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GC-MS/MS Based Metabolite Profiling and Evaluation of Antimicrobial Properties of *Emblica officinalis* Leaves Extract.

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Abstract

Ever since, traditional plants and their products were consumed by human beings in diverse health matters to heal variety of infections. At the moment, in all over globe, the scientific attention and focuses are escalating with regard to phytomedicine. This study explores the value of secondary metabolites of *Emblica officinalis* leaves extract (EOLE) identified through GC-MS/MS profiling in different solvents against microbial strains. *In-vitro* antimicrobial activity was established by means of an agar well diffusion scheme at various loaded concentrations (25 µg/mL-1000 µg/mL). GC-MS/MS profiling confirmed the presence of major accredited compounds characterized as PDMTFM (Rt- 9.891 min and peak area-1.77%), P2,6-BDME (Rt-8.588 min, peak area - 100%), P2,5-BDME (Rt - 8.567 min, peak area - 36.78%) and BFTHTM (Rt-9.055 min, peak area - 7.87%) in tag plant. Gram positive bacteria; *Bacillus pumilis*, *Bacillus cereus*, *Staphylococcus pyogenous*, *Bacillus polymyxa* and Gram negative bacteria; *Vibrio cholera*, *Providencia alcalifaciens*, while fungal species; *Neurospora crassa*, *Trichoderma viridae*, *Aspergillus brasileinsis* and *Cladosporium oxysporum* were used during this study. Aqueous extract made known excellent antimicrobial activity than other selected extract. Inhibitory values of aqueous and methanolic extract were measured with reference to ciprofloxacin and amphotericin. Preliminary results of this study implied that EOLE could be considered as strong antimicrobial agent to treat human infectious diseases.

Keywords: Gas Chromatography, Amla leaves, Extraction techniques, Antimicrobial species

1. Introduction

Amla *(Emblica officinalis)* is a traditional herbal drug belonging to Euphorbiaceae family, usually recognised as "amalaki". Plant is small, consisting of spherical fruit with soft barks and leaves which are light green in colour, simple, subsessile, intimately situated by the side of branchlets. Leaves of *E. officinalis* are commonly a good source of secondary metabolites, acknowledged to be used in the management of a variety of maladies like over activity of thyroid, regulation of high blood glucose level, stimulation of antibodies formation, high level of bilirubin, bacterial disease like tuberculosis, dyspepsia as well decrease high cholesterol level (Ramesh *et al.,* 2015). Previous literature showed that the plant possesses several antimicrobial chemicals such as tannins, phenolics, flavanoids, alkaloids , tannins and carotenes (Banji *et al.,* 2018).

Asmawl *et al.,(*1993) and co-worker have reported that leaves of *E. officinalis* are rich in the polyhydroxy phenolics molecules like gallic acid, mallic acid, quercetine, chebulic acid and O-galloyl glucose respectively. The potential of antimicrobial activity of plant base drug has been recommended due to the presence of polyphenolics compounds. Researcher suggested a variety of techniques for the separation of phyto metabolites from the tissues of plants. While the selection of extraction method based on considerable issues specifically efficiency, robustness, resources outlay, running cost and ease of operation (Gulcin *et al.,* 2012).

In a previous study, several customary extraction methods such as Soxhlet, successive extraction, hot extraction, cold extraction, were recommended for extraction of herbal drugs. On the other hand, extraction by UAE is a recent procedure used to extract bioactive molecules from natural source. This is a trustworthy technique that condenses the release of hazardous pollutants by dropping the utilization of organic solvent and is relatively straightforward to operate. It compressed processing period, minimum chances of oxidation and decomposition of plant molecules and gave significant yield in the course of rapid extraction. Ultrasonic assisted extraction (UAE) may be introduced to isolate the critical medicinal constituents of *E. officinalis* (Shen and Shao, 2005). The adequate antimicrobial reports of *E. officinalis* leaves extract (EOLE) were available; on the other hand, inadequate information exist on the topic of mechanism and antimicrobial scope concerned with EOLE. In this

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regard, the intention of present study is to scrutinize the leaves constituents of *E. officinalis* by UAE technique in altered polarity solvents by means of GC-MS/MS profiling as well as to explore the *in vitro* activity of it against the microbial strains.

Nevertheless, herbal medicine remains the best choice of natural sources mining as antimicrobial remedies. In favor of our statement, there are still some microbial families for which antimicrobial activity of EOLE are less exposed in the earlier studies. There is, as a result, a positive call for the investigation of antimicrobial properties *E. officinalis* through sophisticated analytical techniques to encourage its use throughout the community. Consequently, we propose the title plant as potential candidates for further study in folk medicines to establish the better drugs against microbial disease.

2. Materials and methods

2.1. Equipment, chemicals and procurement of plant material assortment

Crude leaves of *Eumblica officinalis* were used in the proposed study. Plant material was purchased from community marketplace of Khari Bavli New Delhi-110006. The specimen (NBPGR/2017-2) was submitted and verified by National Herbarium of Cultivated Plants at Pusa campus, New Delhi-110012-India. Subsequently, leaves were unsoiled, washed down with water and after acclimatization extraction was executed on an ultrasonic system (Power flow-250 W and Frequency 35/60 kHz). GC-MS/MS profiling was deal with Agilent model 7000D (Triple Quad) system. Methanol was made available by Merck (Mumbai, India) and all other experimental chemicals and solvents were of analytical grade.

