

Figure 1. GC MS of bulb of *Eleutherine palmifolia* Linn.

4. Discussion

Amongst the bacterial test pathogenic organisms used gram negative *Shigella sonnei* were found to be most sensitive. *Klebsiella pneumonia* came next, followed by the *Streptococcus* sp. and *Bacillus subtilis*. Among the two fungal pathogens used *Aspergillus flavus* was found to be more sensitive than *Candida albicans*. This antifungal activity is more or less equivalent to the broad spectrum antifungal agent Ketoconazole.

Methicillin-resistant *Staphylococcus aureus* (MRSA) does not show resistance to *Eleutherine palmifolia* bulb ethanol extract and this is the higher activity than the standard antibiotic tested. *Acinetobacter baumannii* was more sensitive to *Eleutherine palmifolia* Linn. bulb ethanol extract than gentamycin.

The activities exhibited by the 100 μl of the *Eleutherine palmifolia* bulb ethanol extract which is more or less equivalent to stronger and broader spectrum antimicrobial compound Gentamycin. Hence elucidation of the drug principle in this extract is subject of significance.

The fragmentation pattern of the major compound (retention time: 17.84 min and 17.39 min) was found to be rather similar to that of Decane and Cyclohexane, (1,2-dimethylbutyl). This compound is observed to consist about 33.02% as a relative percentage amount.The other compounds that was found in less proportion were respectively Cyclohexane, hexyl, 9-Octadecene,1,1-dimethoxy and cyclohexanecarbonyl chloride that eluded at the Retention times 19.14, 19.88 and 16.76. Cyclohexane may be the active drug principle that is responsible for the antimicrobial activity of *Eleutherine palmifolia* Linn. bulb ethanolic crude extract. The other compounds may also be having synergistic effect on enhancing the antimicrobial activity of the cyclohexane.

Our finding is in correlation with the earlier findings of the [5], that derivatives from *Heliotropium filifolium* 3 H-Spiro [1-benzofuran-2, 1′-cyclohexane] had good antimicrobial activity. Thus, this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study. Antimicrobial activity exhibited by the plant extract against Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii* paves a new way for the scientific community in order to develop a new drug against such resistance pathogens. Further investigations into the pharmacological importance of *Eleutherine palmifolia* and their diversity and detailed Phytochemistry may add new knowledge to the information in the traditional medical systems.

Conflict of interest statement

We declare that we have no conflict of interest.

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