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Network Pharmacology-Mediated In-Silico Molecular Docking Analysis of Acute Leukaemia Activity of *Memecylon edule* Leaf Extracts on Human Monocytic THP-1 Cells

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KEYWORDS

Memecylon edule,

THP-1,

Phytochemical analysis,

Cytotoxicity

ABSTRACT:

This study explores the pharmacological potential of *Memecylon edule* (ME), a plant rich in bioactive compounds through *in vitro* cell line studies. The anti-proliferative activity of ME extracts against human cancer monocyte cell line (THP-1) was evaluated. The extracts were further fractionated, and the compounds isolated and characterized. Additionally, network pharmacology identified seven bioactive compounds, predicting their pharmacokinetic properties and toxicity profiles. Molecular docking revealed potential targets related to Acute Myelocytic Leukemia. *In vitro* assays demonstrated significant cytotoxicity, apoptosis induction, and cell cycle arrest by ME extracts, with ethyl acetate extract exhibiting the highest activity. This integrated approach showcases ME as a promising candidate for further investigation in cancer therapeutics.

Introduction

Memecylon edule (ME) is a woody plant that belongs to the family Melastomataceae. They grow as small shrubs or trees growing up to a height of 8-14m having bright blue flowers in tropical and sub-tropical regions of India, Malaysia and Thailand. ME from India is mostly found in Karnataka, Andhra Pradesh and Tamil Nadu and are commonly known as Iron wood tree, kaayam, delekbangas, delek air, miat, and nemaaru. [1] The plant is well known for its use as a mordant in silk dyeing in Northeast Thailand and is traditionally used to treat leucorrhea, gonorrhoea, wound and gastrointestinal ailments. The leaf extract of ME help heal burning wounds without leaving any scars. A decoction of roots and heartwood has also been used to relief fever symptoms of several diseases such as common cold, measles, chicken-pox. [2, 3] Studies report the presence of alkaloids, triterpenoids, saponins, glycosides, tannins and

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flavonoids in ME extracts.[4] Literature reports indicate that ME leaf extracts have anti-oxidant properties.[5] *In vitro*, ME leaf extracts showed ability to enhance the production of interleukin-10 in murine macrophage cell liine. Similarly, active fractions of ME leaf extract showed anti-inflammatory and analgesic activity in ethylphenyl propiolate (EPP)-induced mouse ear edema model. Extract from the leaves of ME also inhibited the growth of human gastric carcinoma cells.[6] Since ME has been in use as a traditional drug for treating various ailments in human subjects the plant is an attractive candidate for investigating its effect on cancer cells. Estimation of cytotoxicity against cancer cells in an in vitro environment is the preliminary screening method for identifying potential anticancer compounds and extracts. Some of the currently used drugs for cancer therapy like Anastrozole, Valrubicin, etc. have side effects like weakness, urinary incontinence, diarrhoea, arthritis, respiratory congestion and even birth defects. One of the main reasons for these side effects is the non-specificity of the drugs to exclusively target the cancer cells. Hence, anticancer drugs having minimum or no side effects, with the ability to selectively induce apoptosis or cytotoxicity are preferred drugs of choice. In this aspect phytochemicals and extracts derived from plants have been investigated for their ability to be used as anticancer drugs.

In the present study, compounds from ME were subjected to network pharmacology-based analysis to identify its potential as an anticancer drug in acute myelocytic leukemia. Also, leaves of ME (LME) were extracted with suitable solvents and the extracts were evaluated for their anti-proliferative activity against human cancer monocyte cell line (THP-1). Further, the extracts were fractionated using column chromatography and the isolated compound was characterized by spectroscopic techniques.

