Research Article

Structure-activity Relationship of Bioactive Compounds of *Phyllanthus urinaria* in Scavenging Free Radicals

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ABSTRACT

Free radicals have been regarded as fundamental cause of different kinds of diseases. It is known that active oxygen species attack proteins, lipids, nucleic acids, and enzymes in the body, independently or in cooperation, and show various toxic effects. They cause biochemical damage in cells and tissues. Targets of free radicals include lipids, nucleic acids, carbohydrates and proteins. Oxidative stress, caused by the free radicals, is associated with damage to the wide range of above molecules. The defense against oxidative damage is the use of antioxidant enzymes to convert excessive free radicals into non-toxic compounds. An imbalance between the amount of free radicals and antioxidant enzymes is a problem for the health. Many antioxidant compounds, naturally occurring in plant sources have been identified as scavengers of free radicals. One such Indian medicinal plant, that provides antioxidants through their bioactive compounds, is *Phyllanthus urinaria* from which eleven bioactive compounds have been selected for analysis. In the present study, to find out the most effective bio active molecule for cancer, molecular docking was carried out using enzymes molecules causing various cancers as receptors and eleven bioactive compounds as ligands. Among the eleven bioactive compounds, geraniin and chebulagic acid exhibited high energy during reaction in the form of e negative values. In order to find out the efficiency of the bio active compounds, the molecular structure was analyzed. The hydroxyl groups on the phenolic rings are essential for the antiproliferative and apoptotic activity in cancer development. The phenolic hydroxyl groups are primarily responsible for scavenging free radicals involved in various kinds of carcinogenesis. The galloyl moiety (three adjacent hydroxyl groups in a phenolic ring) is required for the antiproliferative, apoptotic and antioxidant effects and also in chelating metal ions. This structure-activity relationship of polyphenols facilitates the research on cancer therapy.

Keywords: antioxidants, bio active compounds, geraniin, chebulagic acid, galloyl moiety, molecular docking and *Phyllanthus urinaria*.

INTRODUCTION

The recent development in the knowledge of free radicals and reactive oxygen species (ROS) in biology is making a medical revolution that promises a new age of health and disease management.[1] Oxygen, an element essential for life, under certain situations, has deleterious effects on the human body[2]. Most of the potentially harmful effects of oxygen are due to the formation and activity of a number of chemical compounds known as ROS, which have a tendency to donate oxygen to other substances [3].

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron is a common characteristic feature shared by most radicals which makes these radicals as unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidant or reductant [4]. The most important oxygen-containing free radicals in many disease states are hydroxyl radical, super oxide anion radical, hydrogen peroxide, oxygen singlet, nitric oxide radical and peroxy nitrite radical. Free radicals have been regarded as a fundamental cause of different kinds of diseases, particularly cancers. Targets of free radicals include all kinds of molecules in the body. Among them, lipids, nucleic acids, and proteins are the most important targets [5]. It is known that active oxygen species attack the above molecules in the body and subsequently cause biochemical damage in cells and tissues independently or in cooperation, and produce various toxic effects.

The term oxidative stress is used to describe the condition of oxidative damage resulting when the critical balance between free radical generation and antioxidant defences is unfavourable [6]. Oxidative stress is associated with damage to a wide range of molecular species including lipids, proteins, and nucleic acids [7]. Short-term oxidative stress may occur in tissues injured by trauma, infection and heat injury. These injured tissues produce increased radical generating enzymes producing excess ROS. The initiation, promotion, and progression of cancer, as well as the side-effects of radiation and chemotherapy, have been linked to the imbalance between ROS and the antioxidant defence system. ROS have been implicated in the induction and complications of diabetes mellitus, age-related eye disease, and neurodegenerative diseases such as Parkinson's disease [8]. The present investigation has planned to correlate the activity of bioactive compounds on the scavenging of free radicals which have been regarded as elements responsible for various diseases. The major system of defence against oxidative damage is the use of antioxidant molecules to convert excessive ROS into non-toxic compounds. An imbalance between the amount of ROS and antioxidants is a problem for our

health. This is why the daily intake of foods with antioxidant ingredients is necessary.

