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Natural antimicrobial and bioactive compounds from *Ludwigia parviflora* Roxb

Abstract

In vitro screening method could offer a preliminary observation which is necessary to select crude plant extract with potentially useful properties for further chemical and pharmacological investigations. The present research work was designed to investigate the natural antimicrobial and bioactive constituents from *Ludwigia parviflora* (fruit extract). was tested for antimicrobial activity against four bacterial and three fungal pathogens using the well diffusion method with six different solvent extracts of these, ethyl acetate and ethanol extract of *L. parviflora* showed significant antimicrobial activity against all the tested pathogens. Bioactive constituents, as revealed by quantitative and qualitative. GC-MS analysis identified the presence of ten bioactive compounds. In conclusion, *L. parviflora* fruit extracts might be to treat diverse of human diseases and it could be responsible for the discovery of novel therapeutic drugs.

Keywords: antimicrobial activity, GC-MS, *Ludwigia parviflora*, phytochemical

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Gobalakrishnan R,[1] Bhuvaneswari R,[2] Rajkumar M[1]
[1]District Institute of Education and Training, India
[2]Government Girls Higher Secondary School, India

Correspondence: Rajagopal Gobalakrishnan, District Institute of Education and Training, Pudukkottai – 622 004, India, Tel 04322 270600, +918124800850, Email gobalaldy@gmail.com

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Abbreviations: RT, retention time; MF, molecular formula; MW, molecular weight, PDA, potato dextrose agar; RNA, ribonucleic acid

Introduction

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes[1] and a number of studies have voiced the necessity of developing alternative antimicrobial drugs.[2] Regard this, researchers are increasingly turning their attention to medicine to develop better drugs against microbial infections[3] and application of plant extract which are easily available, non-pollutive, cost effective, non-hazardous and do not disturb ecological balance.[4] Therefore, scientists are striving hard for interest in the investigation of different extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agent.[5]

The *Ludwigia* Linn. genus (including *L. parviflora*) contains 82 species and 23 sections which occur in tropical Africa, and from South America to East Asia and North Australia[6] and *Ludwigia* species are however, useful as ornamental plants because of their showy flowers have yellow in colour which makes them to be on sale in many parts of Europe.[7] They are also used as attachment hosts in the rearing of insects in the laboratory and some used for toxicity assessment.[8] The medicinal use of *Ludwigia* species dates back to decades and earlier studies on several species have revealed it to be of value in traditional medicine. Leaves of some species are used as poultice in wound dressings and as remedy for dysentery. The leaf sap is also taken orally to stave off threatened abortion, flatulence and constipation.[9] In addition, several *Ludwigia* contains many compounds that are probably toxic to ecosystem: anti-diarrhoeal profile, antitumor and antibacterial activity.[10, 11]

Over the last 20 years, many of secondary metabolites from different plant species have been evaluated for their antimicrobial activity. Although the number of the medicinal plant is not known but there is no doubt that most of the plants around us are medicinal.[12] It has been shown that *in vitro* screening method could offer a preliminary observation which is necessary to select crude plant extract with potentially useful properties for further chemical and pharmacological investigations.[13] Considering these facts, the present research work was designed to investigate natural antimicrobial and bioactive compounds of *Ludwigia parviflora* Roxb., fruit (Family: *Onagraceae*, Synonym: *L. perennis Linn.*), popularly known as Neerkirambu in Tamil.

Materials and methods

A. GC-MS analysis

GC-MS analysis ethanolic extract of the *L. parviflora* fruit was performed using Perkin Elmer GC Clarus 500 MS system for different components present in the extract, under the following conditions: column–dimethyl polysiloxane DB-1fused silica capillary column (30mx0.25mmx0.1µm of film thickness); carrier gas–helium (1mL/min); injector temperature–250°C; detector temperature-200°C; column temperature–35-180°C at 4°C/min–then 180–250°C at 10°C/min; MS electron impact 70$_e$V. Identification of the constituents was achieved based on comparison of mass spectra with the library ones (NIST Ver.2.1).

B. Plant material

Fresh *L. parviflora* plants (Figure 1) were collected from various regions of the Pudukkottai district in Tamilnadu, India. Plant was identified using the facility of Rapinat Herbarium, St. Joseph's college, Tiruchirappalli and identified voucher specimen was deposited in the Research and PG Department of Botany, H.H. The Rajah's College, Pudukkottai. From the plants, fruits were separated and thoroughly washed with water and kept in shade dry at room temperature to get rid of moisture, until further analysis.

Figure 1 Photographs displaying distinct as well as closer views of *L. parviflora* fruit.