Materials and Methods

Network Pharmacology

Through the extensive literature survey, a major 07 bioactive compounds have been listed and its pharmacokinetic parameters were esteemed. The pharmacokinetic properties of the compounds were generated via SwissADME online database.[7] Followed by the toxicity profile of the compounds were estimated from ProTox-II online server.[8] With this preliminary screening the targets of the bioactive compounds were generated to perform the network pharmacology. The targets of 07 bioactive compounds were Collected from the Swiss Target prediction Tool.[9] And the target related to Acute Myelocytic Leukemia; C0023467) were generated from Disgenet online tool. Followed by the compound target and Disease target were integrated to estimate the multiple druggable genomes. The 07 compounds were docked with active target of Acute Myelocytic Leukemia. Molecular docking was performed through the CB-dock online tool. The ligands and proteins were optimised before the docking procedure. [10-12]

Collection of Plant material

The leaves of the ME (LME) plant were collected in June, 2018 from the shrubby forest near Potheri village in Kancheepuram district, Tamil Nadu, India. The plants were authenticated at the Herbal Plant Anatomy Research Centre in west Tambaram, 600045.

Preparation of extract

The LME were shade dried and powdered. The powder was then sequentially extracted with solvents of increasing polarity: hexane, ethyl acetate (EtOAc) and ethanol (EtOH) and the supernatant were filtered using 125mm whattman filter paper. The solvent was evaporated using a rotary vacuum evaporator (Superfit Rotavap – PUB-6). The final extract was collected and stored.

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Cell line and chemicals

Human acute monocytic leukaemia cell line THP-1 was purchased from NCCS Pune, RPMI-1640 culture media (Lonza) with 10% Fetal Bovine Serum (Himedia) and 100 U/ml penicillin and 0.1 mg/ml streptomycin (Lonza), Hexane, Ethyl acetate, Ethanol, 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoilium bromide (MTT - Himedia), dimethylsulfoxide (DMSO), Propidium iodide (PI-Sigma), DAPI, Annexin V-FITC, Binding buffer (B.D Biosciences).

Phytochemical screening

Screening of all three LME extracts to identify the phytochemical constituents such as alkaloids, flavanoids, saponins, tannins, glycosides, triterpinoides and essential oils were carried out as per literature reported protocols.[13, 14]

MTT assay

MTT assay was carried out as described previously (Cheeran and M.R. Ganesh). THP-1 cells were seeded in a 24-well plate at a density of 1 x 10^5 cells/ml. The three LME extracts having concentration of 0.01mg/ml to 0.20 mg/ml were added to the wells and incubated for 24 hours at 37ºC. After incubation, 0.05mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution was then added to all the wells and incubated at 37ºC for 4 hours. Media was then removed and 100% DMSO was added and cells were transferred to 96-well plate. Optical density was read at 570nm using an Elisa microplate reader.

Annexin V – PI staining assay

The assay was performed based on the kit protocol (kit catalogue no BDB556547). THP-1 cells were seeded at a density of 5x10^5 cells/ml in a 24 well plate. Concentrations corresponding to LD50 value of each LME extract was added to the respective wells and incubated for 24 hours in a CO2 incubator. After incubation, the cell suspensions were centrifuged and 100µL of 1X Binding buffer was added to the pellet, re-suspended and incubated in the dark for 10 minutes. 5µL of Propidium Iodide (1mg/ml) and 5µL of Annexin V FITC was then added to all samples. After incubation for 30 minutes, 400µL of binding buffer was added and the tubes were incubated in the dark for 15 minutes. The cells were then analysed using Flow cytometry (BD FACS Calibur). The cells were analysed by flow cytometry using BD FACS calibur (Excitation – 488 nm/Emission -530nm, 585 nm). The proapoptotic and apoptotic cells were calculated using BD cell Quest Pro software.

Cell cycle analysis using PI stain

Cell cycle analysis was carried out based on the method described by (Alabsi et al. 2016). Cells at a density of 1X10^6 cells /ml were seeded to a 24 well plate. The LME extracts were added to the wells at a concentration corresponding to the LD50 values and incubated overnight at 37˚C and 5% CO2. The cell suspension was centrifuged and the cell pellet was fixed in 70% ethanol for 24 hours at 4ºC. The suspension was centrifuged at 10,000rpm for 5 minutes and the pellet was washed in 500µL of 1X PBS. 5µl of Propidium Iodide (1mg/ml) was added and the suspension was incubated in dark for 10 minutes at 37ºC. Readings were taken using FACS at FL2 and FL3 at 635 nm.[15]

DAPI staining using confocal microscopy

Effect on morphological changes induced by LME extracts was detected on THP-1 cells (density 1 x 10^6 cells/ml). Cells were cultured and subjected to treatment with LME extracts for 24 hours. After incubation, the cells were centrifuged and the pellet was collected and resuspended in 50µl PBS. 50µl DAPI was added and incubation was carried out for 20 minutes. The stained cells were examined under a confocal microscope and the nuclear DNA damage was assessed (Zeiss LSM-700).