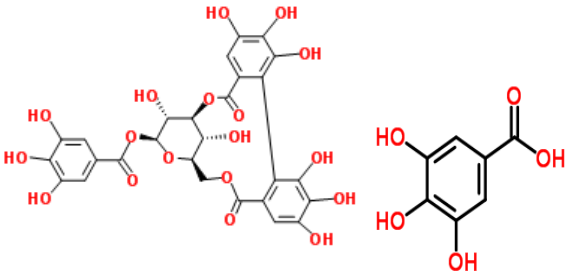
MATERIALS AND METHODS

In the present investigation, in order to analyze bioactive compounds of *P.urinaria*, the molecular structure of bioactive compounds, Gallic acid, Corilagin, Geraniin, Rutin, Quercetin, Pyrogallol, Chebulagic acid, Kaempferol, Quercetin glocoside, Caffeoliquinic acid and Ellagic acid of *P.urinaria* were retrieved form Chemspider database, which contains 3-D structures developed through Swiss Pdb viewer[9]. Molecular structure of receptors was also retrieved from the Protein Data Bank (PDB) which is a repository for the 3-D structural data of large number of biological molecules like proteins. Molecular structures of free radicals like superoxide (O_2), hydrogen peroxide (H_2O_2), hydroxyl radical($OH^{\cdot}$), hydroperoxyl radical and reactive nitrogen species (RNS) such as nitric oxide ($NO^{\cdot}$) and peroxynitrite ($ONOO^{-}$) were retrieved from Chemspider[9]. The retrieved structures of molecules were analyzed using RasMol which is a molecular graphics program intended for the structural visualization of proteins[10]. 3-D structures of proteins are provided by the UniProtKB/Swiss-Prot database [11]. Binding sites of proteins are associated with structural pockets and cavities. CastP provides identification and measurements of surface accessible pockets as well as interior inaccessible cavities, for proteins [12]. Hex, an interactive docking program, written by Ritchie [13] was used to find the energy value of of interaction. Hex understands protein structures in PDB format. Using Hex software, protein-ligand docking is possible. In this docking one molecule (always protein) acts as receptor and the free radicals as ligand.

RESULTS

Reactive oxygen and nitrogen species also called free radicals, such as super oxide anion, hydrogen peroxide, hydroxyl radical, and nitric oxide are acting as oxidants and their biological activity play an important role in carcinogenesis. Their presence in bio system could lead to mutation, transformation, and ultimately cancer. Antioxidants can decrease this oxidative stress induced carcinogenesis by direct scavenging of free radicals. An antioxidant is a stable molecule which could donate an electron to a free radical and neutralizes it and thus reduces its potential to damage biologically important molecules such as DNA, proteins, carbohydrates, and lipids. Many antioxidant compounds, naturally occurring in plant sources, have been identified as scavengers of free radicals. One such Indian medicinal plant, that provides antioxidants through their bioactive compounds, is *P.urinaria* belongs to the family Euphorbiaceae.

The study of structure-activity relationships of polyphenols facilitates the research on cancer therapy. The hydroxyl groups on the phenolic rings are essential for the antiproliferative and apoptotic activity in cancer development. The galloyl moiety (three adjacent hydroxyl groups in a phenolic ring), in addition to the distributed hydroxyl groups, is required for the antiproliferative, apoptotic and antioxidant effects and also in chelating metal ions. Thus the phenolic hydroxyl groups are primarily responsible for scavenging free radicals involved in various kinds of carcinogenesis. In the present investigation eleven bioactive compounds of *P.urinaria* (Figs.1) have been considered for analysis.

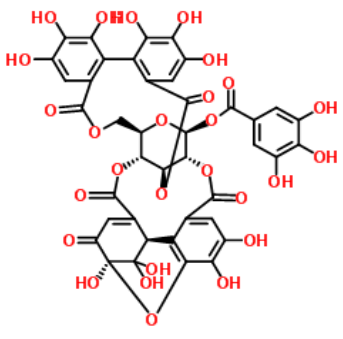


Gallic acid **Corilagin**

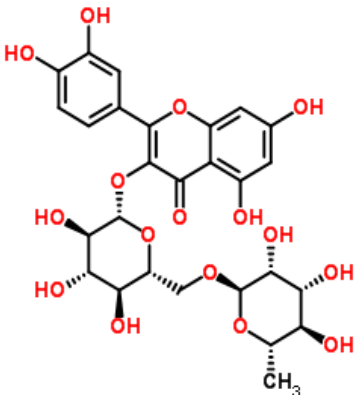
Figure 1: Structure of Bioactive compounds