2.2. Plant extracts preparation by ultrasonic extraction technique

The dried leaves (5gm) were considered for extraction purpose through water and methanol (300 mL) by means of UAE, which connected with water bath, digital gauge and a device to control the temperature. The system was derived at a calculated frequency of 35/60 kHz, fixed ultrasonic input power of 250 W and experiment was repeated for thrice. To avoid excessive heat generation during ultrasonication, the system was carried out at moderate room temperature (25 ^{o}C ± 5). Active extract was recovered and dried by means of rotavapor to remove the trace amount of solvent completely in order to maintain its quality (Tonk *et al.*, 2006).

2.3. GC-MS/MS profiling

2.3.1. Samples preparation and GC-MS/MS background

For GC-MS/MS profiling, the sample was processed by transferring about 5 mg of extract in a graduated measuring flask and attended the volume up to 10 mL with various solvents (water and methanol); subsequently, the mixture was subjected to centrifugation at 6000 rpm for 5 min. Finally, the supernatants were filtered through 0.22 µm syringe filter and the experiment was performed with 1 mL of this solution.

The aqueous and methanolic extracts of *E. officinalis* leaves were submitted to GC-MS/MS (Triple Quadru pole/2010). The GC-MS/MS backgrounds were as follows: system installed with capillary column (5 % phenyl polysiloxane stationary phase x 30.0 m length × 0.25 µm film x 0.25 mm l.d), column oven temperature was set at 60 °C (fixed for 2 min), augmented at 15 °C per min to 270 °C (fixed for 2 min) and at the end fixed at 290 °C for 15 min. The flow rate of the carrier gas, Helium (99.9% purity) was maintained at a speed of 1.5 mL/min. The experiment was executed by loading 1.5 µL of the titled sample through a split ratio of 30: 1 and carried out in electron impact (EI) mode of -70 eV as ionization energy. The injector temperature was asserted at 260 °C (steady). Mass spectra was studied in scanning manner at 25-500 m/z. Sample spectra were analyzed and identified in contrast to spectral configuration documented by means of existing mass spectral database (NIST 14-MS database library).

2.4. Selection of microbial strains for study

2.4.1. Bacterial and .fungal strains

Design protocol is comprised of bacterial and fungal strains that are *Staphylococcus aureus, Bacillus subtilis, Bacillus megaterium, Bacillus pumilis, Bacillus cereus, Staphylococcus pyogenous* and *Bacillus polymyxa* (Gram-positive), *Pseudomonas aeruginosa, Escherichia coli, Vibrio cholera, Salmonella typhi, Shigella dysenteriae, Proteus mirabilis, Providencia alcalifaciens* (Gram negative bacteria) and *Aspergillus niger, Neurospora crassa, Penicillium chrysogenum, Trichoderma viridae, Aspergillus brasileinsis, Candida albicans* and *Cladosporium oxysporum* respectively belonged to fungi category respectively.

2.4.2. Evaluation of antimicrobial and antifungal activity

The antimicrobial assay of EOLE was executed through agar well diffusion method and it included different bacterial pathogens namely (Gram-positive), (Gram-negative) and fungal pathogens respectively (Faiza *et al*., 2012). Once the autoclaving process of media was completed, it kept on cool at 35 ^{o}C-50 ^{o}C. To validate the sterility, ready to use and cooled media was transferred in to glass petri plates of equal diameter (60 mm in diameter), after solidifying of agar media the crude plates were allow to incubate at 35 ^{o}C for 20–24 h. Approximately, 100 µL of the fresh inoculums of bacteria was uniformly dispersed on the smooth surface of solidified agar media while fungal strains were spread on potato dextrose agar media of each petri plate. The wells of identical size (6 mm diameter x 2 mm depth) were crafted by using sterile steel cork borer. About 0.1 mL of various concentrations of EOLE were poured in to the well with sterile glass syringe and set aside to diffuse completely for 2 h at room temperature. Immediately after diffusion, all the petri plate were allowed to incubate in incubator for 24-48 h at 37 ^{o}C used for bacteria strains and for 48-72 h at 30 ^{o}C for fungal strains, respectively. DMSO (0.05 mL) was utilized as a negative control for the study whereas ciprofloxacin (0.1 mg/mL) and amphotericin (0.1 mg/mL) employed as a positive control for bacterial and fungal strains, respectively. The antimicrobial results were documented by measuring the diameter (mm) of zone of inhibition with respect to each well. This study was performed every 3 times for each selected microbial strain.

3. Results

3.1. Selection of ultrasonic extraction technique

The classical extraction techniques are generally based on long exposure of heat reflex, skilled packing of plant material in thimble, demand for longer extraction period, unavoidable consumption and evaporation of solvent during experiment (Shen and Shao, 2005). To prevail these restriction of classical extraction techniques, a non classical ultrasound assisted extraction method has been considered good for the targeted plant sample with lower solvent evaporation and high percentage yield of aqueous (31.16 w/v) and methanolic (10.15 w/v) extracts respectively.

3.2. GC-MS/ MS profiling and spectrum characterization

The GC-MS spectrum of unknown phytomolecules of *E. officinallis* was interpreted in contrast with database of known molecules store in National Institute Standard and Technology (NIST) library. Figure.1 represents the distinct chromatogram of EOLE in targeted solvents. The plants derived bioactive constituents contribute a momentous role in various biomedical rationales. As revealed in Table 1, results of GC-MS profiling of EOLE comprise the retention time, molecular weight, molecular structure and peak area of active phytoconstituents. The major accredited compounds characterized were PDMTFM (Rt - 9.891 min and peak area - 1.77%), P2,6-BDME (Rt - 8.588 min, peak area - 100%), P2,5-BDME (Rt - 8.567 min, peak area - 36.78%) and BFTHTM (Rt - 9.055 min, peak area - 7.87%).

