

Original Research

Phytochemical profiling, antioxidant, 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, chloroform 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 μ g/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 12 ± 0.5 mm for *Escherichia coli*, 12 ± 1.3 mm for *Pseudomonas aeruginosa* and 21 ± 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 petroleum ether extracts.

KEYWORDS: Acute myeloid leukemia, *Bergera koenigii* seeds, THP-1 cells, antioxidant, anticancer, antimicrobial

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.



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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. Singh, 2014 reported that distinct plant extracts have antiviral, anti-inflammatory, antioxidant, antidiabetic, anti-diarrheal, anti-leishmanial, and anticancer effects. *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 Hajam et al., 2022. 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 Szymanska et al., 2018. 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 Abeyasinghe et al., 2021.

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. Khan et al., 2019 suggested that *Bergera koenigii* has anticancer potential and prevents cancer with less toxicity to the human body. The solvent polarity has an impact on how well the antioxidant activity is preserved Kumari et al., 2020. 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.

Materials and Methods

Chemicals and reagents

Aluminum chloride (AlCl₃), ammonia solution (NH₄OH), concentrated sulfuric acid (H₂SO₄), dimethyl sulfoxide (DMSO), 2,2'-diphenyl-1-picrylhydrazyl (DPPH), ferric chloride (FeCl₃), glacial acetic acid, Mayer's reagent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), petroleum ether (C₆H₁₄), sodium bicarbonate

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(NaHCO₃), sodium carbonate (Na₂CO₃), 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.

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) around 28°C 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 further analysis at room temperature.

Extraction of *B. koenigii* seeds

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.42 whatman filter paper and the filtered solvent was concentrated using a rotary evaporator (Buchi R-100) at 40°C. The seed extracts were stored in a refrigerator until further analysis.

Preliminary phytochemical screening

To confirm that the plant's chemical extracts include 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 petroleum ether individually and tested for alkaloids, phenols, terpenoids, flavonoids, steroids, saponins, tannins and reducing sugars, Syame et al., 2022, Ayoola et al., 2008, Gul et al., 2017, Gonfa et al., 2020, Ayoola et al., 2008, Gonfa et al., 2020, Gul et al., 2017, Neelima et al., 2022.

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 Baba and Malik, 2015.

Determination of total flavonoid content

The aluminium 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

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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 Baba and Malik, 2015.

Fourier Transform Infrared Spectroscopy analysis

Bergera koenigii seed 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 Konappa et al., 2020.

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.

Antioxidant profiling

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. The absorbance was measured at 517 nm. The scavenging ability of the seed extract was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = (\text{Abs control} - \text{Abs sample}) / \text{Abs control}$$

Where Abscontrol represents the absorbance of DPPH without sample and Abs sample represents the absorbance of DPPH with sample.

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 Dudonné et al., 2009. The percent suppression of radicals was calculated using the following equation.

$$\text{Suppression activity (\%)} = (\text{Abs control} - \text{Abs sample}) / \text{Abs control}$$

Where Abs control represents the absorbance of ABTS without sample; Abs sample represents the absorbance of ABTS with sample.

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 of cell density 4×10^6 CFU was spread uniformly on the surface of nutrient agar. Commercially available sterile empty and antibiotic discs were purchased (Hi Media, India) and 20 μ L of seed extracts was added in an empty sterile disc without dilution. Empty disc was used as a negative control whereas Streptomycin was used as a positive control. Plates were then incubated at 37°C for 24 h and a zone of inhibition was measured.

Anticancer activity

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 Chanput et al., 2015.

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 μ g/mL). Then, 50 μ L of serum-free media was added to each well, and 50 μ L 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 Al-Nasser et al., 2021.

Statistical analysis

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

Results and Discussion

Qualitative analysis

The preliminary qualitative analysis of the seed extracts revealed the presence of various groups of phyto-chemicals, as shown in Table 1. Alkaloids and glycosides were present in petroleum ether and absent in methanol and chloroform, and terpenoids and flavonoids were present in methanol and absent in chloroform and petroleum ether. Phenols were present in both methanol and petroleum 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 petroleum 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 Parekh and Chanda, 2007; Paswan et al., 2019.

Table 1. Qualitative phytochemical evaluation of *Bergera koenigii* extracts

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

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. The total phenolic content and total flavonoid content of *Bergera koenigii* were determined using the Folin method and aluminium 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 gm) dry weight and as mg quercetin (per gm) 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/gm and 1443.20 mg quercetin/gm, respectively. The chloroform extract and petroleum 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.

