Research Article

In Silico Identification of Natural Drugs for Cancer from the Compounds of *Phyllanthus urinaria* Lin.

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ABSTRACT

Phyllanthus urinaria Linn. has long been used as an anticancer agent in the form of whole plant extract. It is essential to understand the specific compounds responsible for antiproliferative and antiangiogenic activity in the process of tumor development. Previous chemical investigations of this plant, using HPLC, led to the identification of many bioactive compounds. In the present study, all the compounds were made to interact with enzymes, Bcl-2, telomerase and MMP-2 which are involved in the tumor development through *in silico* method. The efficiency of the interaction was calculated based on the e-negative values used at the time of molecular interaction between the bioactive compounds and the enzymes. In this study, the order of anticancer activity of the compounds was geraniin > chebulagic acid > corilagin > rutin > quercetin glucoside > caffeoliquinic acid > quercetin > kaempferol > ellagic acid > gallic acid. Among them, geraniin and chebulagic acid stood first in the anticancer activity comparable to the commercial drug, paclitaxel. Corilagin, rutin and quercetin glucoside formed the second group showing activity comparable to the commercial drug, doxorubicin and the remaining five compounds, caffeoliquinic acid, quercetin, kaempferol, ellagic acid and gallic acid formed third group with low anticancer activity compared to the above two groups.

Keywords: *Phyllanthus urinaria* Linn, *in silico*, geraniin, chebulagic acid, paclitaxel and doxorubicin.

INTRODUCTION

Cancer is a broad group of diseases involving the cells to divide and show uncontrolled proliferation of cells developing into malignant tumors. The tumor development is under the control of various enzymes like Bcl-2, telomerase and MMP2, and these formed the targets for the present anti-cancer study. At present, different kinds of commercial drugs such as paclitaxel, doxorubicin and cisplatin are in use, but at the same time these drugs have some limitations due to serious life threatening side effects and development of drug resistant cancer cells. These drugs have been used as positive control in the present study to compare with bioactive compounds. One effective solution to this problem is the identification of natural anticancer compounds from medicinal plants used in traditional practice. One such potential medicinal plant is *P.urinaria*. It has long been known as folk medicine for the treatment of various cancers, hepatitis B and hepato carcinoma. For long time, without knowing the constituent compounds, the whole plant extract was used as pharmocological agent against various cancers.[1,2] Recently, many reports regarding the bioactive compounds of *P.urinaria* have been appeared.[3,4] Analysis of the aqueous extract of *P.urinaria* by HPLC led to the identification of many compounds like corilagin, geraniin, chebulagic acid, ellagic acid, gallic acid, quercetin, quercetin glucoside and kaempferol.[5]

The aqueous extract of *P.urinaria* showed strong anticancer activity reducing the cell viability of various cancer cell lines and suppression of tumor development. Identification of potential bioactive compounds and new drug development in *P.urinaria* is the task of the present investigation. The decrease in cell viability and tumor growth by *P.urinaria* is associated with the down regulation of BCL-2 and telomerase which resulted in apoptotic induction. P.*urinaria* extract also exhibited anti-angiogenic activity by suppressing MMP2 secretion and inhibition.

MATERIALS AND METHODS

In order to analyze anticancer bioactive compounds from *P.urinaria*, in the present investigation, bioactive compounds Gallic acid, Corilagin, Geraniin, Rutin, Quercetin, Pyrogallol, Chebulagic acid, Kaempferol, Quercetin glucoside, Caffeoliquinicacid, Ellagic acid and commercial drugs Paclitaxel, doxorubicin, cisplatin were retrieved form data bases. The bioactive compounds were retrieved from Chemspider database[6], which was later converted into 3-D structures using Swiss Pdb viewer.[7] The cancer causing receptors Bcl-2, telomerase and MMP-2 were retrieved from Protein databases.[8] The molecular docking between receptor and ligand were performed by using Hex programe [9] to select a better ligand molecule against various cancers.

RESULTS AND DISCUSSION

The present study focused on the molecular docking and analysis of bioactive compounds of *P.urinaria* with cancer causing enzymes like Bcl-2 (Fig.1), telomerase (Fig.2) and MMP-2 (Fig.3). The bio active compounds geraniin (Fig.4), chebulagic acid (Fig.5) and commercial drug paclitaxel (Fig.6) acted as ligands which were docked on the active

sites of the target enzymes. The energy values of the compounds docked were tabulated (Table.1).