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Fractionation by column chromatography

Chromatographic separation of the most bioactive extract was carried out using silica gel (100-200 mesh) as stationary phase with increasing concentrations of Hexane:ethyl acetate as mobile phase. Individual fractions were collected and the fractions showing similar TLC profile were pooled and tested for activity.

Results and Discussion

From the extensive literature survey 07 compounds have been identified in ME leaf such as, Quercetin, Myricetin, Myricetin 3-rhamnoside, Umbelactone, Oleanolic acid, Ursolic acid and beta-Amyrin. The Bioavailability Radar is used to estimate the pharmacological properties. The bioavailability radar result is based on the Six physicochemical properties such as lipophilicity, size, polarity, solubility, flexibility and saturation. The Red colour out of the Pink zone represents the deviation of the specific properties. The chemical structure and pharmacokinetic radar of the compound is depicted in Table 1. The pharmacokinetic profile of the compounds shows that: Quercetin exhibits high gastrointestinal (GI) absorption, suggesting good bioavailability when administered orally. Myricetin and Myricetin 3-rhamnoside show lower GI absorption, indicating potential challenges in achieving systemic circulation through oral administration. Umbelactone displays high GI absorption, suggesting good oral bioavailability. None of the compounds are identified as blood-brain barrier (BBB) permeants, indicating limited penetration into the central nervous system. This is important in assessing their potential for neurological effects. All the compounds are not substrates for P-glycoprotein (P-gp), an efflux transporter that can affect drug absorption. This suggests that these compounds may not be actively pumped out of cells by P-gp. Quercetin, Umbelactone, and beta-Amyrin exhibit inhibitory effects on specific cytochrome P450 (CYP) enzymes (CYP1A2, CYP2D6, and CYP3A4). This information is crucial for predicting potential drug interactions and metabolism. Oleanolic acid, Ursolic acid, and beta-Amyrin demonstrate higher Log Kp values, suggesting better skin permeation.

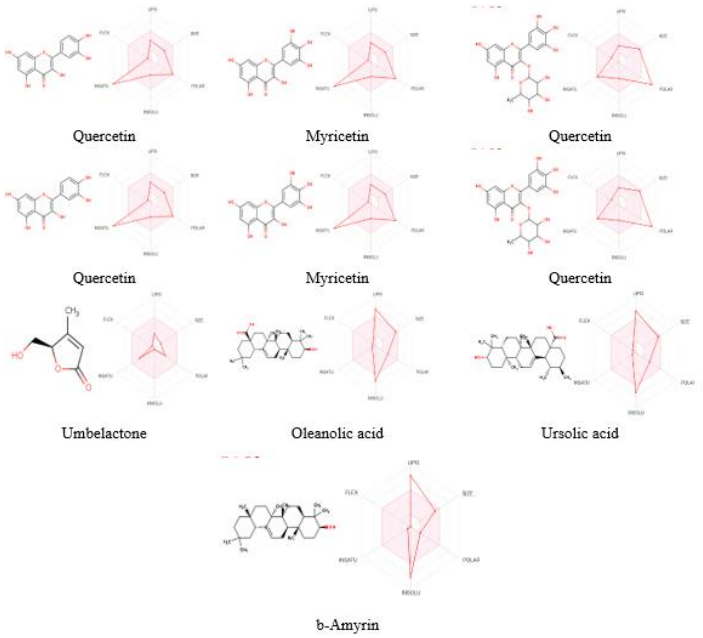

Table 1: Bioavailability radar profile of the bioactive compound.