Molecular docking analysis

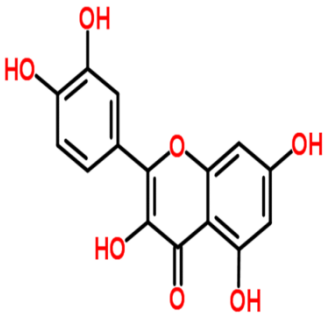
In the present study, different enzyme molecules causing various cancers as receptors and eleven bioactive compounds as ligands, were used in the molecular docking to find out the most effective drug molecule for each cancer. Among the eleven bioactive compounds, geraniin exhibited high e negative values and anticancer activity. It is a most promising ligand molecule showing high binding affinity with receptor molecules in molecular docking (Fig.2). Chebulagic acid also showed highest e negative energy values (Fig.3). Among the eleven bioactive compounds, chebulagi acid and geraniin showed highest e negative values against receptor enzymes (Fig.4). Geraniin exhibited highest energy values (Fig.5) against the following seven cancer causing ezymes such as EGFR tyrosine kinase (Lung Cancer), PAP (Prostate Cancer), THRA1 (Thyroid Cancer), Bcl 2, IL8, NF kappa-B and Mmp-2. The order of the e negative values of receptor enzymes against geraniin in the overall molecular docking was -467.55 PAP (Prostate Cancer) > 454.92 NF kappa-B > 428.09 THRA1 (Thyroid Cancer) > -415.25 EGFR tyrosine kinase (Lung Cancer) > -400.85 MMP 2 > -385.76 IL8 > 299.80 Bcl 2.



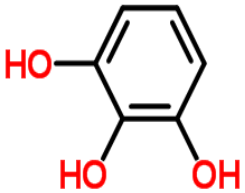
Geraniin



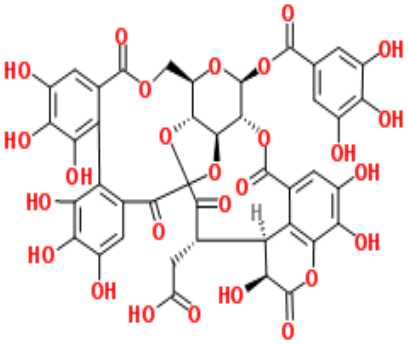
Rutin



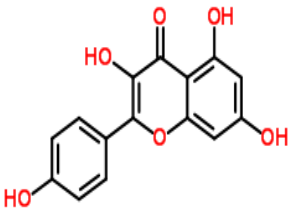
Quercetin



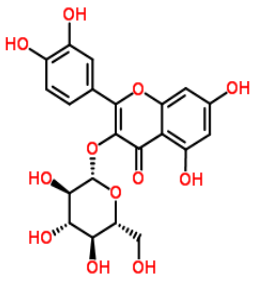
Pyrogallol



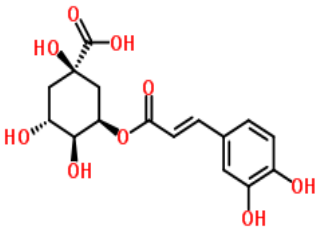
chebulagic acid



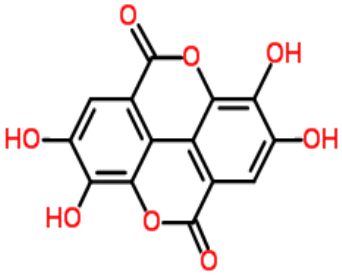
Kaempferol.



