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Research Article

IN VITRO ASSESSMENT OF ANTIOXIDANT AND ANTICANCER ACTIVITIES IN *MURRAYA KOENIGII* (L.): A PHYTOCHEMICAL APPROACH

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ABSTRACT

Objectives: This study presents phytochemical screening and *in vitro* assessment of antioxidant and anticancer activity of *Murraya koenigii* (L.) leaf extract and isolated molecule MKLE-01.

Methods: The ethanol extract was screened for phytochemical analysis by phytochemical screening tests. The antioxidant activity of ethanol extract of *M. koenigii* and isolated compounds (MKLE-01) was evaluated by the 1-diphenyl-2-picrylhydrazyl method, and anticancer activity was carried out by the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) assay method.

Results: The phytochemical screening of ethanol extract showed the presence of flavonoids, alkaloids, glycosides, phytosterols, and glycosides. In terms of their antioxidant activity, our findings demonstrated the ethanolic extracts' significant *in vitro* free-radical scavenging capabilities. The extract and isolated compound half maximal inhibitory concentration (IC_{50}) values of antioxidant activity were found at 154±0.202 µg/mL and 53±0.002 µg/mL, respectively. In comparison with cisplatin (IC_{50}=6±0.001 µg/mL), ethanol extract revealed a moderate and dose-dependent growth diminution of breast cancer cells in the MTT assay at dosages over 100 µg/mL, with an IC_{50} of 72±0.023 µg/mL. The isolated compound displayed a significant effect with an IC_{50} of 34±0.002 µg/mL.

Conclusion: The results we obtained demonstrate that isolated compounds and ethanolic extract could represent promising anticancer agents and effective antioxidants.

Keywords: *Murraya koenigii* (L.), Leaves, *In vitro* anticancer, Antioxidant activities, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide assay, Michigan Cancer Foundation-7 cell lines.

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INTRODUCTION

Biodiversity encompasses the immense variability of life forms across ecosystems, including microbes, plants, and animals. India, with its diverse ecological zones ranging from tropical forests to arid deserts and coastal mangroves, is particularly rich in medicinal plants that have long been utilized in traditional systems of medicine. Among these, *Murraya koenigii* (L.) Spreng, commonly known as curry leaves, has been extensively employed in Ayurveda for its wide spectrum of therapeutic properties, including anti-inflammatory, antimicrobial, and digestive benefits. Recent transcriptomic analyses have revealed the biosynthetic pathways of specialized terpenoids and carbazole alkaloids in *M. koenigii*, underscoring its potential as a reservoir of bioactive compounds with clinical relevance (Saxena *et al*., 2024).

Oxidative stress, driven by reactive oxygen and nitrogen species, plays a central role in the pathogenesis of chronic diseases such as cancer, diabetes, and neurodegenerative disorders. Excessive free radical generation damages biomolecules, thereby accelerating disease progression.

Cancer, in particular, remains a leading cause of global mortality, with conventional therapies such as chemotherapy and radiation often associated with severe side effects and limited long-term efficacy. This has intensified the search for natural phytochemicals with antioxidant and anticancer potential. Phenolic and flavonoid compounds, abundant in medicinal plants, have been reported to inhibit cancer cell proliferation, metastasis, and invasion, while simultaneously modulating oxidative stress pathways (Sisodia and Rathore, 2023). The green leaves of M. koenigii (L.) have many medicinal effects, such as treating piles, inflammation, itching, fresh cuts, dysentery, bruises, and edema [17]. The roots are purgative to some extent [18]. They are stimulating and used for common body aches. The bark helps treat snakebites. M. koenigii (L.) possesses potential secondary metabolites that could be developed as anticancer agents. In one study, the cytotoxic activity was evaluated for three extracts: Hexane, ethyl acetate, and methanol of M. koenigii leaves against the HeLa cell line [19]. Studies also indicated that alkaloid compounds of M. koenigii leaves could induce apoptosis in the mammary cancer cells Michigan Cancer Foundation (MCF7), P388, and HL-60 [20], as well as colon cancer cells HT-29 [21].

