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Research Article

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Phytochemical Profiling, Anti-Oxidant, Antimicrobial and Cytotoxic Evaluation of Bergera Koenigii Seed Extracts Against Leukemic Cancer

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Abstract

Traditional herbs have always been pioneers in the development of new therapeutics. According to Ayurveda, India has always been a hub of many herbs and shrubs that possess numerous polyphenols and flavonoids with promising anticancer, antioxidant, and antibacterial properties. The present study aimed to elucidate the anticancer activity of various methanol, ethanol and petroleum ether extracts of Bergera koenigii seeds against THP-1 cells. In addition to phytochemical analysis, total phenol and flavonoid content (TPC, TFC) and antioxidant assays were also performed to estimate the potential of the Bergera koenigii seeds. Among these, the methanolic extract of Bergera koenigii seeds inhibited leukemic THP-1 cells due to the presence of bioactive compounds such as hexadecanoic acid, octadecadienoic acid, octadecatrienoic acid, tricosanoic acid and γ-sitosterol, as revealed by GC–MS analysis. According to the radical scavenging capacity, both hexadecanoic acid and octadecadienoic acid exhibited the highest anticancer activity, with IC50 values of 15±8.37 and 15±0.23 µg/ml, respectively. Additionally, the methanolic extract had a TPC of 959.97 mg/GAE and a TFC of 1443.20 mg/QE, and the antioxidant activity had an IC50 value of 369.09, Antibacterial activity with 10 ± 0.5 mmEscherichia coli, 8 ± 0.82 mm for Pseudomonas aeruginosa and15 ± 1.7 mm for Staphylococcus aureus. Thus, the above results indicate that the methanolic extracts of Bergera koenigii seeds are more promising drugs against leukemic THP-1 cell lines than are the chloroform and pet ether extracts.

Keywords: Acute Myeloid Leukemia, Bergera Koenigii Seeds, THP-1 Cells, Antioxidant, Anticancer, Antimicrobial

Abbreviations

AML: Acutemyeloid Leukemia; **ABTS:** 2,2′-Azinobis(3-Ethylbenzothiazoline-6-Sulfonic Acid) Diammonium Salt; **DPPH:** 2,2-Diphenyl-1-Picrylhydrazyl; **IC50:** Inhibition Concentration 50; **LPS:** Lipopolysaccharide; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; **TPC:** Total Phenol Content; **TFC:** Total Flavonoid Content; **ZOI:** Zone of Inhibition.

1. Introduction

Plants have been used as medicine since the dawn of humanity. Because traditional medicine is founded on nature, herbs that have the power to lessen the effects of sickness are utilized as treatments. Traditional medicine is rooted in culture, and it is necessary to research the safety, efficacy, and efficiency of its extracts. Drugs used to treat, prevent, and diagnose illnesses make up the majority of pharmaceuticals. The purpose of the medication's adverse effects is to exacerbate the patients' problems. Most adverse pharmaceutical effects may be predicted computationally, and advancements in technology have enabled the synthesis of natural chemical compounds from conventional herbs.

Bergera koenigii, a plant of the Rutaceae family, is extensively dispersed in Eastern Asia, and its medicinal qualities are well documented in Ayurveda, the traditional Indian school of medicine. reported that distinct plant extracts have antiviral, anti-inflammatory, antioxidant, antidiabetic, anti-diarrheal, anti-leishmanial, and anticancer effects [1]. *Bergera koenigii* is indigenous to India, Sri Lanka, and other South Asian countries. In India, *Bergera koenigii* is widespread and can be found in large quantities everywhere from Sikkim to Garhwal, Bengal, Assam, the Western Ghats, and Kerala. Along with South Indian immigrants, it made its way from India to Malaysia, South Africa, and Reunion Island.

Numerous disease pathologies frequently have commonalities with oxidative stress activities. When created at a higher rate, it

causes biomolecule damage, mutational implications, and tissue damage [2]. Oxidative stress is eventually reduced by antioxidant supplements. *Bergera koenigii* has been utilized in many antioxidant activity experiments. Antioxidants, particularly plant-based compounds, provide lead for the prevention and treatment of diseases [3].

Antimicrobial resistance develops among microorganisms as a result of increased use of these drugs. Plant-based compounds could be effective against microorganisms. Phytomedicines work synergistically as therapies by interacting with microorganisms. *Bergera koenigii* shows broad antimicrobial activity against various species [4].

Treatment of cancer with chemotherapy and radiation causes lethality to normal cells and can cause side effects. The active components of plants have paved the way for the development of new therapeutics to treat cancer. Suggested that *Bergera koenigii* has anticancer potential and prevents cancer with less toxicity to the human body [5].

The solvent polarity has an impact on how well the antioxidant activity is preserved [6]. The extract may be poisonous and have negative consequences depending on the solvent. These factors make it crucial to choose the extraction solvent in accordance with the requirements for phytochemicals and the functions of those compounds. The goal of this study was to investigate and elucidate the effects of polar and nonpolar solvents on chemical compound preservation as well as the antioxidant and anticancer properties of *Bergera koenigii* seeds. The anticancer effect of *Bergera koenigii* seeds on the acute myeloid leukemia cell line THP-1 was evident in this study. The seed extracts were initially screened for phytochemicals, after which GC–MS, antioxidant activity, and anticancer activity analyses were performed. The findings highlight the anticancer properties of seed extracts by demonstrating how mahanine, mahanimbine, kenimbine, and other alkaloids play critical roles in preventing the proliferation of the THP-1 cell line.

2. Materials and Methods

2.1 Chemicals and Reagents

Aluminum chloride ($AlCl_3$), ammonia solution (NH4OH), concentrated sulfuric acid (H2SO4), dimethyl sulfoxide (DMSO), 2,2′-diphenyl-1-picrylhydrazyl (DPPH), ferric chloride ($FeCl_3$), glacial acetic acid, Mayer's reagent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), petroleum ether (C_6H_{14}), sodium bicarbonate ($NaHCO_3$), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), the reagents Fehling's solution A and B, ferric chloride, Folin-Ciocalteu reagent, and the solvent methanol (99.8%) were purchased from Thermo Fisher Scientific. Bromine water, nutrient agar, and the solvent chloroform (99.8%) were purchased from HiMedia. The antibiotics dimethyl sulfoxide (DMSO), ethylenediaminetetraacetic acid (EDTA), fetal calf serum (FBS), phosphate-buffered saline (PBS), Roswell Park Memorial Institute (RPMI) 1640 medium and trypsin were purchased from Gibco.

