PHYTOCHEMICAL AND PHYSICOCHEMICAL SCREENING; A BIODIRECTED HPLC AND GC-MS ANALYSIS OF SHOOT PART OF *GUAIACUM OFFICINALE* L.

M. Azeem[1], F. Saleem[*1], J. Iqbal[2], S. S. Hassan[3], S. Shoukat[1], A. A. Shah[1], A. Rashid[4] and Z. Khan[5]

[1]Faculty of Pharmacy, The University of Lahore, 1-KM Defence Road Campus, Lahore-Pakistan.
[2]Leads College of Pharmacy, Lahore Leads University, Lahore-Pakistan.
[3]Imran Idrees College of Pharmacy, 3KM Daska Road, Sialkot-Pakistan.
[4]Johar Institute of Professional Studies, 34-KM, Nabibux stop, Ferozpur Road, Lahore-Pakistan.
[5]Department of Botany, Government College University, Lahore, Pakistan.
Corresponding author email: farooq.saleem@pharm.uol.edu.pk

ABSTRACT: Medicinal plants have a potential source for countless medicinal compounds. The present study explores the therapeutic potential of aerial parts of *Guaiacum officinale*. Various analysis like phytochemical, physicochemical, antioxidant, antibacterial and anticancer of extracts and fractions were performed. Phytochemical results revealed the presence of few metabolites like alkaloids, carbohydrates, saponins and flavonoids whereas others gave moderate to low presence. For antibacterial assay ten bacterial pathogens were tested by well diffusion method and were observed sensitive mostly. Cytotoxic potential was explored through MTT test on BHK (normal) and HepG2 (cancerous) cell lines. Only ethanol extracts of leaf and shoot parts showed significant IC_{50} at 0.89 and 1.37 mg/ml concentrations respectively. Likewise, ethanol fractions like F=1, 3 to 5 predicted IC_{50} at 2.89, 2.96, 2.61 and 1.91 mg/ml respectively. No toxic effects were shown against normal cell line, reflecting the *G. officinale* has safe applications in anti-cancer therapy. The DPPH *in-vitro* analysis explored its profound antioxidant potential; ethanol shoot fraction (F=4) having significant % RSA (69.30 %).

Key words: Aerial parts, *Guaiacum officinale* L., anticancer, antibacterial, antioxidant, GC-MS.

(Received 08.02.2021 Accepted 02.06.2021)

INTRODUCTION

Humanity has been fighting diseases since ancient times and has used various countermeasures such as herbal therapy (Morse, 2001). Among the many resources of remedies, herbal medicines have proved the best, cheap and of high value (Pokharen *et al*., 2011). Even in the current era, 70-80% of the world's population prefers herbal treatment over other available facilities (Kamboj, 2000), because it is cost-effective, easily available, successful, devoid of many side effects and free from strict regulations (Stickel and Schuppan, 2007). Plants have proven themselves as promising new drug source leading to novel drug development (Shakya, 2016).

Oxidative processes in body are initiated by a variety of factors that ultimately can lead to a serious cell damage and many fatal disorders (like coronary heart disease, cancer, aging, etc.) (Devasagayam *et al*., 2004). Redox processes are generally balanced by homeostasis but sometimes there is a greater shift towards oxidation resulting in oxidative stress (Yoshikawa and Naito, 2002). Oxidative damage can be counteracted by antioxidants, available as natural sources like enzymes and non-enzymes, or synthetics sources (Kumar, 2011). However, natural antioxidants are preferred over synthetics due to their carcinogenic potential or other risky effects (Kiran *et al*., 2018). Plants have long been considered a very convincing remedy for several infections and many of them are still used in the treatment of several diseases, caused by oxidative damage (Heinrich *et al*., 2004). In past, the microbial problems were treated with conventional plant extracts. Although in previous decades a vast number of novel antibiotics have been developed. Meanwhile, the drug resistance has also increased at an alarming level both in hospitals and in the community (Nascimento *et al*., 2000). Therefore, it is obligatory to work in antimicrobial domain in the hope of finding novel and promising agents (Cushnie and Lamb, 2005).

Cancer is a fatal condition characterized by unusual, accelerated, unchecked cellular proliferation in any part of the body. Cancerous cells overrun, destroy and sabotage normal cells (Priya *et al*., 2013). Today, cancer is one of the leading public health problems around the world (Zugazagoitia *et al*., 2016). Different anti-cancer therapies are being used, such as chemotherapy, radiation therapy, surgery and immunosuppressive agents (Stewart and Kleihues, 2003). Medicinal plants can serve as a very resourceful alternative in the treatment and management of cancer

because they show much less side effects (Kinghorn and Kinghorn, 1979).

Guaiacum officinale, an evergreen, is generally distributed to many countries like the USA, Honduras, Panama, Ghana, India, and Pakistan (Orwa, 2009). It is used in folklore for a variety of diseases such as sore throat, tonsillitis, gout, arthritis, rheumatism, stomachache, vomiting, cuts and bruises, skin irritations, asthma, hypertension, bladder and kidney disease, diabetes, gonorrhea and syphilis (Halberstein, 1997). *G. officinale* is also reported to have nematocidal, abortifacient, anti-HIV, antidiabetic, molluscicidal, antioxidant, anti-inflammatory and anti- rheumatoid potential (Ibrahim *et al*., 2018; Maneechai and Pikulthong, 2017; Nakano *et al.*, 2017).

The present work focuses on the evaluation of the proximate analysis, antioxidant, antibacterial, and anticancer potential of extracts and fractions of aerial parts of *G. officinale*.

MATERIALS AND METHODS

Chemicals: The chemicals and reagents used during the tests were analytical grade. Ciprofloxacin and methicillin (GlaxoSmithKline) were gifted by Drug Testing Laboratories (DTL), Lahore, Pakistan.

Plant Material (Collection and Identification): The aerial parts of *G. officinale* were collected from Karachi in 2018 during the month of December. The plant was identified by Dr. Zaheer-ul-Deen Khan, Chairman Department of Botany, GCU, Lahore, Pakistan. The competent authority issued the voucher number (GC.herb.bot.3382-A).

Cell lines: BHK and HepG2 cell lines were used for anticancer activity. These were provided by the CRIMM Department, The University of Lahore, Lahore, Pakistan.

Microorganisms: The antibacterial assay was performed on ten bacterial pathogens obtained from the PCSIR (Pakistan Council of Scientific and Industrial Research), including five Gram positive (*Staphylococcus aureus* ATCC29213, *Bacillus cereus* ATCC11778, *Streptococcus pneumoniae* ATCC6303, *Staphylococcus epidermidis* ATCC12228 and *Micrococcus luteus* ATCC4698) and five Gram negative (*Klebsiella pneumoniae* ATCC10031, *Escherichia coli* ATCC25922, *Shigella flexneri* ATCC12022, *Pseudomonas aeruginosa* ATCC27853 and *Salmonella typhi* ATCC14028) strains.

Extraction and Isolation: To avoid the hydrolytic decomposition of phytochemicals, drying phase was completed in the absence of light and moisture. After drying, the material was pulverized and sieved through an 80 mesh sieve. Sequential extraction was achieved using a Soxhlet apparatus (Ahmad *et al*., 2009). From each part, 500 g of powder was taken for experiment. Extraction was continued till the completion of process. The extracts were subjected for the removal of solvent by rotary evaporator and got six dried samples including *n*-hexane leaf extract (4.74 g), chloroform leaf extract (2.68 g), ethanol leaf extract (33.90 g), *n*-hexane shoot extract (1.21 g), chloroform shoot extract (1.17 g) and ethanol shoot extract (22.51 g) (Table-1). From these samples, the active ethanol shoot extract was fractionated by column chromatography which yielded five fractions; chloroform: methanol was run as a gradient elusion (Table-2).

Phytochemical and Proximate Analysis: Standard protocols were followed to conduct the phytochemical and proximate analysis (Tables-3 and 4), including the detection of phytochemicals such as carbohydrates, proteins, lipids, alkaloids, saponins, phenolic compounds etc., and estimations of moisture contents, ash and extractive values (at room temperature and 60°C) (WHO, 1998).

Total Phenolic Contents Assay: The UV spectrophotometric (Shimadzu, Japan) method was employed to evaluate the total phenolic contents of plant extracts using the Folin-Ciocalteu (FC) reagent. 200 µl methanolic extract solution (1mg/ml) was mixed with 1 ml of FC reagent and 9.0 ml of distilled water. After 5 minutes, 10.0 ml of sodium carbonate solution (7%) was added to obtain a final volume of 25.0 ml with distilled water. A blank solution was prepared in the same manner but without extract. Gallic acid was used as standard. Absorbance was recorded after an incubation period of 90 mints at 750 nm wavelength and whole experiment was repeated in triplicate. The calibration curve was constructed based on the absorbance values of standard solutions and was used to determine the total phenolic contents of sample presented as Gallic acid equivalents (mg of GA/g of extract) (Velioglu *et al*., 1998).

Antioxidant Assay: The antioxidant potential was performed by *in-vitro* 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay with slight modifications. Absorbance values of samples and standards were observed after an incubation period of 30 minutes at the wavelength of 588 nm. The following equation was applied to determine the percentage radical scavenging activity (% RSA (Saleem *et al*., 2016).

$$\% \text{ RSA} = \frac{\text{Ac} - \text{As}}{\text{Ac}} \times 100$$

Where, As = Absorbance of sample and Ac = Absorbance of control

Antibacterial Assay: The antibacterial activity was determined by agar well diffusion method with minor changes. Different dilutions of test samples (5, 50 and 100 mg/ml) were prepared in DMSO and tested to explore antibacterial potential according to the

established protocols (Saleem *et al*., 2016). The entire trial was performed in triplicate to obtain average zones of inhibition.

Anticancer Assay: The anticancer activity was evaluated by the MTT (3- (4,5-dimethylthiazol-2-il) -2,5-diphenyltetrazoliuum) assay according to the method selected by Kiran *et al*., 2018 with few altrations (Kiran *et al*., 2018). Two cell lines, HepG2 and BHK, were arranged and refreshed. The concentration that showed IC_{50} was calculated for testing on BHK cell line. The ELISA reader was used to measure absorbance at 570 nm.

$$\text{Cell survival percentage (CSP)} = \frac{\text{absorbance of sample} - \text{absorbsnce of negative control}}{\text{absorbance of positive control or untreated}} \times 100$$

Statistical Analysis: Two-way ANOVA was applied for statistical analysis of the data obtained. IC_{50} values were determined from non-linear regression equations using graph pad prism v6.0.

HPLC Analysis: High performance liquid chromatography (HPLC) was carried out on biologically active fraction (F=3). 2011 Shimadzu HPLC LC-20 equipped with Prominence quaternary continuous LC-20AT pump, analytical front loading injection valve, 20 µl sample loop, manual sampler, 50 µl Hamilton MICROLITRE™ syringe, Prominence variable wavelength UV/Vis detector SPD-20A, Prominence CTO-20A oven, DGU-$20A_{5R}$ degasser was used. Agela analytical column C_{18} (150 x 4 mm: 0.5 µm) was used for separation with Lab Solution software version 5.52.