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

FTIR Analysis

Phytocompounds of *Bergera koenigii* seed extracts were analysed by biomolecule functional group Figure 1. Functional groups such as alcohols, alkanes, amines, alkenes, January 2025 Volume 2 Issue 1 <https://doi.org/10.69936/en04y0025>

phenols, aromatics, and cyclic alkenes were identified in Table 3. 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 Bisht *et al.*, 2016. 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.

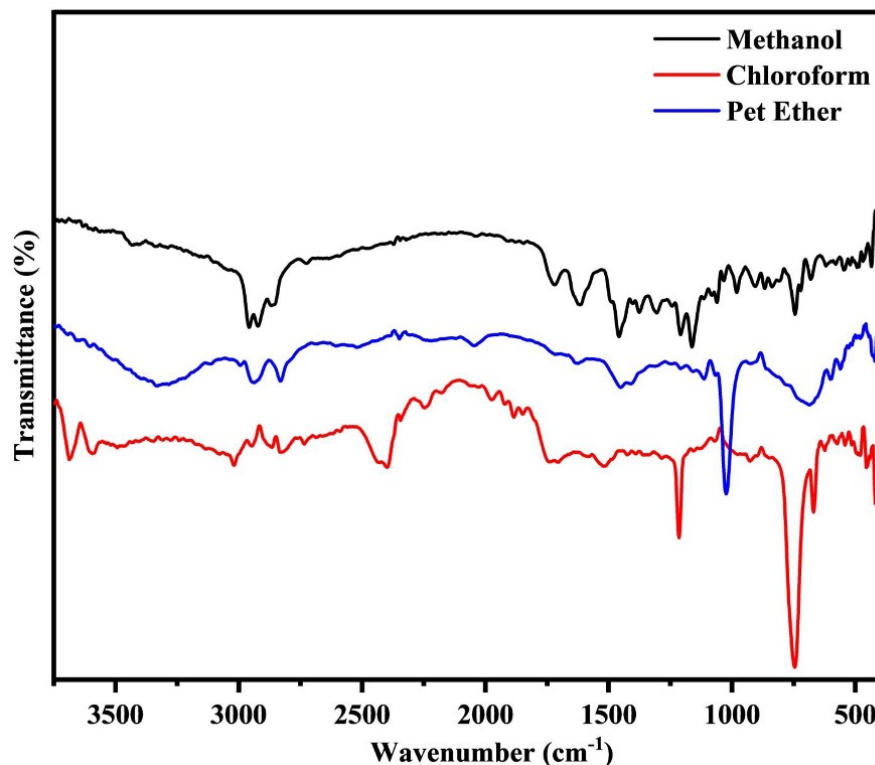


Figure 1. FTIR analysis of the methanol, chloroform and petroleum ether extracts of *Bergera koenigii* seeds

GC–MS analysis

GC–MS analysis of the *Bergera koenigii* extracts revealed the presence of 30, 58 and 35% methanol, chloroform and petroleum 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. 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 Hossain *et al.*, 2014. 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.

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 ^o 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

Some of these compounds are separately isolated in extracts and are used as antimicrobial and radical scavenging agents in medicine formulations Subramanian et al., 2020. 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- α reductase inhibitor activity Kala et al., 2011Saffaryazdi et al., 2020.

Table 4 GC-MS analysis of *Bergera koenigii* seed extract

Peak No	Retention	Formula	Compound name	Total %
Methanol extract				
1	2.760	C ₂ H ₅ NO ₂	Carbamic acid, methyl ester	0.521%
2	56.787	C ₁₇ H ₃₄ O ₂	Hexadecanoic acid, methyl ester	15.052
3	57.487	C ₁₇ H ₃₄ O ₂	Hexadecanoic acid, methyl ester	2.221
4	57.972	C ₁₆ H ₃₂ O ₂	n-Hexadecanoic acid	2.704