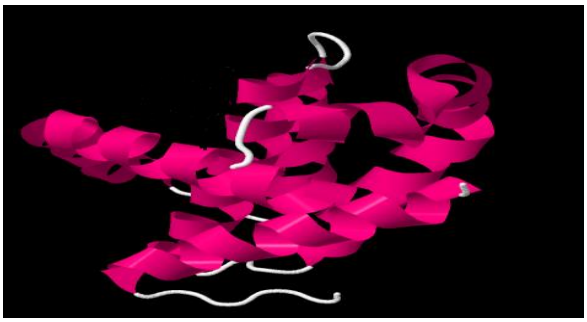

Figure 1: 3D Structure of Bcl2

Figure 2: 3D Structure of Telomerase

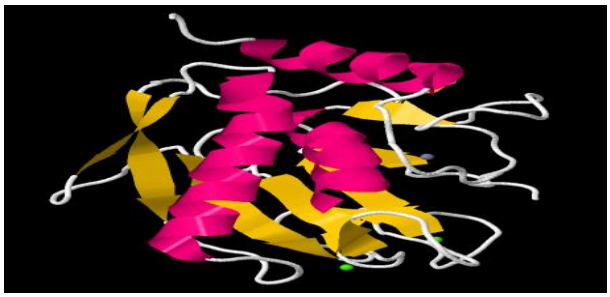

Figure 3: 3D Structure of mmp2

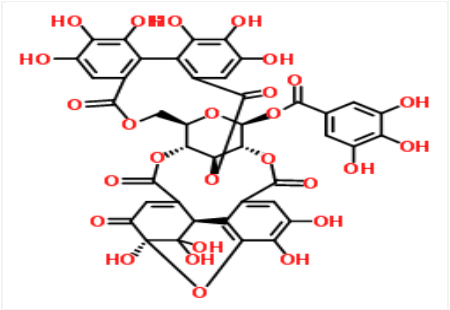

Figure 4: Structure of Geraniin

In this study the e-negative values of the commercial drugs, when docked with the enzymes causing cancers, were taken as the standard values of positive control. Docking BCl-2 with the commercial drugs, paclitaxel and doxorubicin, showed the e-negative values as -299.95 and -261.41 respectively. Bcl-2, on docking with all the bioactive compounds of *P.urinaria* showed energy values ranging from -174.54 to -299.80. Among the various compounds, geraniin stood first showing highest e-value with -299.80 (Fig.7) followed by chebulagic acid (-298.88) in their energy value which was comparable to that of the positive control paclitaxel (-299.95). The e-negative values of rutin (-273.99) quercetin glucoside (-272.99) and corilagin (-263.71) were moderately high comparable to the positive control, doxorubicin with -261.41. Since the other compounds, like caffeoliquinic acid, quercetin, kaempferol, ellagic acid and gallic acid, showed a lower level of e-negative values (-174.54 to – 249.78), were not considered further.

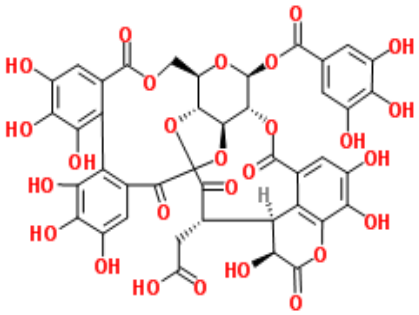

Figure 5: Structure of chebulagic acid

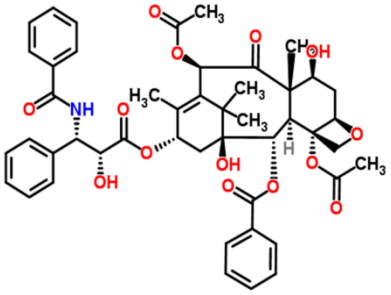

Figure 6: Structure of Paclitaxel.

