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Fatty Acid Derivative of Methanol Extract of *Oldenlandia corymbosa*: A Potential Compound against *K. pneuminiae* and MCF-cell Lines

Archana V[1], Nitha N Thomas[2], Arun A Rauf [3] and Boby T Edwin[4]

[1]Research Fellow, Department of Biotechnology, The Cashew Export Promotion council of India Laboratory and Research Institute, Kollam, Kerala, INDIA

[2]Research Fellow, Department of Biotechnology, The Cashew Export Promotion council of India Laboratory and Research Institute, Kollam, Kerala, INDIA

[3]Asst Prof, Department of Biochemistry, University of Kerala, Thiruvananthapuram, INDIA

[4]Head of the Department, Department of Botany, TKM College of Arts and Sciences, Kollam, Kerala, INDIA

[4]Corresponding Author: bobytedwin2003@gmail.com

ABSTRACT

The acceptance of medicinal plants is increasing progressively for treating human diseases. The phytochemical components; bioactive chemicals present in the plants have a property to protect human body from disease causing agents. The aim of the present study is to screen the methanol extracted fatty acid derivative of *Oldenlandia corymbosa* by GC-MS and to evaluate its anti oxidant, anti bacterial and anti cancer potential. Plants were collected and extracted with Soxhlet apparatus and the extract was subjected for compound separation by column chromatography. Separated compound from plant was subjected to antioxidant, anti bacterial and anticancer study. GC-MS analysis of the compound was done using standard protocol. Methanol fraction of the O.corymbosa showed antibacterial property on K.pneumoniaea only and it has no effect on *E. coli*, *S. typhi* and *S. aureus*. Disc diffusion results revealed that the methanol fraction has effect only on K.pneumoniae with zone of inhibition 16mm. In our study the methanol fraction showed maximum antioxidant property. IC_{50} value was found to be 0.38±0.004 mg/ml. The result shows that there is a concentration and time dependent increase in the percentage of cytotoxicity induced by the compound. The highest anticancer activity on MCF-7 cell line observed with IC50 value of 0.27±0.18 mg/ml. GC-MS study of the Methanol fraction has showed number of phytoconstituents which contribute to the medicinal property of compound. The major constituents present in the compound is methyl stearate (16.62%), Methyl plamitate (14.53%), 1,2-Bezenedicarboxilicacid (6.61%), Trans2-Nonadecene (5.44%) etc. It is only a preliminary study of the anti bacterial and anticancer property of Methanol fraction, an in depth study will provide a good concrete base for pharmacological activity of compound.

Keywords-- Oldenlandia corymbosa, GC-MS, Antioxidant, Anticancer, Antimicrobial.

I. INTRODUCTION

In ancient time people used only herbal medicines to treat all diseases. Hence plants are very useful in human healthcare system and are rich in medicinal properties (1). Plants contain numerous biologically active compounds which exhibit medicinal properties. Knowledge of phytochemicals is desirable for disclosing new sources of drugs (2). The use of medicinal plants as raw materials in production of new drugs is gaining popularity because of the presence of specific constituents of secondary metabolites present in them. The widespread use of antibiotics in clinical medicine leads the development of antibiotic resistance among infectious microbial strains. It reflects very seriously in the treatment of pathogenic microorganisms (3).There are some advantages of using medicinal plants as antimicrobial agents, includes less side effects, better patient tolerance, acceptance due to long history of use and renewable in nature(4).

Cancer is a major disease which severely effects the human population. Research interest is drawing its attention towards naturally derived compounds to treat cancer because it have less toxic and no side effects when compared with chemotherapy(5). Plants have limitless ability to synthesize secondary metabolites, so still more drug molecules present in the plants used to be investigated for isolating the new drugs for increasing efficiency of treatment. Reservoirs of natural antioxidants present in plants are useful for the development of novel drugs. Many plant derived products exhibit chemo preventive activity against animal models. With the success of these products researchers focused on plants to develop novel drugs for the treatment of cancer (6).

Oldenlandia species belongs to Rubiaceae family; more than 20 species of the genus have been used in traditional medicine (7). *Oldenlandia corymbosa* (Rubiaceae) is a weedy herb, widely distributed throughout India. In traditional medicine, the plant is extensively used in gastric irritation, jaundice, liver complaints, skin diseases, cough, bronchitis, necrosis, clear heat and toxins (8-9). It is one of natural resources which revealed to perform anticancer properties. Phytochemical studies reveales the presence of terpenoids, alkaloids, Flavonoids and glycosides in different extracts of *O. corymbosa* (10-11).