5	61.605	C ₁₉ H ₃₄ O ₂	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	15.052
6	62.251	C ₁₉ H ₃₄ O ₂	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	15.052
7	62.439	C ₁₉ H ₃₆ O ₂	9-Octadecenoic acid (Z)-, methyl ester	2.501
8	62.883	C ₁₉ H ₃₂ O ₂	9,12,15-Octadecatrienoic acid, methyl ester	11.557
9	63.045	C ₁₉ H ₃₈ O ₂	Methyl stearate	2.770
10	63.422	C ₁₈ H ₃₄ O ₂	cis-Vaccenic acid	1.421
11	64.081	C ₁₈ H ₃₆ O ₂	Octadecanoic acid	0.703
12	64.740	C ₁₉ H ₃₄ O ₂	Methyl 9-cis,11-trans- octadecadienoate	0.555
13	65.790	C ₁₈ H ₃₂ O ₂	9,12-Octadecadienoic acid	0.344
14	67.929	C ₂₁ H ₄₀ O ₂	11-Eicosenoic acid, methyl ester	1.349
15	68.696	C ₂₁ H ₄₂ O ₂	Eicosanoic acid, methyl ester	0.254
16	69.733	C ₂₀ H ₃₆ O ₂	Ethyl 9.cis.,11.trans.- octadecadienoate	0.328
17	70.029	C ₃₀ H ₅₀ O ₆	Olean-12-ene-3,15,16,21,22,28- hexol, (3β,15α,16α,21β,22α)	1.095
18	71.818	C ₂₀ H ₃₆ O ₂	Methyl 2-octylcyclopropene-1- octanoate	2.163
19	74.012	C ₂₃ H ₄₆ O ₂	Docosanoic acid, methyl ester	0.918
20	74.415	C ₂₄ H ₃₈ O ₄	Diisooctyl phthalate	1.230
21	75.600	C ₂₈ H ₄₈ O	Campesterol	0.570
22	76.515	C ₁₆ H ₄₈ O ₆ Si ₇	Heptasiloxane	4.028
23	78.170	C ₂₉ H ₄₈ O	Stigmasterol	0.724
24	78.950	C ₂₄ H ₄₈ O ₂	Tricosanoic acid, methyl ester	6.212
25	81.817	C ₂₉ H ₅₀ O	γ-Sitosterol	3.290
26	82.557	C ₂₉ H ₄₈	Stigmastan-3,5-diene	1.761

27	84.091	C ₃₂ H ₆₄	1,1,3,6-tetramethyl-2- (3,6,10,13,14-pentamethyl-3-ethyl-pentadecyl)cyclohexane	0.554
28	84.992	C ₂₀ H ₃₃ BrO	1,2-Epoxy cyclopentane, 3- isopropyl-1-methyl-2-[3-(2- bromomethyl-3-methylcyclopentyl)but-3-en-1-yl]	0.720
29	86.755	C ₄₀ H ₅₈ O	Rhodopin	1.058
30	87.361	C ₂₀ H ₄₂ O	-Dodecanol, 2-octyl	0.521
<i>Chloroform extract</i>				
1	25.743	C ₁₂ H ₂₆ O	2-Dodecanol	0.430%
2	26.282	C ₁₂ H ₂₆	Dodecane	0.330%
3	26.632	C ₁₀ H ₂₀ O	Decanal	0.121%
4	30.103	C ₁₀ H ₁₂ O	Benzaldehyde, 4-propyl	1.030%
5	30.736	C ₁₂ H ₂₄	2-Decene, 2,4-dimethyl	0.268%
6	30.843	C ₁₅ H ₃₂	Dodecane, 2,7,10-trimethyl	0.232%
7	31.907	C ₁₄ H ₃₀	Tetradecane	1.179%
8	33.212	C ₁₅ H ₃₂	Dodecane, 2,6,11-trimethyl	0.903%
9	36.347	C ₁₄ H ₂₈	Cyclotetradecane	0.550%
10	36.724	C ₁₄ H ₃₀	Tetradecane	1.065%
11	38.285	C ₁₀ H ₁₈ O ₃	Nonanoic acid, 9-oxo-, methyl ester	0.559%
12	39.496	C ₁₄ H ₂₀ O ₂	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	0.537%
13	40.869	C ₂₁ H ₄₄	Heptadecane, 2,6,10,15-tetramethyl	0.332%
14	41.057	C ₁₅ H ₃₂	Pentadecane	0.345%
15	41.662	C ₁₄ H ₂₂ O	Phenol, 2,4-bis(1,1-dimethylethyl)	1.821%
16	42.726	C ₁₇ H ₃₀ O ₄	Fumaric acid, isobutyl nonyl ester	2.732%
17	43.883	C ₁₂ H ₂₄ O ₂	Dodecanoic acid	0.486%

