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In-Silico and In-Vitro Evaluation of the Anti-cancer Potential of Madhumega Chooranam Extract Against Non-Small Cell Lung Cancer

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Abstract

Background: Madhumega Chooranam is a traditional Siddha polyherbal formulation primarily used in the management of diabetes mellitus. Its phytochemical complexity provides a rationale for exploratory evaluation of biological activities beyond its traditional indication. This study investigated the preliminary cytotoxic effect of Madhumega Chooranam extract (MCE) against A549 human non-small cell lung cancer (NSCLC) cells and examined the predicted interaction of quercetin with fibroblast growth factor receptor 4 (FGFR4).

Methods: The powdered formulation was extracted with 70% methanol. Preliminary phytochemical tests and thin-layer chromatography (TLC) were used to characterize the extract. Molecular docking of quercetin against FGFR4 (PDB ID: 5JKG) was performed using CB-Dock2, with fisogatinib included as a comparator. Cytotoxicity was evaluated by the MTT assay over 10–100 µg/mL, and acridine orange/ethidium bromide (AO/EtBr) staining was used to observe apoptosis-associated cellular changes.

Results: TLC findings were reported as consistent with tannins, flavonoids and glycosides, with a spot assigned to quercetin at an R_f value of 0.48. Quercetin produced a docking score of -7.9, compared with -8.4 for fisogatinib. MCE reduced A549 cell viability in a concentration-dependent manner, from 100% in the vehicle control to 31% at 100 µg/mL. The reported IC₅₀ was 65.55 ± 0.05 µg/mL. AO/EtBr staining at 90 µg/mL showed cellular changes described as early apoptosis, late apoptosis and nuclear fragmentation.

Conclusion: The results provide preliminary in-vitro evidence of concentration-dependent cytotoxicity and computational evidence of a possible quercetin–FGFR4 interaction. The findings do not establish clinical efficacy, tumour selectivity or a confirmed molecular mechanism. Further validation using appropriate normal-cell controls, replicated experiments, quantitative apoptosis assays and in-vivo studies is required.

Keywords: Madhumega Chooranam; Siddha formulation; non-small cell lung cancer; A549; MTT assay; quercetin; FGFR4; molecular docking.

1. Introduction

Cancer is a heterogeneous disease in which accumulated genetic and epigenetic alterations disrupt normal regulation of cellular proliferation, survival, differentiation and programmed cell death. Lung cancer remains a major cause of cancer-related illness and death. Its two broad clinical categories are non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC); NSCLC represents the larger group and

includes adenocarcinoma, squamous cell carcinoma and large-cell carcinoma. Although surgery, radiotherapy, chemotherapy, targeted therapy and immunotherapy have improved management, outcomes continue to be affected by late diagnosis, tumour heterogeneity, metastasis and acquired resistance.

Receptor-mediated signalling pathways are important in cancer biology because they regulate proliferation, survival, migration and angiogenesis. Fibroblast growth factor receptors (FGFRs) are receptor tyrosine kinases that activate downstream signalling networks, including RAS/MAPK and PI3K/AKT. Altered FGF/FGFR signalling has been investigated in several tumour types, including selected NSCLC subgroups. Molecular docking can provide a preliminary hypothesis about how a small molecule may fit within a protein binding cavity; however, a docking score is a computational estimate and does not by itself demonstrate biochemical inhibition or cellular target engagement.

Natural products and traditional polyherbal formulations remain important sources for exploratory pharmacological research. Madhumega Chooranam is a Siddha formulation traditionally associated with the management of Madhumegam, a condition resembling diabetes mellitus. The formulation described in the project contains plant materials including *Cassia auriculata* flowers, *Terminalia chebula* fruits, *Emblica officinalis* fruits, *Terminalia bellirica* fruits, *Picrorhiza kurroa* rhizomes, *Andrographis paniculata*, *Trigonella foenum-graecum* seeds, *Curcuma longa* rhizomes, *Murraya koenigii* leaves, *Cuminum cyminum* fruits and *Tinospora cordifolia* stems. Such multi-ingredient preparations may contain diverse phenolic and other secondary metabolites, but the composition and biological effects require systematic characterization.

The present work combined preliminary phytochemical profiling, TLC, MTT-based cytotoxicity assessment, AO/EtBr staining and molecular docking. The objective was to generate initial evidence regarding the activity of MCE against A549 cells and to explore a possible interaction between quercetin, a phytoconstituent considered in the project, and FGFR4.

2. Materials and Methods

2.1 Preparation of Madhumega Chooranam extract

According to the project report, 100 g of powdered formulation was soaked in 500 mL of 70% methanol in a sealed glass container for seven days with occasional stirring. The mixture was filtered through Whatman No. 1 filter paper. The plant marc was re-extracted twice using fresh solvent (250 mL for each extraction). Combined filtrates were concentrated under reduced pressure using a rotary evaporator at 40°C. The resulting extract was used for subsequent analyses.