The isolation of bioactive compounds present in plant extract is very important. After isolation it is very important to characterize the phytoconsituents of compounds. Determination of phytoconstituent is largely performed by GC-MS, because it is specific in identification (12). Literature survey of this plant revealed that no extensive phytochemical and pharmacological investigation had been carried out. Main objective of the work is to screen the methanol extracted fatty acid derivative of *Oldenlandia corymbosa* by GC-MS and to evaluate its anti oxidant, anti bacterial and anti cancer potential.

II. MATERIALS AND METHODS

Collection and Processing of Plant Material

Collected plants were washed well and dried under hot air oven, powdered and extracted with soxhlet apparatus. Extract was concentrated under pressure and kept in refrigerator for further use. Column chromatographic method was used for the separation of compounds from crude extract. Different solvent systems such as Hexane, Hexane: Chloroform (1:1), Chloroform, Chloroform: Ethyl acetate (1:1), Ethyl acetate, Ethyl acetate: Methanol (1:1) and Methanol were used. Each fraction was collected separately, dried under pressure and kept for further studies.

Anti Oxidant Property by DPPH Assay

DPPH radical scavenging assay was done according to standard method (13). 2ml of DPPH (0.2mM/methanol) was incubated with different concentrations of samples (10, 50, 100, 200, 400, 600, 800, 900µl). The mixtures were shaken and incubated at dark for 30minutes. Ascorbic acid was taken as standard. Absorbance was read at 517nm against blank (2ml methanol with 1ml DPPH solution) using UV-Vis Spectrophotometer117 (Systronics). The free radical scavenging activity was calculated using an equation:

%Inhibition of DPPH Activity=A-B/A×100

Where, A=Optical density of the blank B=Optical density of the sample.

The fraction which shows significant antioxidant property (Methanol fraction) was selected for further studies.

Antibacterial Property

Methanol fraction was analyzed for antibacterial activity by disc diffusion method against *E. coli, Klebsiella pneumoniae, S. typhi and S. aureus.* Muller-Hinton agar plates were freshly seeded with test organisms; sample and standard were impregnated in six mm discs (25µl/disc). Discs were gently placed on agar plates and incubated for 18-24hrs at 37°C. Antibacterial activity of the samples was expressed by measuring zone of inhibition (14).

Minimum Inhibitory Concentration

MIC was analyzed against K. pneumoniae by Agar well diffusion method and broth dilution method.

Agar well Diffusion Method

Similarly that of disc diffusion method agar plates was freshly inoculated with test organism. Then 6mm to 8mm diameter wells were punched aseptically with sterile cork borerora tip. Different concentrations of the compound (1.25, 2.5, 5, 10, 12.5, 15 and 20mg/ml) at desired volume was introduced into the wells (15). The plates were incubated under desirable conditions. After incubation period the zone of inhibition were measured.

Broth Microdilution Method

MIC of the Methanol fraction was determined for K. pneumoniae in triplicate in test tubes. To 2ml of the Nutrient broth in test tubes 0.5ml of varying concentrations of compound 1.25, 2.5, 5, 10, 12.5, 15, 20 µg/ml was added. A loopful of test organism (0.5 Mc Farland turbidity) was introduced into each test tube. Penicillin and streptomycin are used as standards. A test tube with nutrient broth and test organism was served as control. The culture tubes were incubated at 37°C for 24hrs. After incubation period tubes were observed for turbidity to examine the microbial growth (16-17).

Anticancer Property by MTT Assay

The anti carcinogenic property of Methanol fraction was studied by 3-(4, 5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide (MTT) assay. MCF-7cells (breast carcinoma) was procured from National Centre for Cell Sciences (NCCS) Pune and dermal fibroblast cells (juvenile foreskin) from Hi-media laboratories, India were used for control studies. Briefly $5x10^3$ cells were cultured and incubated for 24, 48 and 72 hours. The extract was dissolved in Dimethyl Sulfoxide (DMSO) and used for MTT assay. The extract was added at different concentrations ranging from 1600 µg/ml to 25µg/ml. At the end of incubation period, MTT was added to all the wells and incubated in dark for 2 hours at 37°C. After incubation the lysis solution [20% Sodium Dodecyl Sulphate (SDS) in 50% Dimethyl form amide (DMF)] was added and further incubated for 4 hours in dark. After incubation, the optical density was assessed at 570 nm using a multi well plate reader (18). The percentage of cytotoxicity was calculated.