18	44.785	C ₁₆ H ₃₂	Cetene	1.076%
19	45.094	C ₂₀ H ₄₂	Eicosane	1.263%
20	45.928	C ₁₃ H ₁₀ O	Benzophenone	1.347%
21	47.476	C ₁₇ H ₂₈	Benzene, (1-ethylnonyl)	0.286%
22	48.848	C ₁₇ H ₃₆	Heptadecane	0.843%
23	49.790	C ₁₅ H ₃₀ O ₂	Methyl tetradecanoate	0.837%
24	50.759	C ₂₀ H ₄₂	Hexadecane, 2,6,11,15-tetramethyl	1.120%
25	51.351	C ₁₄ H ₂₈ O ₂	Tetradecanoic acid	1.166%
26	52.414	C ₁₈ H ₃₈	Octadecane	1.128%
27	54.702	C ₁₆ H ₂₂ O ₄	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	1.513%
28	55.953	C ₁₇ H ₃₂ O ₂	9-Hexadecenoic acid, methyl ester, (Z)	0.873%
29	56.492	C ₁₇ H ₂₄ O ₃	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	4.973%
30	56.949	C ₁₇ H ₃₄ O ₂	Hexadecanoic acid, methyl ester	6.804%
31	58.550	C ₂₁ H ₄₄	Eicosane, 2-methyl	5.906%
32	59.035	C ₁₉ H ₃₆ O	12-Methyl-E,E-2,13-octadecadien-1-ol	1.087%
33	59.573	C ₁₇ H ₂₆ O ₃	3,5-di-tert-Butyl-4-hydroxyphenyl propionic acid	1.747%
34	59.896	C ₁₈ H ₃₆ O ₂	Heptadecanoic acid, methyl ester	0.670%
35	62.695	C ₁₉ H ₃₄ O ₂	Methyl 9-cis,11-trans-octadecadienoate	6.804%
36	63.005	C ₁₉ H ₃₂ O ₂	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	3.233%
37	63.314	C ₁₉ H ₃₈ O ₂	Methyl stearate	6.804%

			9-Octadecenoic acid (Z)-, 2-	
38	63.449	C ₂₁ H ₄₀ O ₄	hydroxy-1- (hydroxymethyl)ethyl ester	1.662%
39	64.269	C ₁₈ H ₃₆ O ₂	Octadecanoic acid	4.843%
40	64.512	C ₂₁ H ₄₄	Eicosane, 2-methyl	0.814%
41	64.821	C ₁₉ H ₃₄ O ₂	Methyl 9-cis,11-trans-octadecadienoate	0.803%
			Methyl 9.cis.,11.trans.t,13.trans.-	
42	66.301	C ₁₉ H ₃₂ O ₂	octadecatrienoate	0.867%
			Methyl	
43	66.678	C ₁₉ H ₃₂ O ₂	9.cis.,11.trans.t,13.trans.-octadecatrienoate	0.827%
44	67.526	C ₁₉ H ₃₂ O ₂	6,9,12-Octadecatrienoic acid, methyl ester	2.332%
45	67.983	C ₂₁ H ₄₀ O ₂	11-Eicosenoic acid, methyl ester	1.512%
46	68.737	C ₂₁ H ₄₂ O ₂	Eicosanoic acid, methyl ester	2.305%
47	69.114	C ₁₁ H ₂₀ O	6-Nonenal, 3,7-dimethyl	0.345%
48	70.190	C ₁₁ H ₂₀ O	6-Nonenal, 3,7-dimethyl	2.814%
59	71.859	C ₂₀ H ₃₆ O ₂	Methyl 2-octylcyclopropene-1-octanoate	1.192%
50	72.303	C ₂₁ H ₃₄ O	: Androst-5-en-17-ol, 4,4-dimethyl	0.922%
51	74.052	C ₂₃ H ₄₆ O ₂	Docosanoic acid, methyl ester	2.400%
52	75.627	C ₂₆ H ₅₄	Octadecane, 3-ethyl-5-(2-ethylbutyl)	1.494%
53	76.528	C ₂₄ H ₄₈ O ₂	Tricosanoic acid, methyl ester	1.155%
54	78.197	C ₂₁ H ₃₂ O ₂	Pregn-5-en-20-one, 3-hydroxy	.788%
55	78.964	C ₂₅ H ₅₀ O ₂	Tetracosanoic acid, methyl ester	1.966%
56	81.171	C ₃₀ H ₅₀	Squalene	0.911%
57	84.992	C ₂₇ H ₄₄ O	Cholesta-4,6-dien-3-ol, (3β)	1.855%