2.2 Plant Material

B. koenigii seeds were collected from Mandaiyur, Tiruchirappalli, Tamil Nadu, and India. The seeds were washed thrice with distilled water to remove unwanted debris and then dried at room temperature (RT) for 3 to 4 weeks. The dried seeds were powdered in a blender and sieved. The powder was stored in an airtight, amber-coated bottle for future analysis at room temperature.

2.3 Extraction

The seed powder (50 g) was dissolved in 500 ml of methanol, chloroform, and petroleum ether. The mixture was then incubated on a rotary shaker at 100 rpm for 3 h, followed by mixing in an ultrasonic water bath (Labman LMUC-9) at 50 °C for 50 min. The mixture was then filtered through a No.42whatman filter paper and the filtered solvent were concentrated using a rotary evaporator (Buchi R-100) at 40 °C. The seed extracts were stored in a refrigerator until further analysis.

2.4 Preliminary Phytochemical Screening

To confirm that the plant's chemical extracts includes the anticipated beneficial secondary metabolites, we carried out preliminary phytochemical profiling. Conventional biochemical testing was used to assess the presence or absence of certain phytochemicals in compliance with previously published protocols. The seed extract was dissolved in methanol, chloroform, and pet ether individually and tested for alkaloids, phenols, terpenoids, flavonoids, steroids, saponins, tannins and reducing sugars [7-11].

2.5 Determination of Total Phenolic Content

The Folin-Ciocalteu method was used to determine the total phenolic content of the extracts. Two hundred microliters of 0.1% crude extract (from each solvent) was diluted to 3 ml with distilled water and thoroughly mixed with 0.5 ml of Folin-Ciocalteu reagent for 3 min, after which 2 ml of 20% (w/v) sodium carbonate was added. The mixture was allowed to stand for an additional 60 minutes in the dark, after which the absorbance was measured at 650 nm. The total phenolic content was determined from the calibration curve, and the results are presented as mg of gallic acid equivalent per g dry weight [12].

2.6 Determination of Total Flavonoid Content

The aluminum chloride colorimetric technique was used to determine the total flavonoid content of the crude extract. Fifty microlitres of 0.1% crude extracts (the respective solvents) were diluted to 1 ml with methanol and combined with 4 ml of distilled water. After 5 minutes of incubation, 0.3 mL of a 5% NaNO2 solution and 0.3 mL of a 10% AlCl3 solution were added, and the mixture was allowed to stand for 6 minutes. After that, 2 mL of 1 N NaOH solution was added, and the total volume of the combination was brought to 10 mL using double-distilled water. After 15 minutes, the absorbance was measured at 510 nm. The total flavonoid content was determined from the calibration curve, and the results are presented as mg of quercetin equivalent per g dry weight [12].

2.7 Fourier Transform Infrared Spectroscopy Analysis

Bergera koenigiiseed extract was subjected to Fourier transform infrared (FTIR) spectroscopy (SHIMADZU IRTracer-100) to identify the functional groups present in the extract. Translucent sample discs were prepared by encapsulating 10 mg of extracts in 100 mg of KBr pellets. All spectra were acquired in the range of 4000-400 cm-1 with 4 cm-1 resolution [13].

2.8 Gas Chromatography–Mass Spectrometry

GC–MS analysis of the seed extracts was performed using a Shimadzu QP-2010 Plus thermal desorption system (TD20). Ionization energy of 70 eV was used, and helium gas was used as a carrier with a flow rate of 1.20 ml/min. One microliter of the sample was injected. The GC injector temperature was maintained at 230 °C, and the MS transfer line temperature was maintained at 280 °C. The temperature of the ion source was set at 300 °C. The first oven temperature was set to 50 °C with a hold time of 60 s. Then, the temperature was increased to 300 °C (at 5 °C/min) with a hold time of 5 min and to 235 °C (at 10 °C/min) with a hold time of 10 min. The resulting peaks were analysed using various inbuilt MS libraries, such as WILEY8. LIB and NIST05.LIB.

3. Antioxidant Profiling

3.1 DPPH Assay

The antioxidant activity was characterized using a DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Briefly, different concentrations of seed extract were prepared in methanol, chloroform and petroleum ether. Two hundred microliters of different concentrations of plant extract were mixed with 100 µl of 1 mM DPPH solution (Sigma, India). The mixture was incubated with shaking at room temperature in the dark for 30 min [14]. The absorbance was measured at 517 nm.The scavenging ability of the seed extract was calculated using the following equation:

DPPH scavenging activity (%) = $(Abs_{control} – Abs_{sample})/Abs_{control}$

Where, $Abs_{control}$ represents the absorbance of DPPH without sample and Abs_{sample} represents the absorbance of DPPH with sample.

3.2 ABTS Assay

The ABTS (2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radical cation decolorization assay was used to determine the free radical scavenging ability of the seed extract. The reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1) created the ABTS+ cation radical, which was then kept at room temperature for 12 to 16 hours in the dark. To achieve an absorbance of 0.700 at 734 nm, the ABTS+ solution was diluted with methanol. The absorbance was measured 30 minutes after adding 5 µl of plant extract to 3.995 ml of diluted ABTS+ solution. In each test, a solvent blank was used. All measurements were carried out at least three times. Ascorbic acid was used as a standard. The absorbance at 734 nm [15]. The percent suppression of radical was calculated using the following equation.

Suppression activity (%) = $(Abs_{control} – Abs_{sample}) / Abs_{control}$

Where $Abs_{control}$ represents the absorbance of ABTS without sample; Abs_{sample} represents the absorbance of ABTS with sample.