Although prior investigations have reported apoptosis-inducing effects of *M. koenigii* leaves alkaloids in multiple cancer models, the present work concentrates on MCF-7 cells to elucidate the mechanistic role of *M. koenigii* phytochemicals in modulating oxidative stress, triggering programmed cell death, and inhibiting cellular proliferation.

By focusing on this breast cancer model, the study aims to screen the phytochemical constituents of *M. koenigii* (L.) Leaves and provide targeted insights into their therapeutic potential as natural agents for breast cancer management using water and ethanol extracts.

METHODS

Chemicals, reagents, and cell lines

All the other chemicals were of the highest analytical grade and were procured from certified suppliers. All were obtained from Invitrogen,

Sigma, and HiMedia Laboratories Private Limited, Mumbai, India. The cell line used in the study was the MCF-7 cell line, which was procured from the Centre for Cellular and Molecular Biology, Hyderabad, Telangana, India.

Sample collection and authentication

Plants of *M. koenigii* (L.) were collected from the Visakhapatnam district in Andhra Pradesh. The botanical identity has been authenticated by the Director, Botanical Survey of India. The voucher specimen has been submitted and preserved in the herbarium for future reference.

Processing of plant material

The leaves of *M. koenigii* (L.) were collected, shade dried at room temperature, and then size reduced to get a coarse powder of desired particle size. The powdered drug was passed through a mesh with a size of 80 and stored in an air-tight container. This powdered material was subjected to successive solvent extraction. The powdered form was used for further experiments.

Preparation of seed and leaf powder and extract

The leaves of *M. koenigii* (L.) were washed thoroughly in running tap water to remove soil particles and adherent debris, and finally washed with sterile distilled water and shade dried for 14 days. Materials were ground with the help of a mixer into a fine powder. Plant material (leaves 10 g) was extracted with 250 mL of ethanol at 60°C for 6 h in a Soxhlet extractor. The ethanolic extracts were filtered through Whatman No.1 filter paper. The filtrate was evaporated to dryness at room temperature and stored until further analysis.

Phytochemical screening of the extracts

Phytochemical screening was done using standard procedures as previously described [14]. Samples of the ethanolic and water extracts of leaves of *M. koenigii* (L.) were qualitatively tested for different phytochemical constituents, namely flavonoids, alkaloids, glycosides, phytosterols, and glycoside compounds, by following the standard procedure.

Isolation of plant extract

Leaves of *M. koenigii* (L.) powder are oblong–lanceolate, 6–10×2–4 cm long, acuminate, cuneate at base, shining above. The dried parts of leaves of *M. koenigii* (L.) were powdered, weighed (200 g), and filled in solvent extraction Soxhlet tools. Using petroleum ether, the powdered substance was defatted. The defatted powder was dried at room temperature and extracted using ethanol and water. Solvents were evaporated to get the dried residue of the extract. A small portion of crude ethanol extract was dissolved in chloroform, and the solution was spotted on thin-layer chromatography plates. Then these plates are run by a specific solvent system and viewed individually under ultraviolet (UV) light, and also with the 10% H_2SO_4 reagent. A portion of ethanol extracts (2 g) is subjected to column chromatography (silica gel, 2×90 cm). Finally, the column is eluted with the starting 2:8–4:6 proportions solvent system of n-hexane and ethyl acetate to give a single major compound (250 mg) and the remaining two compounds in small quantities (2 mg and 3 mg). Identification and analytical, standard spectroscopic methods for investigating the structure of natural products-ethanol extracts (leaves of M. koenigii (L.) (MKLE-01) powders comprise nuclear magnetic resonance (NMR), infrared spectroscopy (IR), and these are often combined with mass spectrometry (MS).

