



RESEARCH PAPER

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Determination of effectiveness of berberine, a characteristic phytochemical of *Berberis* species, against human proteome using *in-silico* analysis

Tika Khan^{1*}, Imtiaz Ahmed Khan², Abdul Rehman², Zohra Bibi³

¹Integrated Mountain Area Research Centre, Karakorum International University, Gilgit, Pakistan.

²Department of Biological Sciences, Karakoram International University, Gilgit, Pakistan

³Centre of Bioinformatics, Quaid-i-Azam University, Islamabad, Pakistan

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Abstract

Berberis spp. are important representative plants of Family Berberidaceae. *Berberis pseudumbellata* subsp. *gilgitica* and *Berberis brandisiana* are found in Central Karakorum National Park (CKNP) and rest of Gilgit-Baltistan province of Pakistan (formerly Northern mountainous areas of Pakistan). *Berberis pseudumbellata* subsp. *gilgitica* is an endemic species and has become critically endangered. They are major source of Berberine which is an essential therapeutic phytochemical agent with anticancer, anti-diabetic, anti-AIDS, anti-jaundice, anti-cholera, anti-diarrhea, anti-leprosy and anti-inflammation effects. Studies illustrate inhibitory effect on oncogene-protein expression and therefore considered as an alternative, safer and effective medicine for chemotherapy. Besides being a multi-potent natural agent to combat Alzheimer's disease, its effectiveness against cardiovascular, hypertension, musculoskeletal and ocular ailments cannot be ignored. Present in-silico analysis investigated cancer related Berberine-protein, Berberine lead protein-protein metabolic pathways, interactions and level of effect and role in cellular activities of human metabolic process. Moreover, study also attempts to reflect on action mechanism which is not clear so far. Using FDR= <0.001 as filter molecular function, cellular compartment and Biological processes are shortlisted to identify effect of Berberine on human body. Moreover, analysis is also carried out using FI reactome plugging and pathway predicted to be effected by effectors proteins with FDR= <0.001 score are shortlisted. Study reveals that Berberine has strong positive effect and correlation between Berberine and various carcinomic proteins as exhibited through molecular docking filtered on the basis of Z'-score <-0.05. Study contributes into understanding of Berberine as an alternate potential non-toxic anticancer drug.

*Corresponding Author: Tika Khan ✉ tika.khan@kiu.edu.pk

Introduction

Berberine (C₂₀H₁₈NO₄⁺) is pale yellow quaternary ammonium salt from the protoberberine group of isoquinoline alkaloids (Fig. 1) extracted from plants including *Berberis* and *Coptis chinensis* etc. (ChemSpider, 2014; UofMHealth, 2014; Zhang *et al.*, 2010; Hwang and Jin-Ming *et al.*, 2002; Janbaza and Gilanib, 2000). Berberine differentially found in the roots, rhizomes, stems, bark and berries of these plants (Kulkarni *et al.*, 2012; Tang *et al.*, 2009; Hu *et al.*, 2008; Janbaza and Gilanib, 2000). It has shown significant effect against different types of cancer (Fig. 2&3) including colon, prostate, breast, tongue,

glioblastoma, oral, hepatoma, pancreatic, gastric, epidermoid and leukemia (Aggarwal *et al.*, 2013; Li-Na *et al.*, 2012; Pinto-Garcia *et al.*, 2010; Ho *et al.*, 2009; Auyeung and Ko, 2009; Tang *et al.*, 2009; Kim *et al.*, 2009; Serafim *et al.*, 2008; Fukuda, 1999). However, its mechanism of action is not clearly defined (Zhang *et al.*, 2010). Cytotoxic potency of Berberine found to be lower than camptothecin which is currently used for cancer treatment. Due to lack of undesired side effects, Berberine will significantly replace medicine recently in use for chemotherapy (Kettmann *et al.*, 2004).