2.2 Preliminary phytochemical screening and TLC

Qualitative screening was performed using standard colour or precipitation reactions for selected phytochemical groups. The report describes tests for alkaloids, saponins, tannins, phenolic compounds, flavonoids, coumarins, glycosides, quinones and volatile oils. TLC was used to separate extract constituents. The retention factor (R_f) was calculated as the distance travelled by the sample spot divided by the distance travelled by the solvent front. The project reports TLC findings consistent with tannins, flavonoids and glycosides and assigns an R_f value of 0.48 to a spot considered consistent with quercetin. This assignment should be treated as tentative unless confirmed using a reference standard and an orthogonal analytical method.

2.3 Molecular docking

The molecular docking analysis examined quercetin against FGFR4 using the protein structure identified as PDB ID 5JKG. The project reports use of the CB-Dock2 web server for cavity-guided docking. Fisogatinib was used as a comparator. Docking scores and predicted protein–ligand contacts were reviewed. The reported Vina scores were –7.9 for quercetin and –8.4 for fisogatinib. The docking analysis is computational and should be interpreted as hypothesis-generating rather than as proof of receptor inhibition.

2.4 Cell culture and MTT cytotoxicity assay

A549 human lung carcinoma cells were used for the in-vitro assessment. The report describes culture in DMEM/F-12 medium supplemented with 10% inactivated fetal bovine serum and penicillin–streptomycin, maintained at 37°C in a humidified atmosphere containing 5% CO₂. Cells were seeded in 96-well plates and allowed to attach before exposure to different concentrations of the test extract. MCE concentrations reported for the results ranged from 10 to 100 µg/mL, with vehicle-treated cells as the control.

After treatment, MTT reagent was added and the plates were incubated to permit formation of formazan by metabolically active cells. The formazan was solubilized with dimethyl sulfoxide (DMSO), and absorbance was measured at 590 nm. Cell viability was expressed relative to the vehicle control, and the half-maximal inhibitory concentration (IC₅₀) was reported from the concentration–response data. The project report presents average optical density values with standard error of the mean (SEM).

2.5 AO/EtBr apoptosis staining

Acridine orange and ethidium bromide double staining was used to observe cellular changes associated with apoptosis and loss of membrane integrity. The report describes examination of control cells and cells treated with MCE at 90 µg/mL. The reported observations included early apoptotic cells, late apoptotic cells and nuclear fragmentation. As no quantitative percentages of each cell category are provided in the available results, the observations are described qualitatively.

2.6 Data interpretation

Results are presented descriptively using the values available in the project report. The report notes statistically significant changes in cell viability ($P < 0.001$), but the number of independent experiments, statistical test and multiple-comparison procedure are not clearly specified in the material reviewed. Accordingly, statistical significance is reported as stated by the source and should be confirmed against the original experimental records before publication.

3. Results

3.1 Phytochemical screening and TLC

Preliminary qualitative analysis and TLC suggested the presence of tannins, flavonoids and glycosides in MCE. A TLC spot with an R_f value of 0.48 was considered consistent with quercetin in the project report. These results indicate that the extract contains compounds with chromatographic and qualitative properties compatible with selected phytochemical classes. Since qualitative tests and a single TLC R_f value are not definitive identification methods, confirmation by HPLC, LC–MS or another validated analytical technique is needed.

3.2 Molecular docking with FGFR4

The reported docking score for quercetin was -7.9 , while fisogatinib gave a score of -8.4 against FGFR4 (PDB ID 5JKG). The report describes predicted contacts involving residues including VAL481, ALA501, LYS503, LEU619, ALA629, ASP630 and GLU475 for quercetin. The score for quercetin was numerically close to that of the comparator in this computational setup, but the difference and the predicted contacts do not establish equivalent potency or activity. Biochemical kinase assays and cellular target-engagement studies would be needed to test the hypothesis.

3.3 MTT cytotoxicity assay

MCE showed a concentration-dependent reduction in A549 cell viability. The reported viability declined from 100% in the vehicle control to 82% at 10 µg/mL and 61% at 50 µg/mL. At 70 µg/mL, viability was 47%; at 100 µg/mL, viability was 31%. The reported IC₅₀ was 65.55 ± 0.05 µg/mL.

MCE concentration (µg/mL)	Average OD ± SEM	Viability (%)	Inhibition (%)
Vehicle control	1.113 ± 0.007	100	0
10	0.872 ± 0.010	82	18
20	0.771 ± 0.043	75	25
30	0.705 ± 0.006	70	30
40	0.652 ± 0.032	66	34
50	0.588 ± 0.015	61	39
60	0.484 ± 0.011	54	46
70	0.400 ± 0.018	47	53
80	0.307 ± 0.019	41	59

90	0.204 ± 0.021	33	67
100	0.172 ± 0.020	31	69

The overall trend supports concentration-associated loss of metabolic viability in the A549 assay. The reported IC₅₀ is an estimate for this extract under the reported experimental conditions and should not be interpreted as a clinical dose or as evidence of selectivity for cancer cells.