Table 1. Description and relative composition of bioactive constituents of *E. officinalis* leaves extract by GC-MS/MS.

E. officinalis leaves extract	Name of compound	Nature of compound	Formula	Mol. Wt. (g/mol)	Peaks	RT (min)	% Peak area
Aqueous extract	A. Phen-1,4-diol, 2,3-dimethyl 5trifluoromethy	Phenolic	$C_9H_9F_3O_2$	206	46	9.891	1.77
	B. Phenol, 2,6-bis (1,1-dimethylethyl)	Phenolic	$C_{14}H_{22}O$	206	4	8.588	100
Methanolic extract	C. Phenol, 2,5-bis (1,1-dimethylethyl)	Phenolic	$C_{14}H_{22}O$	206	16	8.567	36.78
	D.2(4H)Benzofuranone,5,6,7,7a-tetrahydro-4,4,7a-trimethyl	Heterocyclic Triterpene	$C_{11}H_{16}O_2$	180	23	9.055	7.87

RT: Retention Time; Mol.Wt.: Molecular weight

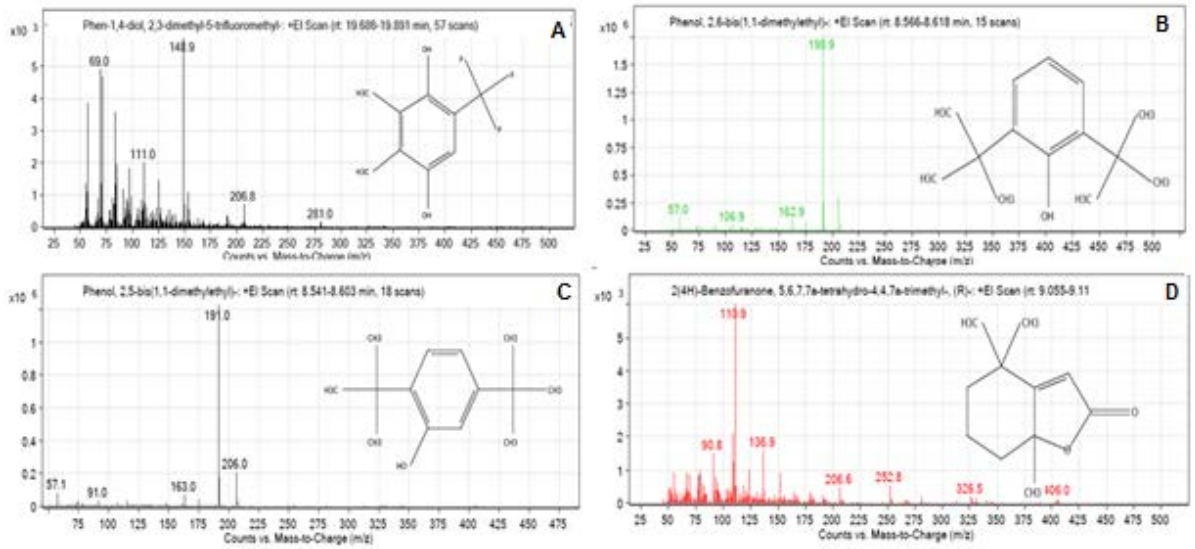

Figure 1. GC-MS/MS Chromatograms, structure and resultant mass peak of *E. officinalis* leaves.

3.3. Antimicrobial activity of designated plant extract

Antimicrobial prospective of different extracts of *E. officinalis* and standard antimicrobial drugs (CPF and AMP) against bacteria and fungi is represented in Table 2-7. Likewise, the zone of inhibition and MIC of standard antibiotics and EOLE were recorded at different concentration aligned with DMSO as negative control. Inhibitory effect of CPF was recorded at 25 µg/mL against all Gram positive and Gram negative bacteria and revealed highest zone of inhibition (15.05 ± 0.17mm) with *S. aureus* and *(*21.13 ± 0.18 mm) in contrast to aqueous and methanolic leaves extract of *E. officinalis* which exhibit no zone of inhibition at 25 µg/mL Table 2. Maximum resultant zone of inhibition against Gram positive bacteria; *B. pumilis* (7.20 ± 0.16mm), *B. megaterium*(5.30 ± 0.24), *B. cereus* (3.37 ± 0.20mm), *B. polymyxa* (3.27 ± 0.19) and Gram negative bacteria; *P. mirabilis* (4.40 ± 0.14mm), *V. Cholera* (4.27 ± 0.29mm), *S. typhi* (3.19 ± 026 mm), *P. aeruginosa* (2.00 ± 0.16) of aqueous extract were recorded at 50 µg/mL, while *B. subtilis*, *S. pyogenous*, *E. coli*, *S. dysenteriaea* and *P. alcalifaciens* respectively were not produced any zone of inhibition at this concentration Table 3. All reported bacteria gave observable zone of inhibition at the concentration (100 µg/mL) except *P. alcalifaciens.* It was observed that concentration ranging from (250 µg/mL–1000 µg/mL) of aqueous extract for the selected Gram positive bacteria and Gram negative bacteria exhibits good results while poor zone of inhibition with similar concentration was experienced with methanolic