***In vitro* antioxidant activity–radical-scavenging activity (1-diphenyl-2-picrylhydrazyl [DPPH] assay)**

DPPH antioxidant evaluation was based on the ability of antioxidants to decolorize 1, DPPH. Briefly, DPPH (100 µg/mL, 0.2 mmol/L) solution was added to MeOH solution of the extract or standard compounds at various concentrations at 5, 10, 30, 40, 50, 100 µg/mL [15-18]. The reaction mixture was shaken vigorously, and the absorbance of remaining DPPH was measured at 517 nm after 30 min at room temperature. Ascorbic acid (Vitamin C) was used as a standard drug. The scavenging activity of the extract against the stable DPPH* was calculated using the following equation:

$$\%\text{Inhibition} = [(AB-AA)/AB]\times 100,$$

$$\text{Scavenging percentage of each extract} = (AB-AA)AB)\times 100$$

Where AB is the absorption of the blank sample, and AA is the absorption of the test sample.

***In vitro* anticancer activity**

The *in vitro* antiproliferative activity of plant extracts was gauged against cancer cell line MCF-7 (breast cancer) by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) assay as done previously [19,20]. Cell suspensions were plated in 96-well microtiter plates at a density of 1×10^5 per mL, and plates were incubated in a 5% CO_2 incubator for 24 and 48 h at 37°C. Then, medium in each well was replaced by different concentrations (25, 50, 75, 100, and 200 µg/mL) of plant extracts, isolated compounds, as well as the negative control dimethyl sulfoxide (DMSO), and the positive control (cisplatin). The previously described tetrazolium-based colorimetric assay was used to detect cell growth inhibition in the studied plant extracts after 24 and 48 h of incubation. After adding 10 µL of MTT to cells in each well for 4 h, 100 µL of solubilizing solution (DMSO) was added, and absorbance was taken at 570 nm using a plate reader (TECAN, Manne Dorf, Switzerland). The cytotoxic potential of each extract prepared in different solvents was calculated as:

$$\%\text{ inhibitory percentage} = (1\text{-absorbance value of treated cell/absorbance value of control})\times 100$$

$$\text{Cell death} = (\text{Control OD - Sample OD})/\text{Control OD} \times 100$$

Data analysis

Quantitative and graphical data were analyzed using the Microsoft Excel package. The results of each series of experiments (performed in triplicate) were expressed as the mean standard deviation. Qualitative data for phytochemicals were also analyzed. The data were presented as mean±standard error of the mean. All the experiments were carried out in triplicate. A value of $p<0.05$ was considered significant. Statistical analyses were performed with GraphPad Prism software (GraphPad Software, San Diego, USA). A value of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Organoleptic study/macroscopical characters of the leaves of the ethanol extract *(M. koenigii* (L.)*).*

Fluorescence analysis

The powdered samples of the leaf part of the *M. koenigii* (L.) were treated with water, 10% NaOH, 50% H_2SO_4, and 1N HCl. The fluorescence of these extracts was observed under ordinary visible light and also under UV light (254 nm) and recorded.

Preliminary phytochemical screening of ethanol extract of leaves of *M. koenigii* (L).

The results obtained from the phytochemical assay are given in Table 2. Standard methods were used for the identification of this preliminary phytochemical screening of the leaf extracts of the plants, which revealed the presence or absence of secondary metabolites. The qualitative analysis showed the presence of flavonoids, alkaloids, glycosides, phytosterols, and glycoside compounds in the various extracts with varying intensities. As shown in Table 2, the majority of these secondary metabolites are present in the ethanolic extract.

Successive solvent extraction

For the preparation of the extracts, a successive solvent extraction was carried out using different solvents in an increasing polarity order. The order of the solvents used was ethanol and water. Packed accurately weighed about 100 g of *M. koenigii* (L.) in muslin cloth and started

Table 1: Fluorescence analysis of powdered leaf of Ethanol extract-*Murraya koenigii* (L.)

S. No.	Samples	Treatment with chemical reagents	Under ordinary light	Under UV light (254 nm)
1	Leaf powder	Powder with water	Dark brown	White
2	Leaf powder	Powder with 1N NaOH	Light green	Dark green
3	Leaf powder	Powder with 50% H_2SO_4	Light brown	Dark green
4	Leaf powder	Powder with 1N HCl	Pale green	Dark brown

Table 2: Preliminary phytochemical screening – ethanol extract of leaves *Murraya koenigii* (L.)