GC-MS Analysis

GC-MS analysis is a common confirmation and effective analysis test. GC-MS analysis of Methanol fraction was detected using Shimadzu QP2010S GC-MS analyzer. Instrument employing the following conditions: Column of Rxi-5Sil MS, 30meter length, 0.25mm ID and .25µm thickness. GC-MS software is GC-MS solutions and Library is NIST 11 and WILEY8.

Statistical Analysis

The data were subjected to statistical analysis. All the tests were recorded in triplicates and the values were expressed as mean ±SD. IC_{50} value was calculated by plotting the graph with percent value on y-axis and concentration on x-axis.

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III. RESULTS

DPPH Assay

DPPH is a nitrogen-based marker which is used to determine the antioxidant free radical scavenging activity of the test materials that contain antioxidants. When reaction occurs the DPPH radical reacts with hydrogen donor groups of the antioxidant substances then the purple colour of the solution change into yellow. In our study the methanol fraction showed maximum antioxidant property. IC_{50} value was found to be 0.38±0.004mg/ml. Fig.1 illustrates the antioxidant activity of compound.

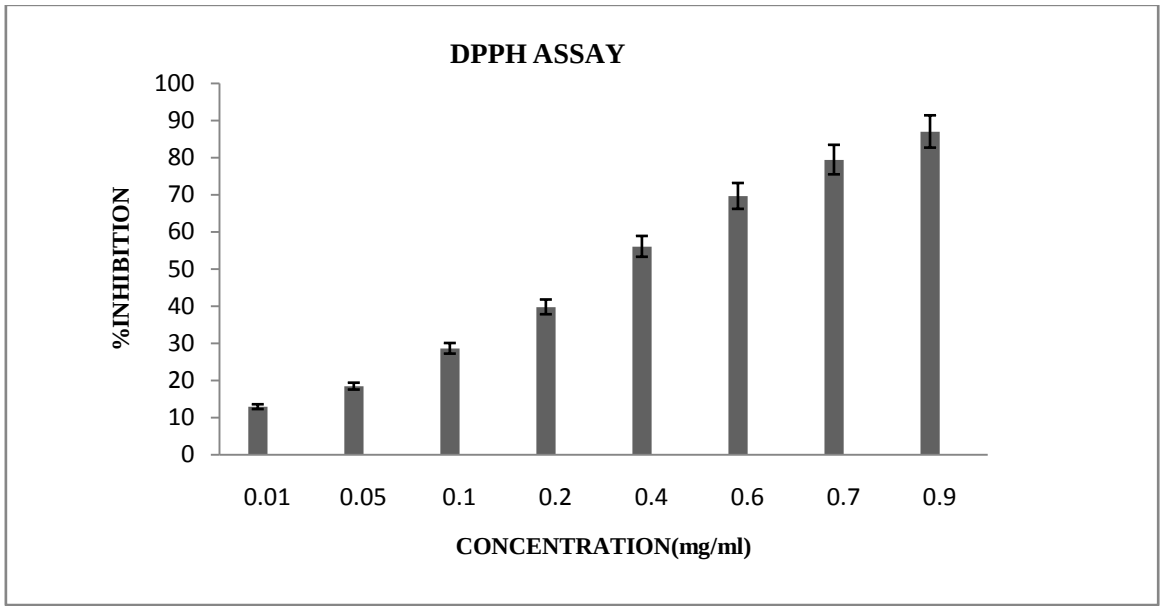

Figure 1: Antioxidant Activity of Methanol Fraction Antibacterial Studies

Methanol fraction of the O.corymbosa showed antibacterial property on K.pneumoniaea only and it has no effect on *E.coli*, *S.typhi* and *S.aureus*. Penicillin and Tetracyclin were used as the positive control. As shown in table 1 the disc diffusion results revealed that the methanol fraction has effect only on K.pneumoniae with zone of inhibition 16mm. The negative control methanol did not show inhibition zone on any organism. Positive controls showed significant inhibition zones.

