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RESEARCH ARTICLE

INVITRO ANTIOXIDANT, ANTI-INFLAMMATORY, *INOVO* ANTI-ANGIOGENIC ACTIVITIES AND VIRTUAL SCREENING OF PHYTO CONSTITUENTS OF *CHROMOLENA ODORATA*

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ABSTRACT

Chromolena odorata roots are used by traditional healers in Western Ghats for treating inflammation and tumor suppression. Hence the study tries to validate the use, by invitro antioxidant, anti-inflammatory and *inovo* anti-angiogenic activities. Virtual screening of active constituents of *C.odorata* was done using admet SAR an online web tool followed by molecular docking using autodock. Plant extract had a total phenol content of 30mg/g GAE. In comparison to the standard Ascorbic acid plant extract exhibited significant antioxidant activity by scavenging DPPH (IC_{50} 104µg) and ABTS ions (IC_{50} 84µg), reducing Ferric ions (IC_{50} 50µg) and a Total Antioxidant Capacity of 27.5 mg/g Ascorbic Acid Equivalent. Percentage protection of HRBC by 300mg of plant extract was equivalent to that of 200mg of standard Diclofenec indicating anti-inflammatory activity with an IC_{50} value of 70µg. In *inovo* anti-angiogenic assay 500mg of extract disrupted the process of angiogenesis. Virtual screening highlighted the possibility of Chalcone, Kaempferide, Tamarixetin and Eupatilin becoming leads in future anti-inflamatory and anti agiogenic drugs.

INTRODUCTION

Oxygen has double-edged properties, being essential for life; it can also aggravate the damage within the cell by oxidative events (Shinde *et al*., 2006). Free radicals generation through both aerobic and anaerobic process leads to the various health problems including cancer, aging, heart diseases, gastric problems etc. At molecular level these free radicals are important mediators of damage to cell structures, nucleic acids, lipids and proteins resulting in inflammation and oncogenesis. The stimulated polynuclear cells at the site of inflammation are capable of producing reactive oxygen species in turn causes tissue damage and could be the reason for developing a chronic process. In this context, the use of medicinal plants with anti-inflammatory and anti-oxidant activity is a classic remedy for preventing and treating inflammations so that they do not become chronic (Ríos *et al*., 2000, Schinella *et al*., 2002). During cancer progression, the newly formed tumor-associated blood vessels serve first as feeding/nurturing tubes for a growing tumor and next, as conduits for dissemination of tumor cells that escaped from an established primary tumor. Therefore, control of tumor angiogenesis has become a central issue in the fight against cancer progression since anticancer therapy could be ineffective once tumor cells reach favored secondary organs and generate metastatic foci (Elena *et al*., 2008).

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Traditional healers of Western Ghats seem to have inherited expertise in the usage of medicinal herbs. Precedents showed that herbal formulations used by the traditional healers of Shimoga District Karnataka have immense anti-cancer potential (self communication). Researchers have observed a correlation between anti-oxidant, anti-inflammatory and apoptotic activities in the plant extracts (Ramchoun *et al*., 2012). With this perspective, the study aims to validate the use of invasive weed *Chromolena odorata* roots by traditional healers in Western Ghats for treating inflammation and tumor suppression.

MATERIALS AND METHODS

Plant Collection and Extraction

The plant and its roots were collected from the location 77.70775^0E and 13.02558^0N, Karnataka State, India. The plant herbarium was prepared and it was authenticated as *Chromolena odorata* (L.) King & Robinson belonging to the family Asteraceae (Reference No. RRCBI-13668) by Dr.Shiddamallayya N, National Ayurvedic Dietetics Institute, Bangalore. The roots were shade dried, powdered mechanically and stored. About 100g of the powdered material was defatted with petroleum ether and extracted with methanol by soxhlation. The solvent was dried at low temperature. The yield was 15.7 % w/w.

Phytochemical Screening

Qualitative estimations for Alkaloids, Flavonoids, Glycosides, Steroids, Cardiac glycosides, Saponins, Resins, Phenols, Tanins, Terpenoids, Quinones, Oxalates, Lignin and Anthraquinones were done as described in Harbone (1973), Trease and Evans (1989) and Sofowora (1993).