Test	Ethanol extract of *M. koenigii* (L.)
Alkaloids	+
Glycosides	+
Carbohydrates	–
Phenolic compounds	+
Phytosterols	+
Saponins	–
Tannins	–
Proteins and amino acids	–
Flavonoids	+
Terpenoids	–

Table 3: Preliminary phytochemical screening – isolated compound-MKEL-01

Test	MKEL-01
Alkaloids	–
Glycosides	–
Carbohydrates	–
Phenolic compounds	++
Phytosterols	–
Saponins	–
Tannins	–
Proteins and amino acids	–
Flavonoids	–
Terpenoids	–

extraction with the Soxhlet apparatus. Extractions are carried out with solvents of increasing polarity order, such as ethanol and water at temperatures of 62°C, 77°C, 78°C, and 100°C, respectively. The obtained extracts are filtered through filter paper, and the solvent is evaporated to dryness at their respective temperatures, finally obtaining a semi-solid mass.

Characterization of isolated compounds (MKLE-01)

Identification and analytical, standard spectroscopic methods for investigating the structure of natural products-ethanol extracts comprise NMR, IR, and these are often combined with MS.

Physical and chemical characterization

The MKLE-01 was isolated as a light green powder. The m.p. of MKLE-01 is 137–139°C. It gave the following phytochemical tests (Table 3).

Spectral characterization of MKLE-01: IR spectrum of MKLE (ethanolic extract-I)

The IR spectrum exhibited a broad absorption band at 3689 cm^{-1}, corresponding to the stretching vibration of a carboxylic –OH group, and a band at 3289 cm^{-1} indicative of a phenolic –OH group attached to an aromatic ring. The absorption at 2598 cm^{-1} is characteristic of =C–H stretching in alkenes, while the band at 1643 cm^{-1} confirms C=C stretching vibrations of an olefinic group. Additional signals at 1509 cm^{-1} and 1489 cm^{-1} correspond to aromatic C–C and C=C stretching, respectively, supporting the presence of a phenyl ring. The band at 1075 cm^{-1} is assigned to C–O bending vibrations, consistent with methoxy substitution, and the absorption at 786 cm^{-1} suggests the presence of an aryl–Cl bond. Collectively, these IR features confirm the presence of hydroxyl, aromatic, olefinic, and methoxy functionalities.

^{1}H-NMR spectrum of MKLE-01

The ^{1}H NMR spectra was observed at 12.05 (1H, s, carboxylic acid proton), 8.948(1H, s, phenolic proton Ar-OH), 4.645–4.499 (2H, d, -CH=C olefin proton); 3.683–3.600(3H, s, cyclic metoxy protons), 1.997–1.900 (3H, s, -CH_3 protons on aromatic ring), respectively. A singlet at δ 1.997–1.900 ppm (3H, s) corresponds to a methyl group substituted on the aromatic ring. The chemical shifts and splitting patterns support the presence of hydroxyl, methoxy, olefinic, and methyl substituents on an aromatic framework. Mass Spectrum: The mass spectrum showed a molecular ion peak at m/z 256.05 $[M]^-$, with isotopic peaks at m/z 257.51 $[M+1]^-$ and m/z 259.05 $[M+2]^-$, consistent with the presence of chlorine in the molecule. The observed molecular ion corresponds to a molecular formula of $C_{14}H_{12}O_4Cl$, which aligns with a substituted phenolic compound containing hydroxyl, methoxy, and chloro substituents.

Fig. 1: *Murraya koenigii* (L.)

Integration of IR, ^{1}H-NMR, and MS data confirms that MKLE-01 is a phenolic compound bearing hydroxyl, methoxy, olefinic, and chloro substituents on an aromatic ring system. Based on phytochemical and spectroscopic analysis, such as IR, ^{1}HNMR, and mass was confirmed that the isolated compound is a phenolic compound obtained, as shown below.