Table 1: Antibacterial Activity of Compound.each Value Indicates Zone of Inhibition in milli meter(mm)

Sl. No	Organisms used	Positive control		Compound
		Penicillin	Tetracyclin	
1	E.coli	15	18	No Zone of Inhibition
2	**K.pneumoniae**	21	20	**16**
3	S.typhi	16	12	No zone of inhibition
4	S.aureus	17	19	No zone of inhibition

Minimum Inhibitory Concentration

MIC of the methanol fraction was analysed both in agar well diffusion and macrodilution methods. Table.2 shows the result of Agar well diffusion method. 15µg/ml concentration shows significant inhibition zone of 21mm.

Table 2: MIC (agar well diffusion) of Compound Against K. pneumoniae

Sl.No	Concentration(µg/ml)	Zone of inhibition(mm)
1	1.25	6
2	2.5	8
4	5	11
4	10	12
5	12.5	19
6	**15**	**21**
7	20	19

In Macrodilution tubes the turbidity decreases according to concentration. 12.5,15,20µg/ml tubes was clear that of control tubes.

MTT Assay

The result shows that there is a concentration and time dependent increase in the percentage of cytotoxicity induced by the compound. Fig.2 shows the cytotoxicity induced by the compound on MCF-7 cell line at different time interval. Maximum cytotoxicity was observed to be at 72 hours incubation when 1000 µg/ml concentration was used. Comparatively, the cytotoxicity induced by the extract was not significantly cytotoxic when the same concentration was applied to normal fibroblast cells at 48 hours incubation time which reveals the potential of the drug for selective killing of cancer cells. Fig.3 shows cytotoxicity induced by the compound on normal fibroblast cells. The highest anticancer activity on MCF-7 cell line observed with IC50 value of 0.27±0.18 mg/ml.