3.3 Antimicrobial Activity

The antimicrobial activity of methanolic, chloroform and petroleum ether extract of *Bergera koenigii* seeds were determined by disc diffusion assay. The test organisms *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* were obtained from the Department of Microbiology, Bharathidasan University, Tiruchirappalli, India.

Briefly, nutrient agar was prepared and poured onto sterile disposable petri plates and using sterile cotton swabs the bacterial suspension was spread uniformly on the surface of nutrient agar. Commercially available sterile empty and antibiotic disc were purchased (Hi Media, India) and 20 µl of seeds extract was added in an empty sterile disc without dilution. Empty disc was used as a negative control whereas Streptomycin disc was used as a positive control. Plates were then incubated at 37 °C for 24 h and zone of inhibition was measured.

4. Anticancer Activity

4.1 Growth Assay

THP-1 cells were cultivated in medium (RPMI-1640) supplemented with 10% complement-inactivated fetal bovine serum (FBS), 100 IU/mL penicillin, 100 g/mL streptomycin, and 3.7 g sodium bicarbonate/L (Sigma–Aldrich, St. Louis, MO, USA). THP-1 cells were cultivated in 96-well culture plates, and then 100 ng/mL (162 nM) phorbol 12-myristate 13-acetate was added. This activation of THP-1 cell differentiation was achieved. Before every experiment, the cells were washed with RPMI-1640 serum-free media to remove undifferentiated cells. The cells were subsequently exposed to lipopolysaccharide (LPS) (100 ng/mL) from E. coli O55:B5 (L2880 Sigma) for 24 hours to activate macrophage-like cells [16].

4.2 MTT Assay

MTT assays were used to determine THP-1 cell viability. At various times, THP-1 cells were exposed to all three extracts of *B. koenigii* at varying concentrations (100 µg/mL). Then, 50 µL of serum-free media was added to each well, and 50 µL of MTT reagent was added to each well. The cells were cultivated in 96-well plates and incubated for three hours. The absorbance was measured at 590 nm. All experiments were carried out in triplicate [17].

4.3 Statistical Analysis

The analysed data are expressed as the mean ± standard deviation. The statistical analysis was performed by using Microsoft Excel, and GraphPad Prism 10.2.0 software was used to plot the graphs.

5. Results and Discussion

5.1 Qualitative Analysis

The preliminary qualitative analysis of the seed extracts revealed the presence of various groups of phyto-chemicals, as shown in ***Table 1.***

Table 1: Phytochemical Evaluation of *Bergera Koenigii* Extracts

S.No.	Test	Methanol	Chloroform	PetEther
1	Alkaloid	-	-	+
2	Terpenoids	+	-	-
3	Phenol	+	+	-
4	Tannin	+	+	+
5	Reducingsugar	-	-	-
6	Sterols	-	+	+
7	Flavonoids	+	-	-
8	Saponin	+	+	+
9	Glycosides	-	-	+

Alkaloids and glycosides were present in pet and absent in methanol and chloroform, and terpenoids and flavonoids were present in methanol and absent in chloroform and ether. Phenols were present in both methanol and pet ether, tannins and saponins were present in all three extracts, reducing sugars were absent in all three extracts, and sterols were present in chloroform and pet ether but absent in methanol. From the qualitative phytochemical analysis, it was observed that all the solvent extracts possessed a decent phytochemical composition, with methanol and petroleum ether having rich and varied phytochemical compositions. Plants are a source of novel antibiotics, and people choose herbal medicines because of the adverse side effects associated with synthetic antibiotics [18,19].

5.2 Quantitative Analysis

The quantitative analysis of the seed extracts was carried out to determine the total phenolic content (TPC) and total flavonoid content (TFC), as presented in **Table 2.**

Table 2: Quantitative Analysis for Estimation of total Phenols and Flavonoids in *Bergera* Koenigii Seed

Compound	TPC	TFC
Methanol	959.97	1443.20
Chloroform	150.68	-50.625
Petether	146.314	97.79

The total phenolic content and total flavonoid content of *Bergera koenigii* were determined using the Folin method and aluminum chloride method, respectively, using calibration curves of different concentrations of gallic acid and quercetin. The TPC and TFC are expressed as mg gallic acid per g dry weight and as mg quercetin per g dry weight, respectively. Both the TPC and TFC of the seed were found to be greater for the methanol extract, at 959.97 mg gallic acid/g and 1443. 20 mg quercetin/g, respectively. The chloroform extract and pet ether extract of the seeds had lower TFC and TPC, respectively. The quantitative analysis of the *B. koenigii* seed extracts showed that the methanolic extract had the greatest phenolic and flavonoid contents, followed by petroleum ether and chloroform.

5.3 FTIR Analysis

Phytocompounds of *Bergera koenigii* seed extracts were analysed by biomolecule functional group Figure 1.

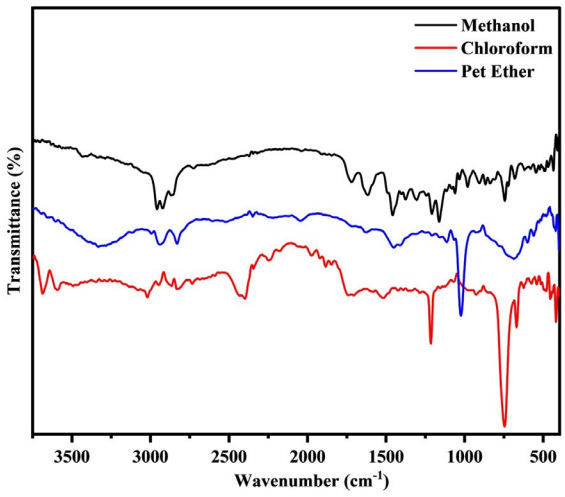


Figure 1: FTIR Analysis of the Methanol, Chloroform and pet ether extracts of *Bergera Koenigii* Seeds

Functional groups such as alcohols, alkanes, amines, alkenes, phenols, aromatics, and cyclic alkenes were identified in ***Table 3.***