58	85.437	C ₂₉ H ₄₆	Stigmasteran-6,22-dien, 3,5-dedihydro	1.704%
<i>Petroleum ether extract</i>				
1	41.636	C ₁₄ H ₂₂ O	Phenol, 2,4-bis(1,1-dimethylethyl)	0.0998%
2	49.185	C ₁₇ H ₃₆	Heptadecane	0.274%
3	51.163	C ₁₄ H ₂₈ O ₂	Tetradecanoic acid	0.364%
4	52.508	C ₁₆ H ₃₄ O	3-Hexadecanol	0.590%
5	53.356	C ₁₇ H ₃₄ O ₂	Isopropyl myristate	0.331%
6	55.940	C ₁₇ H ₃₂ O ₂	9-Hexadecenoic acid, methyl ester, (Z)	0.620%
7	56.532	C ₁₇ H ₃₄ O ₂	Tridecanoic acid, 4,8,12-trimethyl-, methyl ester	1.510%
8	56.720	C ₁₇ H ₃₄ O ₂	Hexadecanoic acid, methyl ester	10.876%
9	57.999	C ₁₆ H ₃₂ O ₂	n-Hexadecanoic acid	4.249%
10	58.927	C ₁₇ H ₂₆ O ₃	3,5-di-tert-Butyl-4-hydroxyphenyl propionic acid	0.629%
11	61.121	C ₁₄ H ₁₄ N ₂ OS	10-Oxo-5,5-dimethyl-5-sila-5,10-dihydro-5H-benzo[e]pyrido[3,4-b]azepine	0.294%
12	61.417	C ₁₉ H ₃₂ O ₂	γ-Linolenic acid, methyl ester	0.491%
13	61.713	C ₁₉ H ₃₄ O ₂	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	1.995%
14	62.049	C ₁₉ H ₃₄ O ₂	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.198%
15	62.345	C ₁₉ H ₃₆ O ₂	trans-13-Octadecenoic acid, methyl ester	1.691%0.346%
16	62.601	C ₁₉ H ₃₂ O ₂	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	0.346%
17	62.776	C ₁₉ H ₃₈ O ₂	Methyl stearate	0.275%

18	63.005	C ₁₉ H ₃₈ O ₂	Heptadecanoic acid, 15-methyl- , methyl ester	10.778%
19	63.206	C ₁₈ H ₃₂ O ₂	9,12-Octadecadienoic acid (Z,Z)-	4.051%
20	64.068	C ₁₈ H ₃₆ O ₂	Octadecanoic acid	1.550%
21	64.418	C ₂₁ H ₄₄	Heneicosane	0.416%
22	66.234	C ₁₉ H ₃₂ O ₂	6,9,12-Octadecatrienoic acid, methyl ester	0.230%
23	66.853	C ₁₅ H ₃₃ ClOSi	1-Dimethyl(3- chloropropyl) silyloxydecane	0.446%
24	67.620	C ₁₉ H ₃₆ O ₃	Methyl 12-hydroxy-9- octadecenoate	0.639%
25	68.024	C ₁₉ H ₃₄ O ₂	10,13-Octadecadienoic acid, methyl ester	0.804%
26	68.697	C ₂₁ H ₄₂ O ₂	Methyl 18- methyl nonadecanoate	0.676%
27	68.885	C ₁₉ H ₃₆ O ₂	16-Octadecenoic acid, methyl ester	1.046%
28	69.221	C ₂₉ H ₆₀	2-methyloctacosane	0.444%
29	70.338	C ₂₁ H ₄₄	Heneicosane	0.252%
30	71.805	C ₁₉ H ₃₂ O ₃	8-Nonenoic acid, 9-(1,3- nonadiene loxy)-, methyl ester	0.336%
31	73.420	C ₂₉ H ₆₂ O ₂ Si	Silane, diethyl heptyloxyoctadecyloxy	12.265%
32	74.012	C ₂₃ H ₄₆ O ₂	Docosanoic acid, methyl ester	0.611%
33	74.429	C ₂₄ H ₃₈ O ₄	Phthalic acid, di(2- propyl pentyl) ester	1.159%
34	78.547	C ₂₀ H ₂₈ O ₃	Benzoic acid, 3,5-dicyclohexyl- 4-hydroxy-, methyl ester	17.263%
35	78.951	C ₂₅ H ₅₀ O ₂	Tetracosanoic acid, methyl ester	0.952%

Compounds such as 3,7,11,15-tetramethyl-2-hexadecen-1-ol (phytol) and squalene also have antimicrobial activities García-Salinas *et al.*, 2018. 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 Garlapati *et al.*, 2013; Ramsden *et al.*, 2015. Since GC–MS analysis revealed only the presence of volatile compounds, phenolic compounds that are nonvolatile were not detected in the GC–MS analysis.

