PHYSICOCHEMICAL SCREENING; IDENTIFICATION OF ANTICANCER, ANTIBACTERIAL AND ANTIOXIDANT PRINCIPLES BY FRACTIONATION AND GC-MS PROFILING OF FRUITS OF *GUAIACUM OFFICINALE L.*

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ABSTRACT: The present work is an attempt to find out biological potential of various extracts and ethanol fractions of *Guaiacum officinale* L. Sequential extraction was performed with different solvents. One of the active extracts (ethanol) was fractionated by column chromatography and the other (*n*-hexane) was profiled by GC-MS. The antimicrobial potential was tested against pathogenic bacteria by a well diffusion process and only one strain (*S. aureus*) displayed a significant resistance against different samples. For the cytotoxic effect, MTT assay was used on normal (BHK) and cancerous (HepG2) cell lines. *n*-Hexane, chloroform and ethanol extracts showed IC_{50} at 3.38, 3.56 and 2.607 mg/ml respectively. Furthermore, two fractions (i.e. F=2 and 3) fractions showed significant IC_{50} at 74.9 and 130.8 µg/ml respectively compared to the standard (Cisplatin, IC_{50} =16 µg/ml). No toxic effect was experienced against the normal cell line, showing it is safe in cancer therapy. The DPPH analysis revealed a significant antioxidant potential of the plant like *n*-hexane, chloroform and ethanol represented 68.67%, 73.00% and 77.67% RSA respectively, whereas F=1 to F=7 along with ascorbic acid showed moderate to less antioxidant effect, i.e. 55.93%, 68.56%, 50.19%, 9.64%, 28.40%, 12.63%, 32.54%, and 81.53%, correspondingly. Based on the above findings, it is highly recommended for further purification, isolation and identification of biomolecules from fruits of *G. officinale* as a possible extension of the present project.

Key words: *Guaiacum officinale* L., physicochemical, MTT assay, well diffusion, DPPH assay, GC-MS.

(Received 05.01.2021 Accepted 26.02.2021)

INTRODUCTION

With the evolution of life, human problems such as the prevalence of serious infections, recurrence, microbial resistance and the emergence of new diseases had focused researchers' attention on the exploration of novel drugs from natural sources such as plants, animals, microbes, etc. Plants are given preference over other sources due to less undesirable effects, structural diversification and ease of access (Gupta & Raina, 1998; Miraj & Alesaeidi, 2016). According to the World Health Organization (WHO), nearly 50,000 clinically important plants are being used in the pharmaceutical industry as traditional medicines (Msomi, N. Z., 2018). *Guaiacum officinale*, one of the traditional medicinal plants is effective against fish poisoning, HIV, as an abortifacient, in angina, tonsillitis, rheumatoid arthritis, mucosal diseases and metabolic abnormalities since ancient times (Ahmad, Bano, & Bano, 1984; Saba, Khatoon, Ali, & Ahmad, 2012). It is a perennial tree, slow-growing and predominantly existing in the West Indies, but are also distributed in different countries such as USA, Honduras, Panama, India and Pakistan (Cooper, 1986; Grandtner, 2005).

An irregular, rapid and uncontrolled division of cells is known as cancer. Nowadays, cancer has become a scary phase in both advanced and developing countries. A wide range of phytochemicals like vincristine, vinblastine, Paclitaxol, Taxol, Docetaxel, deacetyl baccatin III, Podophylotoxin, Demethylpodophylotoxin, α-peltatin, β-peltatin, Topotecan, Vindesine, Vinoselbine etc. have been well documented as anticancer drugs and even more study is going into this area (Kaur, Kapoor, & Kaur, 2011).

The imbalance of redox processes can lead to several life-threatening problems such as cancer, diabetes, atherosclerosis, arthritis, aging, etc. (Farooq Saleem *et al*., 2019). With the practice of natural and synthetic antioxidants, oxidative damage may be reduced. Nevertheless, in practice, synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been limited in food due to

the carcinogenic consequence. Therefore, the usage of natural antioxidants is preferred over synthetic (Kiran *et al*.).

The chemicals/drugs responsible for killing or inhibiting bacterial growth are called antibiotics/antibacterials (Gangrade, Lad, & Bhatia, 2017). In the world, especially in developing countries, healthy life has become a victim of bacterial infections (Nathan, 2004). The natural products being trustworthy and economical than synthetics are used abundantly. Flora is considered the best source for the discovery of novel, effective and therapeutically potential antibacterials (Gupta & Raina, 1998). Present research work was conducted to assess the proximate analysis, anti-cancer, anti-bacterial and antioxidant potential of *G. officinale*.

MATERIAL AND METHODS

Chemicals: All chemicals and reagents used during experimentation were of analytical grade. Ciprofloxacin and methicillin were gifted by Drug Testing Laboratories (DTL), Lahore, Pakistan.

Plant Material (Collection and Identification): The fruits of *G. officinale* were collected from the local Karachi area in Pakistan and were identified by Dr. Zaheer-ul-Deen Khan, chairman, Botanical Department of Government Collage University (GCU), Lahore, Pakistan. The specimen was submitted to the GCU Herbarium to take its voucher number (GC.herb.bot.3382-A) by the competent authority.

Cell lines: Two cell lines i.e. HepG2 (cancer cell line) and BHK (normal cell line) were arranged for anti-cancer tests.

Microorganisms: Ten pathogenic bacterial strains including five Gram-positive (*Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Micrococcus luteus* ATCC 4698 and *Staphylococcus epidermidis* ATCC 12228) and five Gram-negative (*Escherichia* coli ATCC 25922, *Klebsiella pneumoniae* ATCC10031, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhinium* ATCC 14028 and *Pasteurella multocida* ATCC 12945) were gifted by PCSIR (Pakistan Council of Scientific and Industrial Research) of Lahore, Pakistan to perform the antibacterial effect.

Extraction and Isolation: The fruits were dried in the shade (to avoid photochemical degradation), ground and passed through 80 mesh screen to obtain a fine powder. One kg of dry powder was packed in a pre-weighed Soxhlet apparatus and subjected for extraction with *n*-hexane, chloroform and ethanol in chronological order. The solvent extracts were filtered and evaporated under reduced pressure to get dried extracts. Column chromatography (CC) was performed using silica gel 60. The gradient elution was achieved using dichloromethane-methanol (DCM-MeOH) combination in ascending order of polarity. The fractions having similar profiles were pooled and monitored under the UV lamp (254 and 365 nm wavelengths) for spot visualization.