Table 3: FTIR Analysis of *Bergera Koenigii* Extracts

S. No	Frequency Ranges	Bond	Functional groups
1	3336	O-H stretching	Alcohol
2	2924	C-H stretching	Alkane
3	2856	N-H stretching	amine salt
4	2357	C°N stretching	Phenyl or amino
5	1712	C-H bending	aromatic compound
6	1652	C=C stretching	Alkene
7	1610	C=C stretching	cyclic Alkene
8	1452	C-H bending	Alkane Methyl group
9	1035	S=O stretching	Sulfoxide
10	840	C=C bending	Alkene
11	833	1,4-disubstituted or C-H bending	Aromatic compound

FTIR analysis revealed peaks representing various secondary metabolites that indicated the presence of phytoconstituents such as polyphenols, flavonoids, tannins, and terpenoids. Our results contrast with earlier reports in which alkaloids and terpenoids were present in methanol extracts [20]. This may be because of the varied locations, climatic conditions, type of soil where the plant was cultivated, and various other factors that affect the presence and absence of phytoconstituents. These FTIR results are significant according to the preliminary phytochemical analysis.

5.4 GC–MS Analysis

GC–MS analysis of the *Bergera koenigii* extracts revealed the presence of 30, 58 and 35% methanol, chloroform and pet ether extracts, respectively. The analysis led to the identification of several constituents from the GC fractions of the solvent seed extracts presented in ***Table 4.***

Table 4: GC-MS Analysis of Bergera Koenigii seed Extract

Peak No	Retention	Formula	Compound name	Total %
Methanol extract				
1	2.760	C2H5NO2	Carbamic acid, methyl ester	0.521%
2	56.787	C17H34O2	Hexadecanoic acid, methyl ester	15.052
3	57.487	C17H34O2	Hexadecanoic acid, methyl ester	2.221
4	57.972	C16H32O2	n-Hexadecanoic acid	2.704
5	61.605	C19H34O2	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	15.052
6	62.251	C19H34O2	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	15.052
7	62.439	C19H36O2	9-Octadecenoic acid (Z)-, methyl ester	2.501
8	62.883	C19H32O2	9,12,15-Octadecatrienoic acid, methyl ester	11.557
9	63.045	C19H38O2	Methyl stearate	2.770
10	63.422	C18H34O2	cis-Vaccenic acid	1.421
11	64.081	C18H36O2	Octadecanoic acid	0.703
12	64.740	C19H34O2	Methyl 9-cis,11-trans-octadecadienoate	0.555
13	65.790	C18H32O2	9,12-Octadecadienoic acid	0.344
14	67.929	C21H40O2	11-Eicosenoic acid, methyl ester	1.349
15	68.696	C21H42O2	Eicosanoic acid, methyl ester	0.254
16	69.733	C20H36O2	Ethyl 9.cis.,11.trans.-octadecadienoate	0.328
17	70.029	C30H50O6	Olean-12-ene-3,15,16,21,22,28-hexol, (3β,15α,16α,21β,22α)	1.095
18	71.818	C20H36O2	Methyl 2-octylcyclopropene-1-octanoate	2.163
19	74.012	C23H46O2	Docosanoic acid, methyl ester	0.918
20	74.415	C24H38O4	Diisooctyl phthalate	1.230
21	75.600	C28H48O	Campesterol	0.570
22	76.515	C16H48O6Si7	Heptasiloxane	4.028

23	78.170	C29H48O	Stigmasterol	0.724
24	78.950	C24H48O2	Tricosanoic acid, methyl ester	6.212
25	81.817	C29H50O	γ-Sitosterol	3.290
26	82.557	C29H48	Stigmastan-3,5-diene	1.761
27	84.091	C32H64	ethyl-pentadecyl)cyclohexane	0.554
28	84.992	C20H33BrO	1,2-Epoxycyclopentane, 3- isopropyl-1-methyl-2-[3-(2-bromomethyl-3- methylcyclopentyl)but-3-en-1- yl]	0.720
29	86.755	C40H58O	Rhodopin	1.058
30	87.361	C20H42O	-Dodecanol, 2-octyl	0.521
Chloroform extract				
1	25.743	C12H26O	2-Dodecanol	0.430%
2	26.282	C12H26	Dodecane	0.330%
3	26.632	C10H20O	Decanal	0.121%
4	30.103	C10H12O	Benzaldehyde, 4-propyl	1.030%
5	30.736	C12H24	2-Decene, 2,4-dimethyl	0.268%
6	30.843	C15H32	Dodecane, 2,7,10-trimethyl	0.232%
7	31.907	C14H30	Tetradecane	1.179%
8	33.212	C15H32	Dodecane, 2,6,11-trimethyl	0.903%
9	36.347	C14H28	Cyclotetradecane	0.550%
10	36.724	C14H30	Tetradecane	1.065%
11	38.285	C10H18O3	Nonanoic acid, 9-oxo-, methyl ester	0.559%
12	39.496	C14H20O2	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	0.537%
13	40.869	C21H44	Heptadecane, 2,6,10,15-tetramethyl	0.332%
14	41.057	C15H32	Pentadecane	0.345%
15	41.662	C14H22O	Phenol, 2,4-bis(1,1-dimethylethyl)	1.821%
16	42.726	C17H30O4	Fumaric acid, isobutyl nonyl ester	2.732%
17	43.883	C12H24O2	Dodecanoic acid	0.486%
18	44.785	C16H32	Cetene	1.076%
19	45.094	C20H42	Eicosane	1.263%
20	45.928	C13H10O	Benzophenone	1.347%
21	47.476	C17H28	Benzene, (1-ethylnonyl)	0.286%
22	48.848	C17H36	Heptadecane	0.843%
23	49.790	C15H30O2	Methyl tetradecanoate	0.837%
24	50.759	C20H42	Hexadecane, 2,6,11,15-tetramethyl	1.120%
25	51.351	C14H28O2	Tetradecanoic acid	1.166%
26	52.414	C18H38	Octadecane	1.128%
27	54.702	C16H22O4	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	1.513%
28	55.953	C17H32O2	9-Hexadecenoic acid, methyl ester, (Z)	0.873%
29	56.492	C17H24O3	7,9-Di-tert-butyl-1- oxaspiro(4,5)deca-6,9-diene-2,8-dione	4.973%
30	56.949	C17H34O2	Hexadecanoic acid, methyl ester	6.804%
31	58.550	C21H44	Eicosane, 2-methyl	5.906%
32	59.035	C19H36O	12-Methyl-E,E-2,13-octadecadien-1-ol	1.087%
33	59.573	C17H26O3	3,5-di-tert-Butyl-4-hydroxyphenyl propionic acid	1.747%
34	59.896	C18H36O2	Heptadecanoic acid, methyl ester	0.670%
35	62.695	C19H34O2	Methyl 9-cis,11-trans-octadecadienoate	6.804%
36	63.005	C19H32O2	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	3.233%
37	63.314	C19H38O2	Methyl stearate	6.804%