Quercetin glocoside



Caffeoliquinic acid



Ellagic acid

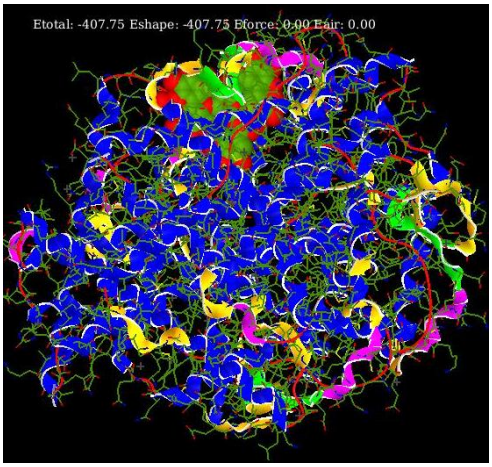


Figure 2: Molecular docking of Erα enzyme with Geraniin

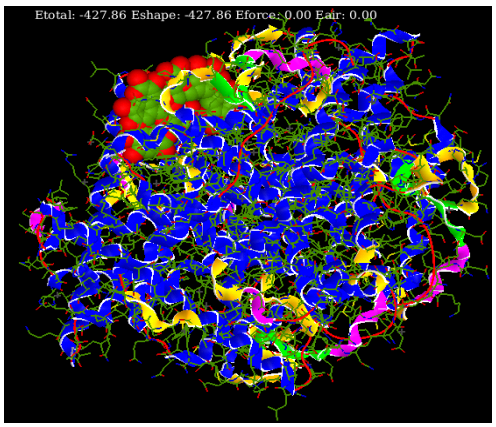


Figure 3: Molecular docking of Erα enzyme with Chebulagic acid

In the oveall molecular docking, the order of the e negative values (Fig.6) of receptor enzymes against chebulagic acid was -517.18 EGFR (ovarian cancer) > -439.62 Stat-3 > -428.47 COX-2 > -427.56 ERα (Breast Cancer) > -423.81 EGFR (Cervical Cancer) > -406.39 ABL (Blood Cancer) > -398.79 HSP90 (Skin Cancer) > -395.76 telomerase > 381.83 BOS D 2 (Gastric Cancer).

The second level group of compounds, corilagin, rutin and quercetin glucoside showed next level of e negative values against all cancer causing enzymes. Most of the enzymes, particularly reacted well with the above three compounds. The order of the e values of the three compounds was corilagin > rutin > quercetinglucoside.

The remaining six compounds, caffeoliquinic acid, quercetin, ellagic acid, kaempferol, gallic acid and pyrogallol, showed low e negative values which were less than that of chebulagic acid, geraniin corilagin, rutin and quercetin glucoside.

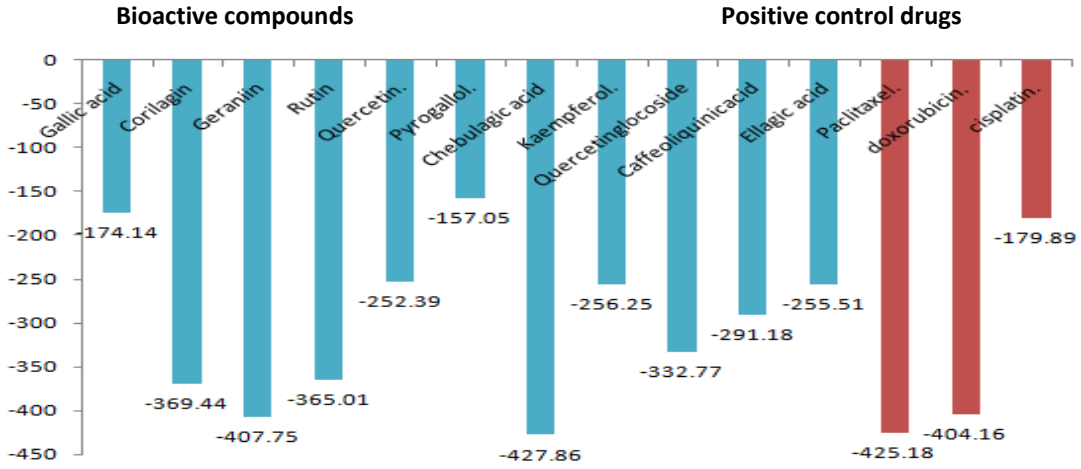


Figure 4: Binding energies used between ERα (Breast Cancer enzymes) and bio active compounds of *P.urinaria*. Note highest e negative values with geranin and chebulagic acid