C. Preparation of extract

Dried fruit materials were powdered with warring blender, at room temperature and 2g of the samples were soaked in 20mL of different solvents (ethanol, ethyl acetate, chloroform, hexane, benzene and water) overnight. Later, the samples were filtered under vacuum using Whatman No.1 filter paper and stored in airtight screw-capped bottles at 5ºC for further analysis.

D. Preparation of inoculums

Seven clinical pathogenic organisms were obtained from the Microbial Clinical Laboratory, KMC Hospital, Tiruchirappalli. Out of the seven, four were bacteria *(Escherichia coli, Salmonella typhi, Bacillus subtilis and Streptococcus pyogenes) and three fungi (Candida albicans, Fusarium solani and Trichophyton rubrum)* and three fungi (Candida albicans, Fusarium solani and Trichophyton rubrum). Stock culture was maintained at 5ºC on slant of nutrient agar for bacteria and potato dextrose agar (PDA) for fungi. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of respective media for respective tested organisms and incubated 37ºC±2ºC for 24hours for antibacterial and 25ºC±2ºC for 48hours for antifungal activity. Muller-Hinton Broth (for bacteria) and PD Broth (for fungi) were prepared for streaking and fresh slant cultures were prepared and stored in refrigerator at 5ºC for future requirements.

E. *In vitro* antimicrobial tests

Spectrum of antibacterial activity was studied by using the techniques described by Bauer et al.[14] Gentamicin sensitivity disc (30mg; Hi-Media) was used as a positive control and respective solvents were taken as negative controls. At the end of incubation period inhibition zones formed around the discs were measured with transparent ruler in millimeter. These studies were performed in triplicate.

F. Biochemical screening

Biochemical tests *viz*. carotenoids, total sugars, total proteins, total free amino acids, total phenols, hydroxyl phenols and lipids[15] were quantitatively revealed of the fresh *L. parviflora* fruit. Secondary metabolites were qualitatively tested by the standard methods of Harborne[16] and Odebiyi and Sofowora.[17]

Results

Present study investigated that the antimicrobial activity of *L. parviflora* fruit using different solvents crude extract against four bacteria (*E. coli, B. subtilis, S. typhi, S. pyogenes*) and three fungi (*C. albicans, F. solani and T. rubrum*) and the results are presented in Table 1. Of these, ethanol and ethyl acetate extract of *L. parviflora* showed significant antimicrobial activity against all the tested strains. Results of antibacterial activity, ethanolic extract had maximum (13.6mm) inhibition against *S. typhi*. Whereas, ethyl acetate extract had maximum (14.9mm) inhibition against *B. subtilis*. In antifungal activity, ethanolic extract had maximum (21.2mm) inhibition against *C. albicans*. Whereas, ethyl acetate extract had maximum (22.9mm) inhibition against *T. rubrum*.

The results of the basic biochemical constituents of the *L. parviflora* fruit extract were presented in Table 2. The secondary metabolites were quantitatively identified *viz*. alkaloids, cardiac glycosides, flavonoids, glycosides, phenolic compounds, *phlobatannins*, steroids and tannins

Table 1 Antimicrobial activity of *L. parviflora* fruit extracts in different solvents

S. No	Tested organisms	Inhibition zone of diameter (mm) after 24 -48hrs.						
	Bacteria	A	B	C	ET	EA	H	Gentamycin
1	E. coli	-	-	-	8.9±0.30	12.7±0.20	13.2±0.40	23.5
2	S. typhi	-	-	-	14.9±0.20	12.8±0.50	-	28.3
3	B. subtitles	-	-	-	9.7±0.04	13.6±0.10	9.2±0.040	25.8
4	S. pyogenes	-	-	-	9.8±0.05	11.3±0.50	-	31.7
	Fungi							
5	C. albicans	14.5±0.03	-	20.9±0.3	21.2±0.20	17±0.04	-	35.6
6	F. solani	13.3±0.02	-	-	14.8±0.10	22.7±0.30	-	28
7	T. rubrum	-	13.4±0.06	-	12.1±0.40	22.9±0.10	-	26.2

a - Values are mean ± standard deviation of three determination. Aqueous (A), Benzene (B), Chloroform (C), Ethanol (ET), Ethyl acetate (EA), Hexane (H), No activity (-).a - Values are mean ± standard deviation of three determination

Table 2 Results of biochemical screening tests

S. No	Biochemical constituents	*L. Parviflora* fruit (mg g^{-1})
1	Carotenoids	0.670 ±0.07
2	Total Sugars	24.50 ±5.01
3	Total Proteins	33.70 ±2.17
4	Total free amino acids	05.95 ±0.43
5	Total phenols	17.03 ±0.31
6	Hydroxyl phenols	9.380 ±0.27
7	Lipids	13.33 ±3.33

Table 3 Compounds identified from fruit extract of *L. parviflora* using GC-M S analysis