38	63.449	C21H40O4	hydroxy-1- (hydroxymethyl)ethyl ester	1.662%
39	64.269	C18H36O2	Octadecanoic acid	4.843%
40	64.512	C21H44	Eicosane, 2-methyl	0.814%
41	64.821	C19H34O2	Methyl 9-cis,11-trans-octadecadienoate	0.803%
42	66.301	C19H32O2	Methyl 9.cis.,11.trans.t,13.trans.-octadecatrienoate	0.867%
43	66.678	C19H32O2	Methyl 9.cis.,11.trans.t,13.trans.- octadecatrienoate	0.827%
44	67.526	C19H32O2	6,9,12-Octadecatrienoic acid, methyl ester	2.332%
45	67.983	C21H40O2	11-Eicosenoic acid, methyl ester	1.512%
46	68.737	C21H42O2	Eicosanoic acid, methyl ester	2.305%
47	69.114	C11H20O	6-Nonenal, 3,7-dimethyl	0.345%
48	70.190	C11H20O	6-Nonenal, 3,7-dimethyl	2.814%
59	71.859	C20H36O2	Methyl 2-octylcyclopropene-1-octanoate	1.192%
50	72.303	C21H34O	: Androst-5-en-17-ol, 4,4-dimethyl	0.922%
51	74.052	C23H46O2	Docosanoic acid, methyl ester	2.400%
52	75.627	C26H54	Octadecane, 3-ethyl-5-(2-ethylbutyl)	1.494%
53	76.528	C24H48O2	Tricosanoic acid, methyl ester	1.155%
54	78.197	C21H32O2	Pregn-5-en-20-one, 3-hydroxy	.788%
55	78.964	C25H50O2	Tetracosanoic acid, methyl ester	1.966%
56	81.171	C30H50	Squalene	0.911%
57	84.992	C27H44O	Cholesta-4,6-dien-3-ol, (3β)	1.855%
58	85.437	C29H46	Stigmastan-6,22-dien, 3,5-dedihydro	1.704%
Petroleum ether extract				
1	41.636	C14H22O	Phenol, 2,4-bis(1,1-dimethylethyl)	0 0.998%
2	49.185	C17H36	Heptadecane	0.274%
3	51.163	C14H28O2	Tetradecanoic acid	0.364%
4	52.508	C16H34O	3-Hexadecanol	0.590%
5	53.356	C17H34O2	Isopropyl myristate	0.331%
6	55. 940	C17H32O2	9-Hexadecenoic acid, methyl ester, (Z)	0.620%
7	56.532	C17H34O2	Tridecanoic acid, 4,8,12-trimethyl-, methyl ester	1.510%
8	56.720	C17H34O2	Hexadecanoic acid, methyl ester	10.876%
9	57.999	C16H32O2	n-Hexadecanoic acid	4.249%
10	58.927	C17H26O3	3,5-di-tert-Butyl-4-hydroxyphenyl propionic acid	0.629%
11	61.121	C14H14N2OS	10-Oxo-5,5-dimethyl-5-sila-5,10-dihydro-5H-benzo[e] pyrido[3,4-b]azepine	0.294%
12	61.417	C19H32O2	γ-Linolenic acid, methyl ester	0.491%
13	61.713	C19H34O2	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	1.995%
14	62.049	C19H34O2	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.198%
15	62.345	C19H36O2	trans-13-Octadecenoic acid, methyl ester	1.691%0.346%
16	62.601	C19H32O2	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	0.346%
17	62.776	C19H38O2	Methyl stearate	0.275%
18	63.005	C19H38O2	Heptadecanoic acid, 15-methyl-, methyl ester	10.778%
19	63.206	C18H32O2	9,12-Octadecadienoic acid (Z,Z)-	4.051%
20	64.068	C18H36O2	Octadecanoic acid	1.550%
21	64.418	C21H44	Heneicosane	0.416%
22	66.234	C19H32O2	6,9,12-Octadecatrienoic acid, methyl ester methyl ester	0.230%
23	66.853	C15H33ClOSi	1-Dimethyl(3-chloropropyl) silyloxydecane	0.446%
24	67.620	C19H36O3	Methyl 12-hydroxy-9-octadecenoate	0.639%

25	68.024	C19H34O2	10,13-Octadecadienoic acid,methyl ester	0.804%
26	68.697	C21H42O2	Methyl 18-methyl	0.676%
27	68.885	C19H36O2	16-Octadecenoic acid, methyl ester	1.046%
28	69.221	C29H60	2-methyloctacosane	0.444%
29	70.338	C21H44	Heneicosane	0.252%
30	71.805	C19H32O3	8-Nonenoic acid, 9-(1,3-nonadiene loxy)-, methyl ester	0.336%
31	73.420	C29H62O2Si	Silane,diethyl heptyloxyoctadecyloxy	12.265%
32	74.012	C23H46O2	Docosanoic acid, methyl ester	0.611%
33	74.429	C24H38O4	Phthalic acid, di(2-propyl pentyl) ester	1.159%
34	78.547	C20H28O3	Benzoic acid, 3,5-dicyclohexyl-4-hydroxy-, methyl ester	17.263%
35	78.951	C25H50O2	Tetracosanoic acid, methyl ester	0.952%