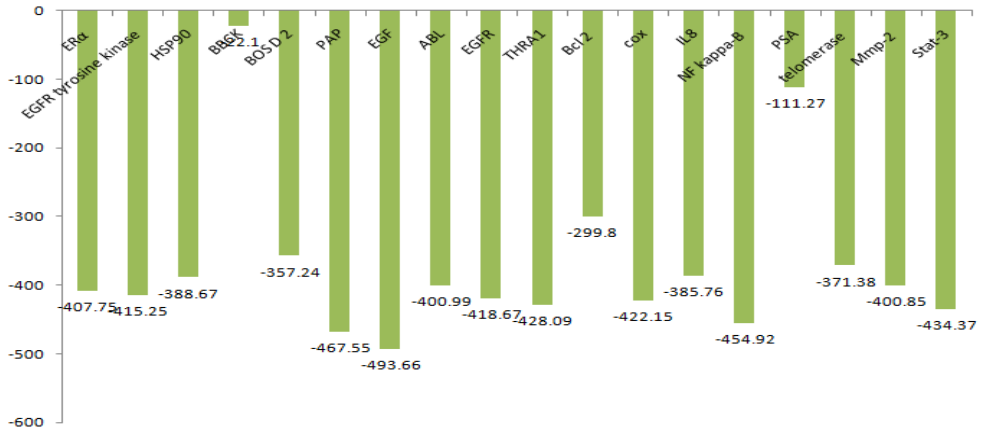


Figure 5: Binding energies of geraniin with various enzymes. Note highest e negative docking values with most of the carcinogenic enzymes

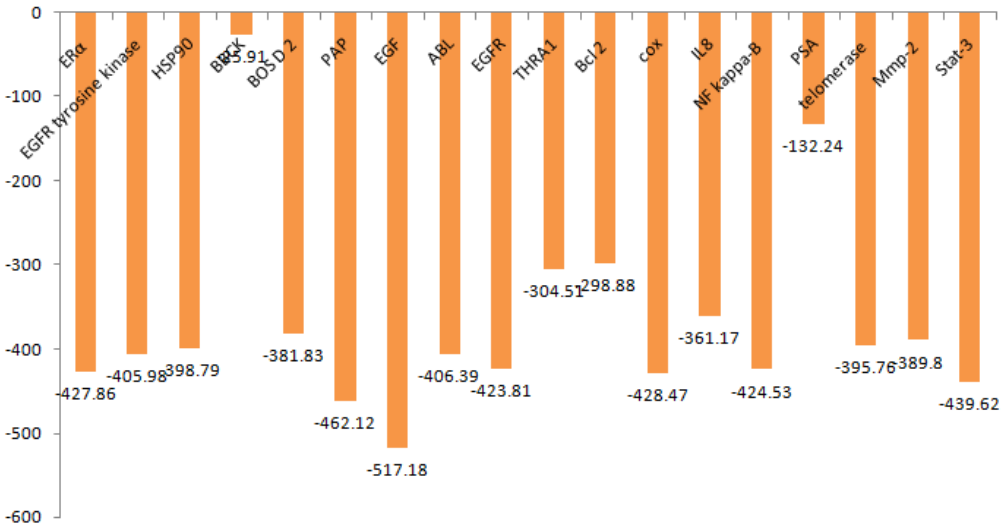


Figure 6: Binding energies of Chebulagic acid with various enzymes. Note highest e negative docking values with most of the carcinogenic enzymes

Structure – activity relationship of bioactive compounds

The molecular structure of the bioactive compounds exhibited a close relationship with the functional activity. In this investigation, the bioactive compounds of *P.urinaria* showed a lot of structural and functional diversity. The structural diversity of the bioactive compounds is mainly due to the presence of hydroxyl groups. Flavan-3-ols are characterized by hydroxylated aromatic rings with multiple hydroxyl groups. The aromatic ring may be monohydroxylated, dihydroxylated or trihydroxylated. The hydroxyl groups on the phenolic ring are essential for the antiproliferative and apoptotic activity. The phenolic hydroxyl groups are primarily responsible for scavenging free radicals. Presence of three adjacent hydroxyl groups in the aromatic ring of the molecule is the galloyl group or galloyl moiety which would be a key factor for enhancing the activity. Thus the compounds having more number of galloyl moieties show more potentiality in the scavenging activity.