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Parameters	Quercetin	Myricetin	Myricetin 3-rhamnoside	Umbelactone	Oleanolic acid	Ursolic acid	beta-Amyrin
GI absorption	High	Low	Low	High	Low	Low	Low
BBB permeant	No	No	No	No	No	No	No
P-gp substrate	No	No	No	No	No	No	No
CYP1A2 inhibitor	Yes	Yes	No	No	No	No	No
CYP2C19 inhibitor	No	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No	No
CYP2D6 inhibitor	Yes	No	No	No	No	No	No
CYP3A4 inhibitor	Yes	Yes	No	No	No	No	No
Log Kp (skin permeation) cm/s	-7.05	-7.4	-8.77	-7.44	-3.77	-3.87	-2.41

Table 2: Pharmacokinetic parameters of the compounds.

Quercetin and Myricetin: Both compounds have LD50 values of 159 mg/kg, indicating a moderate level of toxicity. These values suggest that caution should be exercised when considering dosages, and the compounds may require careful monitoring to prevent adverse effects.

Myricetin 3-rhamnoside: With an LD50 value of 500 mg/kg, this compound demonstrates a relatively lower toxicity compared to Quercetin and Myricetin. It suggests a wider safety margin for this compound.

Umbelactone: With an LD50 value of 4775 mg/kg, Umbelactone shows a lower level of acute toxicity, implying a higher margin of safety in terms of dosing.

Oleanolic acid and Ursolic acid: Both compounds share LD50 values of 2000 mg/kg, indicating a moderate toxicity level similar to Quercetin and Myricetin.

beta-Amyrin: This compound stands out with a notably high LD50 value of 70,000 mg/kg, suggesting a lower acute toxicity and a larger safety margin. This indicates that beta-Amyrin may be relatively safer at higher doses.

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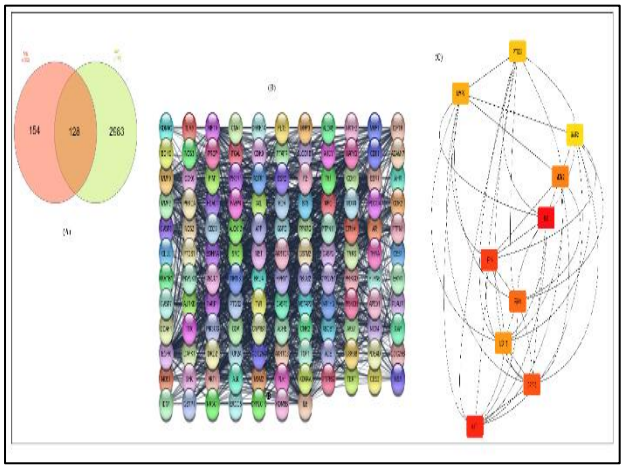

Figure S1: Network construction overview (A) Overlapping of drug and disease target. (B) Protein-Protein interaction of overlapped target. (C) Top-ranked targets of the hub-genes.

The toxicity profile of the compounds was given in Table 3.

By identifying common targets between drug and disease targets, a set of 128 final targets has been meticulously examined. These final 128 targets are regarded as hub genes and were employed for conducting a network analysis of protein-protein interactions.

Table 3: Toxicity Profile of the compounds.

Toxicity Profile	Range	LD50value (mg/kg)
Quercetin	3	159
Myricetin	3	159
Myricetin 3-rhamnoside	5	500
Umbelactone	5	4775
Oleanolic acid	4	2000
Ursolic acid	4	2000
beta-Amyrin	6	70,000

And top ranked targets have been listed. The figure S1 represents the overlapping targets of bioactive compounds and disease followed by network construction. Followed by molecular docking was performed for the 07 compounds towards PDB:60O. The docking scores are represented in terms of energy, specifically in kilocalories per mole (-kcal/mol). Lower binding scores generally indicate stronger binding affinity. The analysis suggests that Oleanolic acid and beta-Amyrin have the lowest binding scores, indicating potentially stronger binding affinity. Additionally, the commonality of amino acid residues among the compounds may suggest some structural and functional similarities. The docking scores and amino acid residues were given in Table 4. The molecular docking image of high binding affinity compounds and its amino acid residues were depicted in Figure S2. Traditionally, medicinal plants have been used across the world as a cure for many diseases. One such plant used in the traditional system of medicine is ME. In this study, extraction of LME yielded LME hexane, LME ethyl acetate (EtOAc) and LME ethanol (EtOH) extracts. The crude extracts were subjected to phytochemical analysis which showed the presence of alkaloids, flavonoids, tannins, glycosides, saponins, steroids and phenolic compounds in all three leaf extracts of ME. Table 5 shows the data of phytochemical analysis.