extract Table 4. The results of our study proposed that aqueous and methanolic leaves extract of *E. officinalis* exhibited no antibacterial activity at the concentration (25 µg/mL). Only two Gram positive bacterial species *B. megaterium* (4.97 ± 0.29 mm), *B. pumilis* (4.83± 0.12 mm) and one Gram negative bacteria *V. cholera* (3.20 ± 0.18 mm) established that the concentration (50 µg/mL) was found appropriate for the activity although it showed no activity for *S. aureus, B. subtilis, B. cereus S. pyogenous, B. polymyxa, E. coli, P. aeruginosa, S. typhi, S. dysenteriae* and *P. alcalifacien.s.* Low inhibitory zone was experienced against all Gram positive and Gram negative bacteria, which required a very high concentration 250 µg/mL–1000 µg/mL excluding *P. alcalifaciens* to which no reaction was observed. The results of (Figure 2) confirmed that concentration (25µg/mL) of aqueous and methanolic extract was not found suitable for the antibacterial activity of the selected species. Although, no response was observed with two Gram positive bacteria; *B. subtilis, S. pyogenous* and three Gram negative bacteria; *E. coli, S. dysenteriaea* and *P. alcalifaciens* at concentration (50 µg/mL) of aqueous leaf extract, in another load five bacterial species *S. pyogenous, E. coli, S. typhi, S. dysenteriae, S. dysenteriae* exhibited no activity at (100 µg/mL) with methanolic extract of *E. officinalis*.

Anti fungal activity of standard amphotericin was not confirmed at (25 µg/mL), while concentration (250 µg/mL-1000 µg/mL) showed different level of zone of inhibitions Tables 5. Aqueous EOLE at (25 µg/mL-50 µg/mL) showed no clear zone of inhibition to *A. niger, N. crassa, P. chrysogenum, T. viridae, A. Brasileinsis, C. albicans* and *C. oxysporum*. Two fungal strain *A. Niger* (7.34 ± 0.9 mm) and *C. oxysporum* (3.35 ± 0.37mm) gave zone of inhibition at (100 µg/mL), while this dose was not satisfactory for other selected fungi. Purely, the growth of fungal strains was inhibited by EOLE at (250 µg/mL-1000 µg/mL). High inhibitory concentration of aqueous extract (100 µg/mL) were observed against *A. niger* (7.19 ± 0.90 mm) while *C. oxysporum* gave the lowest value (3.35 ± 0.17 mm) Tables 6. In comparison to aqueous extract of *E. officinalis*, no satisfactory antifungal activity was found with methanolic extract at (25 µg/mL–100 µg/mL). *A. niger* a single fungi merely gave zone of inhibition (5.27 ± 0.12) at 250 µg/mL. The results made known that a high concentration (500 µg/mL – 1000 µg/mL) of methanolic extract was needed to slow down the growth of concerned fungal species Tables 7. As shown in (Figure 3), the aqueous extract of *E. officinalis* best suited to slow down the fungal growth at a concentration (100 µg/mL) in association to methanolic extract which exhibited minimum antifungal action at higher concentration (250 µg/mL).

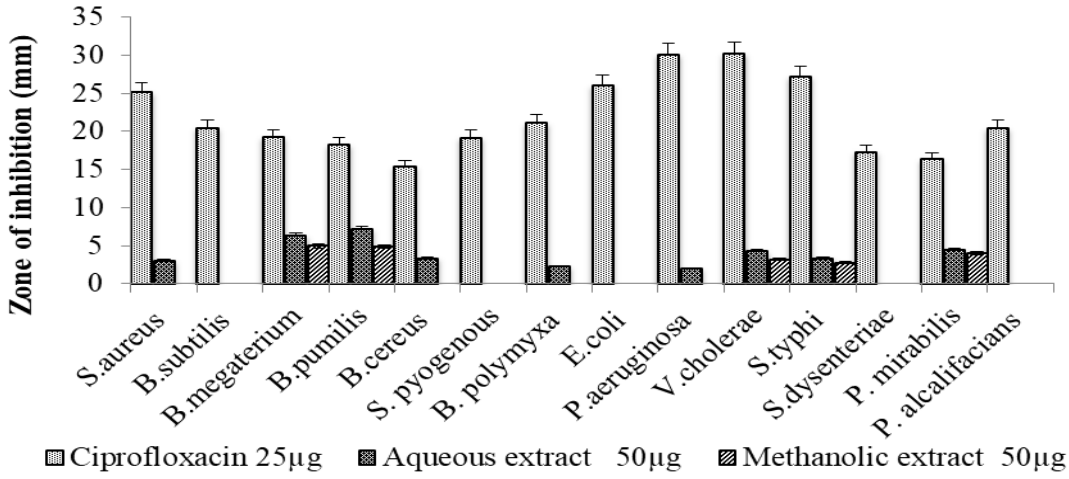

Figure 2. Ciprofloxacin and *E. officinalis* leaves extract which displayed widespread minimum inhibitory concentration (MIC) and name of bacteria against which they were active at different doses.