Among the eleven bioactive compounds of *P.urinaria*, geraniin showed a maximum number of 14 hydroxyl groups in its molecule with four galloyl moieties. Next comes chebulagic acid with 13 hydroxyl groups and three galloyl moieties, followed by corilagin containing 11 hydroxyl groups and three galloyl moieties. Rutin showed 10 hydroxyl groups and two galloyl moieties. Gallic acid, pyrogallol, quercetin glucoside and caffeoliquinic acid were mono galloylcompounds with only one galloyl moiety less number of hydroxyl groups ranging from 3 to 8. Quercetin, ellagic acid and kaempferol molecules were non galloylated compounds with less number of 4 or 5 hydroxyl groups (Figs.1-11; Table.1). Based on the galloyl and hydroxyl groups, the potential of bioactive compounds could be arranged in the following order; geraniin > chebulagic acid > corilagin >rutin > quercetin glucoside> caffeoliquinic acid > quercetin > kaempferol > ellagic acid> gallic acid > pyrogallol.

Table 1: The number of OH in bioactive compounds of *P.urinaria* . Note the maximum number of OH groups in geraniin and chebulagic acid

S.No	Bioactive compounds	Number of OH	Molecular Formula	Average mass	Monoisotopic mass
1	Gallic acid	3	C7H6O5	170.120 Da	170.021530 Da
2	Corilagin	11	C27H22O18	634.453 Da	634.080627 Da
3	Geraniin	14	C41H28O27	952.645 Da	952.081787 Da
4	Rutin	10	C27H30O16	610.518 Da	610.153381 Da
5	Quercetin.	5	C15H10O7	302.236 Da	302.042664 Da
6	Pyrogallol.	3	C6H6O3	126.110 Da	126.031693 Da
7	Chebulagic acid	13	C41H30O27	954.661 Da	954.097473 Da
8	Kaempferol.	4	C15H10O6	286.236 Da	286.047729 Da
9	Quercetinglocoside	8	C21H20O12	464.376 Da	464.095490 Da
10	Caffeoliquinicacid	6	C16H18O9	354.309 Da	354.095093 Da
11	Ellagic acid	4	C14H6O8	302.193 Da	302.006256 Da
12	Paclitaxel.	3	C47H51NO14	853.906 Da	853.330933 Da
13	doxorubicin.	5	C27H29NO11	543.519 Da	543.174072 Da
14	cisplatin.	0	H4Cl2N2	298.035 Da	296.939941 Da

In molecular docking with various receptor enzymes, the order of anticancer activity of the compounds was geraniin > chebulagic acid > corilagin >rutin > quercetin glucoside> caffeoliquinic acid > quercetin > kaempferol > ellagic acid> gallic acid > pyrogallol. Thus the structures of the bioactive compounds are closely related to anticancer activity. The data of molecular mass and the monoisotopic mass of geraniin and chebulagic acid also exhibited high potential among the various bioactive compounds (Table.1).

DISCUSSION

Scavenging of free radicals by flavonoids

Chan *et al.*,[14] demonstrated that flavan-3-ol, (-)-epigallocatechin 3-O-gallate suppresses the production of NO and nitric oxide synthase (iNOS). Haenen *et al.* (1997) (15) observed the peroxynitrite, ONOO-- eliminating activity of flavonoid and pointed out the important role of the catechol group (ring B) and hydroxyl group at position 3 in the manifestation of this scavenging activity.

Green tea tannin components when examined their effects on peroxynitrite, ONOO, it was found that (-)-epigallocatechin 3-O-gallate was the most potent

scavenger; (-)-gallocatechin 3-O gallate and (-)-epicatechin 3-O-gallate also had high scavenger activity. These compounds, with a catechol group and a hydroxyl group with galloyl moiety, were more effective scavengers, due to the presence of gallate group (Haenen *et al*., 1997)[15] On the other hand, gallate-free tannin proved to have low activity, suggesting that, in tannin, gallate might play a major role in the manifestation of scavenger activity (Haenen *et al*., 1997) (15). The scavenger activity was found to be involved not only in the inhibition of ONOO- production but also in the ability to directly scavenge peroxynitrite, ONOO-. Epigallocatechin 3-O-gallate, was also proved to have the ability to scavenge O2 -, hydrogen peroxide (H2O2), hydroperoxyl radical (• O2H) and • OH (Yokozawa *et al*., 1997, 1998)[16, 17] as well as peroxynitrite ONOO-, indicating its potential as a promising natural antioxidant and scavenger molecule having galloyl moiety.