Antioxidant activity

The effect of the solvent used for extraction on the antioxidant activity was studied by the DPPH and ABTS method, as shown in Figure 2, Figure 3. Free radicals can induce biological damage and various pathological events, such as aging, carcinogenesis, and inflammation. Huang *et al.* reported that phenolic compounds are effective against inflammation. Phenols and flavonoids are representative groups of antioxidant compounds Huang *et al.*, 2011. Thus, in our study, the antioxidant activity was evaluated for all the solvent extracts to identify the influence of the solvent used for extraction. Similar to our results, the methanolic seed extracts show high free radical scavenging activity measured through DPPH assay and IC₅₀ was 0.43 mg/ml. And the chloroform extract shows minimal antioxidant potential with IC₅₀ value of 0.79 mg/ml (10.9734/BMRJ/2015/21571). And in another study the methanolic seed extract showed IC₅₀ value of 35.43 µg/ml Nadaf *et al.*, 2020. Our results are in accordance with Zahin *et al.*, 2013 studies in which ethanol was used as a solvent for extraction. Based on the solvent used for extraction, variations in the radical scavenging ability of the *B. koenigii* seed extracts were found.

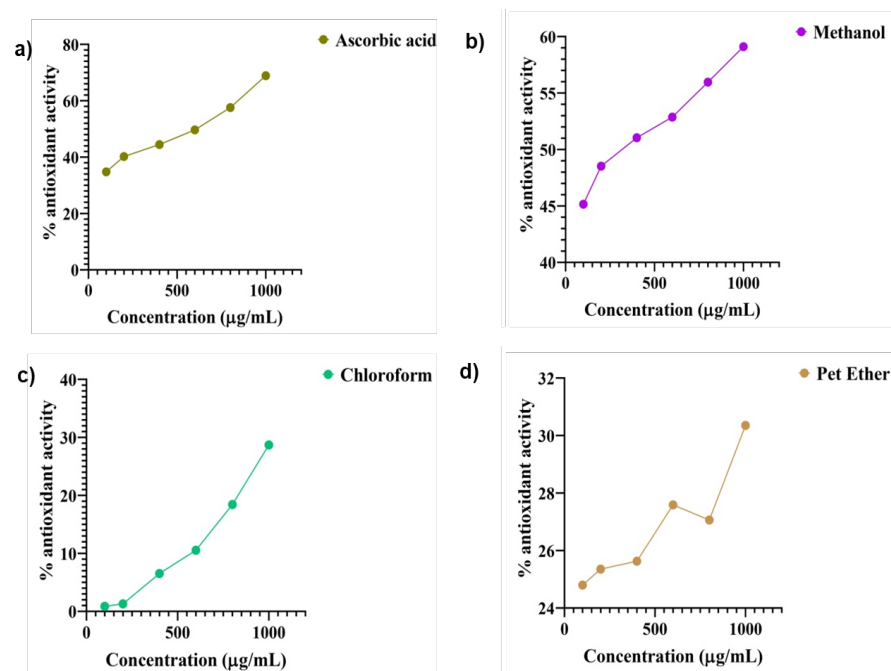


Figure 2. Graphical representation of DPPH scavenging % observed for a) ascorbic acid, b) methanol, c) chloroform and d) petroleum ether extracts of *Bergera koenigii* seeds

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 Carini et al., 2001. 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 *Macademia tetraphylla* peels, selected herbal infusions, *Limnophila aromatica* and *Withania somnifera* (L.), which reported that there is a strong influence of solvent on the antioxidant activity of plant extracts Bhebhe et al., 2016; Dailey and Vuong, 2015; Dhanani et al., 2017; Do et al., 2014.