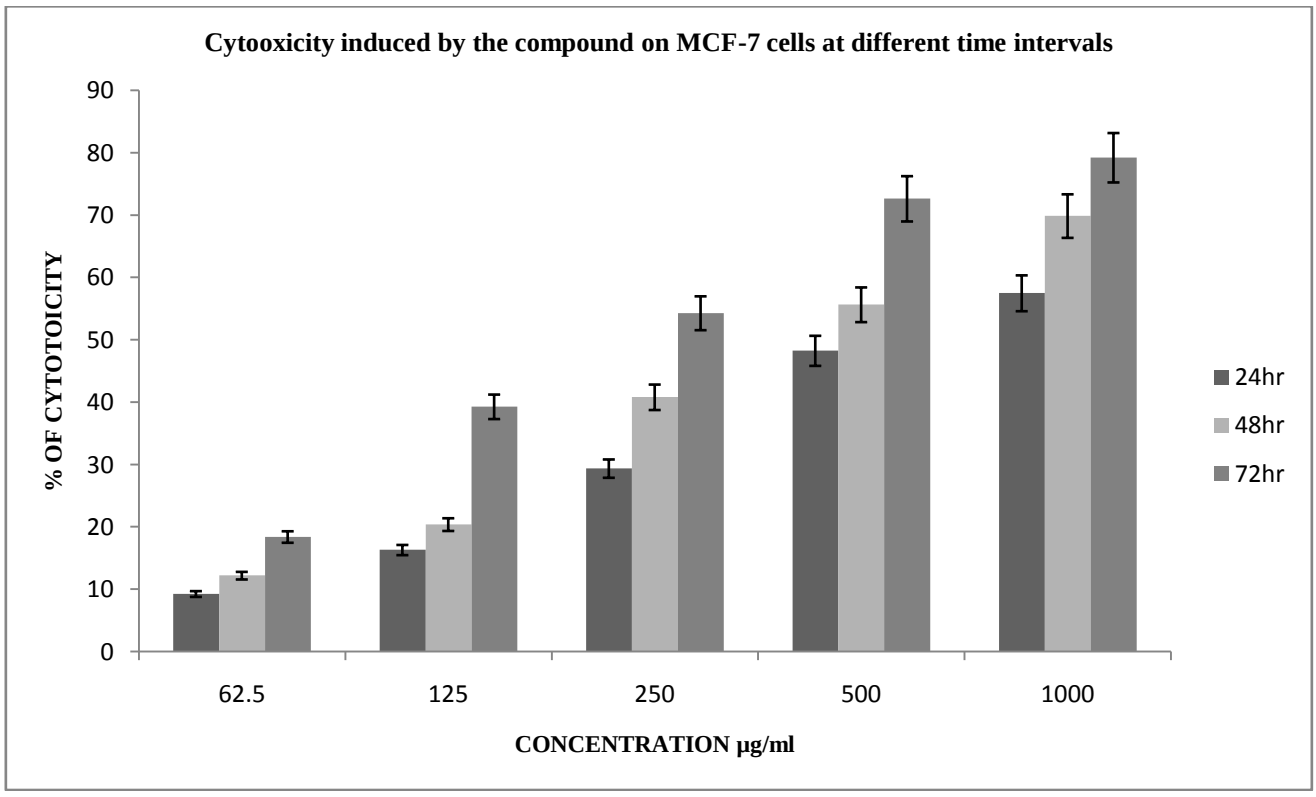

Figure 2: MTT Assay of Methanol Fraction

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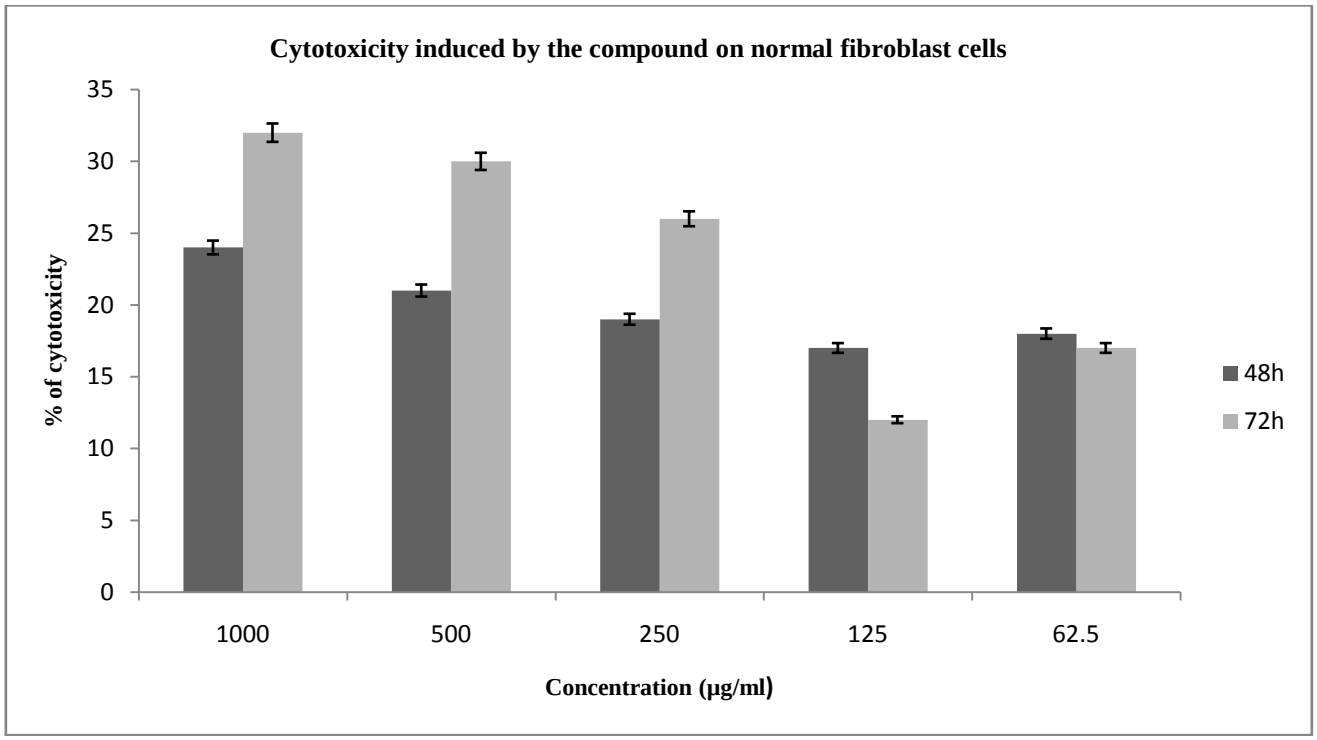

