EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 8
867-877
Year: 2017

IN VITRO AND *IN SILICO* ANTIOXIDANT, ANTI-INFLAMMATORY AND ANTIBACTERIAL ACTIVITY OF METHANOL LEAF EXTRACT OF *RANDIA SPINOSA*

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Article Received on 01/06/2017 **Article Revised on 22/06/2017** **Article Accepted on 12/07/2017**

ABSTRACT

Randia spinosa leaves are using for treating skin diseases and they having important potent bioactive compounds. The methanol leaf extract yielded biologically important compounds viz., glycosides, phytosterol, alkaloids, flavonoids, phenol, tannins, athraquinones. The GC-MS of the same extract yielded nine biologically important compounds. The methanol leaf extract significantly showed strong antioxidant activity in DPPH and FRAP assays. The extract also inhibited the bacteria tested and also strong anti-inflammatory activity by inhibiting the heat induced albumin denaturation and red blood cells membrane stabilization, proteinase inhibition, BSA anti-denaturation and HRBC membrane stabilization. The *in silico* activity, Pyranol[4,3]benzopyron-1,9-dione, 5a-methoxy-9a-methyl-3-(1-propenyl) perhydro) and benzoic acid, 2-[2-methoxyethoxy)-5 (2,2-dimethylpropanolamine showed the highest binding affinity with high score and good hydrogen bond interactions with active site residues. The activities demonstrated by two compounds suggest that they could be useful in management of oxidant molecules, inflammation and growth of the bacteria.

KEYWORDS: *Randia spinosa*, phytochemicals, antioxidant, anti-inflammatory, antibacterial, molecular docking.

INTRODUCTION

Randia spinosa (Poir.) belongs Rubiaceae is a large shrubs or small trees. The plants seeds are using as tonic to treat induce appetite. The plant is given in diarrhoea and dysentery; skin diseases, gastrointestinal; snake and scorpion bite and wound healings.[1] The plant showed the presence of randia acid, saponin, oleanolic acid, leucocyananidin and mannitol, sapogenin, ursosaponin, ursolic acid, triterpenoid saponin, mucilage, resin, scopolectin, d-mannitol, randinoside, galioside, deacetylsperulosidic acid methyl ester, scandoside methyl ester, geniposide, gardenoside, The plant extract exhibited anti-inflammtory.[2,3] antitumor.[4] antimicrobial.[5-7] Mahabaleshwara et al.[8] have worked on phytochemicals analysis in methanol leaf extract of *Randia spinosa*.

The present investigation was aimed to know the role of methanol leaf extract in antibacterial, anti-inflammatory and antioxidant in in vitro and *In silico* methods.

MATERIALS AND METHODS

Collection and processing of plant

Rhe fresh leaves of *Randia spinosa* were collected from hill station of Devarayanadurga, Tumkur district, Karnataka, India in the month of June, 2014 and identified based on the characteristics. The plant material was washed with distilled water, shade-air dried (26 ± 2°C) and pulverized to a coarse powder in a mechanical grinder, passed through a 40 mesh sieve and stored in air tight container for further work.

Preliminary solvent extraction

Dried leaves of *Randia spinosa* extraction was done with methanol at 5 g/15ml (W/V). The methanol extract was dried after extraction and preliminary phytochemical analysis was carried out using standard procedures to identify constituents as described by Harborne.[9] Trease and Evans.[10] and Sofowara.[11]

GC-MS analysis

The methanol extract of *Randia spinosa* bioactive crude extract was separated into various fractions by column chromatography. The column was packed with silica gel (mesh 60 - 120) and run with n-hexane: EtOH (8:2). The earlier stated 6th fraction showed a clear band in TLC and was selected for GC-MS analyses that were performed by the Central Instrumentation Department, Indian Institute of Sciences (IISc), Bangalore. GC-MS measurements were performed with a Shimadzu instrument equipped with GC: Aligent 7890 A, MS: MS detector 5975C, Ionization for MS: Electron Impact Ionization, Mass Analyzer: Quadrupole, Software: Data Analysis, Library: Nist 2008, column: HP 5 ms, Dimensions:30 m (L) x 0.25 mm (ID) x 0.25 µm film thickness, the initial temperature was 0 to 40ºC for 2 min hold time, room temperature was 100ºC to 310ºC for 10 min is the hold time, total time is 34 min, carrier gas was helium, flow (mL/min) was 1.0, split flow:1 mL/min, injection volume: 1 µL, Scan mass range: 30 m/z - 600 m/z and polarity +Ve. GC-MS performed based on the database having more patterns. The spectrum of the unknown compound was compared with the spectrum of the known compounds in the library.

Determination of antioxidant activity

In order to investigate the antioxidant properties of the examined extracts, ferric ion reducing antioxidant power (FRAP), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ABTS assays.

