Investigation on phytochemical composition, anti-oxidant and anticancer properties of methanolic extract of *Phyllanthus niruri* schumach & thonn. And protein modelling and drug docking

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**ABSTRACT**

The study aims to investigate the Phytochemical Composition, Anti-oxidant and Anticancer properties of methanolic extract of *Phyllanthus niruri* Schumach & Thonn And the Protein Modelling and drug docking. The research deals with the methanolic extraction and phytochemical screening, determination of total phenolic and flavonoids contents and anti-oxidant assay. By performing GC-MS characterisation, various active metabolites are analysed. Thin-layer chromatography profiling of the *Phyllanthus niruri* methanolic extract was performed. The IC50 of the *Phyllanthus niruri* methanolic extract against PA-1 Cell lines(Ovarian cancer) was calculated. Docking studies also performed for antitumor activity by using Bioinformatics and Cheminformatics software on corilagin and cisplatin. The results suggested that the methanolic extract of *Phyllanthus niruri* leaves has the anticancer cancer effect on the ovarian cell line. The docking studies also performed that Corilagin interaction with T.F. receptor shows a high binding score when compared to cisplatin. Our future research can be done in this area to optimise anticancer activity efficacy. Our results can be further tested Clinico-pharmacologically to prove its efficiency in human beings.

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**INTRODUCTION**

Phyllanthus niruri (Euphorbiaceae) is commonly found in the tropical regions and an accepted traditional medicine for treating nephrolithiasis and cholelithiasis, hepatic diseases such as jaundice and hepatic carcinoma, tuberculosis, malaria, diabetes. ([Markom et al., 2007](#)) Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Over 100 types of cancers affect humans. ([Cokkinides et al., 2005](#)) Ovarian cancer is cancer that occurs in the ovary of women. They have two ovaries on either side of the uterus(womb). These ovaries in women produce, store and release in eggs. The Ovarian cancer is gynaecological cancer most common in the U.K. and North America women. The consensus of health organisation reported approximately 4400 women die from it around 7000 women diagnosed with ovarian cancer. ([Bankhead et al., 2008](#)) In the early stage of diagnoses, women have an 80-90% survival rate and decrease drastically to 20-30% in the last stage of
diagnosis. (Kosary, 1994) Ovarian cancer is the fifth most common cause of cancer death in worldwide women. (Sharifian et al., 2015) Our docking studies help to identify further that corilagin is a major active component from P. niruri L. extracts and has broad-spectrum antitumor activity, a better antitumor potential but lower toxicity to normal cells.

MATERIALS AND METHODS

 Soxhlet extraction

The collected plant samples were washed thoroughly with running tap water, followed by deionised water. Once washed samples were dried under shade condition until they get full dryness. The shade dried samples were powdered using a milling machine and subjected Soxhlet extraction (Borosil, Mumbai, India) using ethanol as solvent. The powdered samples were made a 50g thimble using handmade filter paper. The resultant solvent extract was condensed using a rotary evaporator (Buchi, Bangalore, India) under reduced temperature in vacuum condition. The resultant precipitant was collected in a glass container for further analysis and storage under -20° C.

Preliminary screening

Phytochemical screening has been done. The total phenolic contents and total flavonoid content was determined according to the method followed by Lin and Tang, 2007 (Jia et al., 2017). Thin Layer Chromatography was carried out to isolate the compounds.

Anti-oxidant Assay

Free radical scavenging ability by the use of a stable D.P.P.H. radical (1,1-diphenyl-2-picrylhydrazyl)

The anti-oxidant assay by D.P.P.H. radical scavenging was estimated according to the procedure described by (Sofowora, 1993)

Anticancer Activity test extract against Ovarian Cancer cell line PA-1

Preparation of cell suspension

A subculture of PA-1 cell lines in Dulbecco’s Modified Eagle’s Medium (D.M.E.M.) was trypsinised separately, after discarding the culture medium. To the disaggregated cells in the flask, 25 mL of D.M.E.M. with 10% F.C.S. was added. The cells suspended in the medium by a gentle passage with the pipette and the cells homogenised.

Seeding of cells

One mL of the homogenised cell suspension was added to each well of a 24 well culture plate along with a different concentration of the sample (Phyllanthus extract) (0 to 200 µg/mL) and incubated at 37° C in a humidified CO₂ incubator with 5% CO₂. After 48 hrs incubation, the cells were observed under an inverted tissue culture microscope. With 80% confluence of cells, the cytotoxic assay was carried out.

Cytotoxicity assay

M.T.T. cytotoxicity assay was carried out with the procedure described by (Evans, 2002)

Docking

Protein Modelling and drug Docking

Background of the current Insilico research investigation:

The main aim of our research is to identify the molecular protein/drug interaction between the Test compound, Corilagin (C.I.D.: 441203) and the control compound, Cisplatin (C.I.D.: 441203) with the receptor of T.F. (Transferrin -Serotransferrin) using advanced Bioinformatics and Cheminformatics software and tools.