The GC–MS results revealed 30 compounds in the methanol extract, 59 in the chloroform extract, and 36 in the petroleum ether extract. Although the chloroform extract contained the greatest number of compounds, chemically or biologically active compounds with important biological applications were found to be the most abundant in the methanol extract. Various studies using GC–MS have revealed the influence of different solvents on the isolation of phytochemical constituents with medicinal value from crude extracts of medicinal plants [21]. Our GC–MS results are in accordance with those previously reported. According to the literature, these compounds are known for their therapeutic properties and have been previously reported in many different medicinal plants. Some of these compounds are separately isolated in extracts and are used as antimicrobial and radical scavenging agents in medicine formulations [22]. For example, compounds such as 9, 12-octadecadienoic acid, methyl ester (linolenic acid), n-hexadecanoic acid (palmitic acid), hexadecanoic acid ethyl ester, hexadecanoic acid methyl ester, tetradecanoic acid, 12-methyl ether, methyl ester, and squalene are known to possess antioxidant and 5-alpha reductase inhibitor activity [23,24]. Compounds such as 3,7,11,15-tetramethyl-2-hexadecen-1-ol (phytol) and squalene also have antimicrobial activities [25]. Compounds such as esters of fatty acids with simple OH groups are of commercial importance; are used as texturizing, emulsifying, antifoam and stabilizing agents; and have applications in cosmetics, detergents and pharma products due to their fragrance [26,27]. Since GC–MS analysis revealed only the presence of volatile compounds, phenolic compounds that are non-volatile were not detected in the GC–MS analysis.

5.5 Antioxidant Activity

The effect of the solvent used for extraction on the antioxidant activity was studied by the DPPH method, and ABTS method as shown in Figure 2 and Figure 3.

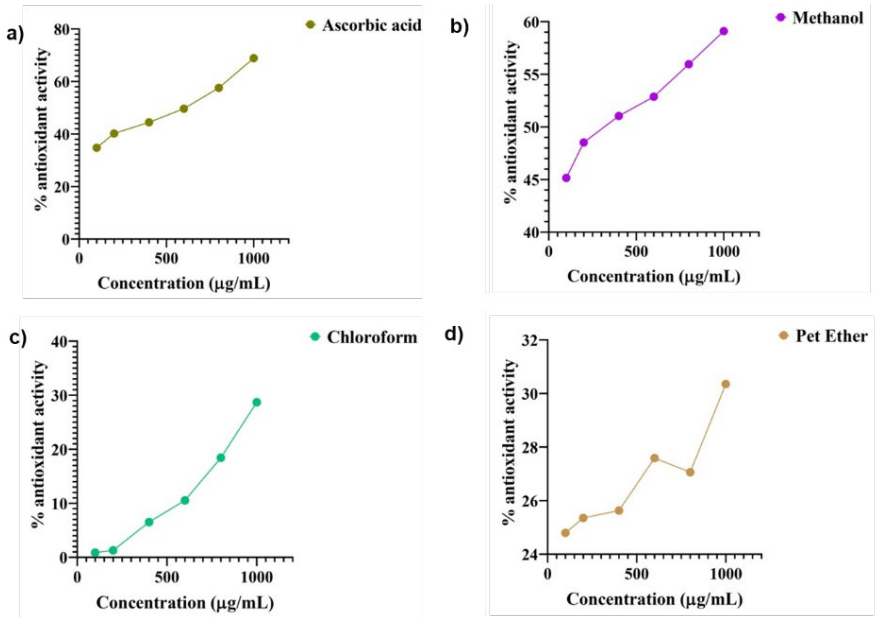


Figure 2: Graphical Representation of DPPH Scavenging % observed for a) Ascorbic Acid, b) Methanol, c) Chloroform and d) Petroleum Ether Extracts of Bergera Koenigiiseeds

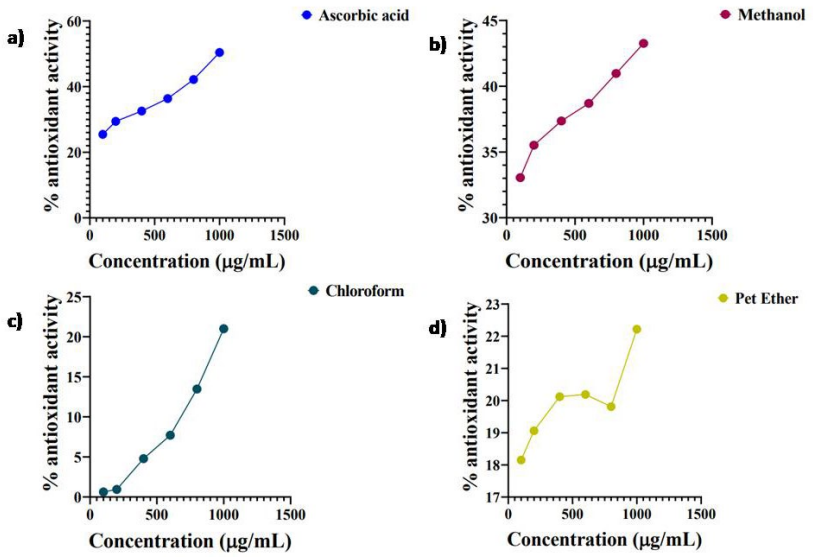


Figure 3: Graphical Representation of ABTS Scavenging % Observed for a) Ascorbic Acid, b) Methanol, c) Chloroform and d) Petroleum Ether Extracts of *Bergera Koenigii* Seeds