S. No	RT	Name of the compounds	Molecular formula	MW	Peak area (%)	Compound nature
1	4.5	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176.25	8.42	Ether compound
2	14.5	3,7,11,15-tetramethyl-2- hexadecen-1-ol	$C_{20}H_{40}O$	296.531	0.49	Terpene alcohol
3	16.3	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.424	11.36	Palmitic acid
4	16.6	Hexadecanoic acid, ethyl ester [Syn: Palmitic acid ethyl ester]	$C_{18}H_{36}O_2$	284.49	1.25	Fatty acid ester
5	18.2	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294.472	0.19	Linoleic acid ester
6	18.3	9-Octadecanoic acid(Z)-, methyl ester	$C_{19}H_{36}O_2$	296.487	0.22	Linoleic acid ester
7	18.7	Phytol	$C_{20}H_{40}O$	296.53	0.29	Diterpene alcohol
8	19.2	Linoleic acid	$C_{18}H_{32}O_2$	280.45	63.26	Fatty acid
9	19.5	Octa decanoic acid [Syn: Stearic acid]	$C_{18}H_{36}O_2$	284.48	13.09	Myristic acid
10	26	1,4-Benzenedicarboxylic acid, dihexyl ester	$C_{20}H_{30}O_4$	334.449	1.43	Plasticizer compound

Gas Chromatography and Mass Spectroscopy analyses were carried out on the ethanolic fruit extract of *L. parviflora* and 10 compounds were identified. Active principles with their Retention time (RT), Molecular formula (MF), Molecular weight (MW), Concentration (%) and nature of the compounds are presented in Table 3. The GC-MS chromatogram showing the peak identities of the compounds have been identified (Figure 2). In the present investigation, a variety of compounds has been detected viz. propane1,1,3-triethoxy, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, n-Hexadecanoic acid, hexadecanoic acid-ethyl ester, 9,12-Octadecadienoic acid, methyl ester (E,E)-, 9-Octadecenoic acid (Z)- methyl ester, phytol, linoleic acid, octadecanoic acid and 1,4-Benzenedicarboxylic acid-dihexyl ester. Among these, linoleic acid has contributed in more percentage level in the total extraction of GC-MS analysis, followed by octadecanoic acid, n-Hexadecanoic acid and propane 1, 1, 3-triethoxy.

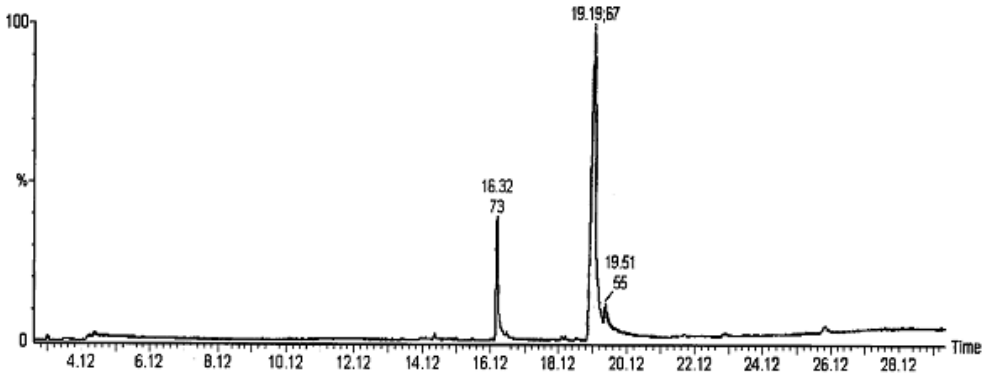

Figure 2 The GC-MS chromatogram showing the peak identities of the compounds.

Discussion

Plants have capacity to produce a variety compounds of known therapeutic properties, which substance that can either inhibit the growth of pathogens or kill them and have no or less toxicity to host cells are considered candidates for developing new antimicrobial drugs.[18] So, present study was carried out antimicrobial activity from the fruit extract of *L. parviflora*.