GC-MS Analysis: GC-MS technique was also carried out to identify the possible chemical components in the same active fraction i.e. F=3. This was achieved using QP 2010 gas chromatography (Shimadzu, Japan) having Shimadzu Technology DB 05 capillary column connected directly to MS detector. Complete separation was attained with a 15 meter long silica capillary column packed with stationary phase comprising of cross linked 95 % dimethyl polysiloxane and 5 % diphenyl polysiloxane. Both the injector and the source were set at a temperature of 200°C. The quantity of sample injected into the column was 1.0 µl with a split ratio of 1:10. Helium gas was applied as the mobile phase at a uniform flow rate of 1.0 ml/min. The oven temperature was initially programmed to be kept at 50°C for the first 3 minutes, then raised to 320°C at a rate of 10°C/min and held for 3 minutes. The mass selective detector (MSD) was upheld at 250°C temperature during this procedure. 50 to 550 was scan mass range and the scan time was 0.5 seconds. Methanol was used as blank. The observations were made in triplicate. The NIST mass spectral library was run to compare the mass spectra of components isolated by GC-MS (Alrumman, 2016). The results have been given in Table-16.

RESULTS AND DISCUSSIONs

Extraction and Isolation: Various extracts of *G. officinale* (leaves and shoots parts) ranging from non-polar to polar (*n*-hexane, chloroform and ethanol) were prepared by Soxhlet apparatus. Furthermore, fractionation of the active ethanolic shoot extract was performed by column chromatography (CC) using chloroform: ethanol in gradient elusion. These fractions were screened for biological activities.

Phytochemical and Proximate Analysis: The phytochemical investigation of *G. officinale* had shown the strongly presence of carbohydrates, saponins, steroids, proteins and phenolic content; moderate existence of alkaloids and weakly presence of glycosides (Table-3). The occurrence of these metabolites indicates a strong biological potential of *G. officinale*.

Powder study of *G. officinale* helped us for proximate analysis (Table-4). The moisture content was found 8.56 % and 9.91 % for leaves and shoots respectively which fell within the normal range (0 - 13%). The above information leads to the conclusion that it was safe to preserve these parts of plants without the risk of excessive degradation and microbial growth. Excessive moisture contents can facilitate the propagation of insects and microbes in herbal materials. Secondly the deterioration processes (because of high moisture contents) can show a low efficacy and toxic effects (Waterman *et al*., 2002). Total ash determined in the leaves was 8.05 % and in the shoots 5.86 %. These values are within the official range (i.e. less than 13 %) and indicate an estimation of the material left after the complete ignition of the plant contents in air. It results in the loss of all organic material from the sample and shows an approximation of the inorganic matter in it. Classically three types of ash values i.e. total ash, water soluble ash and acid insoluble ash, are determined (Sadhu *et al*., 2015). The percentage values of acid insoluble ash in leaves and shoots were 0.87 % and 0.15 % respectively; with official limit of 0.5 - 5.5 %. Insoluble ash value highlights the total silica present in the herbal substance as sand or siliceous earth (Organization, 1998). The acid-soluble ash values were 9.98 % in case of leaves and 5.63 % in case of shoots. Water-insoluble ash value determined in leaves was 5.54 % and in shoots was 3.23 % while water-soluble ash was 2.12 % in leaves and 1.22 % in shoots. 10.96 % and 5.81 % were sulphated ash values in leaves and shoots parts respectively.

The estimation of water-soluble extracts at room temperature showed 8.81 % in leaves and 2.19 % in shoots, whereas the estimation at 60°C gave 9.61 and 3.55 % values for leaves and shoots accordingly. The alcohol-soluble extraction values for both at room temperature were 2.82 and 4.07 %. Furthermore, these values at a temperature of 60°C were ranged up to 6.40 %

for leaves and 5.02% for shoots (Table-4). Both water-soluble and alcohol-soluble extractive values were higher at 60°C temperature than at room temperature. Besides, it was observed that water-soluble extractives were higher than alcohol-soluble in case of leaves while the opposite trend was observed in case of shoots. It revealed that aqueous extraction is superior for leaves and alcohol is a better for shoots of *G. officinale* (Ozarkar, 2005).

Determination of Total Phenolic Contents (TPC): TPC in *G. officinale* leaves and shoots were estimated with Folin-Ciocalteu reagent. The calibration curve of Gallic acid was constructed to get a straight line equation ($y=0.0039x-0.0076$, $R^2=0.9897$) (Figure-1). The straight line equation was used to calculate the TPC represented as mg GAE/g of sample (Table-5, Figure-2). According to the results, the TPC of *n*-hexane, chloroform and ethanol leaves was respectively 10.25, 46.2 and 28.95 mg of GAE/g of extract whereas those of the shoots were 16. 06, 61.31 and 48.03 mg of GAE/g of extract respectively. It was evident that the highest TPC was in chloroform extracts of both parts followed by those of ethanol and *n*-hexane extracts. Observations also indicated that overall shoots were richer in phenolic contents than leaf extracts. Present findings can be correlated to the works performed by Maneechai and Pikulthong (Maneechai and Pikulthong, 2017). The presence of such phytochemicals is responsible for the strong antioxidant potential of *G. officinale* plant (Gan *et al*., 2017).

Antioxidant Activity: The antioxidant potential of *G. officinale* was explored by DPPH method. The free radical scavenging ability (RSA) is estimated by diminishing color intensity of the DPPH solution and measurement of the absorbance. % RSA of *n*-hexane, chloroform and ethanol extracts of leaves were found to be 33.15, 65.40 and 50.76 % respectively while those of shoots were 41.80, 78.23 and 69.55 % correspondingly. Vitamin C was used as a standard and its % RSA was 87.27 % (Table-6, Figure-3). Overall, plant displayed significant antioxidant potential. These conclusions can be compared with the findings of Maneechai and Pikulthong (Maneechai and Pikulthong, 2017).

The DPPH protocol was also used for determination of antioxidant potential of ethanol fractions. Among five fractions, F=4 showed the maximum radical scavenging potential (69.30 % RSA) compared to left four fractions i.e., F=1-3 and 5 with 40.33, 33.54, 49.23, and 52.75 % RSAs respectively (Table-7, Figure-4). The ability to donate hydrogen could be the reason for the antioxidant potential of the samples (Kim, 2012).

Antibacterial Assay: The antibacterial activity of aerial parts of *G. officinale* has not been previously reported. So present work was planned to explore its antibacterial effects against ten bacterial pathogens. The activities were assessed by measuring the zones of inhibition and compared with standard antibiotics (CIP and MET) (Figures-5 and 6). Various concentrations (i.e. 5, 50 and 100 mg/ml) of *n*-hexane extract of leaves didn't show any zone of inhibition against *Bacillus cereus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Shigella flexneri*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* but displayed moderate to significant result against *Micrococcus luteus*, *Streptococcus pneumoniae* and *Salmonella typhi* (Table-8). Chloroform leaf extract was found moderately active against *Bacillus cereus*, *Staphylococcus epidermidis*, *Shigella flexneri* and *Streptococcus pneumoniae* and significantly active against *Staphylococcus aureus* only (Table-8). All dilutions of ethanol extract displayed less significant effect against *Staphylococcus epidermidis* and *Shigella flexneri* and relatively stronger one against *Bacillus cereus* and *Staphylococcus aureus* (Table-8). Likewise, *n*-hexane shoot extract demonstrated moderate to significant effects against six out of ten strains, namely *Staphylococcus epidermidis*, *Micrococcus luteus*, *Streptococcus pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (Table-9). There was moderate activity of chloroform shoot extract against *Staphylococcus aureus* although it was highly effective against *Staphylococcus epidermidis*, *Shigella flexneri*, *Streptococcus pneumoniae*, *Escherichia coli* and *Klebsiella pneumoniae* (Table-9). Ethanol shoot extract revealed the zone of inhibition only against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Shigella flexneri*, *Pseudomonas aeruginosa* and *Escherichia coli* (Table-9).

The antibacterial potential of ethanol extract may be due to the presence of alkaloids and polyphenols (Mabhiza *et al*., 2016). The above discussion showed that all *G. officinale* extracts demonstrated moderate to strong antibacterial effects against selected human pathogens. Overall shoot extracts have been found to have stronger antibacterial potential than leaf extracts. Among shoot extracts, ethanol shoot extract showed the strongest effects followed by chloroform and *n*-hexane extracts. Further research on ethanolic extract could lead to the exploration of biologically active constituents following the principles of bio-guided isolation.

Based on the biological potential, the ethanol shoot extract was selected for further fractionation by column chromatography (CC). CC yielded five fractions which were tested against six pathogenic strains (*Bacillus cereus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*) with two different concentrations (10 and 20 mg/ml) and then compared with standard antibiotics (Figure-7). The antibacterial results revealed that all fractions were totally unproductive against *Bacillus cereus* and *Staphylococcus aureus* (Table-10) whereas showing different zones of inhibition against

Klebsiella pneumoniae, *Escherichia coli* and *Pseudomonas aeruginosa*. Four fractions (F=2, 3, 4 and 5) were effective against *Staphylococcus epidermidis* (Table-10). The results can be correlated with antibacterial study conducted on *Guaiacum Spp.* (Niakan *et al*., 2017) and on selected plants of the *Zygophyllaceae* family by Dastagir (Dastagir *et al*., 2012).

Anticancer Activity: The anti-cancer/cytotoxic activity of samples including extracts and ethanol fractions of *G. officinale* against HepG2 (cancer cell line) and BHK (normal cell line) were also investigated that indicated a dose-dependent response. IC_{50} of test samples were calculated using Graph Pad Prism v6. IC_{50} represents the concentration at which 50 percent cells are killed. Cisplatin was taken as standard cytotoxic drug that showed IC_{50} at 14.14 µg/ml concentration against HepG2. The ethanol extracts of leaf and shoot parts showed IC_{50} at 0.89 and 1.37 mg/ml respectively, whereas *n*-hexane and chloroform extracts of both parts presented mild to partial effect against HepG2 (Tables-11 and 12, Figures-8 and 9).

The antitumor effect of ethanol fractions against HepG2 was also explored (Table-13). Four fractions i.e. F = 1, 3, 4 and 5 gave IC_{50} at 2.89, 2.96, 2.61 and 1.91 mg/ml respectively (Figure-10).

The cytotoxic effect of above mentioned samples was also tested against normal cell line (Table-14). But no reasonable decay was observed, reflecting the plant is safe to use. These findings are in accordance with the previous investigations by Karla Claudio Campos and B.S., Janibeth Hernández Rivera, *et al*, whereas *G. officinale* showed significant activity against different cancerous and normal cell lines (Campos *et al*., 2015). The strongest anticancer potential was shown by polar extract (ethanol) of both parts which could be attributed to the presence of alkaloids and polyphenols (Carocho and CFR Ferreira, 2013).

HPLC and GC-MS Analysis: One of the biologically active fractions (F = 3) was analyzed by HPLC and GC-MS techniques. Mainly three components were eluted at different retention times while using methanol: water in gradient elution (Table-15, Figure-11). The same fraction was also evaluated by GC-MS to identify unknown components using NIST library (Table-16, Figure-12). Total fifteen compounds, belonging to different classes were eluted and identified as seven hydrocarbons (i.e. 2-methyldecane; 2-methylhexadecane; eicosane; 10-methyl nona- decane; 2-methyleicosane; 2-methyloctadecane; and 2-methylnonadecane), four esters (i.e., diisooctyl phthalate; 1,4-benzenedicarboxylic acid, bis (2-ethylhexyl) ester; L-proline, 1-methyl- methyl ester and sulfurous acid butyl decyl ester), two heterocyclic compounds (i.e., 3-methyl-2-(2-oxo propyl) furan and L-proline 1-methyl-, methyl ester), one peptide (L-proline, 1-methyl-, methyl ester), two sulfur containing compounds (i.e., 2,2-dimethyl-propyl 2,2-dimethyl-propane sulfinyl sulfone and sulfurous acid, butyl decyl ester), one ether (hexyl octyl ether) and one haloalkane (1-iodo-2-methylundecane). Literature survey of these compounds depicted that 2,2-dimethyl-propyl 2,2-dimethyl- propane- sulfinyl sulfone possessed antioxidant activity, (Jeyam *et al*., 2013) and anti-seborrheic, antiprotozoal, antiviral and cytoprotectant effects (Bobby *et al*., 2015). 3-methyl-2-(2-oxo propyl) furan was found to exhibit antibacterial activity and being a bio-surfactant can cause anti-biofilm effects (Gupta *et al*., 2019; Singh *et al*., 2016). 10-Methyl nonadecane, a component of the essential oil extracted from *Catha edulis*, has shown antioxidant activity (Hailu *et al*., 2017). Eicosane, 2-methyl- has been reported as antioxidant and is used in Ayurveda preparations for treatment of amenorrhea (Phillips *et al*., 2015). Similarly, Sulfurous acid, butyl decyl ester has insecticidal and nematocidal properties (Harris and Zukel, 1958) whereas Diisooctyl phthalate has revealed the anti-androgenic effects in rats (Saillenfait *et al*., 2013).