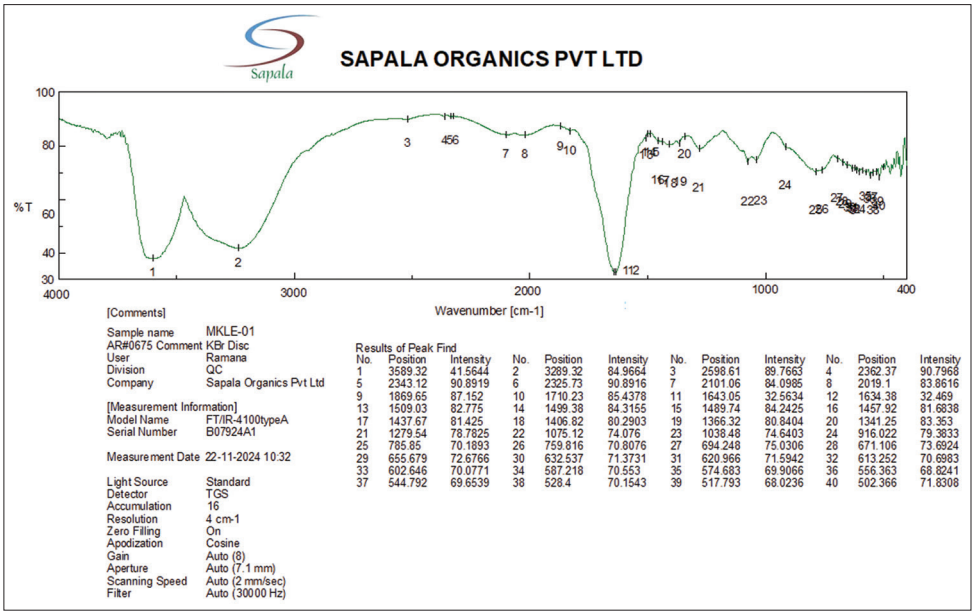
***In vitro* antioxidant activity**

The test for the antioxidant activity of the ethanol extracts of *M. koenigii* (L.) and isolated compounds, such as MKLE-01, bio-molecule compounds contained in the ethanol extract of *M. koenigii* (L.) leaves, was carried out by the free radical scavenging method. The use of the compound 1,1-diphenyl-2- picrylhydrazyl is a free radical that has an unpaired nitrogen atom. The reaction between 1,1-diphenyl-2-picryhydrazyl and hydrogen atoms in the antioxidant will cause a color change from purple to yellow. The results of the antioxidant activity of the extract and pure phenolic compounds are in Table 4.

The results showed that the inhibitory concentration 50% value of the extract was lower than that of the pure compound, but this difference was significantly different from the inhibitory concentration 50% value compared to pure compounds. These results indicate that the antioxidant activity of the pure compounds is higher. Hence, the use of pure compounds is very effective and efficient.

***In vitro* anticancer activity-MTT assay method**

The powerful free-radical-scavenging activity of the ethanol extract prompted us to investigate its *in vitro* cytotoxic potential in a cancer cell line. The cytotoxic activity of the extracts and isolated pure compounds



No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3589.32	41.5644	2	3289.32	84.9664	3	2598.61	89.7663	4	2362.37	90.7968
5	2343.12	90.8919	6	2325.73	90.8916	7	2101.06	84.0985	8	2019.1	83.8616
9	1869.65	87.152	10	1710.23	85.4378	11	1643.05	32.5634	12	1634.38	32.469
13	1509.03	82.775	14	1499.38	84.3155	15	1489.74	84.2425	16	1457.92	81.6838
17	1437.67	81.425	18	1406.82	80.2903	19	1366.32	80.8404	20	1341.25	83.353
21	1279.54	78.7825	22	1075.12	74.076	23	1038.48	74.6403	24	916.022	79.3833
25	785.85	70.1893	26	759.816	70.8076	27	694.248	75.0306	28	671.106	73.6924
29	655.679	72.6766	30	632.537	71.3731	31	620.966	71.5942	32	613.252	70.6983
33	602.646	70.0771	34	587.218	70.553	35	574.683	69.9066	36	556.363	68.8241
37	544.792	69.6539	38	528.4	70.1543	39	517.793	68.0236	40	502.366	71.8308

Fig. 2: Infrared spectroscopy spectrum of MKLE-01 (isolated compound from ethanol extract – leaves of *Murraya koenigii* (L.)