Previously, Selim.,[19] studied a broad spectrum antimicrobial activity from the leaves and stem extract of the *L. adscendens,* similarly Ahmed et al.,[20] found that the methanolic extracts of leaf and stem parts of *L. adscendens* possesses a strong antibacterial activity and used against various skin diseases. Oyedeji et al.,[9] evaluated antibacterial activity from *L. abyssinica* and *L. decurrens* against different bacteria included *E. coli, S. typhi*, and *B. subtilis*. They opined that, water extracts of both *Ludwigia* species not showed inhibitory effects while as ethyl acetate extract had shown inhibitory activity. This echoed with the present results also not showed inhibitory effects on water extract while ethyl acetate extract had shown inhibitory activity. Aliyu et al.,[21] reported antibacterial activity against *E. coli, K. pneumoniae, B. subtilis, S. pyogenes, S. aureus,* and *S. typhi* from the *L. suffruticosa* with ethanol and ethyl acetate fraction. In present study *L. parviflora* fruit of ethanol and ethyl acetate extract had an inhibitory potential against seven human pathogens included *E. coli, S. typhi, B. subtilis* and *S. pyogenes, C. albicans, F. solani* and *T. rubrum*. It was however observed that the antimicrobial activity of crude ethanol and ethyl acetate extracts are higher than those of solvent fraction, which indicate that the activity may be due to influences of synergetic effects.[22] In addition, it might be different solvents have varying solubility capacities for phytoconstituents,[23] and also organic solvents had more responsible for antimicrobial activities and predominant antimicrobial activity,[24] so organic solvents had used frequently for dissolve varieties of active compounds.[25]

The preliminary biochemical tests are significant and helpful in finding chemical constituents in the plant materials that might be lead to the source of pharmacologically active compounds.[26] Previously biochemical study were reported from various species of *Ludwigia* such as *L. octovalvis, L. repens, L. prostrate, L. adscendens, L. suffruticosa, L. abyssinica and L. decurrens*.[27–29] In present study, *L. parviflora* fruit revealed the presented some active compounds such as alkaloids, cardiac glycosides, flavonoids, glycosides, phenolic compounds, phlobatannins, steroids and tannins. Similarly, various authors reported these biochemical constituent acts as antimicrobial drugs viz. alkaloids, saponins, tannins, flavonoids and phenolic compounds,[30] sterols,[31] phlobatannins,[32] and cardiac glycosides.[33] They interfere with processes such as deoxyribonucleic (DNA) replication and ribonucleic acid (RNA) transcription which are vital to microorganisms. Other mechanisms are disruptions of protein synthesis, stability of biomembranes and metabolically important enzymes.[34] In the case of fungi, they had interference with molecular targets in their organs, tissues and cells. The major targets include biomembrane, proteins and nucleic acid and these are still regarding as a valuable pool for discovering novel mode of action.[35]

Knowledge of chemical constituents of plants is desirable because such information will be importance for synthesis of chemical substances,[36] it could be qualified for application in pharmaceutical industry.[26] Therefore, present study revealed that the fruit extract of *L. parviflora* were identified ten different compounds by GC-MS. Especially, linoleic acid is one of the polyunsaturated fatty acids important and essential for health,[37] which was obtained 63.26% from the *L. parviflora* fruit extract. Linoleic acid directly acts against cancer development via its effects on body composition: e.g., reducing body fat, enhancing lean body mass,[38] control of obesity,[39] decreased prostate cancer cell proliferation[40] and possess beneficial properties on the skin.[41] While, the high intake of linoleic acid were significantly increased platelet aggregation,[42] and also increased in polyunsaturated fatty acid content of the platelet membrane.[43] Previously, Praveen Kumar et al.[44] reported linoleic acid can be act as an anti-inflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antiarthritic, anticoronary, 5- alpha reductase inhibitor and insectifuge.

Octadecanoic acid is called as stearic acid, which showed 13.09% from the *L. parviflora* fruit extract. Dr.Duke's Phytochemical and Ethnobotanical databases [online] mentioned octadecanoic acid acts as antiandrogenic, antiarthritic and anticoronary. Hema et al.,[45] reported octadecanoic acid act as 5-alpha-reductase-inhibitor, cosmetic, flavor, hypocholesterolemic, lubricant, perfumery, propecic and suppository.

The *L. parviflora* fruit extract showed 11.36% of n-Hexadecanoic acid was extracted from, which acts as an anticancer drug,[46] antioxidant and antimicrobial activity.[47] Similarly, Praveen Kumar et al.[44] reported that n-Hexadecanoic acid plays as an antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavour, hemolytic and 5- Alpha reductase inhibitor. Propane 1,1,3-triethoxy, was extracted 8.42% from the *L. parviflora* fruit extract. Which was already reported from various plant extract such as, *Euphoria longan, Vitex negundo, Delonix regia, Cadaba trifoliate, Andrographis paniculata, Wattakaka volubilis and Vigna mungo* but activity was not reported clearly.

Conclusion

In present investigation revealed that justification for the use of the plant *L. parviflora* as a folk medicine. Antimicrobial potential and GC-MS analysis of the plant *L. parviflora* fruit is the first of its kind. In present study, ethanolic and ethyl acetate crude extracts had wide ranges of antimicrobial activity as well as contains many biologically active compounds. Especially, GC-MS analysis identified ten bioactive compounds from ethanolic extract, which may serve as candidate for the discovery of novel drugs in the treatment of diverse of human diseases.

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Conflicts of interest

The author declares that there are no conflicts of interest.

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