Determination of Total Phenol by Folin-Ciocalteu Assay

The total phenolic content of crude methanolic extract was determined by the Folin-Ciocalteu method (Singleton *et al*., 1974). 1 ml of extract of various concentrations was added to 5 ml of 1:10 diluted FC reagent followed by 4 ml of 1 M Sodium carbonate solution. After 30 minutes of incubation in dark at room temperature, the absorbance was measured at 750 nm using UV-visible spectrophotometer. A calibration curve was constructed using different concentrations of standard Gallic acid. All readings were performed in triplicates and the level of Total Phenol in the extract was calculated from the standard calibration curve. Results were expressed in gallic acid equivalents (mg GAE/g).

Total Antioxidant Assay (Phosphomolybdenum)

0.3 ml of various concentration of plant extract (100 – 500 µg) was added to 3 mL of reagent mixture (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) followed by incubation at 95ºC for 60 min. After cooling absorption of reaction mixture was measured at 695 nm and the antioxidant activity was expressed as the number of gram equivalents of ascorbic acid (El-Sayed *et al*., 2012).

Evaluation of DPPH free radical scavenging activity

The free radical scavenging activity of extract was studied by DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay (MS Blois. 1958). 2 ml of DPPH (100 µM) was mixed with various dilution ranging from 20µg to 100µg of extract. After 10 minutes of incubation in dark, the absorbance was measured at 517 nm. All readings were performed in triplicates and the free radical scavenging activity was calculated from equation 1. The concentrations of plant extract and Rutin standard was plotted in X-axis against respective percentage inhibition in Y-axis and their IC_{50} values were calculated by extrapolating the graph.

Equation 1: $\%\ \text{Inhibition} = \frac{(A*-A)\times 100}{A*}$

A* is the absorbance of reagent blank and A is the absorbance of the test sample.

ABTS radical cation decolorization assay

To generate ABTS (2,2'-azino-bis, 3-ethylbenzothiazoline-6-sulphonic acid) radical cation, 50 ml of 2 mM ABTS and 0.3 mL of 17 mM potassium persulfate were mixed together and incubated in the dark for 12-16 h to develop prussian blue colored $ABTS^{\cdot+}$ solution which has an absorption maxima at 734 nm (Roberta *et al*., 1999). To determine scavenging activity of extracts, 400 µl of plant extracts of different concentrations (20 – 100 µg) were added to 320 µl of $ABTS^{\cdot+}$ solution. The absorbance was measured at 734 nm after 10 minutes incubation at room temperature. All readings were performed in triplicates and the free radical scavenging activity was calculated from equation 1. The percentage inhibition of plant extract and Rutin standard was plotted against respective concentrations used and their IC_{50} values were calculated by extrapolating the graph.

Ferric Reducing Antioxidant Power

Plant extract with varying concentrations ranging in between 100 - 200 µg was made to react with 2.5 ml of 1% potassium ferricyanide. The mixture was incubated in a water bath for 20min at 50ºC. The resulting solution was cooled rapidly and 2.5ml of 10% trichloroacetic acid was added followed by centrifugation at 3000rpm for 10min. 5ml of supernatant was mixed with 5ml of distilled water and 1ml of 1% ferric chloride. After 10min absorbance was measured at 700nm (Oyaizu. 1986).

$$\text{Reducing Power} = \frac{\text{Absorbance test} - \text{Absorbance blank}}{\text{Absorbance blank}} \times 100$$

Invitro Anti-Inflammatory Activity

The Human Red Blood Cell (HRBC) membrane stabilization method Fresh whole human blood (5 mL) was collected and transferred to the centrifuged tubes containing EDTA to prevent clotting. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline. The reaction mixture consists of 1.0 mL of test sample of different concentrations (50µg – 400 µg) in normal saline and 0.5 mL of 10% HRBC suspension, 1 ml of 0.2 M phosphate buffer, 1 ml hyposaline were incubated at 37^0 C for 30 min and centrifuged at 3,000 rpm for 20 min and the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac was used as standard and a control was prepared without extracts. The percentage of HRBC hemolysis and membrane stabilization or protection was calculated by using the following formula (Murugesh *et al*., 1981).