Proximate analysis: Proximate/physicochemical analysis was conducted to find the values of moisture content: total ash**,** acid-insoluble ash**,** acid-soluble ash, water -insoluble ash, water-soluble ash, sulphated ash**,** alcohol-soluble extractives (at room temperature and 60^{o}C) and water-soluble extractives (at room temperature and 60^{o}C) (Organization, 1998).

Antioxidant assay: The antioxidant potential of *G. officinale* samples was tested by 2,2-diphenyl-1-picryl hydrazyl (DPPH) model with minor modification. The radical scavenging activity (RSA) was calculated by the following formula (Farooq Saleem *et al*.,2016).

$$\% \textbf{ RSA} = \frac{\boldsymbol{A_c - A_s}}{\boldsymbol{A_c}} \times \mathbf{100}$$

Where, A_s = Absorbance of sample, A_c = Absorbance of control

Antibacterial assay: The antibacterial assay of samples was carried out by well diffusion method with minor changes. Different dilutions of plant extracts, and fractions (i.e. 5 mg, 50 mg and 100 mg/ml) and standard antibiotics i.e. ciprofloxacin and methicillin (1 mg/ml) were prepared with DMSO (dimethyl sulfoxide) and tested against selected strains. Efficacy was evaluated by measuring the zones of inhibition (Frooq Saleem, Khan, Mumtaz, Khan, & Jamshaid, 2008).

Anticancer assay/*In-vitro* cytotoxicity (MTT test): The Rate of cell proliferation can be measured by MTT assay. MTT test (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliuum bromide) was performed to find out antitumor effect of test samples (Kiran *et al*.). Stock solutions (5 mg/ml) of samples were prepared with methanol. Two cell lines HepG2 as cancerous and BHK-21 as normal were arranged by CRIMM department of The university of Lahore, Pakistan. The HepG2 and BHK-21 were incubated and given exposure with test samples for 24h and 48h at 37°C respectively. The incubated layer of cells was treated with PBS (pH 7.4) and each well of 96-well plate was loaded with 100 µl medium having 25 µl MTT solution. In living cells MTT solution was turned into purple-colored formazan after 3h incubation and then was solubilized with 10% sodium dodecyl sulphate (SDS). Cisplatin, as an anticancer drug (11) was used here as a positive control at concentrations of 14, 16 and 18 µg/ml. Lastly, the absorbance of the solution was observed at 570 nm by ELISA reader. Cell

survival percentage (CSP) was calculated using the following equation (Kiran *et al*.).

CSP = (Mean optical density of test chemical − mean optical density of negative control x 100)/ Mean optical density of positive control: Statistical analysis: For statistical analysis, Two-way ANOVA was applied. While the percentage of cell survival was assessed by non-linear regression equation on the graph pad prism 7.0.

GC-MS Analysis: The analysis was carried out on biologically active non-polar extract (i.e. *n*-hexane) of *G. officinale*. QP 2010 gas chromatography (Shimadzu, Japan), equipped with Shimadzu Technology DB 05 capillary column was directly coupled to the detector MS. A 15 m long capillary silica column was used, bonded with 5% crosslinked diphenyl and 95% dimethylpolysiloxane (as stationary phase) to achieve maximum separation. The temperature of injector and source were set at 200°C and about 1 µl of the sample was injected into the column with a split ratio of 1:10. Helium gas (mobile phase) was used at a constant flow rate of 1.0 ml/min. The oven temperature was programmed as starting temperature = 50°C, held for 3 minutes then increased to 320°C at a ratio of 10°C/min and maintained for 3 minutes. Throughout the operation, the temperature of the mass selective detector (MSD) was maintained at 250°C. The mass range for scanning was 50-550 at a scanning time of 0.5 sec. Each sample was run in triplicate and *n*-hexane solvent was used as a blank. The components eluted during GC were identified by comparison of their mass spectra with spectra of NIST (2010) mass spectra library.

RESULTS AND DISCUSSION

Extraction and Isolation: The extraction process with *n*-hexane, chloroform and ethanol yielded 72.24 (7.22%), 3.38 (0.34%) and 83.14 g (8.31%) dried extracts respectively (Table 1). Plenty of literatures confer the presence of various bioactive saponins in G. *officinale* fruits with fair solubility in ethanol (Ahmad *et al*., 1984). This diverted our attention towards initial extractions with *n*-hexane and chloroform to separate non-polar and partially polar constituents, leaving behind saponin-rich plant residue which was extracted with ethanol.

The dried ethanol extract was fractionated through column chromatography and collected seven fractions. Two bioactive fractions (F=2 and 3) were further chromatographed to obtain seven and seventeen sub-fractions respectively. The sub-fractionation of both fractions was achieved with $CHCl_3$: MeOH combination starting with 100% $CHCl_3$.

Proximate Analysis: Proximate analysis is the first account of *G. officinale* and its results are summarized in Table 2. It encloses moisture content, total ash, acid-insoluble ash, acid-soluble ash, water-insoluble ash, water-soluble ash, sulphated ash, alcohol soluble extractive (at 25°C and 60°C) and water-soluble extractive (at 25°C and 60°C) values. These results were compared with standard WHO methods. According to the results, the moisture content was about 19.23%, which did not fall within the standard range, i.e. 0 - 13%. This estimation determines that the plant is hygroscopic in nature, so it should be stored safely to avoid microbial growth and hydrolytic degradation. Because moisture tends to accelerate microbiological contamination and hydrolytic damages (Waterman *et al*., 2002). The total ash contents or ignition value was about 5% and is in the range (i.e. less than 13%). Part of the herbal material that remains after ignition, known as ash, and can be calculated in three ways, i.e. acid-insoluble, water-soluble and total ash (Sadhu *et al*., 2015). Total ash includes both physiological as well as non-physiological ash, which reflects the diagnosed purity of the plant. Burning living cells/tissues of plant results in physiological ash, while environmental pollutants deposited on the plant surface represent non-physiological ash (Organization, 1998). The percentages of acid-insoluble and acid-soluble ash were 1.13% and 4.03%, respectively, within the official limit (0.5-5.5%). The value of insoluble ash reflects the percentage of inorganic impurities in plants (Organization, 1998). Since the value of insoluble ash is greater than that of acid-soluble ash, the plant is rich in the environmental contents. 4.02% and 2% were respectively water-insoluble and soluble ashes. Water-soluble extractives at 60°C and 25°C were found 52.26% and 50.50% respectively which reflect the nature of phytochemicals in plant. It was observed that the alcohol-soluble extractive values were respectively 19.83% and 17.30% at 25°C and 60°C. Since the values of water-soluble extractive at 25°C and 60°C were high compared to the values of alcohol-soluble extractive which indicates the water is a better solvent for the extraction of *G. officinale* (Ozarkar, 2005).