Free radicals can induce biological damage and various pathological events, such as aging, carcinogenesis, and inflammation reported that phenolic compounds are effective against inflammation [28]. Phenols and flavonoids are representative groups of antioxidant compounds [28]. Thus, in our study, the antioxidant activity was evaluated for all the solvent extracts to identify the influence of the solvent used for extraction. Our results are in accordance with study in which ethanol was used as a solvent for extraction [29]. Based on the solvent used for extraction, variations in the radical scavenging ability of the *B. koenigii* seed extracts were found. This shows that the solvent significantly influences the antioxidant activity of *B. koenigii* seed extracts. Flavonoids are a widespread group of phenolic compounds in plants that are widely reported for their antioxidant potential [30]. According to the phytochemical analysis, the chloroform and petroleum ether extracts had minimum flavonoid contents, which would have impacted their minimum ROS scavenging activities. These findings were supported by previous studies on *Macademiatetraphylla* peels, selected herbal infusions, *Limnophila aromatic* and *Withnaiasomnifera*(L.), which reported that there is a strong influence of solvent on the antioxidant activity of plant extracts [31-34]. This variation in antioxidant activity may be associated with plant compounds such as phenolic constituents, which are soluble based on the polarity of the solvents used for extraction. Thus, the antioxidant activity of extracts obtained from polar solvents is more vital [28,35]. Because of their strong radical scavenging activity, alcoholic extracts of *B. koenigii* seeds are potential sources of natural antioxidants that can be used to ameliorate the effects of oxidative stress. Antioxidant activity in plants can occur through several mechanisms, such as chain initiation prevention, binding of transition metal ion compounds, peroxide decomposition, inhibition of continued hydrogen abstraction, reduction, and scavenging of radicals [36,37].

5.6 Antimicrobial Activity

The antibacterial activity of Bergera koenigii seeds extract was evaluated against clinical pathogens and the inhibition of microbial growth was seen **Figure 4.**

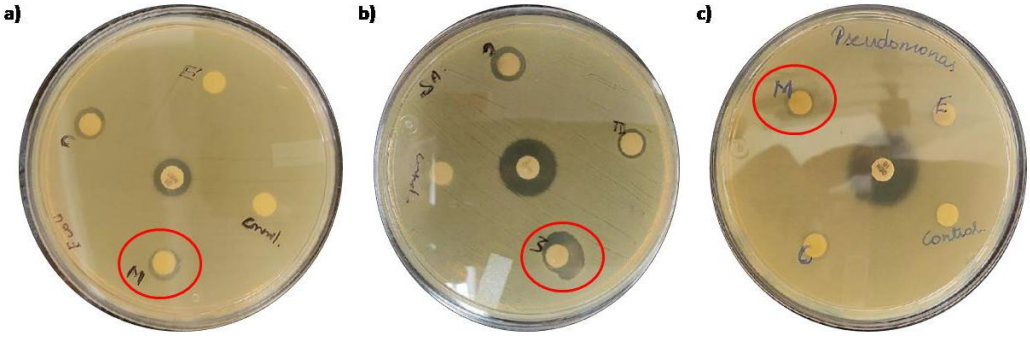


Figure 4: Zone of Inhibition observed for a) *E. coli*, b) *S. aereus* and c) *P. aeruginosa*, with Extracts of *Bergera Koenigii* Seeds

The zone of inhibition of all the solvent extracts on the bacterial strains was measured and given in **Table 5.**

Table 5: Observation of Zone of Inhibition of *Bergera Koenigii* among Different Bacterial Species

	Zone of inhibition (in mm)					
S. No.	**Bacteria**	**Methanol**	**Chloroform**	**PetEther**	**Standard**	**Control**
1	*Escherichia coli*	10±0.5	9±1.5	0	13±0.9	0
2	*Pseudomonas aeruginosa*	8±0.82	0	0	25±0.6	0
3	*Staphylococcus aureus*	15±1.7	10±1.2	7±1.1	28±1.4	0

Plant-based drugs or antimicrobial agents are mitigating the side effects of synthetic antimicrobials. Several reports are available on herbal extracts against -multidrug-resistant pathogens. Plant secondary metabolites (alkaloids, steroids, phenols, and tannins) exert their action by resembling endogenous metabolites, signal transduction, ligands, hormones, and neurotransmitters. Plants also provide boundless prospects for the discovery of new drugs via different ways depending on the availability of chemical diversity. The plants are a source of novel antibiotics and people choose herbal medicines because of the adverse side effects associated with synthetic antibiotics [18,19].With this insight, we analyzed the antibacterial efficiency of *Bergera koenigii* extracts. The highest zone of inhibition was observed in methanol extract with 15 ± 1.7 mm against the Gram-positive organism *S. aureus.* The zone of inhibition was significantly comparable with the commercial antibiotic Ciprofloxacin which is 28 ±1.4 mm. A ZOI of8 ± 0.82 and 10 ± 0.5 mm was observed against gram-negative organisms such as *Pseudomonas aeruginosa* and *E. coli,* respectively. Whereas chloroform and petroleum ether extract showed minimum inhibition against both organisms.The bioactive compound present in methanol extract inhibits microbial growth by binding to their cell surfaces. The bioactive compound adsorbed by bacterial cell membrane leads to the generation of hydroperoxides, disruption, and then cell leakage [38]. For instance, the cell wall synthesis of microbes was inhibited by tannins by irreversible complexes with proline rich proteins [39]. Leakage of protein and some enzymes is caused by the saponins [40]. Terpenoids weakening the membranous tissue of microbes, which results in the dissolution of cell wall [41]. Flavonoids inhibit microbial growth by forming a complex with extracellular, soluble proteins, and cell walls. Gram-negative bacteria cell wall is complex compared gram-positive organisms, which act as a diffusion barrier and make less susceptible to extract [42].