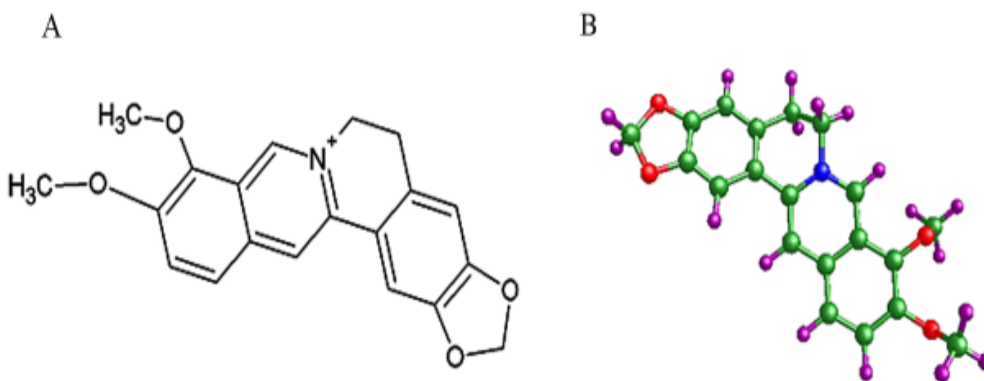


Fig. 1. Structures of Berberine (A) 2D chemical structure of Berberine (B) 3D chemical structure of Berberine green, red, blue and purple colors correspond to carbon, oxygen, nitrogen and hydrogen atoms respectively.

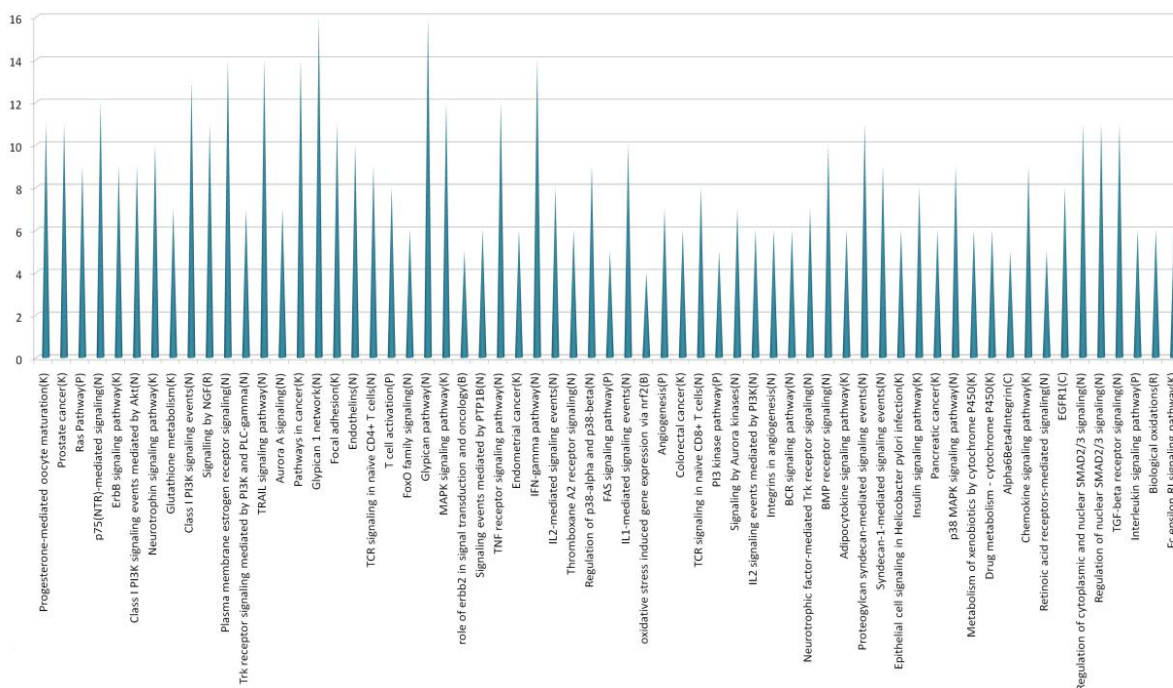


Fig. 2. Berberine-protein pathways.