The structure -activity relationship of bio active compounds

The development of structure-activity relationships of polyphenols facilitates the research on cancer therapy which attracted the attention of many scientists. Flavan-3-ols are involved in multiple metabolic pathways that induce inhibition of cell proliferation. Flavan-3-ols are characterized by hydroxylated aromatic rings with multiple hydroxyl groups.

The aromatic ring may be monohydroxylated, dihydroxylated or trihydroxylated. The hydroxyl groups on the phenolic ring are essential for the antiproliferative and apoptotic activity. The phenolic hydroxyl groups of catechins are primarily responsible for scavenging free radicals, whereas the galloyl moiety is involved in chelating metal ions. The hydroxyl groups from the different aromatic rings also enhance the inhibition of ROS. This may be explained by the presence of the ortho-trihydroxyl group in the B ring, which is important for scavenging super-oxide anion, whereas the galloyl moiety is responsible for quenching the hydroxyl radicals. Presence of three adjacent hydroxyl groups (galloyl group) in the aromatic ring of the molecule would be a key factor for enhancing the activity. Thus the compounds having galloyl moiety showed more potentiality in the scavenging activity (Weisburg *et al*., 2004) .[18.]

Generally, the health promoting activities of catechins, including the antiproliferative effect, are mainly attributed to their antioxidant capacity and ability to scavenge ROS. These properties are due to the presence of the phenolic hydroxy groups on the B ring in ungalloylated catechins and on the galloylated catechins. The presence of the 3,4,5-trihydroxy ring of flavan-3-ols has been shown to be significant for the antioxidant and radical scavenging activities.Thus, it is suggested that the presence of the gallate group plays the most important role in their free radical-scavenging abilities and an additional insertion of a hydroxyl group at the 5′ position in the ring also contributes to their scavenging activities (Chobot *et al*., 2009) [19].

The galloylated catechins showed stronger effects than those of non-galloylated structures. The galloyl moiety appears to be required for the antiproliferative, apoptotic and antioxidant effects. The EGCG contains two gallate moieties, which could explain the highest antiproliferative effect observed in the present study.The difference between EGCG on one side and, the C and EC on the other side is determined by the gallate group. The EGCG treated cells showed the best antiproliferative activity due to this gallate. The structural differences in flavan-3-ols might explain the differences in the antiproliferative responses. It has been established that hydroxyl group and galloyl moiety of the catechins are the main contributing factors to their scavenging activities and the presence of the ortho-dihydroxyl group and the galloyl moiety are important in maintaining the effectiveness of the radical scavenging ability (Braicu *et al*., 2011).[20]

Several studies have evaluated the antiproliferative effect of EGCG, EGC, C and EC, and showed that the gallate moiety is a key component. The present study revealed a potentially important role of the galloyl structure in the antiproliferative and pro apoptotic responses. Thus the galloylated catechins showed stronger antiproliferative effects than those of non-galloylated structures in which the galloyl moiety appears to be essential requirement for the antiproliferative, apoptotic and antioxidant effects.

Green tea extract showed the occurrence of six active flavan-3-ols; epicatechin (EC), epigallocatechin (EGC), epigallocatechin gallate (EGCg), gallocatechin (GC), epicatechin gallate (ECg), gallocatechin gallate (GCg), together with inactive glycosides of kaempferol and quercetin.

Pyrogallol was much more efficient in scavenging O2-* than catechol. The superiority of pyrogallol over catechol in much higher O2-* scavenging activity is due to 3',4',5'-trihydroxyl substitution in the B-ring, compared to quercetin and epicatechin, which contain 3',4'-dihydroxyl substitution. It can be concluded that the pyrogallol moiety is an active component of flavonoids for displaying high O2-* scavenging activity (Furuno *et al*., 2002) [21].

CONCLUSION

The molecular structure of the bioactive compounds exhibited a close relationship with the functional activity. In this investigation, the bioactive compounds of *P.urinaria* showed a lot of structural and functional diversity. The structural diversity of the bioactive compounds is mainly due to the presence of hydroxyl groups.