Antibacterial assay: Fresh microbial cultures were used to detect the antibacterial potential of test samples. Two samples, i.e. *n*-hexane and ethanol extracts (as well as few fractions) displayed zones of inhibition between 10-18 mm at a concentration of 100 mg/ml against some strains. The *n*-hexane extract did not show zone of inhibition against *K. pneumoniae*, *S. aureus* and *P. aeruginosa* at any dose, although its significant effect was observed with respect to the remaining bacteria compared to standard antibiotics (table 3). The antibacterial effect of *n*-hexane extract may be due to the presence of Celesticetin. Celesticetin exists as one of the natural antibiotics (Hanada *et al*., 1980) and was also identified during GC-MS analysis of *n*-hexane extract (Table 13).

The chloroform extract revealed moderate to significant antibacterial effect in terms of zone of inhibition against *S. typhus, E. faecalis* and *P. multocida* and could be related to the presence of slightly polar components such as flavonoids (Xie *et al*., 2015). Like *n*-hexane, ethanol extract also displayed significant outcomes against *B. cereus, S. typhus, E. faecalis, P. multocida, P. aeruginosa, E. coli* and *K. pneumoniae*. Ethanol fractions were tested against those bacterial strains that were susceptible to ethanol extract. Of these three fractions (F=1 to F=3) gave moderate to significant effects against certain bacteria except *K. pneumoniae*, while the rest of the fractions (F=4 to F=7) did not display any effect (Table 4). The antibacterial potential of the ethanol extract and its fractions could be related to the presence of highly polar classes of secondary metabolites, such as alkaloids and polyphenols (Mabhiza *et al*., 2016, Hanada *et al*., 1980, Bahadori *et al*., 2015).

Methicillin, a narrow-spectrum β-lactam antibiotic and structural analogs of D-alanyl-alanine, competitively inhibits transpeptidase enzyme which in turn inhibits cross-linkage between the linear peptidoglycan polymer chains that make up a major component of the cell wall of bacteria, substantially inhibiting the synthesis of cell walls (Gladwin *et al*., 2004). Whereas Ciprofloxacin is a broad-spectrum bactericidal antibiotic belonging to fluoroquinolone class and functions by inhibiting bacterial cell division (Drlica *et al*., 1997). The data reflected that all extracts and ethanol fractions at the dose of 100 mg/ml showed significant zones of inhibition against both Gram positive and Gram negative bacteria as compared to 5 and 50 mg/ml doses. Thus, it can logically be concluded that the antibacterial activity of the studied samples can be related to inhibition of the cell wall or cell division in the same way as the function of ciprofloxacin and methicillin. No antibacterial activity of *G. officinale* had previously been reported but one of the species of *Guaiacum* (i.e. *G. coulteri*) was shown to have anti-tuberculosis and anti-Halictobectar activity (Robles-Zepeda *et al*., 2013, Wang, 2014).

Antioxidant Activity: The DPPH assay was applied to assess the antioxidant potential of *G. officinale*. The Extent of discoloration of DPPH solution gives an estimation of radical scavenging activity (RSA) of test samples (Saleem *et al*., 2016) .After testing, the potential was calculated as % RSA at a concentration of 50 µg/ml. Mostly samples revealed significant outcomes as compared to ascorbic acid (standard). Extracts of *n*-hexane, chloroform and ethanol represented 68.67%, 73.00% and 77.67% RSA respectively, whereas F=1 to F=7 along with ascorbic acid showed moderate to less antioxidant effect, i.e. 55.93%, 68.56%, 50.19%, 9.64%, 28.40%, 12.63%, 32.54%, and 81.53%, correspondingly (Table 5, Figure 1). The findings indicate the plant is rich in antioxidant potential and can be correlated with the work of Suthira Maneechai (Mabhiza *et al*., 2016). The presence of phytochemicals, such as alkaloids and polyphenols, could be the possible reasons for the stronger antioxidant potential of *G. officinale* (Gan *et al*., 2017).

Uncontrolled and imbalanced reactive oxidative species (ROS) are produced in living things which cause oxidative damage to biomolecules including proteins, lipids, lipoproteins and DNA. These ROS cause several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, and neurodegenerative diseases. Antioxidants, in different concentrations and combinations are used to prevent this damage (Sies, 1986, Sies, 1993). To cope with such challenges, plants have been focused on the attainment of antioxidant entities. Polyphenols have been reported to be potent phytochemicals which donate hydrogen to the DPPH radicals due to their unique structural chemistry (Siddhuraju, 2006). Owing to the strong antioxidant results of *G. officinale*, the plant can be tested for further activities.

Anticancer Activity: Research has shown that there is a strong relationship between antioxidants and anticancer drugs. Since antioxidants neutralize ROS which in turn protect living organisms from DNA/protein damage and lipid peroxidation, various human diseases such as cancer, gout, atherosclerosis, etc. are cured by them (Li *et al*., 2007).

Due to the strong antioxidant behavior of *G. officinale*, its anticancer potential was also tested against HepG2 (cancer cell line) and BHK (normal cell line) and showed a dose-dependent response. The concentration at which 50% of the cells are killed is called IC_{50} (Kiran *et al*.). The IC_{50} was calculated by graph pad 7.0 prism. Both HepG2 and BHK-21 cell lines were given the treatment of *n*-hexane, chloroform, ethanol and its fractions at various concentrations. Cisplatin (positive control) depicted IC_{50} at 16 µg/ml (table 7(b), fig 3). Compared to the standard drug, the *n*-hexane extract was most effective in inhibiting HepG2 cell lines, followed by the ethanol and chloroform extracts. IC_{50} of *n*-hexane, chloroform and ethanol extracts were 3.38, 3.56 and 2.607 mg/ml (table 6, fig 2) against HepG2 cell line with a better safety profile against a non-cancer cell line (BHK). The anti-tumor activity of ethanol fractions was also carried out to calculate IC_{50} by a graphic pad. These fractions showed anomalous behavior against cancerous cell lines. IC_{50} of F=1, 4, 5, 6 and 7 was found in mg/ml, i.e. 3.56, 2.705, 3.99, 4.83 and 4.95 respectively, while for F=2 and 3 it was observed in µg/ml (i.e. 74.9 and 130.8) and has been given in table 7(a & b), fig 4 & 5).