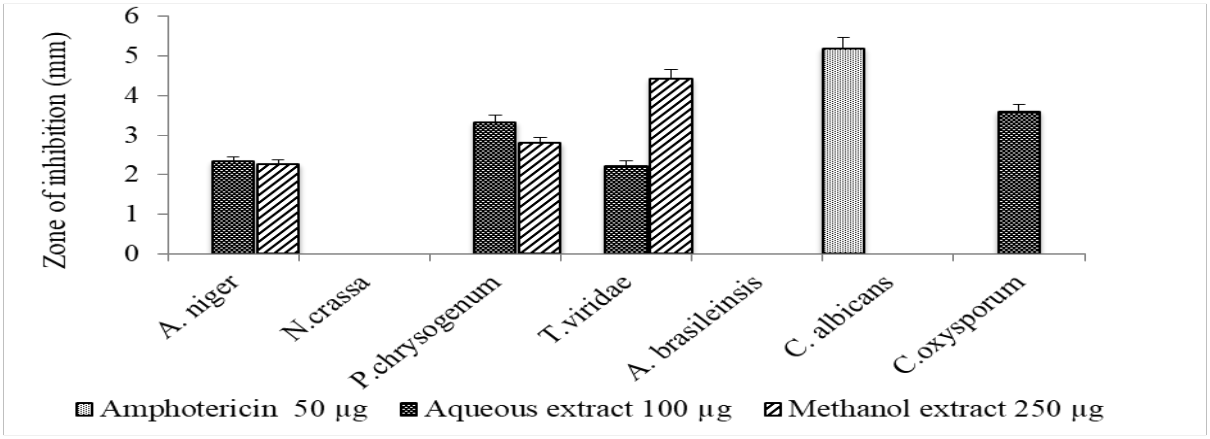

Figure 3. Amphotericin and *E. officinalis* leaves extracts which displayed widespread minimum inhibitory concentration (MIC) and the name of fungi against which they were active at different concentration.

Table 2. Antibacterial activity of standard (Ciprofloxacin) on Gram positive and Gram negative bacteria.

Types and name of Organisms		Concentration of standard (µg/mL) and Zone of inhibition (mm)[a] ± SD						
		25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
Gram positive	*S. aureus*	15.05 ± 0.17	15.63± 0.21	17.24 ± 0.11	17.33 ± 0.11	19.33 ± 0.31	*	25
	B. subtilis	9.14 ± 0.17	9.15 ± 0.21	9.27 ± 0.13	10.20 ± 0.13	10.96 ± 0.15	*	25
	B. megaterium	6.17 ± 0.15	8.23 ± 0.19	9.10 ± 0.15	9.75 ± 0.17	10.20 ± 0.12	*	25
	B. pumilis	13.10 ± 0.17	13.17 ± 0.15	14.12 ± 0.19	14.95 ± 0.01	15.10 ± 0.22	*	25
	B. cereus	11.51 ± 0.14	12.21 ± 0.23	12.55 ± 0.23	13.11 ± 0.29	14.37 ± 0.15	*	25
	S. pyogenous	9.25 ± 0.03	9.35 ± 0.24	9.47 ± 0.12	10.00 ± 0.17	10.15 ± 0.09	*	25
	B. polymyxa	8.03 ± 0.21	8.15 ± 0.10	8.75 ± 0.71	9.13 ± 0.11	9.0 ± 0.12	*	25
Gram negative	*E. coli*	17.15 ± 0.12	17.23 ± 0.03	17.35 ± 0.33	18.15 ± 0.22	18.37 ± 0.21	*	25
	P. aeruginosa	7.13 ± 0.05	7.41 ± 0.15	7.50 ± 0.17	8.25 ± 0.01	8.59 ± 0.71	*	25
	V. cholerae	10.19 ± 0.14	10.25 ± 0.09	10.75 ± 0.22	10.90 ± 0.43	11.05 ± 0.18	*	25
	S. typhi	9.13 ± 0.17	9.35 ± 0.21	9.53 ± 0.15	9.75 ± 0.11	10.00 ± 0.02	*	25
	S. dysenteriae	17.29 ± 0.23	18.42 ± 0.02	18.70 ± 0.04	18.90 ± 0.42	19.50 ± 0.03	*	25
	P. mirabilis	20.26 ± 0.21	20.39 ± 0.90	21.45 ± 0.19	21.50 ± 0.15	21.90 ± 0.01	*	25
	P. alcalifacians	19.43 ± 0.18	21.35 ± 0.07	21.50 ± 0.01	21.81 ± 0.22	22.25 ± 0.06	*	25
DMSO	Negative control	-	-	-	-	-	-	-

MIC µg: Minimum inhibitory concentration in microgram; SD: Standard deviation; DMSO: Dimethyl sulfoxide; CPF: Ciprofloxacin; Values are expressed as mean ± SD from three experiments ([a]n = 3); * Zones could not be measured due to margining.

Table 3. Antibacterial activity of aqueous leaves extract of *E. officinalis* on Gram positive and Gram negative bacteria.