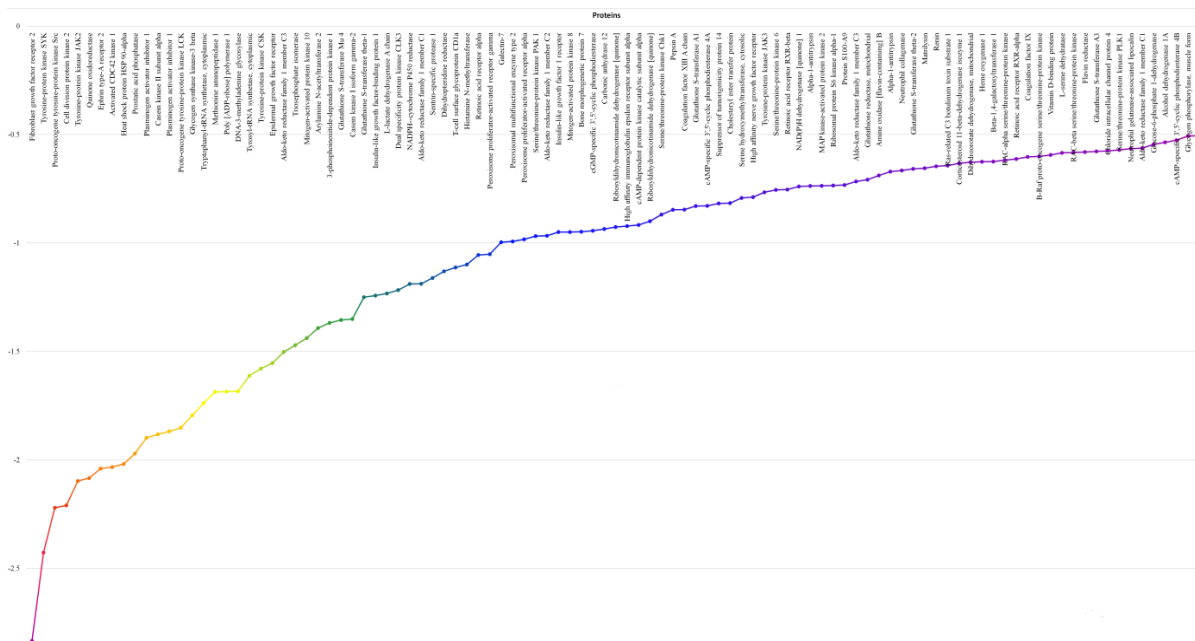


Fig. 3. Berberine affected various human proteins.

Berberine besides having anticancer properties, is an essential therapeutic phytochemical agent (Kulkarni *et al.*, 2012; Asif *et al.*, 2007; Bhandari *et al.*, 2000; Stermitz *et al.*, 2000a; Chandra and Purohit, 1980) with anti-diabetic, anti-AIDS, anti-jaundice, anti- cholera, anti-diarrhea, anti-leprosy and anti- inflammation effects (Khan *et al.*, 2014; Khan *et al.*, 2013; Popov, 2011; Imanshahidi and Hosseinzadeh, 2008; Asif *et al.*, 2007; Tang *et al.*, 2006; Fatehi *et al.*, 2005; Caraballo *et al.*, 2004; Kuo *et al.*, 2004; Kettmann *et al.*, 2004; Kuo *et al.*, 2004; Villinski *et al.*, 2003; Racková *et al.*, 2003; Hwang and Jin-Ming, *et al.*, 2002; Stermitz *et al.*, 2000; Janbaza and Gilanib, 2000; Fukuda, 1999; Ivanoska and Philipov, 1996; Iwasa *et al.*, 1996; Koo and Seang, 1996; Chopra *et al.*, 1981; Chandra and Purohit, 1980).

Studies illustrate inhibitory effect of Berberine on oncogene-protein expression (Domadia *et al.*, 2008; Cao *et al.*, 2011; Li *et al.*, 2011; Tsang *et al.*, 2009; Li *et al.*, 2008) and therefore considered as an alternative medicine for chemotherapy. Besides being a multi-potent natural agent to combat Alzheimer’s disease, its effectiveness against cardiovascular, hypertension, musculoskeletal, cholesterol-lowering, Berberine and human serum albumin interaction (Fig. 4) and ocular ailments cannot be ignored (Marszalek *et al.*, 2013; Hu *et al.*, 2010; Kong *et al.*, 2009; Abidi *et al.*, 2005; Ji and Shen, 2011; Jung *et al.*, 2009; Zhu and Qian, 2006; Li *et al.*, 2005; Kong *et al.*, 2004; Kong *et al.*, 2004). Reports reveal that Berberine is non-toxic in clinical situations and lacks genotoxic, cytotoxic or mutagenic activity (Birdsall and Kelly, 1997; Diogo, 2011; Imanshahidi and Hosseinzadeh, 2008; Hu *et al.*, 2009).