3.4 AO/EtBr staining

At 90 µg/mL, AO/EtBr staining was described as showing early apoptotic changes, late apoptotic changes and nuclear fragmentation compared with the control. These observations are compatible with cellular injury and possible apoptosis, but representative images alone cannot establish the proportion of cells undergoing apoptosis or distinguish apoptosis conclusively from other forms of cell death. Quantitative image analysis and complementary assays would strengthen this interpretation.

4. Discussion

This study provides an initial assessment of the anti-cancer potential of MCE using complementary chemical, computational and cell-based approaches. The phytochemical and TLC findings suggest the presence of several classes of secondary metabolites. Polyphenolic compounds, including flavonoids, are frequently investigated for antioxidant, anti-inflammatory and cell-signalling effects. Nevertheless, a complex extract contains multiple constituents, and the present screening does not determine their concentrations or establish which constituent is responsible for the observed cytotoxicity.

The MTT data show a consistent decrease in A549 cell viability as extract concentration increases. Viability was approximately half of the control around the 60–70 µg/mL range, consistent with the reported IC₅₀ of 65.55 ± 0.05 µg/mL. The highest tested concentration, 100 µg/mL, produced 69% inhibition according to the reported table. These findings support further investigation of MCE in this cell model. However, MTT measures cellular metabolic activity and may be influenced by extract colour, reducing compounds or other assay interference. Appropriate extract-only blanks and orthogonal viability assays are important when evaluating complex botanical samples.

The AO/EtBr observations at 90 µg/mL are consistent with apoptotic-type morphology, including nuclear fragmentation. Because these observations were qualitative, further experiments should quantify apoptotic and necrotic populations using flow cytometry or image-based analysis and confirm cell-death pathways with independent markers. Cell-cycle analysis and assays of caspase activation could help clarify whether apoptosis contributes materially to the viability reduction.

The molecular docking results offer a possible route for investigating quercetin–FGFR4 interactions. Quercetin's score (–7.9) was less favourable numerically than the fisogatinib score (–8.4) in the reported docking run. Docking scores depend on the algorithm, protein preparation, ligand protonation, cavity selection and scoring function. They cannot be compared directly with experimental potency or treated as proof of target engagement. In addition, the report's TLC-based assignment of quercetin requires analytical confirmation. Future work should therefore validate the identity and amount of quercetin in MCE, reproduce the docking with documented preparation settings, and test FGFR4 inhibition experimentally.

A further limitation is the absence of a reported normal human lung cell comparator. Without normal-cell testing, a selectivity index cannot be calculated and it is not possible to determine whether the observed effect is preferential for cancer cells. The source also does not provide sufficient detail about independent biological replicates, the statistical test, or a full quantitative apoptosis analysis. These items should be checked against laboratory records and reported transparently in any journal submission. Results from one cell line cannot be generalized to all NSCLC subtypes or interpreted as evidence of therapeutic benefit in humans.

Taken together, the findings justify further preclinical research but do not establish that Madhumega Chooranam treats lung cancer. Follow-up studies should include standardized extract preparation, chemical fingerprinting and quantification, assay-interference controls, dose–response replication, comparison with non-malignant cells, mechanistic experiments, and appropriate in-vivo safety and efficacy evaluation.

5. Limitations and Future Directions

The work is exploratory and has several limitations: (i) the polyherbal extract is not fully chemically standardized; (ii) the reported TLC identification is tentative; (iii) cytotoxicity is evaluated in a single cancer cell line; (iv) normal-cell selectivity is not reported; (v) AO/EtBr observations are qualitative; (vi) statistical methods and independent replicate numbers require clarification; and (vii) docking predictions have not been confirmed by biochemical or cellular target-engagement experiments.

Future investigations should standardize the formulation and extraction yield, profile the extract using validated chromatographic and mass-spectrometric methods, repeat cytotoxicity assays with biological replicates, include a non-malignant lung cell line and a suitable positive control, and quantify apoptosis using flow cytometry or validated image analysis. FGFR4 activity should be examined through a kinase assay or another target-specific method. Studies in additional NSCLC models and appropriately designed in-vivo investigations would be required before drawing conclusions about therapeutic potential.

6. Conclusion

Madhumega Chooranam extract demonstrated concentration-dependent reduction in A549 cell viability in the reported MTT assay, with a reported IC₅₀ of 65.55 ± 0.05 µg/mL. TLC findings were reported as consistent with tannins, flavonoids and glycosides, and quercetin showed a predicted FGFR4 docking score of -7.9 compared with -8.4 for fisogatinib. AO/EtBr staining at 90 µg/mL showed qualitative cellular changes associated with apoptosis. These findings represent preliminary evidence only. Confirmation of phytochemical identity, assay validity, cancer-cell selectivity, mechanism of action and in-vivo safety and efficacy is necessary before MCE can be considered a potential candidate for further anti-cancer development.

Declarations

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Editorial note: This manuscript has been reorganized from the supplied B.Pharm project report into a journal-style draft. Before submission, authors should verify all experimental details and references against the original records, add complete bibliographic details for studies cited in the project but not fully listed here, and adapt formatting to the selected journal's author guidelines.