$$\%\ \text{Hemolysis} = \frac{\text{Optical density of Test sample}}{\text{Optical density of Control}} \times 100$$

$$\%\ \text{Protection} = 100 - \frac{\text{Optical density of Test sample}}{\text{Optical density of Control}} \times 100$$

Anti-Angiogenesis Assay Using Chick Chorioallantoic Membrane

Fertilized hens eggs were procured from Indian Veterinary Research Institute (IVRI) (Bill No-28384), Bangalore, and surface sterilized using 70% alcohol. The eggs were incubated in fan assisted humidified incubator at 35-37^0C. On the 4th day in the laminar flow cabinet, the eggs were wiped with 70% alcohol and shell was cracked out into thin film of the hammock, Egg preparation was covered with sterile glass plate and returned to the incubator. On 5th day the plant extract

100µg, 250µg and 500µg was loaded on the filter paper disc, placed over the blood vessel and eggs were returned to the incubator. Results were observed next day (Domenico Ribatti. 2010).

Virtual Screening of phyto constituents of *C.odorata.*

Ligand preparation

3D structures of active constituents present in *C.odorata* (Phan et al., 2001) viz. 2',4'-Dihydroxy-2,3',6'-trimethoxychalcone (CID 21636239), 4',5,6,7-tetramethoxyflavone (CID 96118), 4-coumaric acid (CID 637542), 9-tigloylretronecine (CID 5352410), 4-hydroxybenzoic acid (CID 135), 7-Angeloy lretronecine (CID 5352406), Intermedine N-Oxide (CID 340066), beta-MSH (CID 114843), Rinderine 1-acetate (CID 185847), Eupatilin (CID 5273755), Ferulic acid (CID 445858), Kaempferide (CID 5281666), Protocatechuic acid (CID 72), Sinensetin (CID 145659), Tamarixetin (CID 5281699) and Vanillic Acid (CID 8468) were downloaded from Pubchem. By using JSDraw V1.3.2 inbuilt in admetSAR all the compound's smiles formats were retrieved and used for virtual screening.

ADMET Screening

Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) properties of drug candidates or environmental chemicals play a key role in drug discovery and environmental hazard assessment. The ADMET structure-activity relationship server, entitled admetSAR, is a comprehensive knowledge and tool for predicting ADMET properties of drug candidates and environmental chemicals. In this server, over 200000 ADMET annotated data points for about 96 thousands of unique compounds have been manually curated from large literatures. The admetSAR server provides a user-friendly interface to easily search a chemical profiles, by CASRN, common name and similarity search. In addition, 30 high predictive QSAR models were implemented in admetSAR for new chemical ADMET properties in silico filtering. admetSAR helps in Optimizing ADMET profiles of Hits or Leads in drug discovery and predicting QSAR-based ADMET properties, toxicity properties and in Environmental Hazard Assessment (Feixiong *et al*., 2012). Only the compounds which had properties viz., negative blood brain barrier, positive Human Intestinal Absorption, non-carcinogenic, non-toxic and non-substrate to metabolic enzymes were selected for molecular docking studies.

Anti-Inflammatory and Anti-Angiogenic Drug Targets

Nuclear Factor kappa B Receptor

NF-kB regulates many genes involved in the promotion of cancer i.e. clonal expansion, growth, diversification, angiogenesis, adhesion, extravasations, degradation of extracellular matrix and also in constitutive activation. The most direct strategy for blocking activation of NF-kB is to block its DNA binding activity (Garg *et al*., 2002). Hence Structure of the NF-kappa B p50 homodimer, PDB id: 1SVC (Muller *et al*., 1995) was downloaded from Protein Data Bank and processed for docking studies.