The significant biological potential of *G. officinale* fraction can be correlated with various identified compounds (GC-MS analysis) as the antioxidant potential could be due to the presence of compounds such as 2,2-dimethyl-propyl 2,2-dimethyl-propane sulfinyl sulfone; 10-Methyl nonadecane; Eicosane, 2-methyl- and other phenolics. 2,2-dimethyl-propyl 2,2-dimethyl- propane sulfinyl sulfone and 3-methyl-2-(2-oxo propyl) furan may be responsible for antimicrobial effects of the plant. Likewise, anticancer effects could be related with the presence of antioxidants and toxic compounds such as phthalates; 2,2-dimethyl-propyl 2,2-dimethyl- propane sulfinyl sulfone; alkaloids; polyphenols and other strongly polar compounds.

These findings can lead to the chemical characterization of the ethanol fraction and therefore demonstrate an evidence of biological potential of the plant. Based on these results, we can establish a strong correlation between the phytochemicals and the therapeutic use of *G. officinale* in a number of ailments (such as bacterial infections, tumors, oxidative stress etc.). However, it is suggested that the ethanol fraction can be further purified by chromatographic technique to obtain pharmacologically active pure constituents followed by the bio-guided isolation principle.

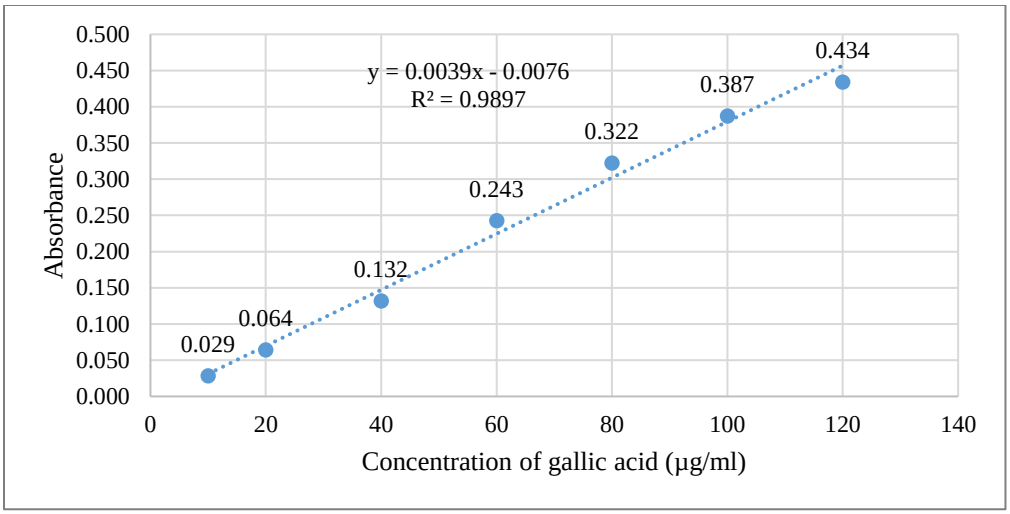


Figure-1: Gallic acid calibration curve.

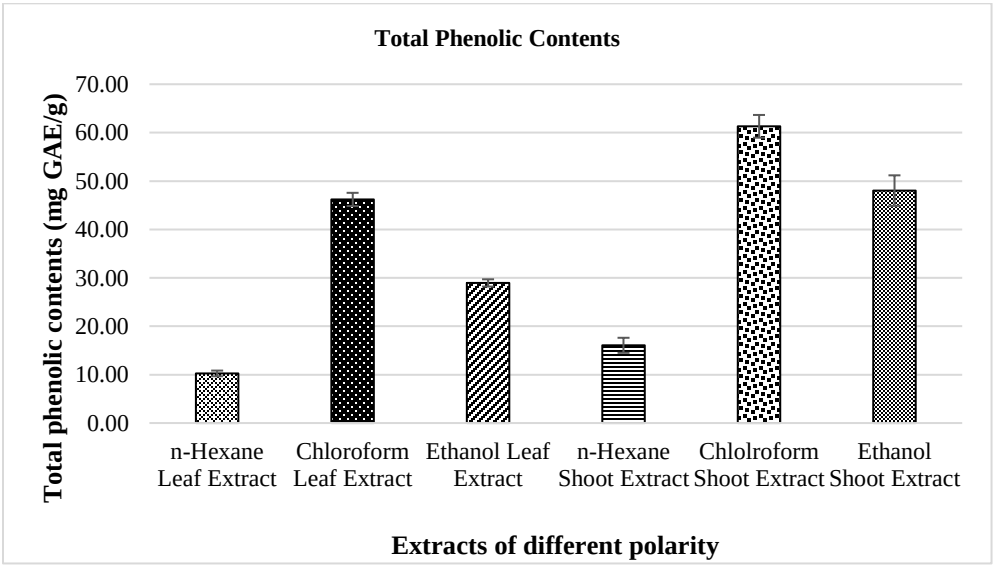


Figure-2: Total phenolic contents of extracts.

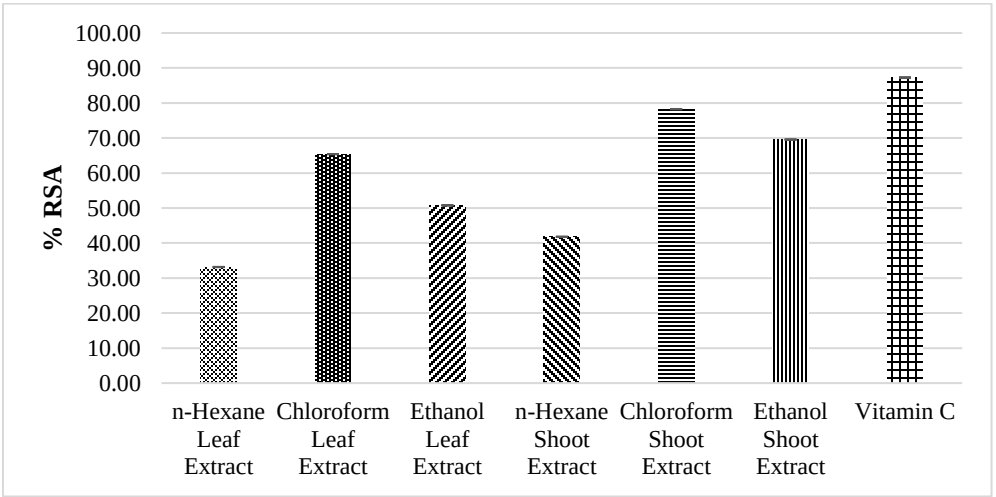


Figure-3: Antioxidant activity of extracts.

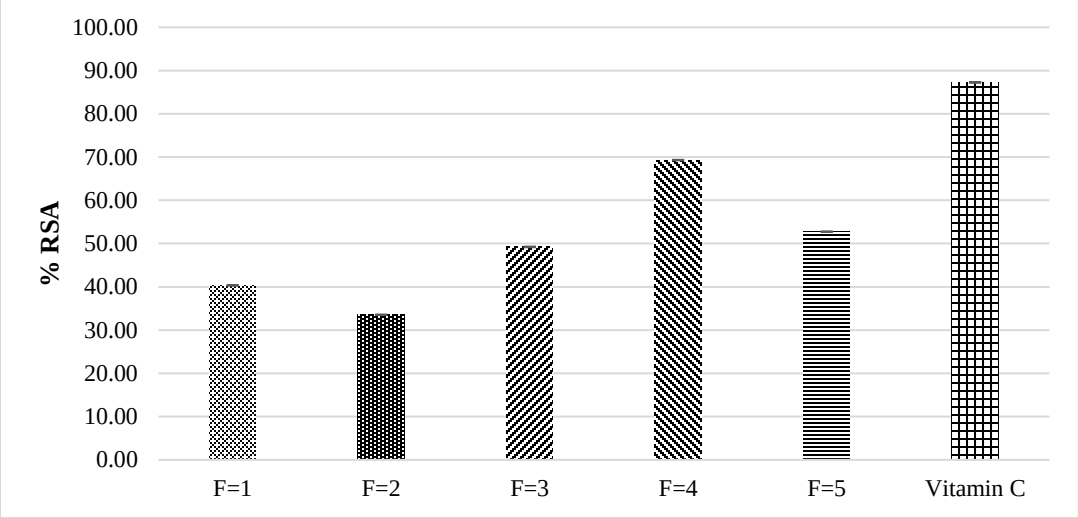


Figure-4: Antioxidant activity of ethanol shoot fractions.

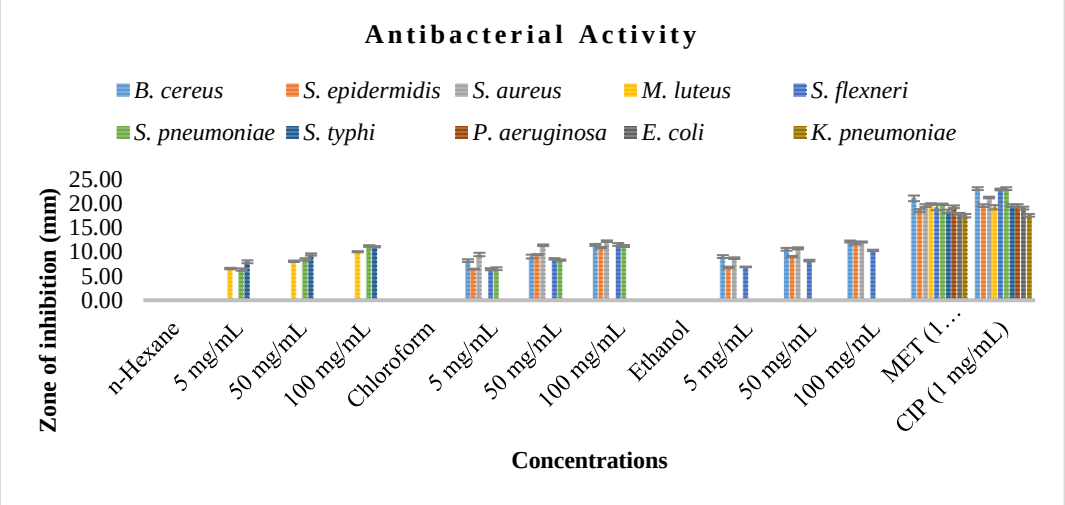


Figure-5: Antibacterial activity of leaf extracts.

