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SUMMARY AND CONCLUSION

Recent research has led to the discovery of new anticancer drugs, including plant-derived compounds that target malignant cells directly while reducing adverse effects. Many phytochemicals and plant-derived compounds have been identified as possible anticancer candidates. Ethnopharmacology knowledge on the use of some natural plant-derived compounds can be used to develop newer, safer, and more mild drugs. The protein / gene expression techniques pave way to study the molecular targets in cancer cells and the plant-derived bioactive compounds as a promising anticancer mechanism. Plants rich in syringic acid have been shown to possess several biological activities, including anticancer property against many cancer types. Though the cancer preventive and antiproliferative effects of plant extract have been studied earlier, the molecular mechanisms underlying their anticancer activities in lung adenocarcinoma A549 cells using SAF have not been previously reported. With this background, for the present study, *Plectranthus amboinicus* belonging to Lamiaceae family was chosen as the candidate plant. It is rich in phenolic acids and possess numerous pharmacological properties. Thus the present study aimed to probe the presence of bioactive components in the methanolic extract of *Plectranthus amboinicus* and to investigate the anticancer activity of the isolated syringic acid fraction along with the standard syringic acid and the drug paclitaxel.

The present study entitled **"Validating the potential of *Plectranthus amboinicus* against lung cancer - *In silico, in vitro* and *in vivo* approaches"** has been performed in six phases and the findings are summarised below:

Phase I

In Phase I, preliminary screening and qualitative phytochemical analysis of *Plectranthus amboinicus* leaf extracts using petroleum ether, methanol and water as solvents were assessed. Results revealed that the methanol extract indicated

the presence of carbohydrates, proteins and amino acids, phenols, saponins, flavonoids, alkaloids, sterols, glycosides and the absence of tannins, terpenoids, quinones/ anthroquinones, anthocyanin and leucoanthocyanin. On contrary, in the aqueous extract alkaloids, phenols, flavonoids and sterols were present and the rest of the secondary metabolites were absent. The non-polar solvent, petroleum ether indicated the presence phenols, terpenoids and sterols whereas all the other phytoconstituents were absent. The yield of the extracts varied according to the character of solvent used for extraction and maximum yield was attained in methanolic extract.

Phase II

The antioxidant potential of methanolic extracts of *Plectranthus amboinicus* leaves were evaluated in Phase II utilizing a battery of radical scavenging assays such as DPPH, ABTS, nitric oxide, hydrogen peroxide, and superoxide at varying concentrations. The findings indicated that the methanolic extract improved scavenging activity in the tested assays in a dose-dependent manner. Hence, *Plectranthus amboinicus* offers persuasive antioxidant components which might lead to the exclusion of Reactive Oxygen Species mediated ailments by scavenging free radicals or preventing the oxidation of biomolecules.

Phase III

In Phase III, the phytocompounds present in the methanolic leaf extract of *Plectranthus amboinicus* was screened through GC-MS analysis and validated for its anticancancer potential through *in silico* approach. GC-MS screening of methanolic leaf extract of *Plectranthus amboinicus* revealed the presence of seven major phytocompounds namely carvacrol, thymol, alpha pinene, limonene, vanillin, cineole and syringic acid. These phytocompounds were chosen to validate the anticancer potential against apoptotic regulator target proteins (PDB IDs 3MK8, 3PK1, 4IDT, 4ZBF, 5FMI, 5LAY and 5MW7) through step wise *in silico* validation procedure. The phytocompounds were subjected to drug likeness and ADMET screening to reveal its physico-chemical, pharmacological and pharmacognostic profile. It was observed that all the phytocompounds possess

good agreement with drug likeness and ADMET properties. Seven important apoptotic regulator target proteins were docked with the phytocompounds and they show good binding affinity in the range of -4.8 to -6.7kcal/mol. Phytocompound, carvacrol shows good binding affinity with all target proteins possessing appreciable bonded and non-bonded interactions with amino acid residues of binding pockets of target proteins followed by thymol and syringic acid. Orbital energy calculations were made for the phytocompounds through DFT analysis which revealed good binding affinities towards the selected protein targets. All screened phytocompounds possess high softness, low hardness and less energy gap, thereby revealing its stability and biological activity. The bioactivity score of the phytocompounds predicted its potency with moderate biological activity. Collectively, the present *in silico* based characterization demonstrated *Plectranthus amboinicus* as a potential source of anticancer compounds and fetch novel improvement for alternative medicine in the prevention and treatment of cancer.