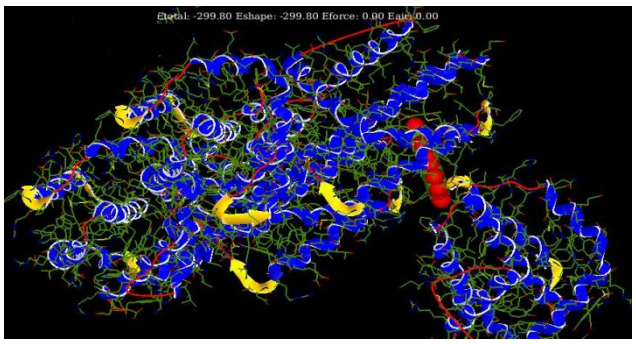

Figure 7: 3 D view of Molecular docking of Bcl 2 with Geraniin

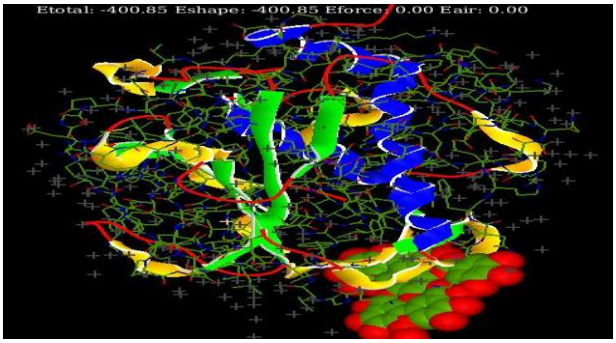

Figure 8: 3 D view of Molecular docking of Mmp-2with Geraniin

When all the compounds of *P.urinaria* and the commercial durgs like paclitaxel and doxorubicin were docked with telomerase, the e-negative values obtained were in the range between -151 -86 and 401.99. The highest e-negative values were found in chebulagic acid (-395.76) followed by geraniin (-371.38) which were very nearer to the value of the positive control, paclitaxel (-401.99). The e-negative value of the other positive control, doxorubicin (-328.55) was comparable to some bio active compounds like corilagin (-354.17) rutin (-334.23) and quercetin glucoside (-300.32). The remaining compounds of *P.urinaria* were not comparable to either with paclitaxel or with doxorubicin and therefore not considered further.

Table 1: Showing docking results of bioactive compounds from *P.urinaria* against Bcl 2, telomerase and Mmp-2

S.No	Bioactive compounds	Bcl 2	telomerase	Mmp-2
1	Gallic acid	-174.54	-151.86	-176.05
2	Corilagin	-263.71	-354.17	-331.38
3	Geraniin	**-299.80**	-371.38	**-400.85**
4	Rutin	-273.99	-334.23	-325.70
5	Quercetin.	-229.50	-238.70	-243.05
6	Pyrogallol.	-147.26	-140.11	-151.38
7	Chebulagic acid	-298.88	**-395.76**	-389.80
8	Kaempferol.	-211.67	-236.82	-242.13
9	Quercetinglocoside	-272.99	-300.32	-307.05
10	Caffeoliquinicacid	-249.78	-264.78	-264.85
11	Ellagic acid	-196.87	-211.14	-220.11
12	Paclitaxel.	**-299.95**	**-401.99**	**-420.80**
13	Doxorubicin.	-261.41	-328.55	-317.01
14	cisplatin.	-112.43	-204.98	-218.39

The enzyme MMP2, which is related to the formation of new blood vessels in the developing tumors (angiogenesis), was docked with the bioactive compounds of *P.urinaria,* paclitaxel and doxorubicin (Table.1). The e-negative values obtained were from -176.05 to -420.80. Among the bio active compounds, geraniin stood first with -400.85 (Fig.8) followed by chebulagic acid (389.80) which were comparable to that of the positive control, paclitaxel (-420.80). The e-negative values of some other compounds like corilagin (-331.38) rutin (-325.70) and quercetin glucoside (-300.32) were comparable to that of the other positive control doxorubicin (-317.01). Since the e-negative values of the remaining compounds were less than the values of the both the positive control, neglected for further consideration.

From the above study geraniin and chebulagic acid were identified as best bio active compounds showing higher e-negative values by which effective molecular interaction comparable to that of the best commercial drugs paclitaxel, the compounds corilagin, rutin and quercetin glucoside exhibited second level of e-negative energy values comparable to that of doxorubicin. The e-negative values of other compounds like quercetin, caffeo liquinic acid, gallic acid, ellagic acid and kaempferol were less than that of both the positive control and therefore rejected.