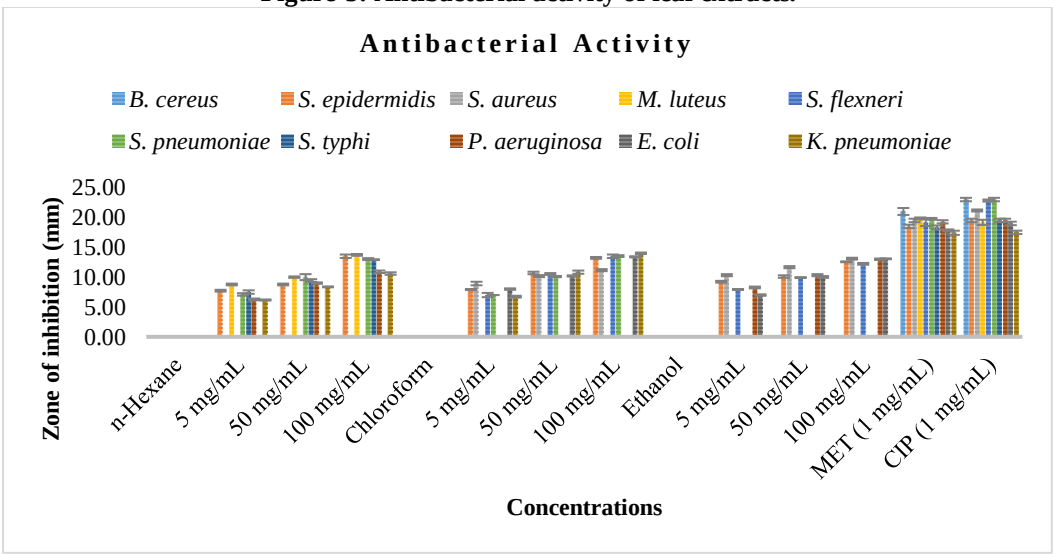


Figure-6: Antibacterial activity of shoot extracts.

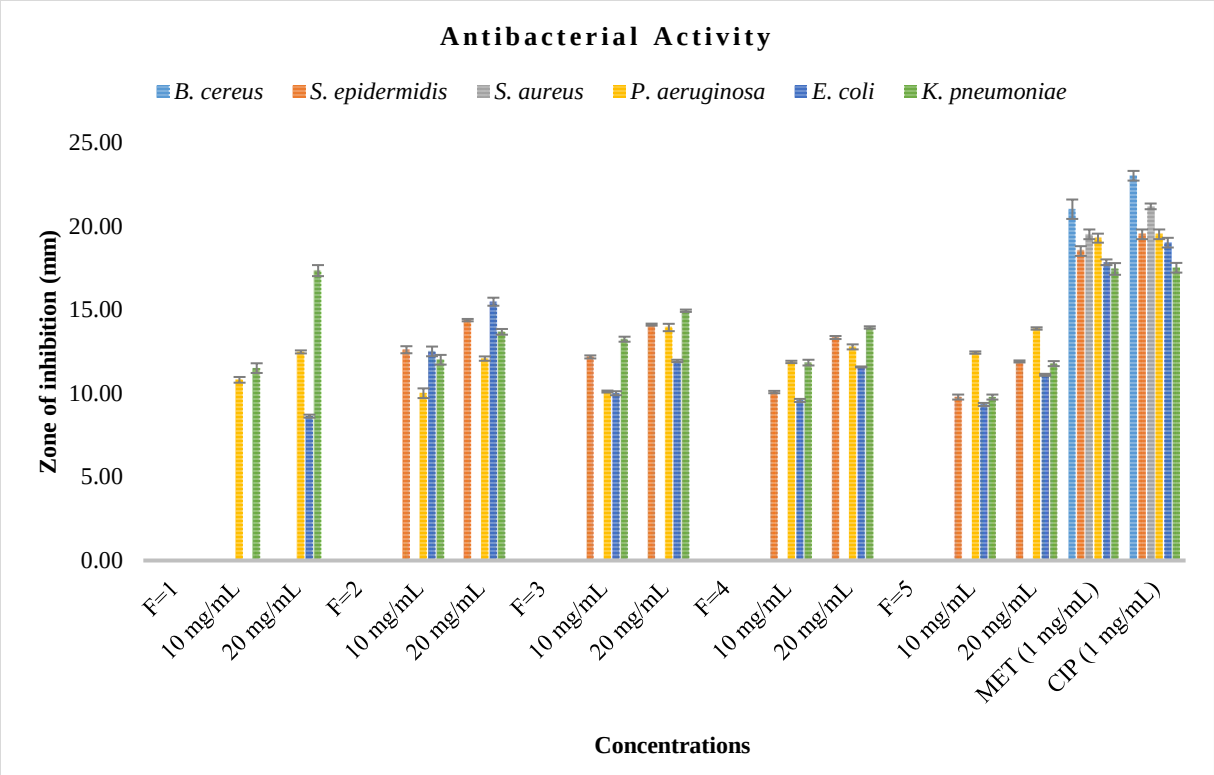


Figure-7: Antibacterial activity of ethanol shoot fractions.

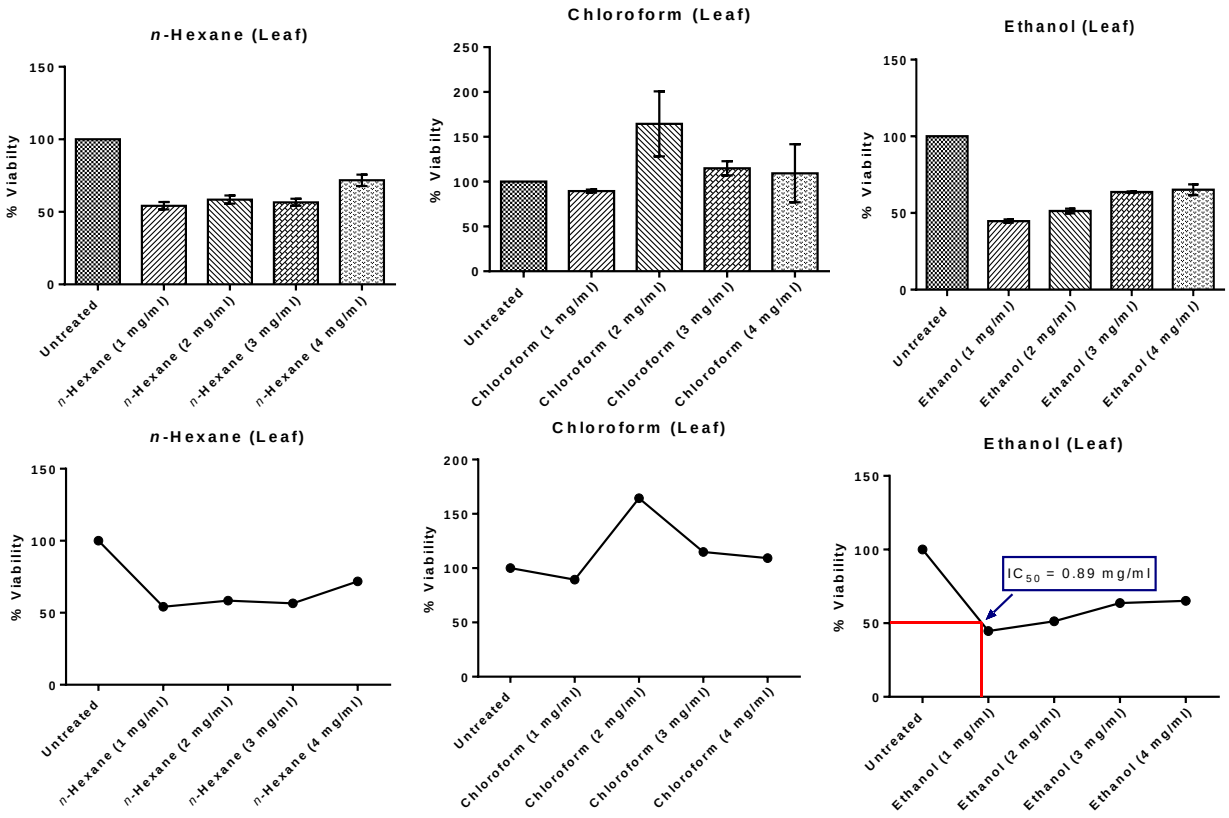


Figure-8: *IC50* of leaf extracts.

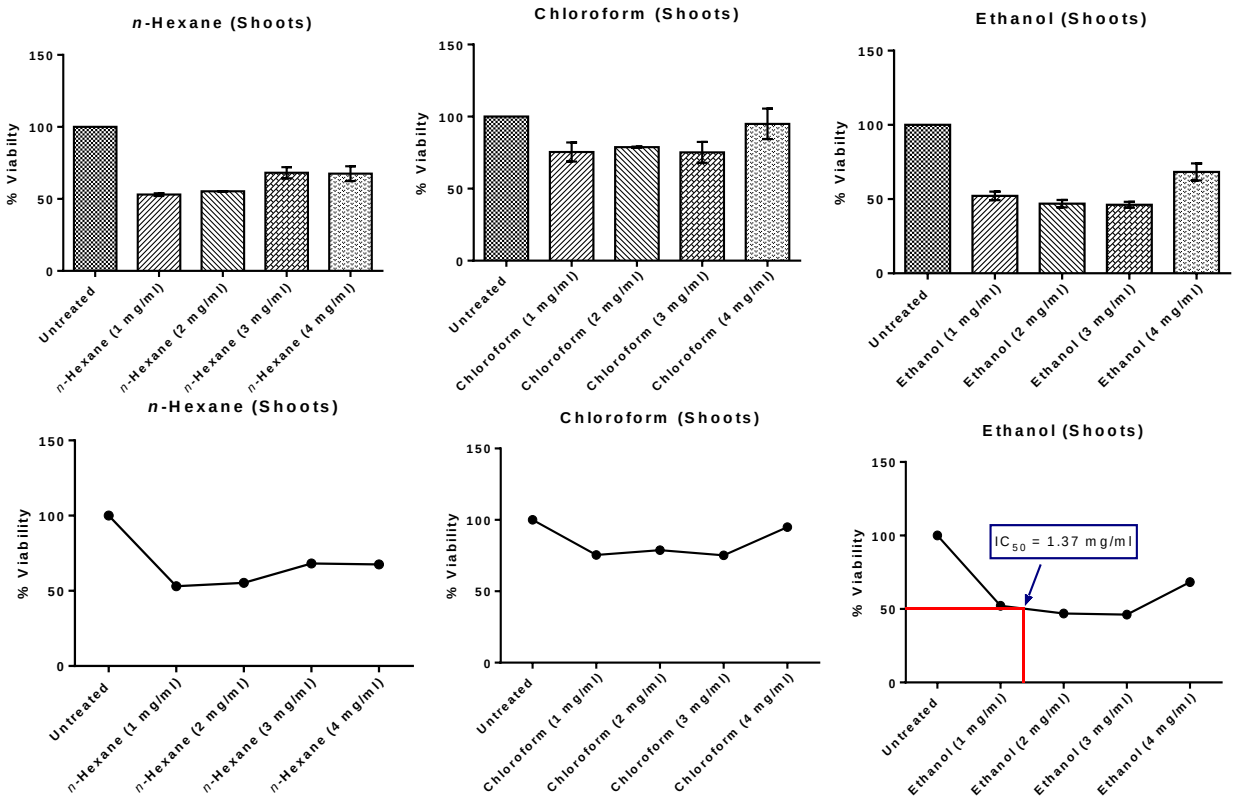
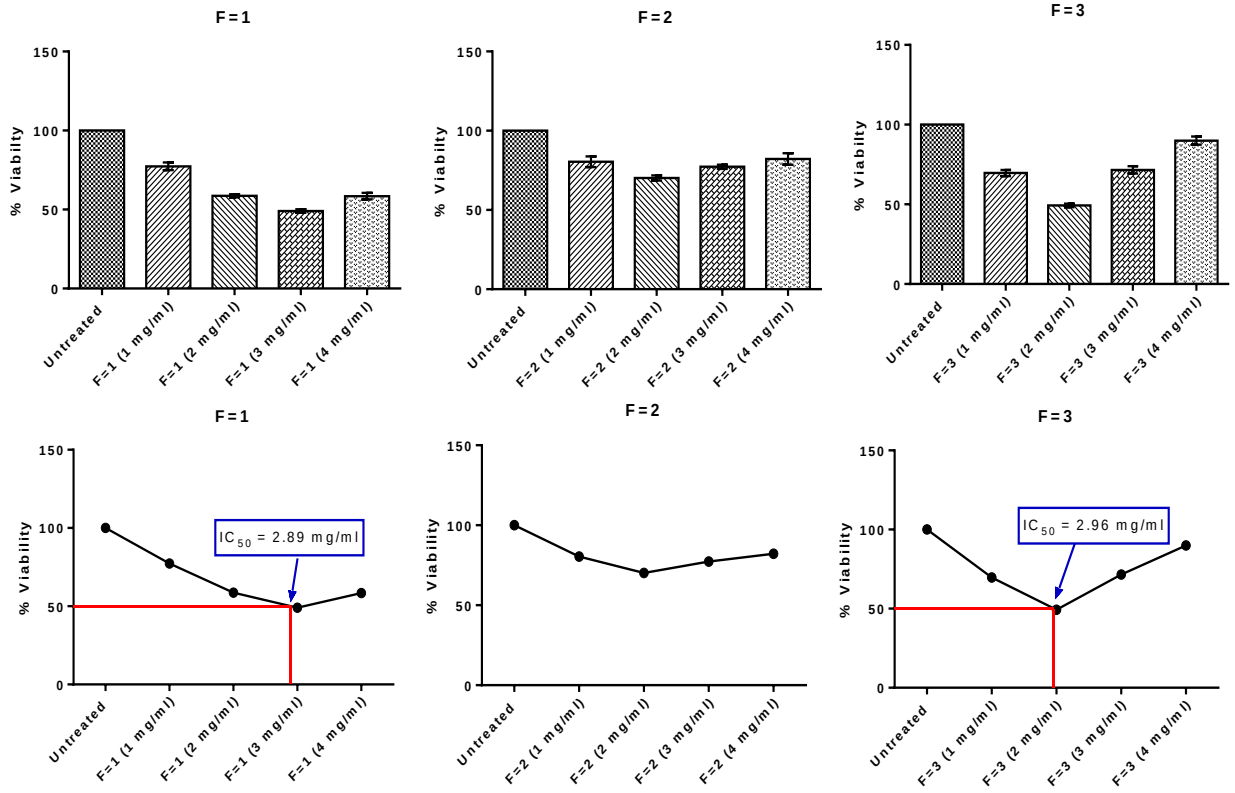