Tyrosine kinase Receptor

Tyrosine kinases are important cellular signaling proteins that have a variety of biological activities including cell proliferation and migration. Multiple kinases are involved in angiogenesis, including receptor tyrosine kinases such as the vascular endothelial growth factor receptor. Inhibition of angiogenic tyrosine kinases has been developed as a systemic treatment strategy for cancer (Kristy *et al*., 2010). Crystal structure of c-Kit receptor protein-tyrosine kinase in complex with Imatinib/Gleevec, PDB Id: 1T46 (Clifford *et al*., 2004) was downloaded from Protein Data Bank and processed for docking studies.

Human Vascular Endothelial Growth Factor-B

Pathological angiogenesis has a pivotal role in sustaining tumor growth and chronic inflammation. Vascular endothelial growth factor-b (VEGF-B) is a member of the VEGF family of growth factors that regulate blood vessel and lymphatic angiogenesis. Crystal structure of human vascular endothelial growth factor-b, PDB id: 2C7W (Iyer *et al*., 2006) was downloaded from protein data bank and processed for docking studies.

Molecular docking using Autodock

By using Dock Prep function in UCSF Chimera software (Pettersen *et al*., 2004) all the PDB files were processed for docking simulation. The Graphical User Interface program Auto-Dock Tools was used for docking studies (Michel *et al*., 1999). Polar hydrogens were added followed by computing Gasteiger Charges and non polar hydrogen's were merged into the receptor PDB file. The grid box size was set at 62, 62 and 62 A° (x, y, and z) to include all the amino acid residues that are present in the active site of rigid macromolecules which was identified by inbuilt program Auto ligand. Auto Grid 4.2 Program was used to produce grid maps. The spacing between grid points was 0.375 angstroms. The Lamarckian Genetic Algorithm (LGA) was chosen search for the best conformers. During the docking process, a maximum of 10 conformers was considered. The population size was set to 150 and the individuals were initialized randomly. Maximum number of energy evaluation was set to 250000, maximum number of generations 27000, maximum number of top individual that automatically survived set to 1, mutation rate of 0.02, crossover rate of 0.8, Step sizes were 0.2 Å for translations, 50.0° for quaternion and 50.0° for torsions. Cluster tolerance 2.0Aº, external grid energy 1000.0, max initial energy 0.0, max number of retries 10000 and 10 LGA runs were performed. Auto dock results were analyzed to study the interactions and the binding energy of the docked structure.

RESULTS AND DISCUSSION

Preliminary Screening

The phytochemical screening of the plants studied showed the presence of Alkaloids, Flavonoids, Terpenoids, Phenols,

Tanins, Saponins, cardiac glycosides, Steroids and Glycosides; absence of anthraquinones, lignin, oxalate, quinines and Resins.

Antioxidant Activity

In the present investigation, it was found that methanolic extract of *C.odorata* had total phenolic content 30 mg/g GAE exerting antioxidant effect as free radical scavengers. Compared to the standard Ascorbic acid, methanolic extract exhibited good IC_{50} values (Table 1) at 104µg/ml in DPPH free radical scavenging assay, 84µg/ml in ABTS radical cation decolorization assay, 50 µg/ml in FRAP assay and mg/g of Ascorbic acid equivalent in Phosphomolybdenum assay.

Table 1. Anti oxidant activity of methanolic root extract of *C. odorata* and Ascorbic acid

Assay	IC_{50} values of *C.odorata*	IC_{50} values of Ascorbic acid
DPPH Assay	104 µg/ml	98 µg/ml
ABTS Assay	84µg/ml	66 µg/ml
FRAP Assay	50 µg/ml	60 µg/ml
Total Phenol Content	30 mg/g Gallic Acid Equivalents	
Total Antioxidant Activity	27.5 mg/g Ascorbic Acid Equivalent	

Invitro Anti-Inflammatory Activity

The plant extract showed significant anti-inflammatory activity by protecting HRBC in hypotonic solution (Table 2). Standard diclofenac results 64.04% and 73.03% at 100 and 200 µg were comparable with that of 200 and 300 µg of plant extract. 70 µg/ml and 40 µg/ml were the IC_{50} values of plant extract and standard Diclofenec respectively. The plant extract exhibited membrane stabilization effect by inhibiting hypotonicity induced lyses of RBC membrane. The RBC membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes. Stabilization of lysosomal membrane plays an important role in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release (Rajendran *et al*., 2008).