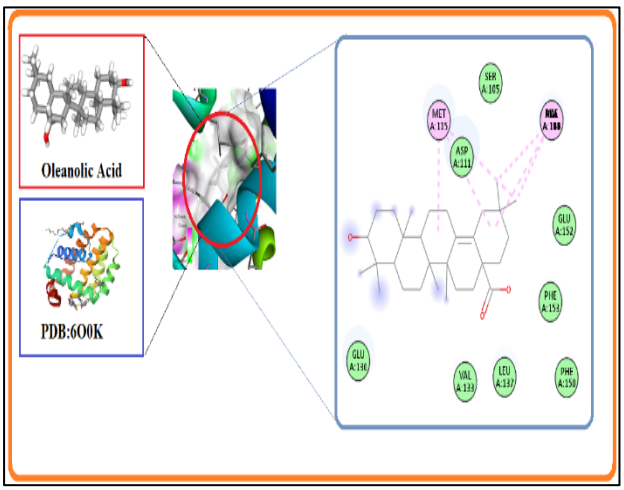

Figure S2: Molecular docking images of **Oleanolic acid** toward PDB:60OK.

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Compound and towards PDB:60OK	Binding Score (-kcal/mol)	Amino acid residues
Quercetin	-7.1	PHE104 ASP111 PHE112 MET115 GLU136 LEU137 PHE138 ARG139 ASP140 ARG146 VAL148 ALA149 PHE150 GLU152 PHE153
Myricetin	-7.0	PHE104 ASP111 PHE112 MET115 GLU136 LEU137 PHE138 ARG146 ALA149 PHE150 GLU152 PHE153
Myricetin-3-rhamnoside	-7.4	PHE104 ASP111 PHE112 MET115 GLU136 LEU137 PHE138 ARG139 ASP140 ARG146 VAL148 ALA149 PHE150 GLU152 PHE153
Umbelactone	-4.7	PHE104 SER105 ASP111 PHE112 MET115 VAL133 LEU137 ARG146 ALA149 PHE150 GLU152 PHE153 VAL156
Oleanolic acid	-8.9	PHE104 SER105 TYR108 ASP111 PHE112 MET115 GLN118 VAL133 GLU136 LEU137 ALA149 PHE150 GLU152 PHE153 VAL156
Ursolic acid	-7.7	VAL134 GLU135 PHE138 ARG139 VAL142 TYR180 HIS184 LEU185 THR187 TRP188 ASP191
b-Amyrin	-8.1	PHE104 TYR108 ASP111 MET115 VAL133 LEU137 ASN143 GLY145 ARG146 ALA149 PHE150 GLU152 PHE153

Table 4: Molecular docking analysis report of bioactive compounds towards PDB:60OK

Se. No.	Phytoconstituents	LME Hexane	LME EtOAc	LME EtOH
1.	Alkaloids	-ve	+ve	+ve
2.	Glycosides	-ve	+ve	-ve
3.	Flavanoids	-ve	+ve	+ve
4.	Tannins	+ve	+ve	+ve
5.	Saponin	+ve	+ve	+ve
6.	Steroids	-ve	-ve	+ve
7.	Phenol	+ve	+ve	+ve
8.	Proteins	-ve	-ve	-ve
9.	Carbohydrates	-ve	-ve	-ve

Table 5: Phytochemical analysis of LME extracts

The cell viability and the toxicity of LME were investigated by the MTT assay. In the present study, THP-1 cells were treated with LME-Hex, LME-EtOAc and LME-EtOH extracts in increasing concentrations from 0.01mg/ml to 0.20 mg/ml. Percentage cell toxicity of LME extracts in THP-1 cell line were depicted in Figure S3. The LD50 value was calculated using linear regression analysis for LME-EtOAc (0.0891 mg/ml), LME-Hex (0.1041 mg/ml) and LME-EtOH (0.1253 mg/ml). LME-EtOAc extract showed the most cytotoxic activity at all the tested concentrations compared to LME-Hex. Similarly LME-Hex also showed significantly higher cytotoxicity compared to the LME-EtOH.