Types and name of Organisms		Concentration of extract (µg/mL) and Zone of inhibition (mm)[a] ± SD						
		25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
Gram positive	*S. aureus*	NZI	3.00 ± 0.16	9.13 ± 0.26	11.50 ± 0.34	12.20 ± 0.16	12.63 ± 0.20	50
	B. subtilis	NZI	NZI	5.23 ± 0.21	7.77 ± 0.20	8.1.9 ± 0.08	9.63 ± 0.33	100
	B. megaterium	NZI	5.30 ± 0.24	6.47 ± 0.34	7.33 ± 0.24	8.83 ± 0.12	9.33 ± 0.25	50
	B. pumilis	NZI	7.20 ± 0.16	9.47 ± 0.29	10.50 ± 0.16	11.29 ± 0.25	12.87 ± 0.26	50
	B. cereus	NZI	3.37 ± 0.20	5.67 ± 0.21	6.87 ± 0.12	9.6 ± 0.21	11.23 ± 0.25	50
	S. pyogenous	NZI	NZI	3.13 ± 0.21	4.27 ± 0.20	8.63 ± 0.58	11.00 ± 0.62	100
	B. polymyxa	NZI	3.27 ± 0.19	6.13 ± 0.46	7.00 ± 0.29	7.40 ± 0.22	9.30 ± 0.29	50
Gram negative	*E. coli*	NZI	NZI	2.13 ± 0.25	4.57 ± 0.25	6.27 ± 0.25	7.50 ± 0.37	100
	P. aeruginosa	NZI	2.00 ± 0.16	5.90 ± 0.29	6.33 ± 0.17	7.03 ± 0.37	8.25 ± 0.17	50
	V. cholerae	NZI	4.27 ± 0.29	4.60 ± 0.29	4.97 ± 0.17	6.47 ± 0.33	7.07 ± 0.09	50
	S. typhi	NZI	3.19± 026	5.27 ± 0.30	6.13 ± 0.26	6.37 ± 0.33	9.30 ± 0.22	50
	S. dysenteriae	NZI	NZI	2.17 ± 0.17	5.43 ± 0.25	6.27 ± 0.21	10.25 ± 0.37	100
	P. mirabilis	NZI	4.40 ± 0.14	7.67 ± 0.29	9.87 ± 0.17	12.63 ± 0.43	13.43± 0.25	50
	P. alcalifacians	NZI	NZI	NZI	4.13 ± 0.20	6.10 ± 0.29	8.50 ± 0.46	250
DMSO	Negative control	-	-	-	-	-	-	-

MIC µg: Minimum inhibitory concentration expressed in microgram; NZI: No zone of inhibition; SD: Standard deviation, DMSO: Dimethyl sulfoxide; Data are expressed as mean ± SD as of three experiments ([a]n = 3)

Table 4. Antibacterial activity of methanolic leaves extract of *E. officinalis* on Gram positive and Gram negative bacteria.

Types and name of Organisms		Concentration of extract (µg/mL) and Zone of inhibition (mm)[a] ± SD						
		25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
Gram positive	*S. aureus*	NZI	NZI	7.83 ± 0.02	9.20 ± 0.43	11.13 ± 0.19	13.23 ± 0.16	100
	B. subtilis	NZI	NZI	4.80 ± 0.22	6.13 ± 0.09	9.90 ± 0.72	12.90 ± 0.70	100
	B. megaterium	NZI	4.97 ± 0.29	5.07 ± 0.25	6.33 ± 0.47	8.07 ± 0.25	9.37 ± 0.52	50
	B. pumilis	NZI	4.83 ± 0.12	5.40 ± 0.20	7.10 ± 0.05	7.90 ± 0.33	8.90 ± 0.08	50
	B. cereus	NZI	NZI	4.40 ± 0.06	4.90 ± 0.26	7.93 ± 0.38	9.93 ± 0.86	100
	S. pyogenous	NZI	NZI	NZI	2.60 ± 0.54	6.80 ± 0.59	10.20 ± 0.16	250
	B. polymyxa	NZI	NZI	4.90 ± 0.09	8.07 ± 0.17	11.33 ± 0.25	12.30 ± 0.24	100
Gram negative	*E. coli*	NZI	NZI	NZI	4.10 ± 0.30	6.23 ± 0.01	7.30 ± 0.20	250
	P. aeruginosa	NZI	NZI	5.07 ± 0.13	8.97 ± 0.12	10.50 ± 0.81	11.07 ± 0.25	100
	V. cholerae	NZI	3.20 ± 0.18	5.50 ± 0.05	9.03 ± 0.17	11.33 ± 0.47	13.93 ± 0.14	50
	S. typhi	NZI	NZ	NZ	2.65 ± 0.25	4.27 ± 0.38	5.96 ± 0.37	250
	S. dysenteriae	NZI	NZI	NZI	3.70 ± 0.24	5.73 ± 0.11	7.30 ± 0.22	250
	P. mirabilis	NZI	3.63 ± 0.33	3.97 ± 0.29	5.57 ± 0.01	8.63 ± 0.46	10.23 ± 0.01	50
	P. alcalifacians P. alcalifacians	NZI	NZI	NZI	NZI	3.57 ± 0.37	5.23 ± 0.71	500
DMSO	Negative control	-	-	-	-	-	-	-

MIC µg: Minimum inhibitory concentration expressed in microgram; NZI: No zone of inhibition; SD: Standard deviation, DMSO: Dimethyl sulfoxide; Data are expressed as mean ± SD as of three experiments ([a]n = 3)

Table 5. Antifungal activity of amphotericin.

Name of Organism	Concentration of AMT (µg/mL) and Zone of inhibition (mm)[a] ± SD						
	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
A. niger	NZI	NZI	11.15 ± 0.01	13.30 ± 0.09	16.13 ± 0.19	17.11 ± 0.05	100
N. crassa	NZI	NZI	13.85 ± 0.01	15.54 ± 0.12	17.55 ± 0.03	18.57 ± 0.07	100
P. chrysogenum	NZI	NZI	NZI	13.57 ± 0.11	16.27 ± 0.17	16.51 ± 0.01	250
T. viridae	NZI	NZI	NZI	14.54 ± 0.15	17.52 ± 0.01	18.20 ± 0.14	250
A. brasileinsis	NZI	NZI	11.35 ± 0.01	12.51 ± 0.51	12.71 ± 0.05	14.17 ± 0.37	100
C. albicans	NZI	3.40 ± 0.05	5.17 ± 0.01	11.13 ± 0.05	14.13 ± 0.15	19.21 ± 0.05	50
C. oxysporum	NZI	NZI	NZI	9.15 ± 0.13	10.15 ± 0.24	15.25 ± 0.28	250
DMSO	-	-	-	-	-	-	-