The antitumor activity of *n*-hexane extract may be due to the synergistic effect of secondary metabolites. Literature survey reveals that ethanolic extracts are

always rich in secondary metabolites that become responsible for the anticancer potential (Grigalius and Petrikaite, 2017). There is a massive difference between the doses of standard drug and extracts tested, logically reflecting the involvement of different mechanisms of action in the anti-tumor activity. These results are in accordance with previously conducted research work by Karla Claudio Campos, B.S. where ethanolic/methanolic leaf extracts of *G. officinale* depicted cytotoxicity against the breast adenocarcinoma cell lines MCF-7, ZR-75-1 and T47D. While *Artemia salina* was considered as a normal cell line (Campos *et al*., 2015).

The antitumor results can be linked with spirocyclic lignans, ramonanins A–D (7–10), isolated from *G. officinale* and *G. sanctum*. These ramonanins are cytotoxic against human breast cancer cell lines MDA-MB-231 (Campos *et al*., 2015).

Cytotoxic Effect against Normal Cell Line: The maximum concentrations that showed IC_{50} against HepG2 cell line, were also tested against BHK (normal) cell line. All test samples of *G. officinale* did not show any marked cytotoxic effect against normal cell line which reveals its safe use for normal body cells. Rather for fractions F=6 and 7, the % viability was raised above 100%, reflecting that both fractions provided a suitable environment for the propagation of normal body cells. Because of high antioxidant and strong anticancer potential, *G. officinale* should be focused on further scientific research. The results are summarized in Table 8.

GC-MS analysis: The active *n*-hexane fraction was profiled by GCMS to find the possible components responsible for its biological potential**.** Gas chromatogram was shown in Figure 5. For identification and characterization of separated components during gas chromatography, spectra were compared to known compounds available in the library (NIST), and further authentication was completed in terms of the name, molecular weight, retention time, area percentage and structure of each separated (table 9). A total of nine compounds were identified during GCMS analysis, and of the five were major (i.e. di-n-octyl phthalate; heptadecane, 2-methyl-; phenol, 2-methoxy-5- (1-propenyl)-, (E)-; 7-hexadecenoic acid, methyl ester, (Z) and 1-octanol, 2-butyl-). According to the literature review, identified esters possess good antioxidant potential (Aragão *et al*., 2006). A number of commercial uses are related to methyl ester compounds (Foresti *et al*., 2005). Celesticetin, one of the established natural antibiotics (Mabhiza *et al*., 2016, Hanada *et al*., 1980, Bahadori *et al*., 2015) has also been traced during the analysis. The identified compounds have strong potential roots in pharmaceutical fields, so it can be suggested for further isolation and purifications to get pure bioactive molecules.

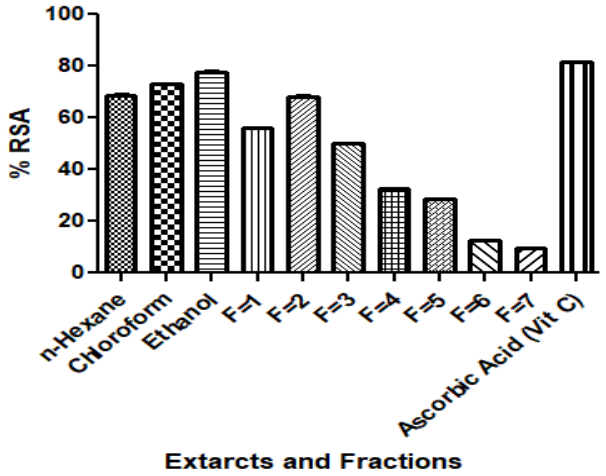


Figure 1. Antioxidant activity of extracts and ethanolic fractions of *G. officinale* using DPPH method. Each value of mean is average of three repeated experiments ± standard error (S.E.).