Among the eleven bioactive compounds of *P.urinaria*, geraniin showed a maximum number of 14 hydroxyl

groups in its molecule with four galloyl moieties. Next chebulagic acid comes with 13 hydroxyl groups and three galloyl moieties, followed by corilagin containing 11 hydroxyl groups and three galloyl moieties. Rutin showed 10 hydroxyl groups and two galloyl moieties. Based on the number of galloyl and hydroxyl groups, the bioactive compounds could be arranged in the following order; geraniin > chebulagic acid > corilagin >rutin > quercetin glucoside> caffeoliquinic acid > quercetin > kaempferol > ellagic acid> gallic acid > pyrogallol.

In support of the above study, the molecular docking results, molecular mass and the monoisotopic mass of geraniin and chebulagic acid go hand in hand with the results of the structure-activity relationships of the bio active compounds of *P.urinaria.*

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REFERENCE

1. Aruoma OI. Methodological consideration for characterization for potential antioxidant actions of bioactive components in plants foods. Mutat Res. 532, 2003; 9–20. [PubMed]
2. Bagchi K, Puri S. Free radicals and antioxidants in health and disease. East Mediterranean Health Jr. 4, 1998, 350–60.
3. Aruoma OI. Nutrition and health aspects of free radicals and antioxidants. Food Chem Toxicol. 32, 1994, 671–83. [PubMed]
4. Cheeseman KH, Slater TF. An introduction to free radicals chemistry. Br Med Bull. 49, 1993, 481–93.[PubMed]
5. Young IS, Woodside JV. Antioxidants in health and disease. J Clin Pathol. 2001, 54, 176–86.[PMC free article] [PubMed]
6. Rock CL, Jacob RA, Bowen PE. Update o biological characteristics of the antioxidant micronutrients- Vitamin C, Vitamin E and the carotenoids. J Am Diet Assoc. 96, 1996, 693–702. [PubMed]
7. Mc Cord JM. The evolution of free radicals and oxidative stress. Am J Med. 108, 2000, 652–9. [PubMed]
8. Rao AL, Bharani M, Pallavi V. Role of antioxidants and free radicals in health and disease. Adv Pharmacol Toxicol. 7, 2006, 29–38.
9. http://www.chemspider.com/
10. http://www.umass.edu/microbio/rasmol
11. http://www.uniprot.org/
12. http://sts.bioengr.uic.edu/castp/
13. http://www.hex.loria.fr/dist50/
14. Chan, MM. Fong, D. Ho, CT. and Huang, HI. Inhibition of inducible nitric oxide synthase gene expression and enzyme activity by epigallocatechin gallate, a natural product from green tea. *Biochem Pharmacol*., 54(12), 1997, 1281-6.
15. Haenen, G.R. Paquay, J.B. Korthouwer, R.E. and Bast, A.. Peroxynitrite scavenging by flavonoids. *Biochemical and biophysical research communications*,. 236(3), 1997591-593.
16. Yokozawa, T. Dong, E. Liu, Z.W. and Shimizu, M.."Antioxidative activity of flavones and flavonols *in vitro*." *Phytotherapy Research*., 11(6), 1997, 446-449.
17. Yokozawa, T. Dong, E. Nakagawa, T. Kashiwagi, H. Nakagawa, H. Takeuchi, S. and Chung, H. Y. *In vitro* and *in vivo* studies on the radical-scavenging activity of tea. *Journal of agricultural and food chemistry*, 46(6), 1998, 2143-2150
18. Weisburg, J.H. Weissman, D.B. Sedaghat, T. and Babich, H. *In vitro* cytotoxicity of epigallocatechin gallate and tea extracts to cancerous and normal cells from the human oral cavity. *Basic Clin. Pharmacol. Toxicol*., 95, 2004. 191-200
19. Chobot, V. Huber, C. Trettenhahn, G. and Hadacek, F. (±)-Catechin: Chemical weapon, antioxidant, or stress regulator. *J. Chem. Ecol*., 35, 2009, 980-996.
20. Braicu, C. Pilecki, V. Balacescu, O. Irimie, A. and Berindan Neagoe, I. The Relationships between Biological Activities and Structure of Flavan-3-Ols. *International Journal of Molecular Sciences*., 12(12), 2011, 9342-9353.
21. Furuno, K. Akasako, T. and Sugihara, N. The contribution of the pyrogallol moiety to the superoxide radical scavenging activity of flavonoids. *Biological and Pharmaceutical Bulletin*, 25(1), 2002, 19-23.

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