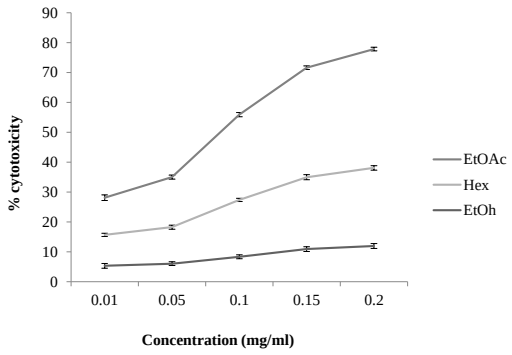

Figure S3. Percentage cell toxicity in THP-1 cell line when treated with different LME extracts.

The Annexin V-FITC/ Propidium Iodide flow cytometric assay was carried out to investigate

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the effect of LME on the apoptosis of monocytic cells. The auto-fluorescence profile attained is illustrated by the Annexin V FITC/PI fluorescence for untreated THP-1 monocytic cells which confirmed that LME induces apoptosis in monocytes. In the present study, LME-EtOAc extracts showed 20.37% cells in the necrotic stage (R4) and 78.77% cells in the late apoptotic stage (R3). Similarly, LME-Hex shows 0.23% and 98.5% cells whereas LME-EtOH shows 0.41% and 93.58% cells in the R4 and R3 stages respectively. Hence, LME-EtOAc treatment for 24h at a concentration corresponding to LD50 value showed an increase in Annexin V binding and PI binding by 71.8 fold and 344 fold respectively when compared to the unlabelled sample. Annexin/FITC -PI staining of THP-1 monocytic cells reports were depicted in Figure S4.

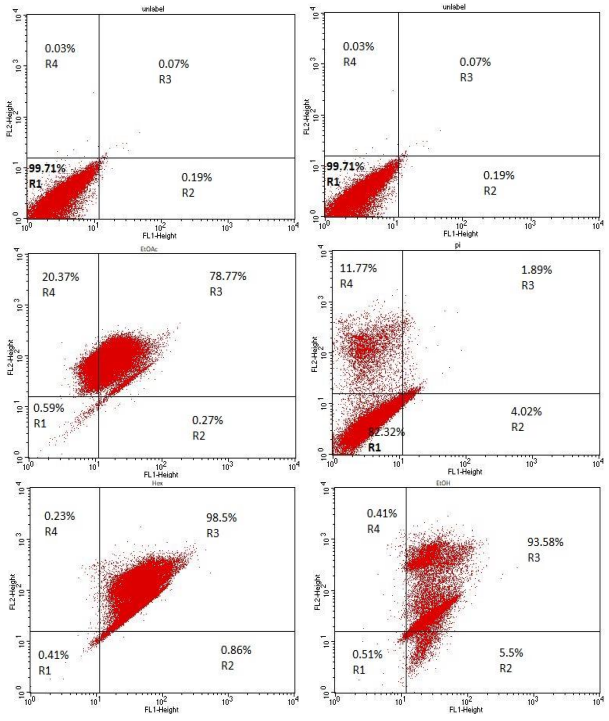

Figure. S4. Annexin/FITC -PI staining of THP-1 monocytic cells (a) Unlabelled cells (b) Cells with PI (c) Cells with Annexin V (d) Cells treated with LME-Hex (e) Cells treated with LME-EtOAc (f) Cells treated with LME-EtOH. [R1: Annex –ve, PI –ve; R2: Annex +ve PI –ve; R3: Annex +ve, PI +ve; R4: Annex –ve, PI +ve]