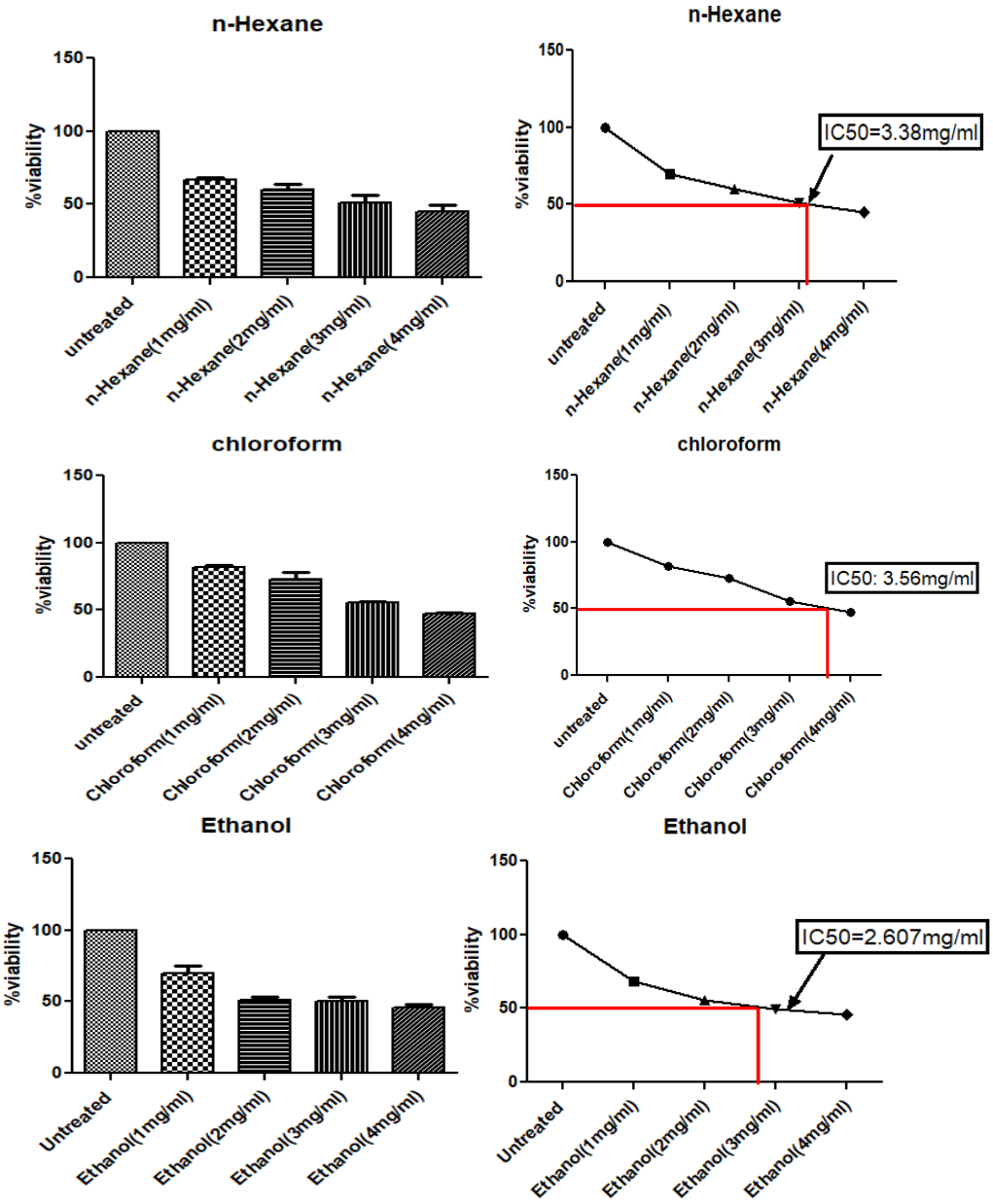


Figure 2. ***IC*$_{50}$ of *n*-hexane, chloroform and ethanol extracts**

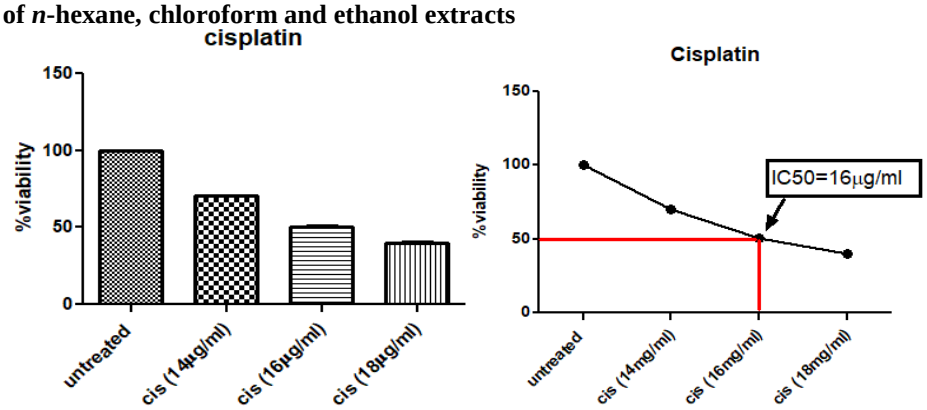


Figure 3. ***IC*$_{50}$ of Cisplatin against HepG2.**

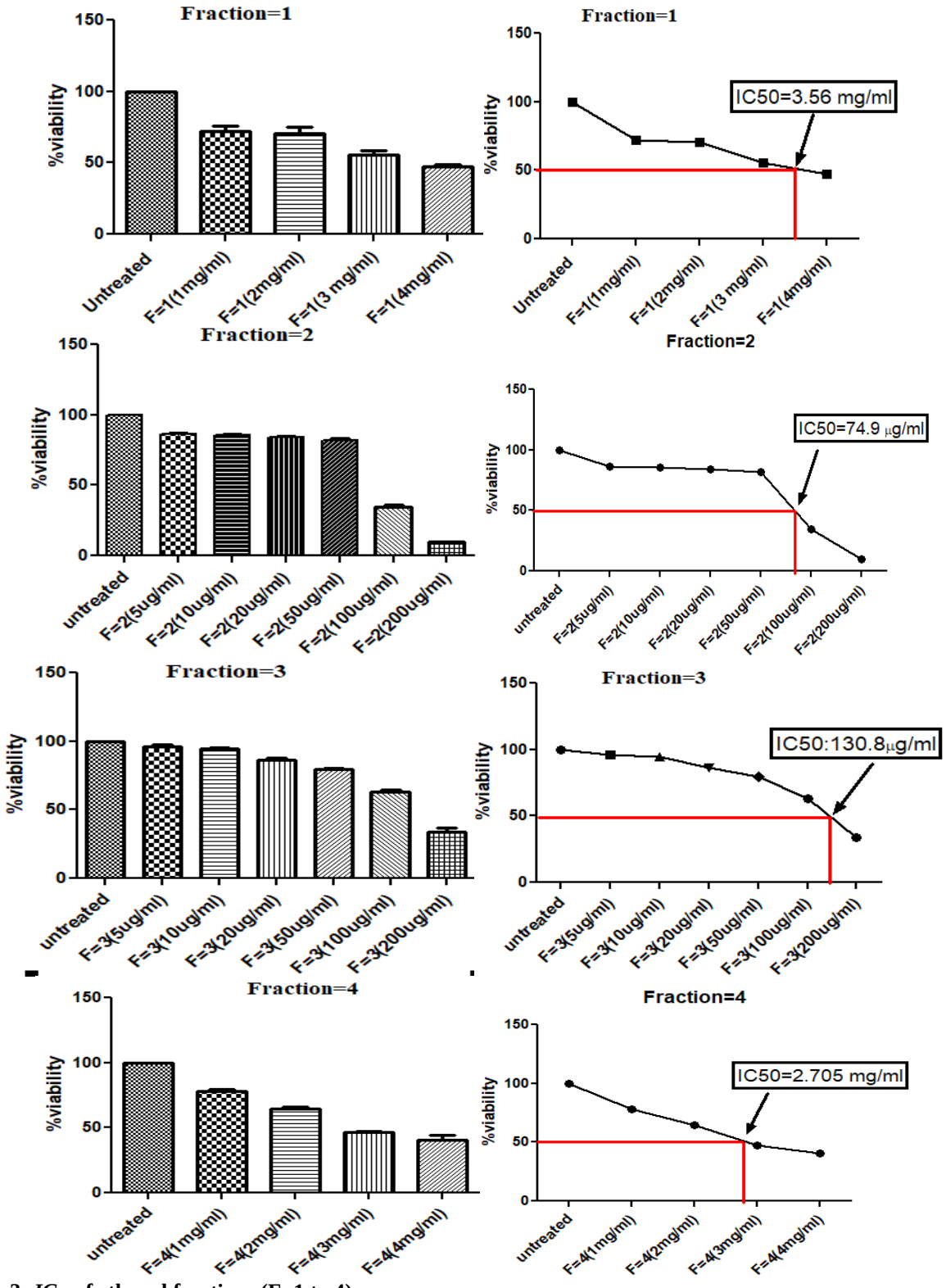