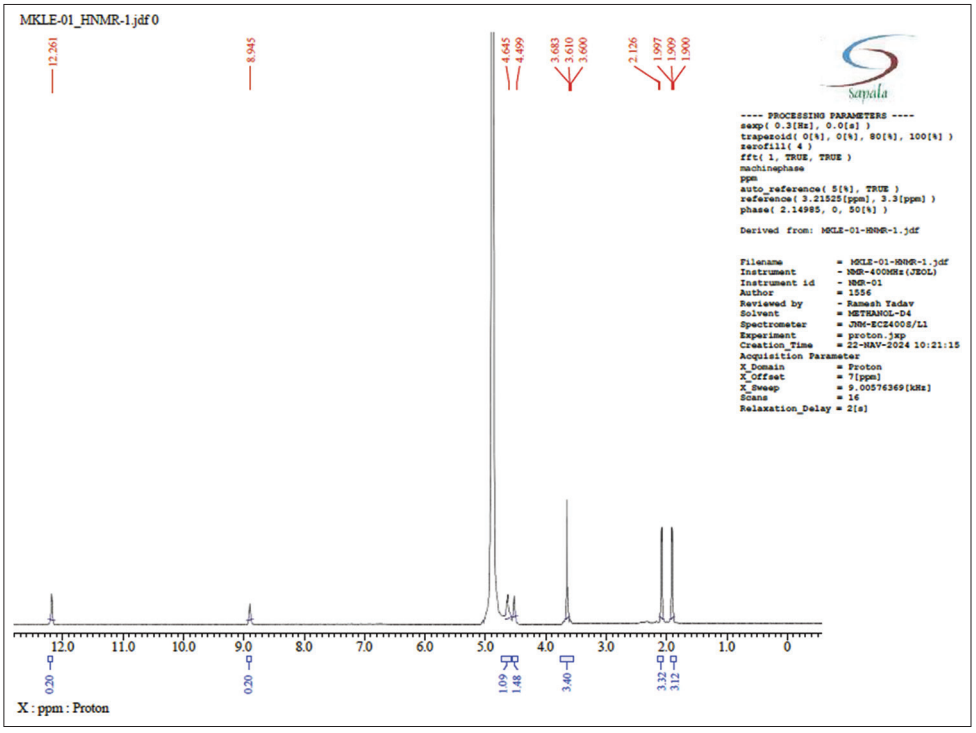


Fig. 3: ^{1}HNMR spectrum of MKLE-01 (isolated compound from ethanol extract – leaves of *Murraya koenigii* (L.)

Table 4: The results of antioxidant activity of extracts and pure phenolic compounds

S. No.	Test samples	IC50 (µg/mL)
1	Ethanol extracts of *Murraya koenigii* (L.)	154±0.202 µg/mL
2	MKLE-01	53±0.002 µg/mL
3	Acerbic acid	12±0.001 µg/mL

All the values are expressed as the mean±standard error of the mean; n=3. Statistical significance: **p<0.005

was investigated through the MTT assay. The results revealed an increase in cell lysis in a dose-dependent manner.

The chemotherapy medication used to treat the MCF-7 cancer cell line in this test is cisplatin (half maximal inhibitory concentration [IC_{50}]=6±0.001 µg/mL); ethanol extract of M koenigii (L.) presented IC50=72±0.023 µg/mL, however, the pure phenolic compounds showed higher IC50=34±0.002 µg/mL values(Table 5 and Fig. 7).