MIC µg: Minimum inhibitory concentration expressed in microgram; NZI: No zone of inhibition; SD: Standard deviation, DMSO: Dimethyl sulfoxide; AMT: Amphotericin; Data are expressed as mean ± SD as of three experiments ([a]n = 3)

Table 6. Antifungal activity of aqueous leaves extract of *E. officinalis.*

Name of organism	Concentration of extract (µg/mL) and Zone of inhibition (mm)[a] ± SD						
	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
A. niger	NZI	NZI	7.19 ± 0.9	9.05 ± 0.38	9.75 ± 0.21	10.53 ± 0.33	100
N. crassa	NZI	NZI	NZI	6.51 ± 0.16	8.54 ± 0.14	10.23 ± 0.29	250
P. chrysogenum	NZI	NZI	NZI	5.63 ± 0.17	7.27 ± 0.48	10.11 ± 0.12	250
T. viridae	NZI	NZI	NZI	7.61 ± 0.22	8.67 ± 0.29	11.27 ± 0.20	250
A. brasileinsis	NZI	NZI	NZI	5.80 ± 0.33	6.87 ± 0.20	9.45 ± 0.71	250
C. albicans	NZI	NZI	NZI	6.85 ± 0.25	7.1 ± 0.30	8..35 ± 0.65	250
C. oxysporum	NZI	NZI	3.35 ± 0.17	5.67 ± 0.17	6.21 ± 0.31	9.80 ± 0.12	100
DMSO	-	-	-	-	-	-	-

MIC µg: Minimum inhibitory concentration expressed in microgram; NZI: No zone of inhibition; SD: Standard deviation, DMSO: Dimethyl sulfoxide; Data are expressed as mean ± SD as of three experiments ([a]n = 3)

Table 7. Antifungal activity of methanolic leaves extract of *E. officinalis.*

Name of organisms	Concentration of extract (µg/mL) and Zone of inhibition (mm)[a] ± SD						
	25 µg	50 µg	100 µg	250 µg	500 µg	1000 µg	MIC µg
A. niger	NZI	NZI	NZI	5.27 ± 0.12	6.80 ± 0.41	8.89 ± 0.37	250
N. crassa	NZI	NZI	NZI	NZI	4.50 ± 0.41	7.43 ± 0.33	500
P. chrysogenum	NZI	NZI	NZI	NZI	5.20 ± 0.16	8.37 ± 0.32	500
T. viridae	NZI	NZI	NZI	NZI	4.39 ± 0.26	7..25 ± 0.20	500
A. brasileinsis	NZI	NZI	NZI	NZI	6.45 ± 0.41	8.43 ± 0.45	500
C. albicans	NZI	NZI	NZI	NZI	5.37 ± 0.12	7.97 ± 0.24	500
C. oxysporum	NZI	NZI	NZI	NZI	5.53 ± 0.17	7.13 ± 0.20	500
DMSO	-	-	-	-	-	-	-

MIC µg: Minimum inhibitory concentration expressed in microgram; NZI: No zone of inhibition; SD: Standard deviation, DMSO: Dimethyl sulfoxide; Data are expressed as mean ± SD as of three experiments ([a]n = 3)

4. Discussion

4.1. Ultrasonic extraction technique

Plant and plantconstituents give their important contribution to the wellbeing from the time immortal. Plant base metabolites are procured by various traditional extraction techniques namely filtration, maceration, decoction and soxhlet extraction while non-traditional modes of extractions are microwave-assisted extraction, supercritical fluid extraction and ultrasonic-assisted extraction. Ultrasonic extraction technique is an easy, straightforward, uncomplicated and unsophisticated technique. The competence of phyto extraction depends on various issues, including temperament of plant base metabolites, method of separation, particle size of crude drug to be extracted, extraction period, temperature of unit, pH of solvent, compatibility of solute to solvent and solvent polarity (Gonbad *et al*., 2015). In an existing study, ultra sonic extraction technique was used for the extraction of plant constituents from the leaves of *E. officinalis*, and their extractive yield was calculated. High extraction yield was achieved with water 3.7 g while methanol yielded 3.0 g respectively. High extractive value showed better extractive efficiency of aqueous solvent than methanol and this result was established to be analogous with the existing study . The reported technique is a proficient that needed a very short duration of run period and consume a smaller amount solvent. It condenses the load of harmful toxicants of organic solvent to the extract; the technique is robust, effective, precise and relatively straightforward to manage. The prospects of solvent oxidation, crumbling and rotting of plant metabolite are very less to this extraction technique (Khaled *et al.,* 2018). Due to these qualities, UAE attended more attention than other traditional extraction techniques, to fulfil the extraction demand of the plant metabolite. The proposed mechanism behind robustness of UAE process extraction efficiency was increased in surface areas, speed of mass transfer and assembly of several bubbles which generate significant interior heat and load on tissues causing cell destruction of plant tissues (Nostro *et al.,* 2000).