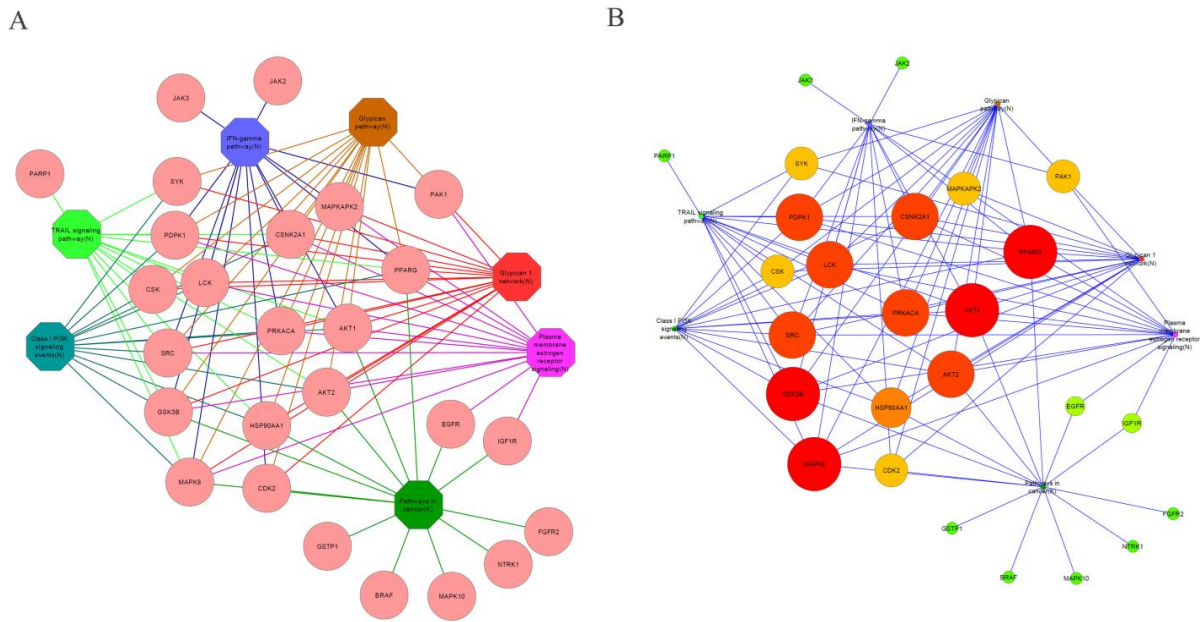


Fig. 4. A&B: Berberine-Cancer-Protein interaction network.