Fig. 8 illustrates the dose-dependent morphological alterations in MCF-7 breast cancer cells following 48-h treatment with the pure phenolic compound MKLE-01 at concentrations of 5, 15, 25, 50, 75, and 100 µg/mL. Fluorescence microscopy reveals a progressive decline in green fluorescent signal intensity and cell density with

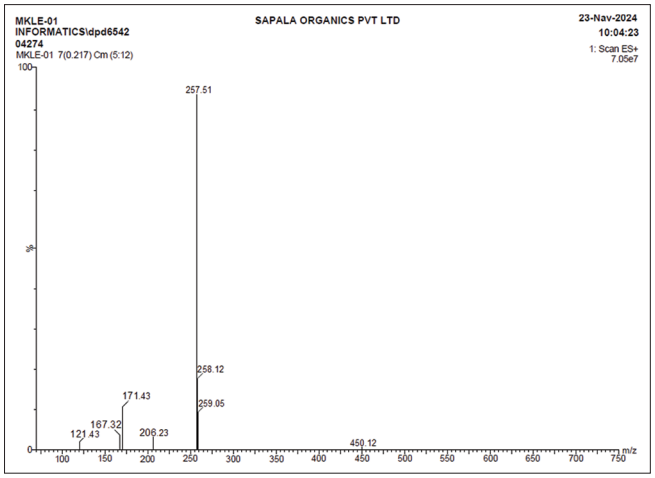


Fig. 4: MASS spectrum of MKLE-01 (isolated compound from ethanol extract – leaves of *Murraya koenigii* (L.)

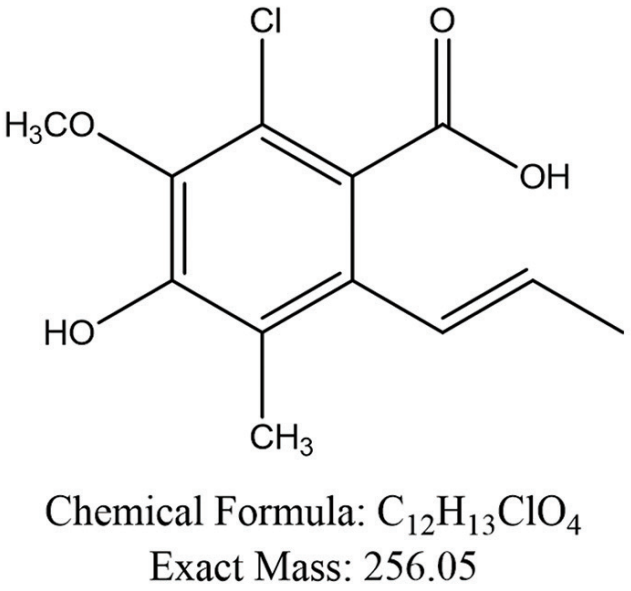


Fig. 5: Isolated phyto molecules: Phenolic compounds MKLE-01

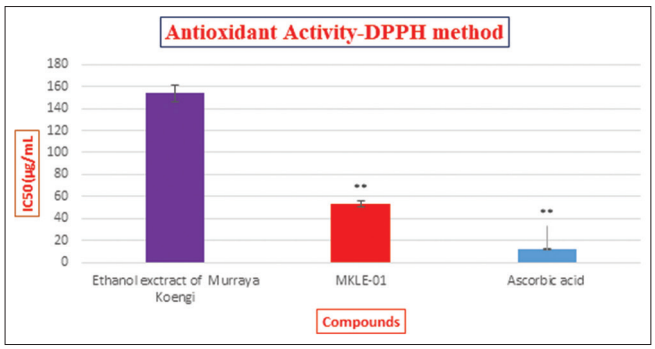


Fig. 6: Antioxidant activity of ethanol extract and isolated compound-MKLE-01 with half-maximal inhibitory concentration values.

increasing compound concentration. At lower doses (5–15 µg/mL), cells retain their typical epithelial morphology and confluency, with