Bioinformatics

Protein modelling and visualisations

The T.F. (Transferrin -Serotransferrin) (Uniprot Id: P02787) gene-coded protein sequence was applied into an automated homology modelling server (CPH 3.0 model server) http://www.cbs.dtu.dk/services/CPHmodels/ (Markom et al., 2007; Cokkinides et al., 2005) to convert the amino acids sequence into a 3D structure. The modelled protein 3D structure was viewed with the help of the molecular visualisation software, Discovery studio software.

Cheminformatics

Drug 3D prediction

In this project, we chose a Test molecule, Corilagin (C.I.D.: 441203) (https://pubchem.ncbi.nlm.nih.gov/) and a controlled drug, Cisplatin (C.I.D.: 441203) retrieved from NCBI –P.U.B.C.H.E.M. compound chemical database to perform molecular drug docking studies. The 2D drug was converted into a 3D structure using Discovery studio software for drug docking protocols.

Molecular Drug Docking

Molecular drug docking studies were performed using an automated molecular drug docking server, PATCHDOCK https://bioinfo3d.cs.tau.ac.il/PatchDock/. (Bankhead et al., 2008; Kosary, 1994) From the results obtained from drug docking studies, we analyse the binding affinities and ligand-protein interactions between the modelled protein target,
T.F. (Transferrin -Serotransferrin) and the selected chemical molecules, Corilagin, the test compound (C.I.D.: 441203) and cisplatin, the control drug (C.I.D.: 441203).

RESULTS

Result of plant extract

Extraction of Plant crude metabolites

The powdered sample was successfully extracted with methanol using Soxhlet extractor. About 5.61 g of extracts were obtained in the given 50 g of *Phyllanthus niruri* leave powder.

Phytochemical Screening of the *Phyllanthus niruri* methanolic Extract

The Phytochemical analysis of the *Phyllanthus niruri* methanolic Extract showed many Phyto-constituents presence, and few they were absence (Table 1).

| S.No. | Phyto-constituents | Availability |
|-------|--------------------|--------------|
| 1     | Alkaloids          | -            |
| 2     | Anthraquinones     | +            |
| 3     | Coumarins          | -            |
| 4     | Glycosides         | -            |
| 5     | Flavonoids         | +            |
| 6     | Phenols            | +            |
| 7     | Tannins            | +            |
| 8     | Terpenoids         | +            |
| 9     | Saponins           | +            |
| 10    | Steroids           | +            |

+ Presence, – Absence

Total phenol and flavonoids content

Total phenol and flavonoids content of the *Phyllanthus niruri* methanolic extract was determined using folin-phenol reagent and aluminium chloride method, respectively. It was estimated that, 971.33 mg Gallic acid equivalent per gram of dried extract. The total phenolic content of the sample was compared with standard gallic acid. The flavonoids content of the *Phyllanthus niruri* methanolic extract was estimated at 87.5 mg Quercetin equivalent per gram dried extract (Table 2).

Anti-oxidant activity of *Phyllanthus niruri* methanolic extract against D.P.P.H. free radicals

Anti-oxidant activity of *Phyllanthus niruri* methanolic extract against D.P.P.H. free radicals was tested under assay conditions. The ability of the plant extract against D.P.P.H. free radical scavenging was indicated that the given sample scavenged excellently with the D.P.P.H. free radicals at all the tested concentrations. The highest tested concentration, 0.5 mg of the crude extract showed 87.92% inhibition against D.P.P.H. free radical. It was calculated that IC50 of the *Phyllanthus niruri* methanolic extract against D.P.P.H. free radicals was 0.27 mg/ml. (Table 3)

T.L.C. profiling of the *Phyllanthus niruri* methanolic extract

The T.L.C. profiling of the test sample *Phyllanthus niruri* methanolic extract was done using chloroform and methanol at 9:1 (v/v) ratio. There were eight different major compounds were seen in the *Phyllanthus niruri* methanolic extract with the Rf value viz., 0.90, 0.83, 0.78, 0.73, 0.68, 0.60, 0.50 and 0.38. (Figure 1)

GC-MS profiling of the *Phyllanthus niruri* methanolic extract

The various active metabolites availability were analysed using GC-MS profiling with *Phyllanthus niruri* methanolic extract showed many compounds presented in the test report. There were 18 different metabolites seen in the extract with various proportions. Among them, Cinerin II was found more abundantly as 20.12% of peak area coverage. Other notable secondary metabolites such as Metribuzin DA (15%), Disulfoton (10.10%) and Cyromazine (9.69%).