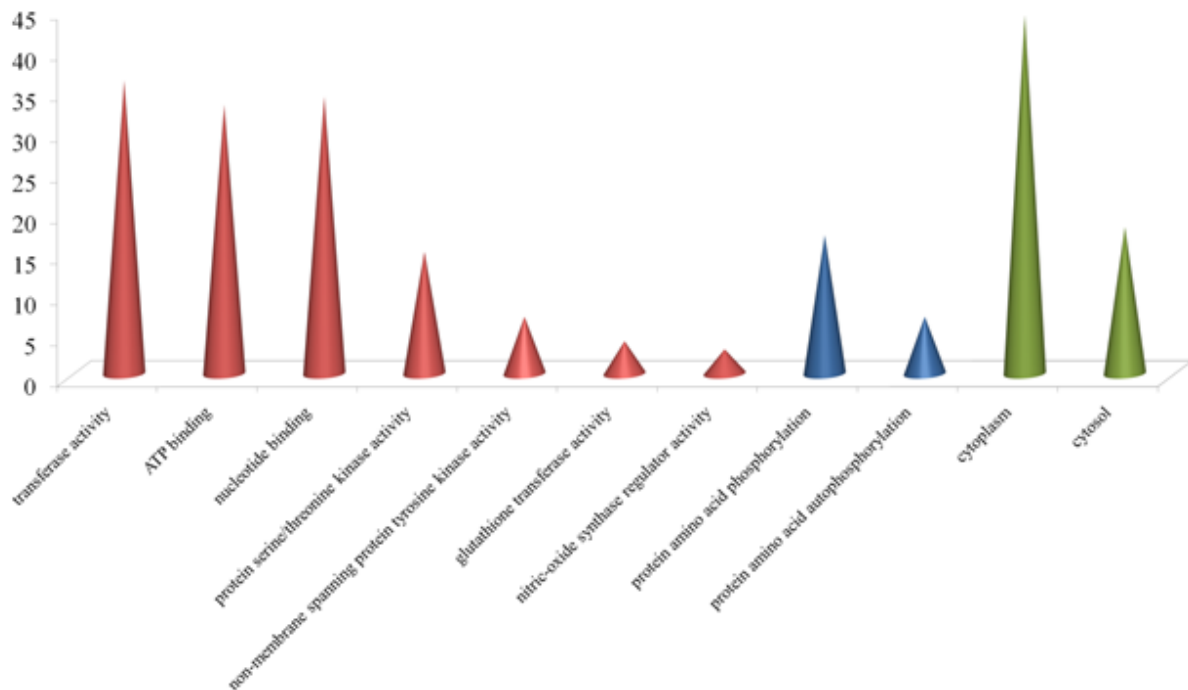


Fig. 5. Role of Berberine in various cellular activities.

In vitro studies show that Berberine inhibits biosynthesis of UNA, RNA, protein, lipids and oxidation of glucose. In S 180 cells, glucose and Berberine behave antagonistically and Phlorizin inhibit Berberine intake and nucleic acid (Creasey, 1979).

Berberine may suppress the proliferation and promote the apoptosis of IMCE cells. The mechanisms may relate to the inhibition of the phosphorylation of EGFR and Akt (Cao *et al.*, 2011). Berberine arrested cell growth and inhibited cell migration in various cancer cell lines (Tsang *et al.*, 2009).

Various computer based software and databases like INVDOCK, Cytoscape FI-reactome plugin, protein data bank, Drug Repositioning and Adverse Reaction via Chemical-Protein Interactome (DRAR-CPI) server, PubChem Compound database, Reactome database, GeneGo, DrugBank, BioAssay Knowledgebase, Vega-ZZ, molecular docking, ChemMine, Tide (Target Identification) etc. (Burley *et al.*, 1999; Lindley, 1999; Brenner *et al.*, 1997; Wells *et al.*, 1993; Stigers *et al.*, 1999; Clackson and Wells, 1995; Lengauer and Rarey, 1996) have helped in understanding of molecular action dynamics and effects in metabolic pathways. These drug discovery approaches by screening known targets (Drews, 2000) are critically helpful in automated identification of potential alternative medicine. These software use both forward and reverse approaches to utilize the identified chemicals as “research tools” for determining the functions, interactions, and architecture of cellular networks in living organisms (Girke *et al.*, 2005; Borderies *et al.*, 2003). 60% of computer-identified potential therapeutic protein targets and 27% of computer-identified potential toxicity targets have been implicated or confirmed by experiments (Chen and Ung, 2002). Drug repositioning has emerged as an efficient way of maximizing their potential. Also, it helps in minimizing adverse drug reaction which is one of the leading causes of death among hospitalized patients (Luo *et al.*, 2011).

Present in-Silico study helps for clarifying the mechanism of Berberine’s anti-tumor effect and might be supportive to find therapy-target for treatment of human carcinoma (Lu *et al.*, 2010).

Present in-Silico investigation was aimed at identification of Berberine’s effect on human proteome and its role in cellular metabolic dynamics with a special reference to carcinogenesis.