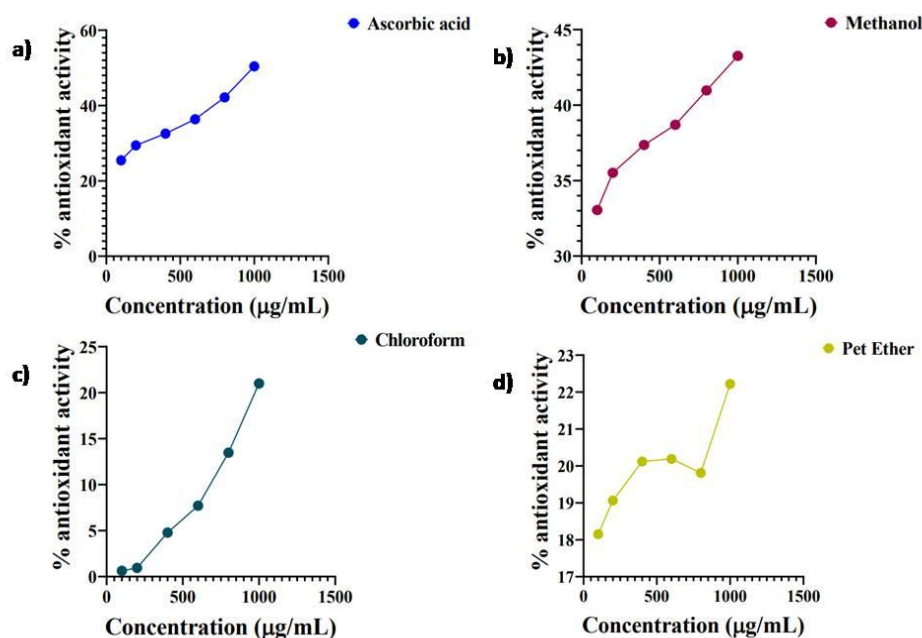


Figure 3. Graphical representation of ABTS scavenging % observed for a) ascorbic acid, b) methanol, c) chloroform and d) petroleum ether extracts of *Bergera koenigii* seeds

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 Huang et al., 2011; Mohaddesi et al., 2015. 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 Kasote et al., 2015; Kumaran and Joel Karunakaran, 2006.

Antibacterial activity

Drugs or antimicrobial agents derived from the plant are mitigating the side effects of synthetic antimicrobials and antitoxins. Numerous studies suggest that multi-drug resistant pathogens are susceptible to the herbal extracts. Plant secondary metabolites

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 such as alkaloids, steroids, phenols, and tannins which resembles the action of endogenous metabolites and performs signal transduction. Plant components also provide boundless prospects for the discovery of new drugs via different ways depending on the availability of chemical diversity.

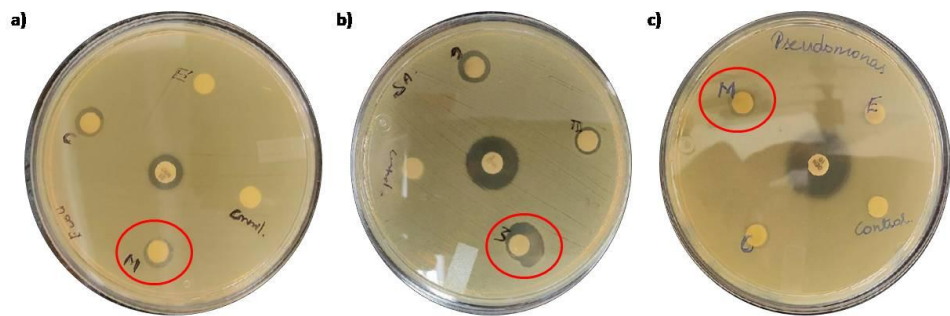


Figure 4. Zone of Inhibition observed for a) *E. coli*, b) *S. aureus* and c) *P. aeruginosa*, with extracts of *Bergera koenigii* seeds.

Table 5 Observation of Zone of inhibition of *Bergera koenigii* among different bacterial species

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

The plants are a source of novel antibiotics and people choose herbal medicines because of the adverse side effects associated with synthetic antibiotics Paswan et al., 2020. The methanolic seed extracts of *Murraya koenigii* showed effective antibacterial activity against multi-drug resistant *Acinetobacter baumani* with the highest ZOI 21 ± 1 mm El-Shiekh et al., 2024. With this insight, we analysed the antibacterial efficiency of *Bergera koenigii* extracts for 20 µl were shown and presented in Figure 4 and Table 5. The highest zone of inhibition was 15 ± 1.7 mm, observed in the methanol extract against the Gram-positive organism *S. aureus*. The zone of inhibition was 28 ± 1.4 mm, significantly comparable with the commercial antibiotic Ciprofloxacin. A ZOI of 8 ± 0.82 mm and 10 ± 0.5 mm was observed against gram-negative organisms such as *Pseudomonas aeruginosa* and *Escherichia coli*, respectively. The chloroform and petroleum ether extract showed inhibition with ZOI 10 ± 1.2 mm and 7 ± 1.1 mm against *Staphylococcus aureus* and minimum to no inhibition against other organisms. Similar

to our results the methanolic extract shows antibacterial activity against *Staphylococcus aureus* with ZOI 7.03 mm at 200 mg/ml Jakhar et al., 2015. 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 Rameshkumar et al., 2013. For instance, the cell wall synthesis of microbes was inhibited by tannins through its interaction with the cell wall or inactivating synthesis enzymes Dong et al., 2018. Leakage of protein and some enzymes is caused by the saponins Zablotowicz et al., 1996. Terpenoids weakening the membranous tissue of microbes, which results in the dissolution of cell wall Guimarães et al., 2019; Hernández et al., 2000. Flavonoids inhibit microbial growth by forming a complex with extracellular, soluble proteins, and cell walls. Gram-negative bacteria cell wall are complex compared to gram-positive organisms, which act as a diffusion barrier and make them less susceptible to extract.