Anticancer Activity of *Phyllanthus niruri*
methanolic extract

Corilagin

Corilagin \((\beta - 1 - O - galloyl - 3,6 - (R) - hexahydroxydiphenoyl - d - glucose)\) is a tannin isolated from Phyllanthus niruri (Figure 3) (Moreira et al., 2013)

Table 2: Quantitative analysis of Phyllanthus niruri methanolic extract total phenol and flavonoids

| S.No | Phyto-constituents | Availability         |
|------|-------------------|----------------------|
| 1    | Total Phenol      | 971.33 mg G.A.E./g DE |
| 2    | Flavonoids        | 87.5 mg Q.E./g DE    |

GAE: Gallic acid equivalent, DE: Dry Extract, Q.E.: Quercetin equivalent

Drug docking

The binding affinity score is given by atomic contact energy (A.C.E.) value for Cisplatin at T.F. (Transferrin -Serotransferrin) receptor at T.F. (Transferrin-Serotransferrin) is -421.15. The interacting amino acids were more with Cisplatin at T.F. (Transferrin -Serotransferrin) receptor, but the binding affinity was lesser. The hydrogen bond and hydrophobic interactions formed by Cisplatin at T.F. (Transferrin-Serotransferrin) receptor. Cisplatin interacts with T.F. receptor through hydrogen bond at the following binding sites. Among these amino acids, the H-bond interaction of cisplatin is directly involved in the receptor of T.F. (Figure 4)

Phyllanthus niruri is a herbal plant having various pharmacological properties. The result obtained for our project has proven that the Phyllanthus niruri species is effective against the cancer cell
Table 3: D.P.P.H. free radical scavenging ability of *Phyllanthus niruri* methanolic extract

| S.No. | Concentration (mg) | Absorbance @ 515 nm | % Inhibition |
|-------|-------------------|---------------------|--------------|
| 1     | 0                 | 0.621               | 0.00         |
| 2     | 0.1               | 0.564               | 9.18         |
| 3     | 0.2               | 0.357               | 42.51        |
| 4     | 0.3               | 0.254               | 59.10        |
| 5     | 0.4               | 0.124               | 80.03        |
| 6     | 0.5               | 0.075               | 87.92        |

Table 4: In vitro cytotoxicity effect of sample *Phyllanthus niruri* methanolic extract against PA-1 Cell lines

| Sample Conc. (mg/mL) | % Cell Viability |
|----------------------|------------------|
| 0                    | 100.00           |
| 1.625                | 91.03            |
| 3.125                | 79.32            |
| 6.25                 | 69.90            |
| 12.5                 | 62.97            |
| 25                   | 54.34            |
| 50                   | 46.07            |
| 100                  | 32.43            |
| 200                  | 16.23            |

spread ability in the human body. In the study, the leaves of *Phyllanthus niruri* was extracted by using methanol as solvent. (von Gadow et al., 1997; Yen and Duh, 1994) The phytoconstituents present in the methanolic extract of *Phyllanthus niruri* leaves are Anthraquinines, Flavonoids, Phenol, Tannins, Terpenoids, Saponins and Steroids. The total phenol content and flavonoid content present in *Phyllanthus niruri* leaves methanolic extract, which was determined by using folin phenol reagent and aluminium chloride method are 971.33mg G.A.E./gDE and 87.5mg Q.E./gDE, respectively. (Abondanza et al., 2008; Mosmann, 1983; Singleton and Rossi, 1965) The *Phyllanthus niruri* leaves methanolic extract sample has scavenged extraordinary with the D.P.P.H. free radicals at all tested concentration. The higher tested concentration 0.5mg of crude drug extract showed 87.92% inhibition against D.P.P.H. free radical. In the T.L.C. profiling test, eight major compounds were identified. (Lin and Tang, 2007; Yang et al., 1984) By G.C. — M.S. profiling the *Phyllanthus niruri* methanolic extract 18 different metabolic were identified as 20.12% peak area coverage. (Park et al., 1985) The in-vitro cytotoxicity activity of *Phyllanthus niruri* extract against PA-1 cell line studies was showed growth-inhibiting on increasing the sample concentration. (Lund et al., 2002; Nielsen et al., 2010) It is observed that the sample. *Phyllanthus niruri* extra shows 16.23% cytotoxic activity against PA-1 cell line when tested at a high concentration as 200mg/ml. In our investigation reported that the IC50 of the *Phyllanthus niruri* leaves methanolic extract against PA-1 cell lines was 76.26mg/ml then standard drug cisplatin shows IC50 as 8.56mg/ml against PA-1 cell lines. It so, It proves that the *Phyllanthus niruri* leaves methanolic extract contains cytotoxic effect as low as in the Ovarian cancer cell line, which is PA-1 cell lines. (Duhovny et al., 2002; Schneidman-Duhovny et al., 2005) The result suggested that the methanolic extract of *Phyllanthus niruri* leaves as the anti-cancer cancer effect on ovarian cancer.
CONCLUSIONS

As per the report of our investigation, it was found that methanolic extract of *Phyllanthus niruri* leaves has the good anti-oxidant effect on Ovarian cancer, which has significant inhibition against the harmful cancerous cells and their functional growth. Future research can be done to optimise the efficacy of anticancer activity. Our Insilico results strongly conclude that Corilagin can act as a potential therapeutic agent for ovarian cancer. Our results can be further tested Clinico-pharmacologically to prove its efficiency in human beings.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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