Figure-9: *IC50* of shoot extracts.



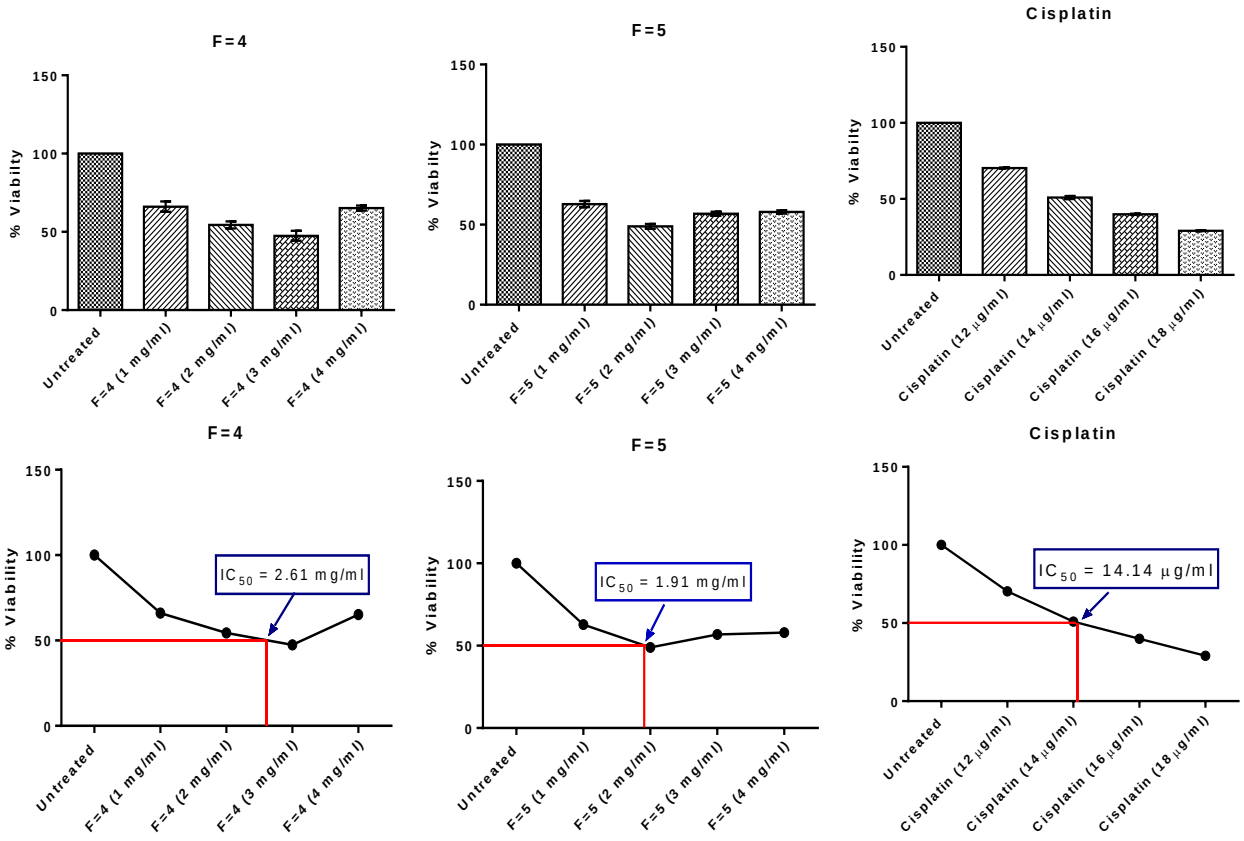


Figure-10: *IC50* of ethanol shoot fractions and Cisplatin

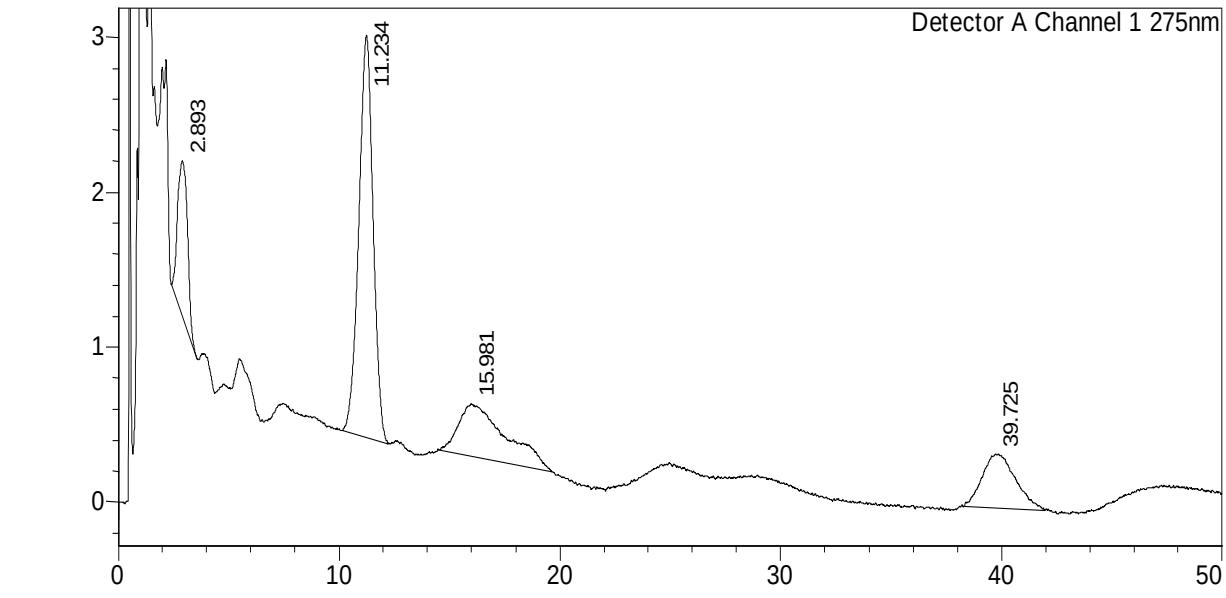


Figure-11: HPLC chromatogram of ethanol fraction (F=3)

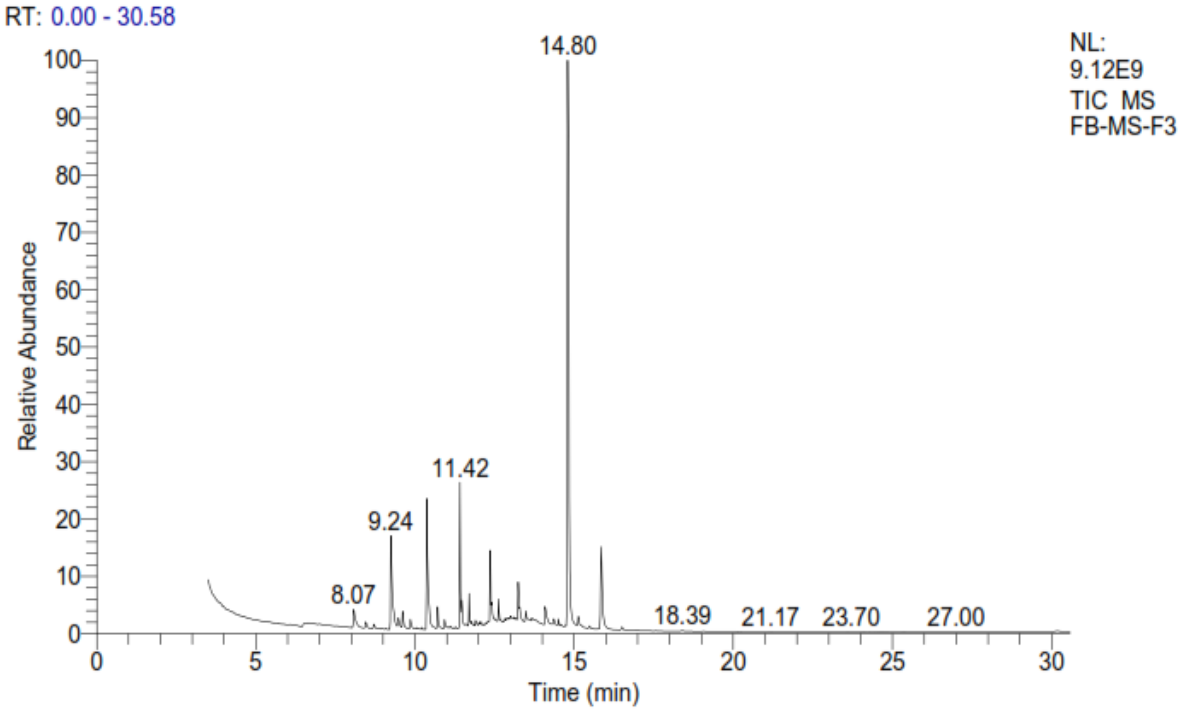


Figure-12: Gas chromatogram of ethanol fraction (F=3)

Table-1: Table of dried extracts obtained by continuous extraction.