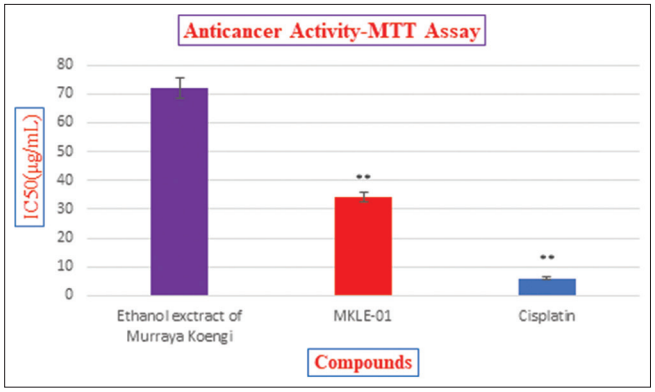


Fig. 7: Anticancer activity of ethanol extract and isolated compound-MKLE-01 with half-maximal inhibitory concentration values

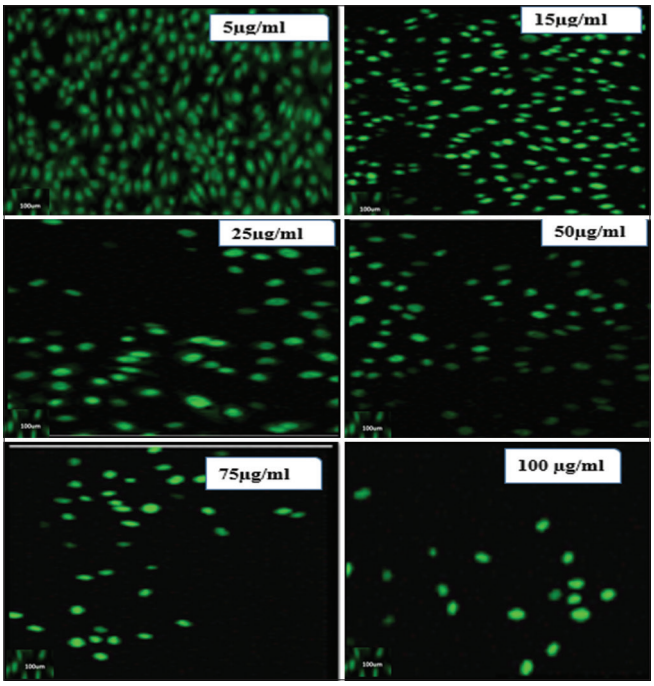


Fig. 8: Morphological changes of Michigan Cancer Foundation-7 cells treated with the pure phenolic compound – MKLE-01 at different concentrations (5, 15, 25, 50, 75, and 100 µg/mL) for 48 h

Table 5: The results of *in vitro* anticancer activity of extracts and pure phenolic compounds

S. No.	Test samples	IC_{50} (µg/mL)
1	Ethanol extract of *Murraya koenigii* (L.)	72±0.023 µg/mL
2	MKLE-01	34±0.002 µg/mL
3	Cisplatin	6±0.001 µg/mL

All the values are expressed as the mean±standard error of the mean; n=3. Statistical significance: **p<0.005

minimal signs of cytotoxicity. However, at intermediate concentrations (25–50 µg/mL), cells begin to exhibit membrane blebbing, shrinkage, and reduced adherence, indicative of early apoptotic events. At higher concentrations (75–100 µg/mL), a marked reduction in cell number and fluorescence is observed, suggesting advanced apoptosis or complete loss of viability.

CONCLUSION

This is the first investigation to report on the cytotoxic and antioxidant properties of pure phenolic compounds and extracts from *M. koenigii* (L.). *M. koenigii* (L.) has numerous advantages that render it a remarkable ecological treasure. When compared to other extracts, the ethanolic extract's biological activities may be determined by the presence of phenolic substances in it. Based on the present studies, the plant has been used to treat a variety of diseases. It focuses attention on each component of the herb and provides researchers with the inspiration they need to continue experimenting with creating its numerous formulations, which could eventually help both people and animals.

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AUTHORS CONTRIBUTION

Debapriya Subhadarshan Behera: Conceptualization, literature search, writing - original draft. Nihar Ranjan Kar: Data curation, visualization, proofreading.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING SOURCE

Nil.

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