Figure 3: *IC*$_{50}$ of ethanol fractions (F=1 to 4)

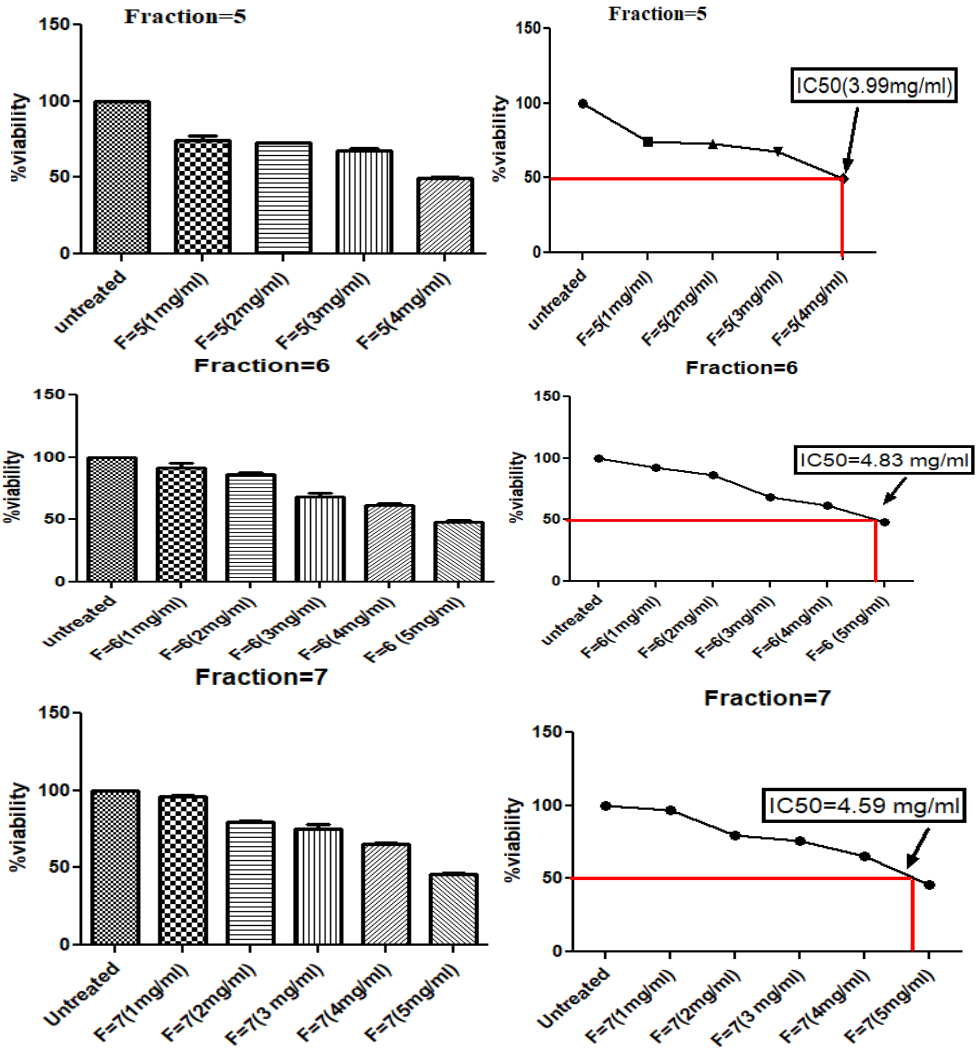


Figure 4: *IC*$_{50}$ of ethanol fractions (F=5 to 7).

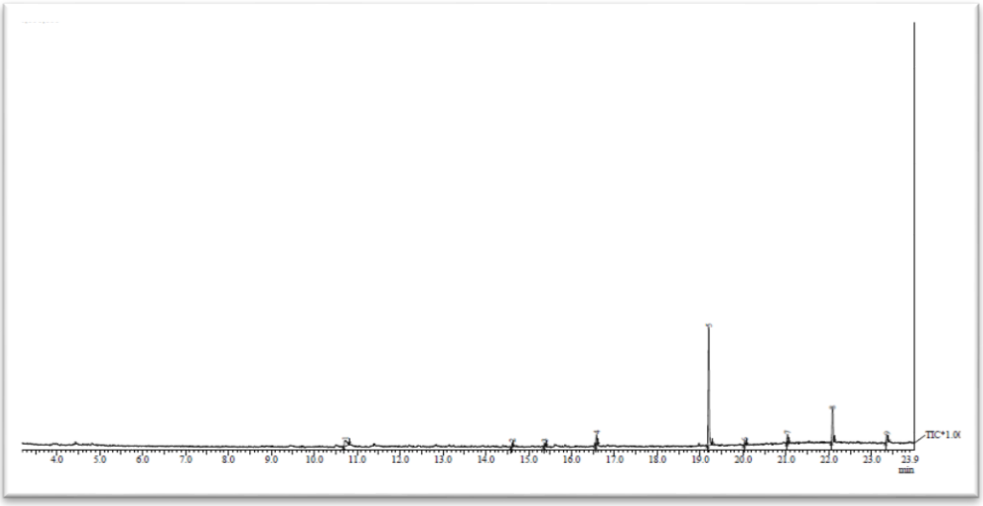


Figure 5. Gas chromatogram of *n*-hexane extract

Table 1. General table of dried extracts.