Sr. No.	Plant Part	Extract	Quantity (grams)	Percentage
1	Leaves	*n*-Hexane	4.74	0.948
2	Leaves	Chloroform	2.68	0.536
3	Leaves	Ethanolic	33.90	6.780
4	Shoots	*n*-Hexane	1.21	0.242
5	Shoots	Chloroform	1.17	0.234
6	Shoots	Ethanolic	22.51	4.502

Table-2: Fractions of ethanol shoot extract.

Serial No.	Fraction No.	Solvent System	Ratio
1	F=1	Chloroform:Ethanol	49:01
2	F=2	Chloroform:Ethanol	19:01
3	F=3	Chloroform:Ethanol	03:01
4	F=4	Chloroform:Ethanol	03:02
5	F=5	Ethanol	100%

Table-3: Phytochemical studies.

Groups	Phytochemical Tests	Results	
		Leaves	**Shoots**
1) Alkaloids	i) Mayer's test	+	+
	ii) Wagner's test	++	++
	iii) Hager's test	-	-
2) Carbohydrates	i) Molisch's test	+++	++
	ii) Barfoed's test	++	+
	iii) Benedicts test	+++	++
3) Protein and amino acids	i) Biuret test	+++	++

	ii) Millon's test	++	+++
4) Phenolic compounds	i) Ferric chloride test	+	++
	ii) Gelatin test	++	++
	iii) Lead acetate test	+++	+++
5) Saponins	i) Foam test	+++	+++
6) Steroids	i) Salkowski's test	++	+
	ii) Liebermann's test	++	++
	iii) Sulpher test	+++	++
7) Terpenoids	i) Salkowski's test	++	+
	ii) Liebermann's test	++	++
8) Lipids	i) Spot test	-	-
9) Glycosides	i) Borntrager's test	+	+

- = Absent, + = weakly present, ++ = moderately present, +++ = strongly present.

Table-4: Physicochemical assessment.

S. No.	Properties	Leaves		Shoots	
		% age	Mean ± S. E.	% age	Mean ± S. E.
1	Moisture contents	08.04	08.56 ± 0.420	10.01	09.91 ± 0.080
		09.40		09.75	
		08.25		09.97	
2	Total ash	08.06	08.05 ± 0.270	05.75	05.86 ± 0.060
		07.58		05.95	
		08.50		05.87	
3	Acid insoluble ash	00.95	00.87 ± 0.040	00.16	00.15 ± 0.010
		00.80		00.15	
		00.85		00.13	
4	Acid soluble ash	10.10	09.98 ± 0.070	05.65	05.63 ± 0.040
		09.87		05.70	
		09.96		05.55	
5	Water insoluble ash	05.54	05.54 ± 0.040	03.05	03.23 ± 0.090
		05.46		03.36	
		05.61		03.28	
6	Water soluble ash	02.04	02.12 ± 0.060	01.04	01.22 ± 0.110
		02.23		01.21	
		02.10		01.42	
7	Sulphated ash	10.52	10.96 ± 0.230	05.56	05.81 ± 0.150
		11.31		06.08	
		11.05		05.79	
8	Water soluble extractive value (RT)	08.88	08.81 ± 0.080	02.05	02.19 ± 0.080
		08.91		02.32	
		08.65		02.19	
9	Water soluble extractive value (60^{o}C)	09.62	09.61 ± 0.190	03.47	03.55 ± 0.040
		09.94		03.62	
		09.27		03.55	
10	Alcohol soluble extractive value (RT)	02.62	02.82 ± 0.120	04.26	04.07 ± 0.100
		02.81		04.05	
		03.04		03.90	
11	Alcohol soluble extractive value (60^{o}C)	06.24	06.40 ± 0.100	04.86	05.02 ± 0.090
		06.58		05.05	
		06.37		05.15	

RT = Room Temperature

Table-5: Total Phenolic Contents of extracts.

Extracts	Leaves	Shoots
	Mean TPC (mg GAE/g) ± S.E.	**Mean TPC (mg GAE/g) ± S.E.**
n-Hexane	10.25 ± 00.59	16.06 ± 01.56
Chloroform	46.20 ± 01.37	61.31 ± 02.35
Ethanol	28.95 ± 00.75	48.03 ± 03.15

TPC = Total phenolic contents, GAE = Gallic acid equivalents

Table-6: Antioxidant activity of extracts.

Plant Part	Extract/ Standard	Absorbance	DPPH Activity	
			% RSA	**Mean% RSA ± S.E.**
Leaves	*n*-Hexane	1.047	33.15	33.15 ± 0.048
		1.046	33.24	
		1.048	33.07	
	Chloroform	0.660	65.34	65.40 ± 0.028
		0.659	65.42	
		0.659	65.42	
	Ethanol	0.835	50.79	50.76 ± 0.028
		0.835	50.79	
		0.836	50.70	
Shoots	*n*-Hexane	0.943	41.80	41.80 ± 0.048
		0.942	41.89	
		0.944	41.72	
	Chloroform	0.506	78.15	78.23 ± 0.48
		0.505	78.23	
		0.504	78.31	
	Ethanol	0.610	69.50	69.55 ± 0.055
		0.610	69.50	
		0.608	69.66	
	Ascorbic Acid	0.396	87.30	87.27 ± 0.073
		0.398	87.13	
		0.395	87.38	

Table-7: Antioxidant activity of fractions of ethanol shoot extract.

Fraction/ Standard	Absorbance	DPPH Activity	
		% RSA	**Mean% RSA ± S.E.**
F=1	0.959	40.47	40.33 ± 0.073
	0.962	40.22	
	0.961	40.31	
F=2	1.043	33.49	33.54 ± 0.055
	1.043	33.49	
	1.041	33.65	
F=3	0.855	49.12	49.23 ± 0.073
	0.852	49.37	
	0.854	49.20	
F=4	0.611	69.42	69.30 ± 0.055
	0.613	69.25	
	0.613	69.25	
F=5	0.812	52.70	52.75 ± 0.55
	0.812	52.70	
	0.810	52.86	

Ascorbic Acid	0.396	87.30	87.27 ± 0.073
	0.398	87.13	
	0.395	87.38	

Table-8: Antibacterial activity of leaf extracts.

	Mean zones of inhibition of Bacterial Strains in mm ± S.E.									
Extract/ Concentration	***B. cereus***	***S. epidermidis***	***S. aureus***	***M. luteus***	***S. flexneri***	***S. pneumoniae***	***S. typhi***	***P. aeruginosa***	***E. coli***	***K. pneumoniae***
n-Hexane										
5 mg/mL	-	-	-	6.53 ±0.15	-	6.33 ±0.24	7.93 ±0.30	-	-	-
50 mg/mL	-	-	-	8.03 ±0.15	-	8.43 ±0.23	9.43 ±0.23	-	-	-
100 mg/mL	-	-	-	10.0 ±0.12	-	11.17 ±0.17	11.03 ±0.15	-	-	-
Chloroform										
5 mg/mL	8.17 ±0.27	6.43 ±0.07	9.43 ±0.35	-	6.40 ±0.21	6.50 ±0.29	-	-	-	-
50 mg/mL	9.00 ±0.40	9.40 ±0.10	11.33 ±0.17	-	8.53 ±0.15	8.27 ±0.15	-	-	-	-
100 mg/mL	11.40 ±0.21	10.87 ±0.07	12.17 ±0.17	-	11.50 ±0.29	11.17 ±0.20	-	-	-	-
Ethanol										
5 mg/mL	9.00 ±0.29	6.77 ±0.15	8.67 ±0.17	-	6.87 ±0.07	-	-	-	-	-
50 mg/mL	10.50 ±0.29	9.00 ±0.12	10.70 ±0.21	-	8.17 ±0.17	-	-	-	-	-
100 mg/mL	12.10 ±0.21	11.77 ±0.26	12.00 ±0.12	-	10.27 ±0.15	-	-	-	-	-
MET (1 mg/mL)	21.00 ±0.58	18.5 ±0.29	19.5 ±0.29	19.83 ±0.17	19.17 ±0.60	19.77 ±0.15	18.33 ±0.33	19.27 ±0.27	17.83 ±0.17	17.43 ±0.35
CIP (1 mg/mL)	23.00 ±0.29	19.5 ±0.29	21.17 ±0.17	19.17 ±0.44	22.83 ±0.17	23.00 ±0.29	19.50 ±0.29	19.50 ±0.29	19.00 ±0.29	17.50 ±0.29

- = No zone of Inhibition, MET = Methicillin, CIP = Ciprofloxacin *B. = Bacillus, S. = Staphylococcus, S. = Staphylococcus, M. = Micrococcus, S. = Shigella, S. = Streptococcus, S. = Salmonella, P. = Pseudomonas, E. = Escherichia, K. = Klebsiell*

Table-9: Antibacterial activity of shoot extracts.

	Mean zones of inhibition of Bacterial Strains in mm ± S.E.									
Extract/ Concentration	***B. cereus***	***S. epidermidis***	***S. aureus***	***M. luteus***	***S. flexneri***	***S. pneumoniae***	***S. typhi***	***P. aeruginosa***	***E. coli***	***K. pneumoniae***
n-Hexane										
5 mg/mL	-	7.73 ±0.15	-	8.77 ±0.15	-	7.10 ±0.21	7.50 ±0.29	6.27 ±0.15	-	6.17 ±0.09
50 mg/mL	-	8.77 ±0.15	-	10.00 ±0.12	-	10.00 ±0.50	9.43 ±0.23	9.00 ±0.12	-	8.37 ±0.07
100 mg/mL	-	13.50 ±0.29	-	13.73 ±0.15	-	13.03 ±0.15	12.93 ±0.07	10.90 ±0.21	-	10.60 ±0.21
Chloroform										
5 mg/mL	-	7.93 ±0.07	8.93 ±0.30	-	7.00 ±0.36	7.00 ±0.00	-	-	8.00 ±0.12	6.73 ±0.15
50 mg/mL	-	10.70 ±0.21	10.17 ±0.17	-	10.53 ±0.15	10.10 ±0.10	-	-	10.20 ±0.10	10.83 ±0.27
100 mg/mL	-	13.23 ±0.15	11.17 ±0.17	-	13.50 ±0.29	13.53 ±0.15	-	-	13.40 ±0.10	14.00 ±0.12
Ethanol										
5 mg/mL	-	9.23 ±0.15	10.33 ±0.17	-	7.93 ±0.07	-	-	8.27 ±0.15	7.00 ±0.12	-
50 mg/mL	-	10.10 ±0.21	11.67	-	9.93	-	-	10.33	10.03	-

			±017		±0.07			±0.17	±0.25	
100 mg/mL	-	12.57 ±0.07	13.10	-	12.23	-	-	13.00	13.07	-
			±0.15		±0.15			±0.12	±0.12	
MET (1	21.00	18.5 ±0.29	19.5	19.83	19.17	19.77 ±0.15	18.33	19.27	17.83	17.43 ±0.35
mg/mL)	±0.58		±0.29	±0.17	±0.60		±0.33	±0.27	±0.17	
CIP (1	23.00	19.5 ±0.29	21.17	19.17	22.83	23.00 ±0.29	19.50	19.50	19.00	17.50 ±0.29
mg/mL)	±0.29		±0.17	±0.44	±0.17		±0.29	±0.29	±0.29	

- = No zone of Inhibition, MET = Methicillin, CIP = Ciprofloxacin *B. = Bacillus, S. = Staphylococcus, S. = Staphylococcus, M. = Micrococcus, S. = Shigella, S. = Streptococcus, S. = Salmonella, P. = Pseudomonas, E. = Escherichia, K. = Klebsiella*

Table-10: Antibacterial activity of ethanol shoot extract fractions.