Figure 3: Cytotoxicity Induced by Methanol Fraction on Normal Fibroblast cells

GC-MS Analysis

GC-MS study of the Methanol fraction has showed number of phytoconstituents which contribute to the medicinal property of compound. The major constituents present in the compound is methyl stearate (16.62%), Methyl plamitate (14.53%), 1,2-Bezenedicarboxilicacid (6.61%), Trans2-Nonadecene (5.44%) etc. GC-MS result reveals that the compound is a fatty acid derivative. Table.1 shows the components present methanol fraction. Fig.4 shows the GC-MS chromatogram of the fraction.

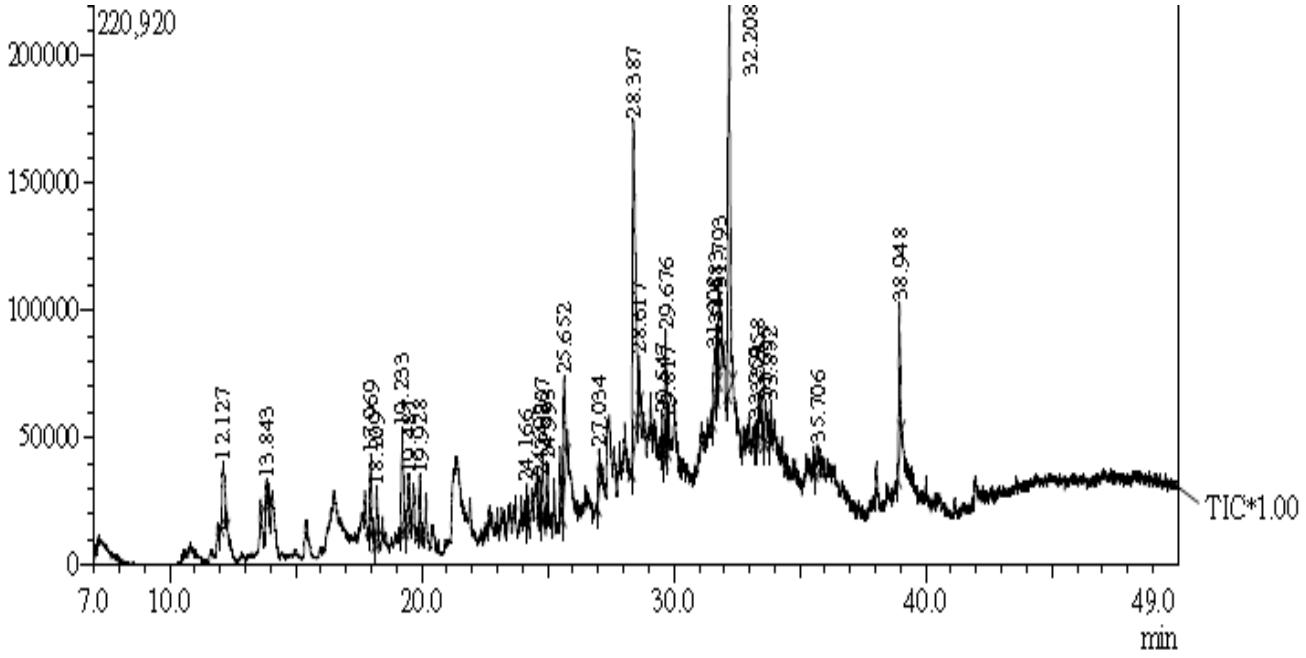

Figure 4: GC-MS Chromatogram of Methanol Fraction

Table 3: Phytoconstituents in Methanol Fraction

Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	12.127	195221	4.11	26062	2.85	Benzene, 1,3-bis(1,1-dimethylethyl)-	175.15
2	13.843	34542	0.73	9583	1.05	1-Undecene, 7-methyl-	69.05
3	17.969	132366	2.79	30593	3.34	Sulfurous acid, hexyl octyl ester	73.05
4	18.209	82578	1.74	21650	2.37	HEXANE, 2,3,4-TRIMETHYL-	57.00
5	19.233	195094	4.11	40125	4.38	Phenol, 3,5-bis(1,1-dimethylethyl)-	191.10
6	19.487	136609	2.88	22966	2.51	1-TRIDECANOL	69.05
7	19.928	82845	1.74	23141	2.53	DIISODECYL ETHER	57.05
8	24.166	32746	0.69	14034	1.53	Oxalic acid, 6-ethyloct-3-yl ethyl ester	57.05
9	24.692	49867	1.05	13664	1.49	Oxalic acid, allyl pentadecyl ester	111.15
10	24.807	114372	2.41	28002	3.06	DIISODECYL ETHER	69.10
11	24.993	68468	1.44	20781	2.27	2-Isopropyl-5-methyl-1-heptanol	57.10
12	25.652	205709	4.33	44777	4.89	(TRANS)-2-NONADECENE	55.05
13	27.034	69388	1.46	16837	1.84	Phthalic acid, cis-hex-3-enyl heptadecyl ester	149.00
14	28.387	1004427	21.14	132963	14.53	Methyl palmitate	74.05
15	28.617	81294	1.71	20908	2.28	TETRATRIACONTANE	57.05
16	29.547	34866	0.73	13992	1.53	11-Methyldodecanol	70.05
17	29.676	190793	4.02	49773	5.44	(TRANS)-2-NONADECENE	57.05
18	29.817	60347	1.27	10875	1.19	TETRATRIACONTANE	57.05
19	31.608	136424	2.87	24030	2.63	2,11-Dodecadiene, 4-chloro-	67.05
20	31.683	114477	2.41	28516	3.12	DIMETHYL TRIDECANEDIOATE	84.10
21	31.793	203433	4.28	33994	3.71	tert-Hexadecanethiol	57.05
22	32.208	801257	16.87	152105	16.62	METHYLSTEARATE	174.05
23	33.259	39264	0.83	9830	1.07	1-OCTANOL, 3,7-DIMETHYL-	70.05
24	33.358	178134	3.75	20299	2.22	n-Tetracosanol-1	56.00
25	33.635	106393	2.24	16992	1.86	OCTADECANOIC ACID, 2-HYDROXY-, METHYL ESTER, (.+-.)-	99.10
26	33.852	40418	0.85	15921	1.74	4-Chlorobutyric acid, octadecyl ester	69.10