Sr. No.	Extracts	Quantity (g)	% age
1	*n*-Hexane	72.24	7.224
2	Chloroform	3.379	0.337
3	Ethanol	83.14	8.314

Table 2. Proximate analysis of *G. officinale*.

Sr. No	Properties	Mean±S.E.
1	Moisture contents	19.23±0.14
2	Total ash	05.00±0.00
3	Acid insoluble ash	01.13±0.08
4	Acid soluble ash	04.02±0.01
5	Water insoluble ash	04.02±0.01
6	Water soluble ash	02.00±0.00
7	Sulphated ash	13.40±0.45
8	Water soluble extractive value (R.T.)	50.50±0.00
9	Water soluble extractive value (60^{o}C)	52.26±0.14
10	Alcohol soluble extractive value (R.T.)	19.83±0.03
11	Alcohol soluble extractive value (60^{o}C)	17.30±0.09

Table 3. Antibacterial activity of different concentrations (5, 50 and 100 mg/mL) of *n*-hexane, chloroform and ethanol extracts of *G. officinale.* Each value represents the mean ± standard error of diameter of zone of inhibition (mm) in triplicates.

	Bacterial Strains Mean of zones of inhibition ± S.E									
Extracts	***B. cereus***	***S. typhus***	***S. aureus***	***M. luteus***	***E. faecalis***	***P. multocida***	***S. epidermidis***	***P. aeruginosa***	***E. coli***	***K. pneumoniae***
***n*-Hexane**										
5 mg/Ml	16.16±0.60	10.83±0.83	-	15±0.57	11.33±0.66	14±0.99	14.66±0.33	-	12.16±0.16	-
50 mg/mL	17.83±0.44	11.16±0.16	-	15.5±0.28	15.33±0.33	15.16±0.16	16±0.57	-	13±0.28	-
100 mg/mL	20.16±0.16	16.83±0.60	-	17.66±0.33	16.66±0.16	17.33±0.33	18.33±0.88	-	13.06±0.23	-
Chloroform										
5 mg/mL	-	10.83±0.44	-	-	10±0.57	13±0.57	-	-	-	-
50 mg/mL	-	15.83±0.44	-	-	13.66±0.33	14±0.57	-	-	-	-
100 mg/mL	-	17.16±0.44	-	-	`16±0.00	15.06±0.06	-	-	-	-
Ethanol										
5 mg/mL	16.5±0.28	13.5±0.28	-	-	10.83±0.44	13.33±0.33	-	12.00±0.57	12.1±0.20	10.16±0.33
50 mg/mL	18.16±0.16	14±0.00	-	-	14±0.57	14.33±0.66	-	12.5 ± 0.28	12.83±0.44	10.33±0.60
100 mg/mL	12.5±1.32	15±0.00	-	-	14.66±0.33	17.66±0.33	-	13.16± 0.16	13.83±0.44	14.66±0.16
MET (1 mg/mL)	26.33±0.33	-	23.66±0.33	24.66±0.33	20 ± 0.00	24 ± 0.00	-	24.33±0.33	20.66±0.33	20.66±0.33
CIP (1mg/mL)	28.66±0.33	-	26.00±0.00	20.33±0.33	28±0.00	28±0.00	-	24.33±0.33	22.33±0.33	20.66±0.33

Table 4. Antibacterial activity of ethanolic fractions. Each value represents the mean ±standard error of diameter of zone of inhibition (mm) in triplicates.

	Bacterial Strains Mean of zones of inhibition ± S.E						
Fractions no.	***B. cereus***	***S. typhus***	***E. faecalis***	***P. multocida***	***P. aeruginosa***	***E. coli***	***K. pneumoniae***
F=1							
5 mg/mL	12.30±0.21	-	10.44±0.44	-	-	-	-
50 mg/mL	12.50±0.11	-	12.10±0.57	10.23±0.33	-	12.83±0.44	-
100 mg/mL	13.16±0.10	-	13.66±0.33	13.43±0.66	-	13.83±0.22	-
F=2							
5 mg/mL	11.12±0.21	13.25±0.28	-	10.83±0.44	12.22±0.23	11.20±0.21	12.15±0.21
50 mg/mL	13.50±0.32	13.01±0.00	13.16± 0.16	13.33±0.33	12.50 ±o.11	12.50±0.32	12.55±0.02
100 mg/mL	19.16±0.16	14.51±0.00	16.66±0.33	14.66±0.16	13.16±0.16	14.50±0.47	14.23±0.01
F=3							
5 mg/mL	10.16±0.33	12.54±0.45	-	12.43±0.12	-	10.48±0.57	12.22±0.11
50 mg/mL	15.54±0.45	15.27±0.22	12.64±0.11	13.48±0.42	-	13.54±0.18	12.65±0.43
100 mg/mL	18.64±0.28	17.54±0.32	15.54±0.45	15.43±0.13	-	15.54±0.19	13.16±0.32
F=4							
5 mg/mL	-	-	-	-	-	-	-
50 mg/mL	-	-	-	-	-	-	-
100 mg/mL	-	-	-	-	-	-	-
F=5							
5 mg/mL	-	-	-	-	-	-	-
50 mg/mL	-	-	-	-	-	-	-
100 mg/mL	-	-	-	-	-	-	-
F=6							
5 mg/mL	-	-	-	-	-	-	-
50 mg/mL	-	-	-	-	-	-	-
100 mg/mL	-	-	-	-	-	-	-
F=7							
5 mg/mL	-	-	-	-	-	-	-
50 mg/mL	-	-	-	-	-	-	-
100 mg/mL	-	-	-	-	-	-	-
MET (1 mg/mL)	26.33±0.33	-	20.00± 0.00	24.00± 0.00	24.33±0.33	20.66±0.33	20.66±0.33
CIP(1 mg/mL)	28.66±0.33	-	28.00±0.00	28.00±0.00	24.33±0.33	22.33±0.33	20.66±0.33

Table 5: Antioxidant activity of extracts and ethanolic fractions of *G. officinale* using DPPH method. Each value of mean is average of three repeated experiments ± standard error (S.E.).