	Mean zones of inhibition of Bacterial Strains in mm ± S.E.					
Extract/ Concentration	***B. cereus***	***S. epidermidis***	***S. aureus***	***P. aeruginosa***	***E. coli***	***K. pneumoniae***
F=1						
10 mg/mL	-	-	-	10.80 ±0.17	-	11.50 ±0.29
20 mg/mL	-	-	-	12.47 ±0.09	8.63 ±0.09	17.33 ±0.33
F=2						
10 mg/mL	-	12.60 ±0.21	-	10.00 ±0.29	12.50 ±0.29	12.00 ±0.29
20 mg/mL	-	14.37 ±0.07	-	12.07 ±0.13	15.47 ±0.24	13.67 ±0.17
F=3						
10 mg/mL	-	12.17 ±0.09	-	10.10 ±0.06	10.00 ±0.12	13.23 ±0.15
20 mg/mL	-	14.10 ±0.06	-	13.93 ±0.22	11.93 ±0.07	14.93 ±0.07
F=4						
10 mg/mL	-	10.07 ±0.07	-	11.87 ±0.07	9.57 ±0.09	11.83 ±0.17
20 mg/mL	-	13.33 ±0.09	-	12.77 ±0.15	11.57 ±0.03	13.93 ±0.07
F=5						
10 mg/mL	-	9.77 ±0.15	-	12.43 ±0.07	9.33 ±0.09	9.77 ±0.15
20 mg/mL	-	11.90 ±0.06	-	13.87 ±0.07	11.10 ±0.06	11.77 ±0.15
MET (1 mg/mL)	21.00 ±0.58	18.5 ±0.29	19.5 ±0.29	19.27 ±0.27	17.83 ±0.17	17.43 ±0.35
CIP (1 mg/mL)	23.00 ±0.29	19.5 ±0.29	21.17 ±0.17	19.50 ±0.29	19.00 ±0.29	17.50 ±0.29

- = No zone of Inhibition, MET = Methicillin, CIP = Ciprofloxacin
B. = Bacillus, S. = Staphylococcus, S. = Staphylococcus, P. = Pseudomonas, E. = Escherichia, K. = Klebsiella

Table-11: Anticancer activity of leaf extracts.

Extracts	**Concentrations**	**% Viability**	**Mean % Viability ± S.E.**	***IC*$_{50}$**
***n*- Hexane**	1 mg/ml	59.42	54.2 ± 42.60	-
		50.98		
		52.48		
	2 mg/ml	60.03	58.49 ± 02.79	
		53.18		
		62.49		
	3 mg/ml	53.78	56.62 ± 02.39	
		54.75		
		61.39		
	4 mg/ml	79.66	71.76 ± 03.96	
		68.78		
		67.00		
Chloroform	1 mg/ml	87.77	89.48 ± 01.74	-
		87.76		
		92.97		
	2 mg/ml	103.81	164.49 ± 36.34	
		229.25		

		157.40		
	3 mg/ml	129.86	114.90 ± 07.85	
		103.12		
		112.28		
	4 mg/ml	173.73	109.33 ± 32.36	
		86.20		
		69.30		
Ethanol	1 mg/ml	42.71	44.74 ± 01.02	00.89 mg/ml
		45.50		
		45.99		
	2 mg/ml	52.91	51.32 ± 01.48	
		48.41		
		52.78		
	3 mg/ml	62.80	63.65 ± 00.44	
		63.85		
		64.28		
	4 mg/ml	71.67	65.23 ± 03.49	
		64.39		
		59.69		
Untreated	No concentration	100.00	100.00 ± 00.00	
		100.00		
		100.00		
Cisplatin	12 µg/ml	69.80	70.34 ± 00.31	14.14 µg/ml
		70.88		
		70.34		
	14 µg/ml	49.91	50.97 ± 01.01	
		50.00		
		52.99		
	16 µg/ml	39.34	39.99 ± 00.33	
		40.22		
		40.41		
	18 µg/ml	29.22	29.09 ± 00.32	
		28.48		
		29.56		

Table-12: Anticancer activity of shoot extracts.

Extracts	Concentrations	% Viability	Mean % Viability ± S.E.	IC_{50}
***n*- Hexane**	1 mg/ml	54.19	53.01 ± 00.92	-
		53.63		
		51.20		
	2 mg/ml	55.11	55.31 ± 00.16	
		55.62		
		55.18		
	3 mg/ml	68.93	68.18 ± 03.92	
		74.43		
		60.92		
	4 mg/ml	67.92	67.52 ± 05.11	
		75.98		
		58.30		
Chloroform	1 mg/ml	62.40	75.44 ± 60.57	-
		83.89		
		79.61		
	2 mg/ml	79.44	78.77 ± 00.43	
		77.98		
		78.94		

	3 mg/ml	89.77	75.20 ± 07.27	
		69.24		
		66.89		
	4 mg/ml	83.45	94.98 ± 10.52	
		85.76		
		116.09		
Ethanol	1 mg/ml	57.93	52.19 ± 02.86	01.37 mg/ml
		48.87		
		49.93		
	2 mg/ml	49.00	46.94 ± 02.46	
		42.13		
		49.90		
	3 mg/ml	50.22	46.15 ± 02.02	
		44.28		
		44.04		
	4 mg/ml	75.49	68.26 ± 05.74	
		72.28		
		56.88		
Untreated	No concentration	100.00	100.00 ± 00.00	
		100.00		
		100.00		
Cisplatin	12 µg/ml	69.80	70.34 ± 00.31	14.14 µg/ml
		70.88		
		70.34		
	14 µg/ml	49.91	50.97 ± 01.01	
		50.00		
		52.99		
	16 µg/ml	39.34	39.99 ± 00.33	
		40.22		
		40.41		
	18 µg/ml	29.22	29.09 ± 00.32	
		28.48		
		29.56		

Table-13: Anticancer activity of fractions of ethanol shoot extract.

Fraction	Concentration	% Viability	Mean % Viability ± S.E.	IC_{50}
F=1	1 mg/ml	74.15	77.37 ± 2.466	02.89 mg/ml
		75.80		
		82.23		
	2 mg/ml	57.21	58.68 ± 0.983	
		58.28		
		60.55		
	3 mg/ml	49.14	49.09 ± 1.094	
		47.20		
		50.99		
	4 mg/ml	57.22	58.49 ± 2.036	
		55.84		
		62.52		
F=2	1 mg/ml	83.63	80.44 ± 3.366	-
		73.81		
		84.17		
	2 mg/ml	71.74	70.20 ± 1.578	
		71.78		
		67.03		
	3 mg/ml	79.60	77.25 ± 1.324	

		77.14		
		75.02		
	4 mg/ml	83.66	82.16 ± 3.639	
		75.37		
		87.74		
F=3	1 mg/ml	71.63	69.64 ± 1.923	02.96 mg/ml
		71.45		
		65.77		
	2 mg/ml	50.01	49.33 ± 1.122	
		47.18		
		50.90		
	3 mg/ml	76.28	71.60 ± 2.329	
		69.64		
		69.00		
	4 mg/ml	84.78	89.98 ± 2.595	
		92.04		
		93.00		
F=4	1 mg/ml	69.06	66.11 ± 3.345	02.61 mg/ml
		59.54		
		70.02		
	2 mg/ml	59.01	54.44 ± 2.297	
		51.53		
		52.92		
	3 m g/ml	41.14	47.48 ± 3.161	
		51.18		
		49.94		
	4 mg/ml	67.66	65.18 ± 1.591	
		65.67		
		62.21		
F=5	1 mg/ml	65.69	62.84 ± 1.945	01.91 mg/ml
		59.15		
		63.83		
	2 mg/ml	51.56	48.95 ± 1.461	
		48.78		
		46.51		
	3 m g/ml	54.97	56.82 ± 1.281	
		59.26		
		56.13		
	4 mg/ml	56.06	57.97 ± 1.029	
		58.23		
		59.59		
untreated	No concentration	100.00	100.00 ± 0.000	
		100.00		
		100.00		
Cisplatin	12 µg/ml	69.80	70.34 ± 00.31	14.14 µg/ml
		70.88		
		70.34		
	14 µg/ml	49.91	50.97 ± 01.01	
		50.00		
		52.99		
	16 µg/ml	39.34	39.99 ± 00.33	
		40.22		
		40.41		
	18 µg/ml	29.22	29.09 ± 00.32	
		28.48		
		29.56		

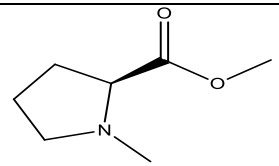
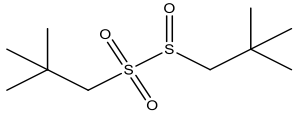
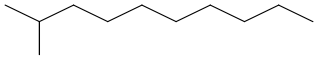
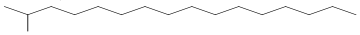
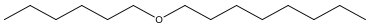
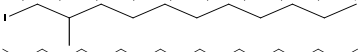
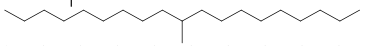
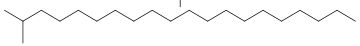
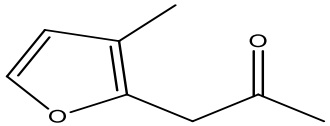
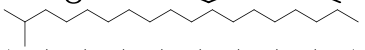
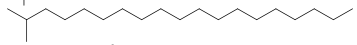
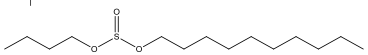
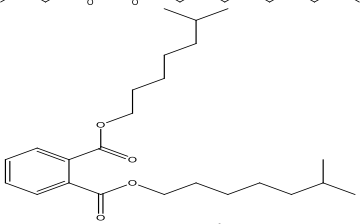
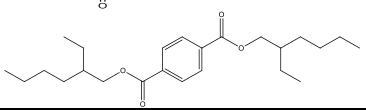
Table-14: % viability against BHK cell line.

Part	Name of extract	Concentration	% viability
	Untreated	No treatment	100.00 %
Leaves	*n*-Hexane	3.57 mg/ml	91.60 %
	Chloroform	3.76 mg/ml	86.80 %
	Ethanol	3.65 mg/ml	98.00 %
Shoots	*n*-Hexane	3.51 mg/ml	88.25 %
	Chloroform	3.95 mg/ml	92.50 %
	Ethanol	3.84 mg/ml	96.20 %
Ethanol Shoot Fractions	F=1	3.03 mg/ml	96.72 %
	F=2	2.87 mg/ml	98.25 %
	F=3	3.25 mg/ml	85.57 %
	F=4	3.88 mg/ml	105.43 %
	F=5	3.35 mg/ml	101.20 %

Table-15: Showing RT of components isolated from F=3.

Detector A Channel 1 275nm				
Peak #	**Ret. Time**	**Area**	**Height**	**Conc.**
1	02.893	31648	1003	19.964
2	05.483	9919	242	06.257
3	11.234	116955	2594	73.778
Total		158522	3839	

Table-16: Names of compounds with their RT, name, molecular mass, % Area and structural formulae.