5.7 Anticancer Activity

The cytotoxic effects of the crude extracts of *B. koenigii* seed extract on the leukemic monocyte THP-1 cell line were investigated by the MTT assay, as shown in ***Figure 5.***

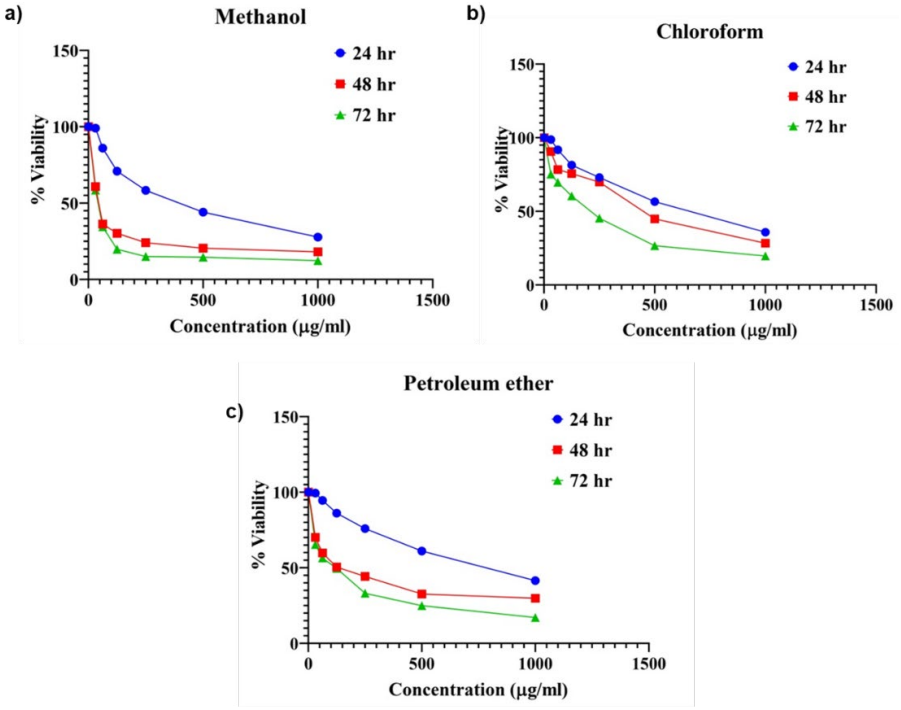


Figure 5: Anticancer Activity of a) Methanol, b) Chloroform and c) Pet Ether Extracts of B. Koenigii Seeds Against Leukemic THP-1 Cell Line observed by MTT Assay

The results showed significant concentration-dependent cytotoxic activity in all the crude extracts. After 24 h of treatment with the methanol extract, the Thp-1 cell line showed significant concentration-dependent cytotoxicity in the range of 99.07 ± 0.88 – 27.73 ± 2.21% viability at concentrations ranging from 31.25 – 1000 mg/mL. After 24 h of chloroform extraction, 98.71 ± 1.05 – 35.81 ± 2.06% viability was observed at the tested concentrations. Similarly, after 24 h of petroleum ether extract treatment, 99.36 ± 0.21 – 41.50 ± 1.76% viability of the THP-1 cells was observed at the concentrations used. At the same concentrations, after increasing the treatment duration to 48 h, the viability decreased to 60.74 ± 1.40 – 13.07 ± 0.23%, 90.55 ± - 28.36 ± 1.54%, and 94.07 ± 0.03 – 39.83 ± 1.77% in the presence of the methanol, chloroform, and petroleum ether extracts, respectively. After 72 h, there were no significant changes in the % viability, indicating equilibrium. The *B. koenigii* (L) seed extracts exhibited cytotoxicity against the THP-1 cell line. The presence of hexadecanoic acid, octadecadienoic acid, octadecatrienoic acid, tricosanoic acid and γ-sitosterol in methanol makes it effective against leukemic THP-1 cells. Reported that stigmasterol exhibited anticancer activity against the ovarian cancer cell lines ES2 and OV90 by reducing cell migration and inhibiting the PI3K/MAPK signalling cascade [43]. Stigmasterols suppress skin cancer by preventing DNA damage and increasing the levels of lipid peroxides [44]. Octadecadienoic acid and octadecatrienoic acid exhibit anticancer activity by inducing apoptosis through caspase 3 activation [45,46]. hexadecanoic acid, a hexane solvent isolated from *Turbinaria ornate*, has potential anticancer activity against HT-29 human colon cancer cells through apoptosis induction and cell cycle arrest. γ-Sitosterol exhibited potential anticancer activity through cell cycle arrest, growth inhibition and apoptosis [47]. Thus, the combination of all bioactive compounds makes the methanolic extract of *Bergera koenigii* seeds a potential anticancer drug.

Authors Contributions

Balaji G- Conceptualization, methodology, investigation, data analysis and original draft preparation.

Trini Mary Infanta–Investigation, methodology, data analysis and original draft preparation

Murugappan KM–Methodology and draft preparation

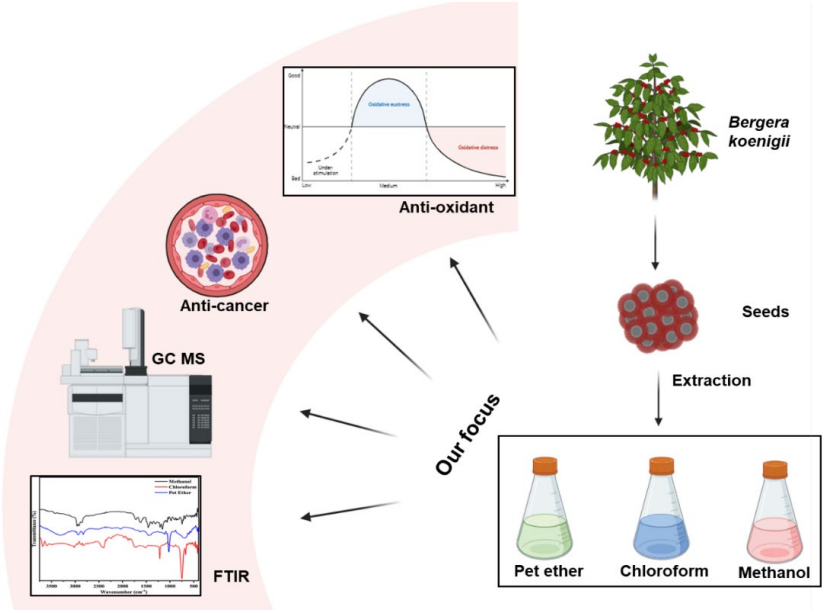
Availability of Data and Material

All the data are presented in the manuscript.

Acknowledgements

The graphical abstract was prepared by using Biorender software.

Graphic Abstract



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