Table 2. % of Hemolysis and Protection of Diclofenec and methanolic extract of *C. odorata*

Conc of in µg/ml	% of Hemolysis of Extract	% Protection of Extract
100	46.06	53.9
200	33.71	66.3
300	25.80	74.2
400	20.22	79.7

Inovo anti-angiogenic activity

As plausible in the Fig. 1. 500µg of plant extract disrupted the process of angiogenisis in CAM assay. Angiogenesis is necessary for tumor growth and distribution of tumor cells. Inhibiting angiogenesis can be helpful in arresting tumor progression. Sorafenib (NEXAVAR) is a multi targeted inhibitor of Vascular Endothelial Growth Factor and RAF kinase that has been approved in the USA and European Union (Hood *et al*., 2002).

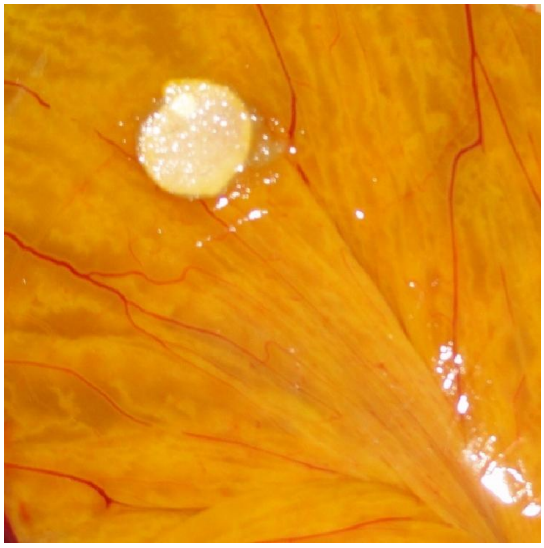

Figure 1. 500µg of extract disrupting the process of angiogenesis

Virtual Screening of phyto constituents on *C.odorata*

Among the phyto constituents screened only Chalcone, Kaempferide, Tamarixetin and Eupatilin were qualified to be future anti-inflamatory and anti agiogenic drugs. The qualified compounds were unable to cross blood brain barrier, positive to human intestinal absorption, Non-Substrates for metabolic enzymes (Cyp450 2C9, CYP450 2D6, CYP450 3A4) non carcinogenic and non mutagenic (Table 3). In Molecular docking studies, their low binding energy, total internal energy, inter molecular energy and the number of hydrogen bonds formed indicated their effectiveness in binding anti-inflamatory and anti agiogenic drug target protein (Table 3).

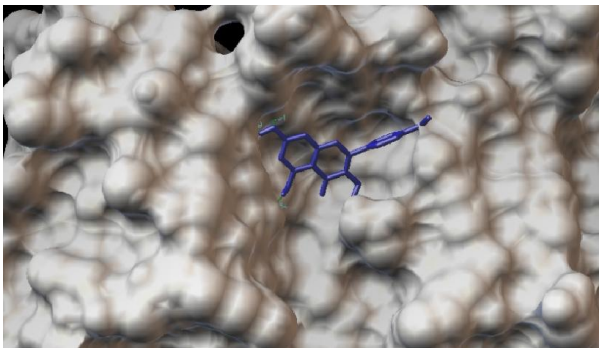

Figure 2. Kaempferide bound in the active site of Receptor Tyrosine Kinase

Plant derived medicines are based upon the premise that they contain natural substances that can promote health and alleviate illness, also the chemical constituents present in the herbal medicine or plant are a part of the physiological functions of living flora and hence they are believed to have better compatibility with human body (Saikat *et al*., 2010). Present investigation not only validates the use of *C.odorata* by