4.2. Characterization of potential compounds by GC-MS/MS

Characterization of phytoconstituents has been done by GC-MS/MS technique, a versatile analytical tool which is repeatedly the choice of many researchers, adopted due to its robustness. Different types of phenolics compound are characterized in plant extracts; in this view Dharni *et al.,* 2014, proposed 2,4-Di-tert-butylphenol (2,4-DTBP) as a potent antibacterial and antifungal natural compound characterised by GC-MS analysis. In an additional report, Sangmanee and Hongpattarakere (2014) suggested that low concentration (100 µg/mL) of (2,4-DTBP) which was detected by GC–MS profiling slows down the speed of spore germination of *F. oxysporum.* Padmavathi *et al.,* 2014, present in their study the antibacterial activity of *S. marcescens*, a Gram-negative bacteria was inhibited by polyphenolic constituents 2,4-DTBP and PBDME. The proposed findings are completely synchronize by means of the earlier facts, in which the phenolic compounds, 2,4-DTBP and PBDME showed good antioxidant aptitude because of their ability to forage free radicals and active oxygen species. Recently, an active constituent BFTHTM was fractioned from the leaves of *Azadirachta indica*, used as an analgesic, antiinflamatory, antidiabetic, antimicrobial agent (Rangel-Sanchez *et al.,* 2014). P2,6-BDME a polyphenolic compound, has also reduced the microbial load and slowed down the spore germination belonging to Enterobacteriaceae family (Mujeeb *et al.,* 2014). Our results corroborate with an earlier study and proposed that certain volatile organic composite P2,6-BDME, BFTHTM, PDMTFM present in EOLE established an antifungal property of EOLE (Yahiaoui *et al.,* 2017).

4.3. Antimicrobial activity

Today, a number of synthetic antibacterial and antifungal drugs are available to the medical field; however, their casual and regular use fixed a drug resistance aptitude (Anurag *et al.,* 2017). To overcome this menace, there is a continuous need to find the appropriate solution. In our findings, we proposed plant source drugs, as the available modern drugs responsible for a number of side effects in an individual. To this fact, we tested antimicrobial activity of EOLE and the growth of all microbes were successfully inhibited by aqueous extract in comparison to methanolic extract. *B. cereus, B. pumilis*

and P. mirabilis causing health issues to a millions of general public all over the globe while data of previous four decades raise the issues of subclinical urinary tract disease by *P. mirabilis* (Armbruster and Mobley, 2012). One more survey publicized that antibodies against proteus microbes were augmented considerably in patients and there are fewer chances for *P. vulgaris* to be isolated commonly in the urine of patients in contrast to other selected microorganism (Drzewiecka, 2016). Consequently, the use of aqueous EOLE may be advantageous towards these diseases sourcing microorganism. The inhibitory pattern of the microorganism showed a discrepancy with the nature of solvent extracts and this may possibly be due to discrepancy in the extraction procedures and microorganism to be tested (Mujeeb *et al.,* 2014). The antibacterial action of the crude extract of EOLE was tested against CPF (positive control) and DMSO as a negative control at different dilution. CPF inhibited the growth of all microbes at 25 µg/mL. To ascertain the antifungal activity of EOLE, AMT was used as standard and the value of inhibitory zone was recorded against DMSO. A high concentration (500 µg/mL–1000 µg/mL) of methanolic EOLE was needed to inhibit the augmentation of all diseases causing fungi whereas the study proposed that the multiplications of the fungus was greatly reduced by means of aqueous extract of *E. officinalis* at low dose (100 µg/mL) while methanolic extract demonstrated nominal antifungal action at higher dose (250 µg/mL). The antifungal action of extracts might be due to the additive outcome of different active phytoconstituents in both extracts. To support our result and mechanism, EOLE was subjected to GC-MS/MS profiling which revealed the existence of PDMTFM, P2,6-BDME and P2,5-BDME 2,4-Di-tert-butyl phenol (2,4-DTBP) which belongs to terpenes and phenolics categories, and predominantly mono and sesquiterpenes are able to inhibit the growth of bacteria, fungi, virus and protozoan (Habtemariam and Gray *et al.,* 1993). The greatest antibacterial and antifungal activity of aqueous extract against all the targeted microbes may possibly be due to high solubility of plant metabolites extracted in the solvent (Kumar and Pandey, 2012). These phytoconstituents, conceivably, may inhibit microbial acceleration efficiently by binding to their cell membrane (Kai *et al.,* 2007). In support of this, the antimicrobial activity of aqueous EOLE can be explained due to the presence of secondary plant metabolites, such as tannins, terpenoids, alkaloids and flavonoids (Siddiqua *et al.,* 2015). Earlier results have assured that the potential antimicrobial properties of EOLE can be most probably associated with the existence of secondary plant metabolites and the collective effect of these compounds absorption onto the cell surface causing its distraction and cell contents leakage and the production of hydroperoxides by polyphenolic compounds (Brul and Coote, 1999). These outcomes validate the healing property of *E. officinalis* leaf extracts. However, the majority of the experiments have appraised antimicrobial and antifungal properties of *E. officinalis* fruits extracts and some degree of magnitude is specified to the extract of other part of the plant

5. Conclusion

It can be concluded that results of present report, focused on the activity of phytoconstituents of aqueous and methanolic extract to their different polyphenolics compounds. The significant inhibitory role of targeted drug against a variety of microbial strains was primarily due to the occurrence of secondary metabolites. GC-MS profiling confirmed the occurrence of a number of active constituents belonging to flavanoids and phenolics compounds. EOLE showed a wide spectrum of biological activity against pathogenic Gram Positive bacteria and Gram negative bacteria as well as fungal species. These conclusions give a reason of choice for antimicrobial study in order to monitor the activity of the extract of traditional herbal medicine *E. officinalis* and their products that may hold rich contents of phenolic compounds.

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