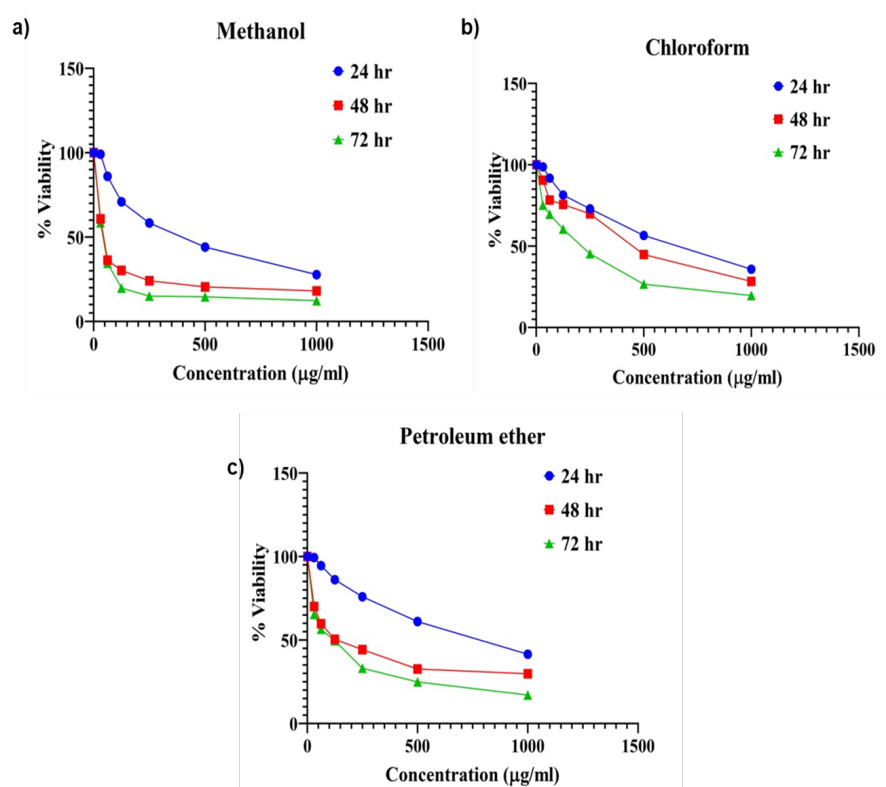


Figure 5. Anti cancer profiling of *Bergera koenigii* seed extracts against THP-1 cell lines, observed for 24 hr, 48 hr and 72 hr.

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. 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 $99.07 \pm 0.88 - 27.73 \pm 2.21\%$ viability at concentrations ranging from 31.25 – 1000 mg/mL. After 24 h of chloroform extraction, $98.71 \pm 1.05 - 35.81 \pm 2.06\%$ viability was observed at the tested

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concentrations. Similarly, after 24 h of petroleum ether extract treatment, $99.36 \pm 0.21 - 41.50 \pm 1.76\%$ 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 $60.74 \pm 1.40 - 13.07 \pm 0.23\%$, $90.55 \pm 28.36 \pm 1.54\%$, and $94.07 \pm 0.03 - 39.83 \pm 1.77\%$ 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. Similar to our results, the cytotoxic effects of the methanolic seed extract against MCF-7 and DLD-1 shows IC₅₀ of 284.02 $\mu\text{g/ml}$ and 269.67 $\mu\text{g/ml}$ Nadaf et al., 2020b. 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. Bae et al., 2020 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. Stigmasterols suppress skin cancer by preventing DNA damage and increasing the levels of lipid peroxides Ali et al., 2015. Octadecadienoic acid and octadecatrienoic acid exhibit anticancer activity by inducing apoptosis through caspase 3 activation Yoo et al., 2007, Bharath et al., 2021. 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 Sundarraj et al., 2012. Thus, the combination of all bioactive compounds makes the methanolic extract of *Bergera koenigii* seeds a potential anticancer drug.

Authorship Contributions and Declaration

BG, TMI and MK conducted the literature survey, analysed the data, initial draft, and wrote the manuscript. MK prepared the graphical abstract. BG conceived, designed, supervised the initial draft. BG and MK edited the manuscript and reviewed editing. MK contributed to preparing the graphical abstract. All authors read and approved the final paper.

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