Table 3. *Insilco* Virtually Screened compounds of *C.odorata*

MODEL	Chalcone	Kaempferide	Tamarixetin	Eupatilin
Blood Brain Barrier	BBB- (0.5255)	BBB- (0.6382)	BBB- (0.6382)	BBB- (0.6825)
Human Intestinal Absorption	HIA+ (0.9740)	HIA+ (0.9783)	HIA+ (0.9783)	HIA+ (0.9693)
Caco-2 Permeability	Caco2+(0.8599)	Caco2+ (0.8866)	Caco2+(0.8866)	Caco2+ (0.8778)
P-glycoprotein Substrate	Substrate (0.5372)	Substrate (0.6384)	Substrate (0.6384)	Substrate (0.6326)
P-glycoprotein Inhibitor	Non-Inhibitor (0.6483) Inhibitor (0.5322)	Non-Inhibitor (0.7108) Inhibitor (0.7878)	Non-Inhibitor (0.7108) Inhibitor (0.7878)	Non-Inhibitor (0.5799) Inhibitor (0.8548)
Renal Organic Cation Transporter	Non-Inhibitor (0.8919)	Non-Inhibitor (0.8979)	Non-Inhibitor (0.8979)	Non-Inhibitor (0.9180)
CYP450 2C9 Substrate	Non-Substrate (0.7241)	Non-Substrate (0.7326)	Non-Substrate (0.7326)	Non-Substrate (0.7599)
CYP450 2D6 Substrate	Non-Substrate (0.8483)	Non-Substrate (0.8942)	Non-Substrate (0.8942)	Non-Substrate (0.8614)
CYP450 3A4 Substrate	Non-Substrate (0.5000)	Non-Substrate (0.5827)	Non-Substrate (0.5827)	Non-Substrate (0.5000)
CYP450 1A2 Inhibitor	Inhibitor (0.8110)	Inhibitor (0.9218)	Inhibitor (0.9218)	Inhibitor (0.8610)
CYP450 2C9 Inhibitor	Non-Inhibitor (0.6023)	Inhibitor (0.7560)	Inhibitor (0.7560)	Non-Inhibitor (0.7484)
CYP450 2D6 Inhibitor	Non-Inhibitor (0.8297)	Non-Inhibitor (0.6993)	Non-Inhibitor (0.6993)	Non-Inhibitor (0.7310)
CYP450 2C19 Inhibitor	Inhibitor (0.6629)	Inhibitor (0.8648)	Inhibitor (0.8648)	Inhibitor (0.5640)
CYP450 3A4 Inhibitor	Inhibitor (0.7310)	Inhibitor (0.7348)	Inhibitor (0.7348)	Inhibitor (0.6154)
CYP Inhibitory Promiscuity	High CYP Inhibitory Promiscuity (0.7925)	High CYP Inhibitory Promiscuity (0.8546)	High CYP Inhibitory Promiscuity (0.8546)	High CYP Inhibitory Promiscuity (0.8068)
Human Ether-a-go-go-Related	Weak inhibitor (0.9738)	Weak Inhibitor (0.9772)	Weak Inhibitor (0.9772)	Weak Inhibitor (0.9817)
Gene Inhibition	Non-Inhibitor (0.8960)	Non Inhibitor (0.8494)	Non Inhibitor (0.8494)	Non Inhibitor (0.8256)
AMES Toxicity	Non AMES Toxic (0.9247)	Non AMES toxic (0.9133)	Non AMES toxic (0.9133)	Non AMES toxic (0.9250)
Carcinogens	Non Carcinogens (0.8568)	Non-carcinogens (0.9423)	Non-carcinogens (0.9423)	Non-carcinogens (0.9184)
Fish Toxicity	High FHMT (0.9551)	High FMHT (0.8612)	High FMHT (0.8612)	High FMHT (0.8520)
Tetrahymena Pyriformis Toxicity	High TPT (0.9986)	High TPT (0.9965)	High TPT (0.9965)	High TPT (0.9942)
Honey Bee Toxicity	High HBT (0.7937)	High HBT (0.6908)	High HBT (0.6908)	High HBT (0.7528)