Plant based herbal medicines plays a major role in identification of lead molecule.[16] From a conceptual standpoint, there are numerous approaches to harness the medicinal potential of plants. The most common application is in the form of herbal teas or other homemade treatments. Pharmaceutical items including tablets, tinctures, powders, and other forms are made using either crude plant extracts or standardized fractions of them. Additionally, a number of bioactive substances that are directly employed as medications have been isolated from plants.[17] Bioactive compounds for direct use as drug, e.g. digoxin. Bioactive compounds with structures which themselves may act as lead compounds for more potent compounds, e.g. paclitaxel from Taxus species. Overall, these pharmacokinetic data provide insights into the absorption, distribution, and metabolic characteristics of the bioactive compounds, aiding in the assessment of their suitability for various routes of administration and potential therapeutic applications. Additionally, the information on enzyme inhibition is essential for predicting possible drug-drug interactions. The varying toxicity levels among the compounds suggest that certain bioactive compounds may be more suitable for therapeutic use than others. Lower toxicity levels and larger safety margins are generally favourable when considering these compounds for medicinal or nutritional purposes. The toxicity profile provides crucial insights into the safety of the bioactive compounds, guiding researchers and practitioners in making informed choices regarding dosage, formulation, and potential applications in the fields of medicine and nutrition. [18] In vitro cell line studies for herbal medicines offer valuable insights into their potential mechanisms of action and therapeutic effects, aiding in the assessment of safety and efficacy before advancing to more complex in vivo models. It also showed a higher percentage of cells in the

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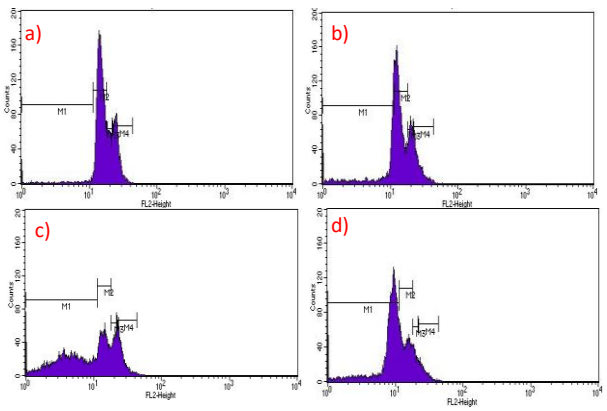
late apoptosis and necrosis stage, indicating increased cell death when compared to LME-Hex and LME-EtOH. Studies report the anti-proliferative and apoptogenic activity of ME showed that the ethyl acetate leaf extract inhibited cancer cell growth by inducing cytotoxicity, activating Caspase 3 and downregulating other genes like Bcl2 leading to loss in mitochondrial potential hence proving that ME induces selective apoptosis in cancer cells (Naidu VGM. et al.,2013). Our results were in line with the previously reported studies.

Cell-cycle analysis using Propidium Iodide is used to examine the effects of the ME extracts on different phases of the cell cycle (G0, G1, S and M). Cell cycle arrest is one of the major characteristic feature in the process of apoptosis. The percentage of cells in a particular cell cycle stage is determined by the degree of fluorescence intensity in each stage (ALabsi et al). The percentage of THP-1 cells undergoing apoptosis increased from 1.82% in control to 57.61% in LME-EtOAc (31.65 folds) whereas LME-Hex and LME-EtOH showed a 22.47 and10.65 fold increase respectively. The cell cycle progression on THP-1 cells of LME extracts were depicted in Figure S5. Cell cycle analysis using PI on THP-1 human monocyte cell line indicated that LME-EtOAc has higher inhibitory effects on THP-1 cancer cell line when compared to the LME-Hex and LME-EtOH.