Peak No.	RT (min)	Compound Name	Molecular mass	% Area	Structure
1	06.65	L-Proline, 1-methyl-, methyl ester	143	04.74	
2	08.10	2,2-Dimethyl-propyl 2,2-dimethyl-propanesulfinyl sulfone	254	03.02	
3	09.27	Decane, 2-methyl-	156	13.27	
4	09.61	Hexadecane, 2-methyl-	240	02.08	
5	09.88	Hexyl octyl ether	214	01.20	
6	10.40	Eicosane	282	12.55	
7	10.71	1-Iodo-2-methylundecane	296	03.78	
8	11.43	10-Methylnonadecane	282	11.18	
9	11.72	Eicosane, 2-methyl-	296	04.00	
10	12.04	3-Methyl-2-(2-oxopropyl)furan	138	03.42	
11	12.37	Octadecane, 2-methyl-	268	17.11	
12	13.26	Nonadecane, 2-methyl-	282	00.28	
13	14.13	Sulfurous acid, butyl decyl ester	278	10.09	
14	14.81	Diisooctyl phthalate	390	00.80	
15	15.87	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	390	12.49	

RT = Retention Time

Conclusions: *G. officinale*, an important medicinal plant, was subjected for phytochemical, physicochemical and biological screening. The presence of some strong phytochemicals revealed that plant has the potential for folk uses. Because of the significant results by all six extracts (*n*-hexane, chloroform and ethanol extracts of leaves and shoots), ethanol shoot extract was selected for fractionation by column chromatography.

Antibacterial, antioxidant and anticancer effects of *G. officinale* samples explored its therapeutic potential. More investigation is suggested to isolate the biologically active compounds from same plant.

Acknowledgments: The authors feel great privilege to acknowledge Prof. Dr. Zaheer-ul-Din Khan, Botany Department of Government College University, Lahore-Pakistan for the identification and authenticity of *G. officinale*. Special regards and thanks to Dr. Sana Javed Awan, Center for Research in Molecular Medicine (CRIMM), The University of Lahore, Lahore-Pakistan and Dr. Muhammad Saeed, Associate Professor, Department of Chemistry, Lahore University of Management Sciences (LUMS), Lahore-Pakistan for providing their skills and laboratory facilities.

REFERENCES

Ahmad, A., Alkarkhi, A. F., Hena, S., and Khim, L. H. (2009). Extraction, separation and identification of chemical ingredients of *Elephantopus scaber* L. using factorial design of experiment. IJC, 1(1), 36.

Alrumman, S. A. (2016). Phytochemical and antimicrobial properties of *Tamarix aphylla* L. leaves growing naturally in the Abha Region, Saudi Arabia. AJSE, 41(6), 2123-2129.

Bobby, M. N., Gnanaraj, W. E., and Alias, J. M. (2015). GC-MS Analysis of *Albizia lebbeck* Benth. WJPR, 11(4), 1284-1304.

Campos, K. C., Rivera, J. H., Gutierrez, J. R., Rivera, I. O., Velez, A. C., Torres, M. P., . . . Millán, C. A. O. (2015). Biological screening of select Puerto Rican plants for cytotoxic and antitumor activities. PRHSJ, 34(1), 25.

Carocho, M., and CFR Ferreira, I. (2013). The role of phenolic compounds in the fight against cancer - a review. Anti-Cancer Agents in Med. Chem., 13(8), 1236-1258.

Cushnie, T. T., and Lamb, A. J. (2005). Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents, 26(5), 343-356.

Dastagir, G., Hussain, F., and Khan, A. A. (2012). Antibacterial activity of some selected plants of family Zygophyllaceae and Euphorbiaceae. J. Med. Plant Res., 6(40), 5360-5368.

Devasagayam, T., Tilak, J., Boloor, K., Sane, K. S., Ghaskadbi, S. S., and Lele, R. (2004). Free radicals and antioxidants in human health: current status and future prospects. JAPI, 52(794804), 4.

Gan, J., Feng, Y., He, Z., Li, X., and Zhang, H. (2017). Correlations between antioxidant activity and alkaloids and phenols of maca (*Lepidium meyenii*). J. Food Qual., 2017.

Gupta, K., Singh, S. P., Manhar, A. K., Saikia, D., Namsa, N. D., Konwar, B. K., and Mandal, M. (2019). Inhibition of *Staphylococcus aureus* and *Pseudomonas aeruginosa* Biofilm and Virulence by Active Fraction of *Syzygium cumini* (L.) Skeels Leaf Extract: *In-vitro* and *In-silico* Studies. Indian J. Microbiol., 59(1), 13-21.

Hailu, Y. M., Atlabachew, M., Chandravanshi, B. S., and Redi-Abshiro, M. (2017). Composition of essential oil and antioxidant activity of Khat (*Catha edulis* Forsk), Ethiopia. Chem. Int., 3, 25-31.

Halberstein, R. (1997). Traditional botanical remedies on a small Caribbean island: middle (grand) Caicos, West Indies. J. Altern. Complement. Med., 3(3), 227-239.

Harris, W. D., and Zukel, J. W. (1958). Organic esters of sulfurous acid. *U.S. Patent No. 2,820,808*. Washington, DC: U.S. Patent and Trademark Office.

Heinrich, M., Barnes, J., Gibbons, S., and Williamson, E. (2004). Fundamentls of pharmacognosy and phytotherapy, Churchill Livingstone, Edinbourgh, London, New York (pp. 245-252): Oxford, Philadelphia, St. Luis, Sydney, Toronto.

Ibrahim, S., Naqvi, S., Perveen, R., Abrar, H., and Akram, Z. (2018). Antidiabetic effect of *Guaiacum officinale*; on exocrine function and histopathology of pancreas in streptozotocin induced diabetic rats. TPMJ, 25(4).

Jeyam, M., Peelaja, R., Shalini, G., and Ravikumar, P. (2013). Evaluation of *Artocarpus hirsutus* fruit pulp against alzheimer's disease. Arch. of pharm. and bio. Sci., 2(1), 30-40.

Kamboj, V. P. (2000). Herbal medicine. Curr. Sci., 78(1), 35-39.

Kim, S. Y. (2012). Comparison of nutritional compositions and antioxidant activities of building blocks in shinseoncho and kale green vegetable juices. Prev Nutr Food Sci, 17(4), 269.

Kinghorn, A. D., and Kinghorn, A. (1979). Cocarcinogenic irritant Euphorbiaceae: Columbia University Press, New York, pp. 137-160.

Kiran, K., Saleem, F., Awan, S., Ahmad, S., Ahmad, S., Malik, A., . . . Peerzada, S. (2018). Anti-Inflammatory and Anticancer Activity of *Pteris*

cretica Whole Plant Extracts. Pak Vet J, 38(3), 225-230.

Kumar, S. (2011). Free radicals and antioxidants: human and food system. Adv Appl Sci Res, 2(1), 129-135.

Mabhiza, D., Chitemerere, T., and Mukanganyama, S. (2016). Antibacterial Properties of Alkaloid Extracts from *Callistemon citrinus* and *Vernonia adoensis* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Int. J. Med. Chem., 2016.

Maneechai, S., and Pikulthong, V. (2017). Total Phenolic Contents and Free Radical Scavenging Activity of *Guaiacum officinale* L. Extracts. Pharmacogn. J., 9(6), 929-931.

Morse, S. S. (2001). Factors in the emergence of infectious diseases Plagues and politics (pp. 8-26): Springer.

Nakano, Y., Nasu, M., Kano, M., Kameoka, H., Okuyama, T., Nishizawa, M., and Ikeya, Y. (2017). Lignans from guaiac resin decrease nitric oxide production in interleukin 1β-treated hepatocytes. J. Nat. Med., 71(1), 190-197.

Nascimento, G. G., Locatelli, J., Freitas, P. C., and Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz. J. Microbiol., 31(4), 247-256.

Niakan, M., Jafary, F., and Abbasi, A. (2017). Antibacterial effect of Guaiacum methanol extracts on clinical isolated *Acinetobacter baumannii*.

Organization, W. H. (1998). Basic tests for drugs: pharmaceutical substances, medicinal plant materials and dosage forms: World Health Organization.

Orwa, C. (2009). Agroforestree Database: a tree reference and selection guide, version 4.0. http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp

Ozarkar, K. (2005). Studies on anti-inflammatory effects of two herbs *Cissus quadrangularis* Linn. and *Valeriana wallichi* DC using mouse model. University of Mumbai, Mumbai.

Phillips, S., Rao, M. R. K., Prabhu, K., Priya, M., Kalaivani, S., Ravi, A., and Dinakar, S. (2015). Preliminary GC-MS analysis of an Ayurvedic medicine Kulathadi Kashayam. J. chem. pharm., 7(9), 393-400.

Pokharen, N., Dahal, S., and Anuradha, M. (2011). Phytochemical and antimicrobial studies of leaf extract of *Euphorbia neriifolia*. J. Med. Plant Res., 5(24), 5785-5788.

Priya, K., Krishnakumari, S., and Vijayakumar, M. (2013). Cyathula prostrata: A potent source of anticancer agent against Daltons Ascites in Swiss albino mice. Asian Pac. J. Trop. Med., 6(10), 776-779.

Sadhu, A., Upadhyay, P., Singh, P. K., Agrawal, A., Ilango, K., Karmakar, D., . . . Dubey, G. P. (2015). Quantitative analysis of heavy metals in medicinal plants collected from environmentally diverse locations in India for use in a novel phytopharmaceutical product. Environ. Monit. Assess., 187(8), 542

Saillenfait, A.-M., Sabaté, J.-P., Robert, A., Cossec, B., Roudot, A.-C., Denis, F., and Burgart, M. (2013). Adverse effects of diisooctyl phthalate on the male rat reproductive development following prenatal exposure. Reprod. Toxicol., 42, 192-202.

Saleem, F., Khan, M. T. J., SALEEM, H., Azeem, M., Ahmed, S., Shahid, N., . . . Altaf, H. (2016). Phytochemical, antimicrobial and antioxidant activities of *Pteris cretica* L. (Pteridaceae) extracts. Acta Poloniae Pharmaceut Drug Res, 73, 1397-1404.

Shakya, A. K. (2016). Medicinal plants: future source of new drugs. Int. J. Herb. Med., 4(4), 59-64.

Singh, N., Sudandiradoss, C., and Abraham, J. (2016). Screening of furanone in *Cucurbita melo* and evaluation of its bioactive potential using in silico studies. Interdiscip. Sci. Comput. Life. Sci., 8(4), 395-402.

Stewart, B. W., and Kleihues, P. (2003). World cancer report (Vol. 57): IARC press Lyon.

Stickel, F., and Schuppan, D. (2007). Herbal medicine in the treatment of liver diseases. Dig. Liver Dis., 39(4), 293-304.

Velioglu, Y., Mazza, G., Gao, L., and Oomah, B. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J. Agric. Food Chem., 46(10), 4113-4117.

Waterman, K. C., Adami, R. C., Alsante, K. M., Hong, J., Landis, M. S., Lombardo, F., and Roberts, C. J. (2002). Stabilization of pharmaceuticals to oxidative degradation. Pharm. Dev. Technol., 7(1), 1-32.

Yoshikawa, T., and Naito, Y. (2002). What is oxidative stress? JMAJ, 45(7), 271-276.

Zugazagoitia, J., Guedes, C., Ponce, S., Ferrer, I., Molina-Pinelo, S., and Paz-Ares, L. (2016). Current challenges in cancer treatment. Clin. Ther., 38(7), 1551-1566.