Biodegradation	Not Ready Biodegradable (0.9290)	Not Ready Biodegradable (0.8952)	Not Ready Biodegradable (0.8952)	Not Ready Biodegradable (0.9651)
Acute Oral Toxicity	III (0.6805)	III (0.7362)	III (0.7362)	III (0.5210)
Carcinogenicity (Three-class)	Not- required (0.6510)	Not-Required (0.6176)	Not-Required (0.6176)	Not-Required (0.6142)
Aqueous solubility	-3.6351 LogS	-3.2219 LogS	-3.2219 LogS	-3.5214 LogS
Caco-2 Permeability	1.1924 LogPapp, cm/s	0.9162 LogPapp, cm/s	0.9162 LogPapp, cm/s	1.1812 LogPapp, cm/s
Rat Acute Toxicity	2.3510 LD50, mol/kg	2.7192 LD50, mol/kg	2.7192 LD50, mol/kg	3.0354 LD50, mol/kg
Fish Toxicity	0.8689 pLC50, mg/L	0.6628 pLC50, mg/L	0.6628 pLC50, mg/L	0.8856 pLC50, mg/L
Tetrahymena Pyriformis Toxicity	2.0641 pIGC50, ug/L	1.3073 pIGC50, ug/L	1.3073 pIGC50, ug/L	1.2355 pIGC50, ug/L
Molecular Docking with Tyrosine kinase Receptor: PDB ID - 1T46				
Binding energy	-4.48	-5.45	-4.71	-4.46
Ligand efficiency	-0.19	-0.25	-0.2	-0.18
Inhibition Constant	516.73μM	100.77μM	352.51μM	538.0μM
Intermolecular energy	-6.87	-6.94	-6.5	-6.25
Electrostatic energy	-0.53	-0.08	-0.38	-0.17
Vdw_hb_desolvation energy	-6.34	-6.87	-6.12	-6.08
Total internal energy	-1.04	-0.65	-1.22	-1.39
Torsional energy	2.39	1.49	1.79	1.79
Unbound energy	-1.04	-0.65	-1.22	-1.39
H-bonds	02	05	03	01
Molecular Docking with Nuclear Factor kappa B Receptor: PDB ID - 1SVC				
Binding energy	-3.82	-5.86	-5.78	-5.96
Ligand efficiency	-0.16	-0.27	-0.25	-0.24
Inhibition Constant	1.58mM	50.95μM	57.89 μM	42.43μM
Intermolecular energy	-6.21	-7.35	-7.57	-7.75
Electrostatic energy	-0.17	-0.37	-0.29	-0.44
Vdw_hb_desolvation energy	-6.04	-6.97	-7.28	-7.32
Total internal energy	-1.28	-0.65	-1.25	-1.41
Torsional energy	2.39	1.49	1.79	1.79
Unbound energy	-1.28	-0.65	-1.25	-1.41
H-bonds	01	02	02	02
Molecular Docking with Human Vascular Endothelial Growth Factor-B: PDB ID - 2C7W				
Binding energy	-6.18	-6.23	-6.3	-6.99
Ligand efficiency	-0.26	-0.28	-0.27	-0.28
Inhibition Constant	29.48μM	27.2 μM	24.26 μM	7.51μM
Intermolecular energy	-8.57	-7.72	-8.09	-8.78
Electrostatic energy	-0.31	-0.06	-0.16	-0.13
Vdw_hb_desolvation energy	-8.26	-7.66	-7.92	-8.65
Total internal energy	-1.2	-1.17	-1.01	-1.3
Torsional energy	2.39	1.49	1.79	1.79
Unbound energy	-1.2	-1.17	-1.01	-1.3
H-bonds	03	03	03	02

traditional healers but also highlights the possibility of Chalcone, Kaempferide, Tamarixetin and Eupatilin becoming the leads in treating inflammation and tumor progression. Further methods of isolation, *invitro* and *invivo* studies, synergism and pathway analysis will prove beneficial in developing potent anti-cancer drug.

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