Phase IV

The identification of bioactive components in *P. amboinicus* was carried out by FT-IR, NMR, UV-Vis absorption spectroscopy, HPLC and HPTLC. The FT–IR analysis revealed the presence of functional groups such as hydroxyl, amines and alkynes in the methanolic extract of *P. amboinicus.* The 1H NMR spectrum revealed the resonating peaks with the presence of hydrogen, methyl and hydroxyl groups and also confirms the presence the syringic acid as compared with NIST library. Further through bioassay guided fractionation 8 similar fractions were pooled and subjected to phenolic estimation. Fraction 4 was found to have high amount of phenol when compared with other fractions. The peaks of UV-Vis spectrum, HPLC and HPTLC of the fraction 4 coincides with the peaks of standard syringic acid and thereby confirming its presence in the isolated fraction.

Phase V

In Phase 5, the cytotoxicity of syringic acid fraction extracted from *Plectranthus amboinicus* leaves was investigated against the human lung cancer

cell line (A549) under *in vitro* conditions and compared with commercial syringic acid and the drug paclitaxel which served as positive controls. MTT assay confirmed that the identified syringic acid fraction had cytotoxicity towards A549 lung cancer cell lines in a dose-dependent mode. The AO/EtBr, DAPI and PI staining revealed the morphological changes such as genomic DNA fragmentation, nuclear condensation and mitochondrial membrane damage in the A549 cells treated with syringic acid fraction. The effect of syringic acid fraction on cell cycle distribution and arrest was assessed through flow cytometer. The syringic acid fraction exhibited a multifaceted chemotherapeutic impact by instigating apoptosis and cell-cycle arrest in comparison with the positive control syringic acid and paclitaxel. The apoptotic protein and gene expressions were further investigated by Western blotting and RT-PCR analyses. Results revealed that, syringic acid fraction down regulated the expression of anti-apoptotic protein Bcl-2 and upregulated the expressions of apoptotic proteins namely cytochrome c, Caspase 3 and p53. The capacity of syringic acid fraction to decrease the growth of lung cancer cells for chemotherapeutic medication may contribute insightful remedial measures for various ailments.

Phase VI

In phase VI, the efficacy of syringic acid fraction (SAF) was assessed for its antitumor activity in benzopyrene induced lung tumor mice and compared with the commercial syringic acid and the standard drug paclitaxel. From the acute toxicity results, 25 and 50 mg/kg of SAF was selected as oral dose for B(a)P induced mice. With tumor growth response factor, the mean survival time for higher and lower dose of SAF was 14.5 and 15 days as comparable with syringic acid (15.66) and standard drug paclitaxel (16.66) and increased life span.

The body weight of mice induced with B(a)P reduced gradually whereas those treated with SAF restored the body weight to near normal as compared with syringic acid and paclitaxel. In contrast, the relative lung weight was found to be high in B(a)P treated mice, whereas it was decreased in mice treated with syringic acid and paclitaxel. The levels of haematological parameters namely RBC, Hb and the platelets were decreased in B(a)P treated mice, whereas its

level were restored to normal in mice treated with SAF, syringic acid and paclitaxel. The levels of WBC and neutrophils significantly increased whereas the monocytes, basophils and lymphocytes decreased in mice treated with B(a)P, and its count levels were normalized in SAF, syringic acid and paclitaxel treated mice. The biochemical parameters such as Aspartate transaminase, Alanine transaminase, Alkaline phosphatase (Liver marker enzymes), urea, uric acid and creatinine (Renal markers) and Gamma glutamyl transferase, Adenine deaminase and Lactose dehydrogenase (Tumor marker enzymes) were observed to be increased in B(a)P treated mice whereas its levels were restored to normal in mice treated with SAF, syringic acid and paclitaxel. The lung tissues of B(a)P stained with hematoxylin and eosin revealed normal alveolar structure whereas SAF, syringic acid and paclitaxel replaced its hyperplastic foci with extensive proliferation of alveolar epithelium.

Thus to conclude, the outcomes of the present study revealed that syringic acid fraction proves to have potential anticancer effect as validated through *in vitro* and *in vivo* approaches and emerged to be a promising candidate for the preparation of an improved traditional medicine for lung cancer.

SUGGESTIONS FOR FUTURE RESEARCH

The outcome of the present study has opened up a number of avenues for future research. Some of them that can be suggested for active research are given below.

- The anticancer activity of the syringic acid fraction can be tested in various cancer cell lines of different tissue origin to know whether the activity is tissue-specific.
- The apoptosis-inducing activity of the syringic acid fraction can be analyzed further for the mechanism involved using other molecular markers of apoptosis.
- The proteomic profile of the cancer cells treated with the syringic acid fraction fraction may be characterized.

- The nanoparticles of the syringic acid fraction can be synthesized and their effect on the various cancer cell lines can be studied.
- The anticancer activity of the syringic acid fraction can be studied on human subjects.