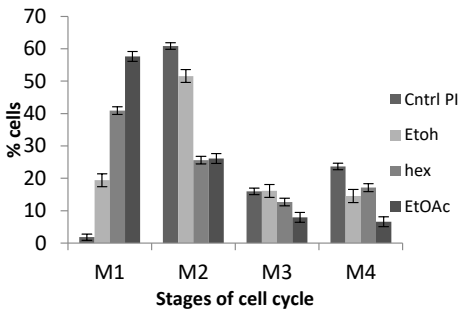


Figure. S5. Effect of LME extracts on cell cycle progression on THP-1 cells. (a) THP-1 control cells (b), (c), (d) THP-1 cells treated with LME-EtOH, LME-Hex, LME-EtOAc extracts respectively after 24h of incubation followed by analysis of cell cycle distribution. [M1 – Sub G1, M2 – G0/G1 , M3 – S phase, M4 – G2/M phase].

The morphological analysis of apoptosis using DAPI was checked under confocal microscope. DAPI(4′,6-diamidino-2-phenylindole) is used in cell death detection, as it enters more effectively and generates fluorescence in dead cells. The untreated cells showed intact nucleus with mild fluorescence. The cells treated with LME showed condensed degenerated nucleus with intense fluorescence. Figure S6 shows the DAPI staining by data by confocal microscopy. These results indicate the LME EtOAc extracts induce effective DNA damage in T cell leukemic cell lines when compared to hexane and EtOH extracts. Comparatively, among the three LME extracts, LME EtOAc extract showed significantly high cytotoxic activity with higher degree of apoptosis in THP-1 monocytic cells. Historically, natural products and their structural analogues have played a significant role in pharmacotherapy, particularly in the treatment of cancer and infectious diseases.

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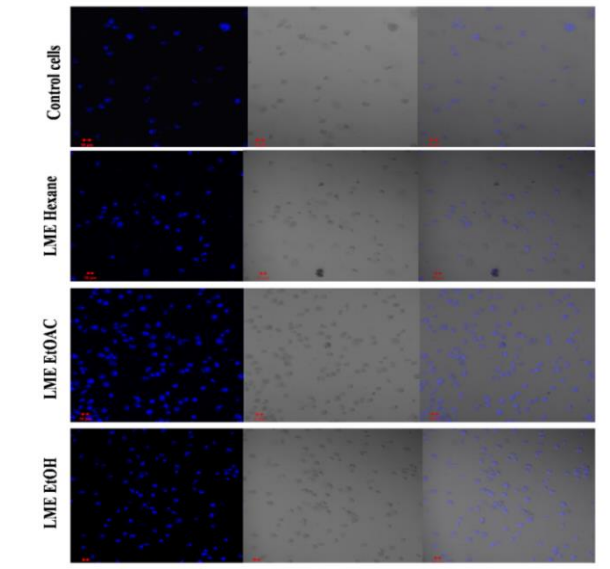

Figure S6. DAPI staining by confocal microscopy (a) Untreated control THP-1 Cells (b) Treated with LME hexane (c) Treated with EtOAc (d) Treated with EtOH.

However, the drug discovery process for natural products has been hindered by various challenges, including technical obstacles in screening, isolation, characterization, and optimization. These challenges led to a waning interest in natural products within the pharmaceutical industry starting from the 1990s. In recent years, advancements in technology and science, such as enhanced analytical tools, genome mining and engineering strategies, and improvements in microbial culturing, have addressed these obstacles and created new avenues for exploration. Consequently, there is a renewed interest in leveraging natural products as potential drug candidates, particularly in the context of combating antimicrobial resistance. This article provides a summary of recent technological developments facilitating natural product-based drug discovery, highlights specific applications, and explores the key opportunities emerging in this revitalized field.

Conclusion

The study demonstrates the basic pharmacokinetic properties and major targets via the network analysis. The network helps in the identification of multiple druggable genomes from the bioactive compounds in ME leaf for the future drug discovery. In this study, the EtOAc extracts showed significantly high cytotoxicity. Also, induction of apoptosis in THP-1 monocytic cell line by the LME extracts was confirmed by Annexin/FITC –PI staining and cell cycle analysis. This in-silico and invitro assisted cell lines studies report helps in the identification of lead molecule at the earlier stages of drug development.

Declarations

Competing interests / COI statement-nothing to disclose

Funding - The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Ethical Approval - All procedures performed involving human participants followed the ethical standards of the research committee of the SRM Institute of Science and Technology (Tamil Nadu, India).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request

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