27	35.706	118573	2.50	12135	1.33	TRIDECANOIC ACID, 4,8,12-TRIMETHYL-, METHYL ESTER	87.00
28	38.948	240587	5.06	60521	6.61	1,2-BENZENEDICARBOXYLIC ACID	149.00
		4750492	100.00	915069	100.00		

IV. DISCUSSION

In present study we analyze the future of *Oldenlandia corymbosa* as antibacterial as well as anticancer agent. In past few decades, the research for new antibacterial agents has occupied. General considerations established for the study of antibacterial activity of plant extracts and the compounds isolated from them(19). Previous study reported that the methanolic decoctions of *Oldenlandia sps* exhibited anibacterial activity against *Klebsiella pneumoniae*(20). In our study the compound shows strong antibacterial activity against *K.pneumoniaea* with zone of inhibition of 16mm in disc diffusion method and the concentration 15µg/ml shows 21mm zone of inhibition in MIC. The result reveals that the compound has significant antibacterial activity against *K.pneumonieae* and further study is necessary.

Previous studies determined the medicinal properties of various extracts of *Oldenlandia corymbosa* plant (21-23). The present study shows that the compound from *Oldenlandia corymbosa* has significant antioxidant as well as anticancer activity. The compound exhibited strong antiproliferative activity against cancer cell line. The compound effectively inhibited the growth of MCF-7 cell line with IC_{50} value of 0.27±0.18 mg/ml. The significant anticancer and antioxidant property of the compound promises it as an effective compound in drug industry. It is only a preliminary study of the anticancer property of Methanol fraction, an in depth study will provide a good concrete base for pharmacological activity of compound.

It is the first time GC-MS analysis of Methanol fraction was done, no previous data are available for same compound. From GC-MS analysis it was clear that compound contains high concentrations of saturated fatty acids (Methyl stearate(16.62%) and Methyl plamitate (14.53%)) hence it may be a fatty acid derivative. Its high anti cancer property may due to the presence of these fatty acid compounds. GC-MS analysis is the first step towards understanding the nature of active principles in compound and it will be helpful for further detailed study.

Conflict of interest

No potential conflict of interest was declared by the authors.

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