Serial no.	Extracts/ Fractions/standard	Concentrations	Mean % RSA ± S.E.
1	*n*-Hexane	50 µg	68.67 ± 0.33
2	Chloroform	50 µg	73.00 ± 0.00
3	Ethanol	50 µg	77.67 ± 0.33
4	F=1	50 µg	55.93 ± 0.30
5	F=2	50 µg	68.56 ± 0.29
6	F=3	50 µg	50.19 ± 0.03
7	F=4	50 µg	09.64 ± 0.00
8	F=5	50 µg	28.40 ± 0.00
9	F=6	50 µg	12.63 ± 0.00
10	F=7	50 µg	32.54 ± 0.01
11	Ascorbic Acid (Vit. C)	50 µg	81.53 ± 0.00

Table 6. Data represents the cell survival percentage of HepG2cell line at the different concentrations of various extracts of *G. officinale* and IC_{50}.

	Mean % Viability.± S.E. at different concentrations				
	4 mg/ml	**3 mg/ml**	**2 mg/ml**	**1 mg/ml**	IC_{50}
***n*- Hexane**	44.83 ± 4.84	51.10 ± 5.62	59.90 ± 4.08	66.70 ± 4.29	3.38 mg/ml
Chloroform	47.60 ± 0.67	55.40 ± 0.99	73.20 ± 5.15	81.60 ± 1.93	3.56 mg/ml
Ethanol	45.70 ± 2.07	50.40 ± 2.92	51.20 ± 1.97	69.80 ± 5.16	2.607 mg/ml

Table 7(a). Data represents the cell survival percentage of HepG2cell line at the different concentrations of ethanol fractions and IC_{50}.

	Mean % Viability.± S.E. at different concentrations				
	4 mg/ml	**3 mg/ml**	**2 mg/ml**	**1 mg/ml**	IC_{50}
F=1	47.40 ± 1.75	55.50 ± 3.11	70.30 ± 4.59	71.8 ± 4.29	3.56 mg/ml
F=4	40.70 ± 4.05	47.00 ± 0.75	64.9 ± 1.19	78.2 ± 1.33	2.705 mg/ml
F=5	49.6 ± 2.80	67.9 ± 0.64	72.5 ± 0.57	74.1 ± 3.40	3.99 mg/ml
F=6	61.8 ± 1.37	68.7 ± 2.50	86.2 ± 1.79	92.0 ± 3.12	4.83 mg/ml
F=7	65.2 ± 1.06	75.5 ± 2.42	79.3 ± 1.08	96.5 ± 0.7	4.95 mg/ml

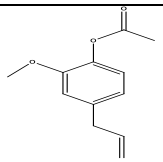
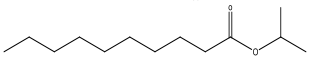
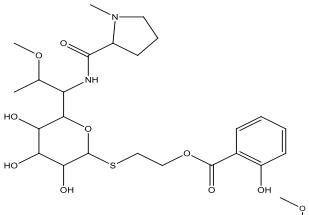
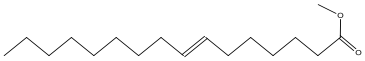
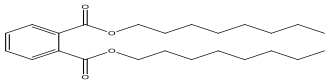
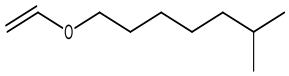
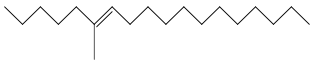
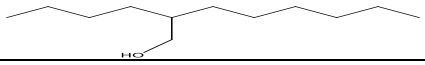
Table 7 (b).

				Mean % Viability.± S.E. at different concentrations			
	200 µg	**100 µg**	**50 µg**	**20 µg**	**10 µg**	**5 µg**	IC_{50}
F=2	9.52 ± 1.86	34.4 ± 0.59	81.6 ± 1.79	83.8 ± 0.80	85.8 ± 0.41	86.7 ± 0.75	74.9 µg/ml
F=3	34.0 ± 2.52	62.8 ± 1.55	79.5 ± 0.92	86.5 ± 1.21	94.5 ± 0.56	96.2 ± 1.19	130.8 µg/ml
Cisplatin							16.0 µg/ml
untreated				100 ± 0.00			

Table 8. % viability against BHK cell line.

Name of extracts	Concentration	% viability
Untreated	No treatment	100.0 %
n-Hexane	3.57 mg	90.00 %
Chloroform	3.76 mg	86.85 %
Ethanol	3.65 mg	100.0 %
F=1	4.03 mg	96.72 %
F=2	1.87 mg	98.25 %
F=3	81.57 µg	81.94 %
F=4	4.25 mg	85.57 %
F=5	79.5 µg	93.30 %
F=6	4.88 mg	115.93 %
F=7	5.35 mg	103.3 %

Table 9. Names of compounds with their RT, names, molecular masses, % area and structures.

Peak No.	RT	Compounds Name	Molecular mass	% Area	Structure
1	10.726	Phenol, 2-methoxy-5-(1-propenyl)-, (E)-	164	7.90	
2	14.624	n-Capric acid isopropyl ester	214	2.03	
3	15.376	Celesticetin	528	1.75	
4	16.583	7-Hexadecenoic acid, methyl ester, (Z)	268	4.48	
5	19.198	Di-n-octyl phthalate	390	55.39	
6	20.041	Isooctane, (ethenyloxy)-	156	1.69	
7	21.036	6-Methyloctadecane	268	3.94	
8	22.087	Heptadecane, 2-methyl-	254	18.19	
9	23.361	1-Octanol, 2-butyl-	186	4.64	

Conclusion: *G. officinale*, an important medicinal plant was subjected to find out its physicochemical and biological potential. The presence of a few promising phytochemical constituents reveal that plant has a great potential for its folk uses. Because of the satisfactory biological outcomes by all extracts, the ethanol extract was chromatographed for fractionation and sub fractionation via chromatography techniques. Antibacterial, antioxidant and anticancer activities by well diffusion, DPPH and MTT essays respectively on extracts and fractions have unfolded that fruits of *G. officinale* could be used safely against various ailments. Further investigation on *n*-hexane and few ethanol fractions could be extended as a future plan to get pure bioactive molecules.

Acknowledgment: I acknowledge with gratitude to Prof. Dr. Zaheer-ul-din Khan, Botany Department of Government College University, Lahore-Pakistan for identification and authenticity of *G. officinale*. I am also grateful for the sincere support of Dr. Muhammad Saeed, Associate Professor, Department of Chemistry, Lahore University of Management Sciences (LUMS), Lahore-Pakistan for providing his skills and laboratory facilities

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