Material and methods

Study scope

Berberis spp. are important source of Berberine belong to Family Berberidaceae. *Berberis pseudumbellata* subsp. *gilgitica* and *Berberis brandisiana* found in Central Karakoram National Park (CKNP) and rest of Gilgit-Baltistan (Khan *et al.*, 2014; CKNP, 2013; Abbas, 2013; Alam and Ali, 2010; Khan and Khatoon, 2007). Gilgit-Baltistan is famous for being home of largest and longest glacial mass outside poles and having meeting point of 3 mighty mountain ranges of the world viz; Himalaya, Hindukush and Karakoram. Study area harbor taxonomically diverse, rich and complex flora and fauna in the world (Abbas *et al.*, 2014; Virk *et al.*, 2003). *Berberis pseudumbellata* subsp. *gilgitica* is an endemic species and has become critically endangered (Khan *et al.*, 2014; Alam and Ali, 2010).

Data retrieval and process

The molecular and structural data along with PubChem ID (CID) of Berberine was obtained from PubChem Compound database (Bolton *et al.*, 2008). The 3-D structure of Berberine was optimized via Vega-ZZ (2014). This optimized structure was saved as .mol2 file and is up loaded to Drug Repositioning and Adverse Reaction via Chemical-protein Interactome (DRAR-CPI) server (Zhang *et al.*, 2013; Jongejan *et al.*, 2005). DRAR-CPI server provided list of predicted effected human proteins via molecular docking. The list of protein obtained from DRAR-CPI are filter on basis of Z'-score <-0.05.

The short-listed proteins genes’ IDs are retrieved from protein data bank (PDB, 2014) and via Cytoscape2.8 (Smoot *et al.*, 2011). A chemical-genes network is generated (Fig. 2) from these proteins’ gene IDs. By employing Cytoscape2.8 FI-reactome plugin (Smoot *et al.*, 2011) molecular function, cellular compartment, Biological process (Fig. 5) and pathways (Fig. 3) are fetched from Reactome database (RDb, 2014) for shortlisted proteins (Fig. 4).

Using FDR= <0.001 as filter molecular function, cellular compartment and Biological processes are shortlisted (Benjamini and Hochberg, 1995) to identify effect of Berberine on human body (Fig. 4). Moreover, analysis is also carried out using FI reactome plugging and pathway predicted to be effected by effectors proteins with FDR= <0.001 score are shortlisted.

Analysis

In-Silico docking techniques are being used to investigate the structural and functional complementarity at the molecular level of a ligand and a protein target for suitable binding (Kroemer, 2007). Analysis is carried out using FI reactome plugging and pathway predicted to be effected by effectors proteins with FDR= <0.001 score are shortlisted.

Results and discussion

Cancer and other pathways

Pathway interaction reveals that Berberine plays its role directly in cancer pathway and interacts with various other pathways including FGFR2, NTRK1, MAPK10, BRAF, GSTP1, CDK2, MAPK8, GSK3B, HSP90AA1, AKT2, AKT1, EGFR and IGF1R (Fig. 2).

Proteins

Similarly, Berberine and various carcinomic proteins as exhibited through molecular docking filtered on the basis of Z'-score <-0.05 exposes that molecular functioning of Berberine shows strong interaction. During such an interaction Berberine interacts with various human proteins involved in carcinogenesis are proto-oncogene tyrosine-protein kinase Sre, Fibroblast growth factor receptor 2, tyrosine-protein kinase SYK, cell division protein kinase 2 and tyrosine-protein kinase JAK2 etc. (Fig. 4).

Metabolic processes

Berberine exhibits its effect in different biological processes including transferase activity, ATP binding and nucleotide binding differentially in cytoplasm

and cytosol. Cytoplasm appears as centre of Berberine lead activities (Fig. 5).

Treatment with several inhibitors suggested that Berberine uptake depended on the ATP level. Some inhibitors of P-glycoprotein, an ABC transporter involved in multiple drug resistance in cancer cells, strongly inhibited Berberine uptake, whereas a specific inhibitor for glutathione biosynthesis and vacuolar ATPase, bafilomycin A1, had little effect (Sakai, 2002).

Berberine potential

These results demonstrate that Berberine has wide physiologic function and has great potential for structural modification as new drug lead. However, there is no systematic review about the study of Berberine and its derivatives up to now. The current review would provide some useful information for further study on structural modification of Berberine for discovering new drug leads based on its pharmaco-dynamic mechanisms (Li *et al.*, 2008).

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