There are many naturally occurring plant extracts recognized to be anticancer agents, used as cancer therapy drugs. In recent years, there has been increasing interest in many bioactive compounds from various medicinal plants which possess potential therapeutic properties. For example, Huang *et al*,[2] reported gallic acid and ellagic acid as the most effective bioactive compounds in *P.urinaria* to induce the cytotoxic effect. The aqueous extract of *P. urinaria* has been demonstrated to have anticancer activity against a variety of cancers. In the past, in clinical applications, many people have used the extract of *P. urinaria* as an anticancer drug for patients suffering from cancers without any side effect.[10] The extract of the plant reduced the cell viability of various cancer cells and suppressed the tumor development by inducing apoptosis through the inhibition of Bcl- 2 and telomerase enzymes.[10, 11] The induction of apoptosis is known to be one of the efficient strategies for cancer therapy. Huang *et al*., [10.] have demonstrated the induction of apoptosis in Lewis lung carcinoma cells by *P. urinaria* treatment without any side effects even after used it for long time. *P. urinaria* extract also showed anti-angiogenic activity in tumor development. Angiogenesis is the development of new blood vessels in the developing tumors for the supply of nutrients and oxygen. This angiogenesis is caused by the enzyme MMP-2. This angiogenic activity is suppressed by *P. urinaria* extract by inhibiting the enzyme, MMP-2. Thus the whole plant extract of *P. urinaria* has been established as a potential natural drug against the

proliferation and angiogenesis in tumor development by Bcl -2, telomerase and MMP-2 enzymes.

Even though, the whole plant extract of *P. urinaria* has been established as a natural drug against cancer, it is essential to identify the specific bioactive compounds to formulate effective drugs comparable to that of paclitaxel and doxorubicin, responsible for the inhibition of the target enzymes, causing cancer. Huang *et al*., [5] identified various bioactive compounds such as gallic acid, corilagin, geraniin, chebulagic acid, rutin, quercetin glucoside, caffeoliquinic acid, ellagic acid, kaempferol and pyrogallol from the aqueous extract of *P. urinaria.* There are various reports available on the bioactive nature of these compounds against different types of cancers. According to Zhong *et al*.,[12] the anti-proliferative effect of *P. urinaria* could be due the occurrence of geraniin, rutin, quercetin and gallic acid. Ellagic acd has been reported as the active compound against the angiogenic activity during tumor development and thereby inhibiting the secretion of MMP-2 protein. Gallic acid inhibited the cell viability of cancer cells by manipulating the cell cycle and induces apoptosis in addition, it also shows the anti-tumor activity against osteocarcinoma. [5,13] Corilagin plays an important role in the inhibition of ovarian cancer while the quercetin leads to the inhibition of growth and induction of apoptosis in pancreatic tumor cells.[14] Similarly, geraniin suppresses the proliferation of lung carcinoma cells by inhibiting Bcl-2 expression.[15] Chebulagic acid shows broad spectrum anticancer effects on colon, breast and prostrate cancers, and chronic myeloid leukemia cells.[16] Thus from the above account, it is clear that ellagic acid, corilagin, gallic acid, quercetin, geraniin, rutin and chebulagic acid are the natural anticancer compounds responsible for the prevention of various cancers. Even though, anticancer activity of various compounds of *P. urinaria* has been reported, the effectiveness of the compounds during molecular interaction has not been studied so far. Hence the present study was carried out.

In the present investigation, the energy level of the bioactive compounds against the target proteins, Bcl-2, telomerase and MMP-2, was determined based on the e negative values obtained during molecular docking. Three groups of bioactive compounds were identified through molecular docking. High level activity, comparable to commercial drug, paclitaxel, was found in geraniin and chebulagic acid while medium level, comparable to doxorubicin, found in corilagin, rutin and quercetin glucoside. Third group comprising quercetin, gallic acid, caffeoliquinic acid, ellagic acid and kaempferol showed low level of activity by showing low level of energy values. From the above findings, geraniin and chebulagic acid were identified as the best bioactive compounds by showing maximum energy level against all the three enzymes tested. Though the energy level of second group of compounds was less, their effectiveness on different types of cancers cannot be ignored as stated above.

CONCLUSION

Cancer cells divide and show uncontrolled proliferation of cells developing into malignant tumors. *P.urinaria* produced bioactive compounds Gallic acid, Corilagin, Geraniin, Rutin, Quercetin, Pyrogallol, Chebulagic acid, Kaempferol, Quercetinglocoside, Caffeoliquinicacid, Ellagic acid and commercial drugs Paclitaxel, doxorubicin, cisplatin were docked against cancer causing receptors Bcl-2, telomerase and MMP-2. From the above study it is concluded that geraniin and chebulagic acid were identified as the best bioactive compounds by showing maximum energy level against above said three enzymes

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