DPPH radical scavenging assay

The free radical scavenging activities of extracts were measured by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH). Briefly, extract concentration of (0.1-20 mg/ml) in methanol (4 ml) was mixed with 1 ml of methanol solution containing DPPH (Sigma) radicals of 0.2 mM. The mixture was shaken vigorously and left to stand for 30 min in the dark and the absorbance was measured at 517 nm against a blank.[12]. EC_{50} value (mg/ml) is the effective concentration at which DPPH radicals were scavenged by 50% and the value was obtained by interpolation from linear regression analysis. BHT was used as standard for the comparison. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{DPPH scavenging effect (\%)} = \left[\frac{\{A_0 - A_1\}}{A_0}\right] \times 100,$$

Whereas A0 is the absorbance of the control reaction and A1 the absorbance in the presence of the sample. The extract concentration providing 50% inhibition (EC_{50}) was calculated was obtained by interpolation from linear regression analysis.

FRAP assay

FRAP reagents was freshly prepared by mixing 25 mL acetate buffer (300 mM, pH 3.6), 2.5 mL 2,4,6-tris (2-pyridyl)-S-triazine (TPTZ) solution (10 mM TPTZ in 40 mM/L HCl) and 2.5 mL $FeCl_3$ (20 mM) water solution. Each sample (150 µL) (0.5 mg/mL) dissolved in methanol was added in 4.5 mL of freshly prepared FRAP reagent and stirred and after 5 min, absorbance was measured at 593nm, using FRAP working solution as blank.[13-14] A calibration curve of ferrous sulphate (100-1000 µmol/L) was used and results were expressed in µmol Fe2+/mg dry weight extract. The relative activity of the samples was compared to L-ascorbic acid.

Determination of antimicrobial activity

Antimicrobial assay

Total of five pathogenic bacterial strains were used namely *Pseudomonas species*, *Shigella species*, *Salmonella species* and *Shewanella putrefaciens* all are of Gram negative bacteria and Gram positive Staphylococcus aureus. The clinical isolates were collected in the above prepared slants from Sagar Hospital (Tilaknagar, Bengaluru).

Paper disc method

Diameter of zone of inhibition was determined using the paper disc diffusion method as described by Lai et al. [14] and Adedapo et al.[15] A swab of the bacteria suspension containing 1 × 108 CFU/ml was spread on to Petri plates containing nutrient agar media. Each extracts were dissolved in ethanol to final concentration of 10 mg/ ml. Sterilized filter paper discs (6 mm in diameter) impregnated with 1 mg of plant extracts were placed on culture plates. The plates were incubated at 37 °C for 24 h. The methanol served as negative control while the standard streptomycin (10 µg) discs were used as positive controls. Antimicrobial activity was indicated by the presence of clear inhibition zone around the discs. The assay was repeated thrice and mean of three experiments was recorded.

In vitro anti-inflammatory activity

Inhibition of albumin denaturation

Method of Sakat et al.[17] was followed, the reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37 °C HCl. The sample extracts were incubated at 37 °C for 20 min and then heated to 51 °C for 20 min. after cooling the samples the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate. Per cent inhibition of protein denaturation was calculated as fallows,

$$\text{\% inhibition} = \left[\frac{\{Abs_{control} - Abs_{sample}\}}{Abs_{control}}\right] \times 100,$$

Where $Abs_{control}$ is the absorbance of the DPPH radical+ solvent, Abs_{sample} is the absorbance of DPPH radical+ sample extract/standard.

Membrane stabilization test

Preparation of red blood cells (RBCs) suspension

Fresh whole human blood (10 ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline.[17]

Heat induced hemolytic

The reaction mixture (2 ml) consisted of 1 ml of test sample solution and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 °C for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Per cent membrane stabilization activity was calculated by the formula mentioned above.[17]

Protein inhibitory action

The test was performed according to the modified method of Sakat et al. [17]. The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml of 20 mM Tris HCl buffer (pH7.4) and 1 ml test sample of different concentrations. The reaction mixture was incubated at 37 °C for 5 min and then 1 ml of 0.8% (W/V) casein was added. The mixture was inhibited for an additional 20 min, 2 ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage of inhibition of proteinase inhibitory activity was calculated.

BSA anti-denaturation assay

Five ml of each extract was dried in vacuum oven and redissolved in 5 ml of isosaline. Then, 1 mg/ml of all extracts were made from the abovementioned stock solution. To 1.8 ml of 1% of BSA solution, 0.2 ml of extract solution in isosaline was added. The pH was adjusted to 6.5 using 1N HCl. This solution was incubated at 37 ºC for 20 minutes and then heated to 57 ºC for 10 to 15 minutes. After cooling, turbidity was measured at 660 nm. Control was taken without the extracts.[18]

HRBC membrane stabilization assay

Blood was collected freshly and mixed with equal volume of Alsever solution. It was then centrifuged at 3000 rpm for 15 minutes. The packed cells were washed with isosaline and a 10% suspension was made with isosaline. To 0.5 mlof extract, 1 ml phosphate buffer, 2 ml hyposaline and 0.5 ml HRBC suspension was added. This was incubated for 30 minutes at 37 ºC and then centrifuged at 3000 rpm for 20 minutes. Absorbance was measured at 560 nm. Control was taken without the extract.[19]

***In silico* molecular interaction studies of the *Randia spinosa* antioxidant, anti-inflammatory and antibacterial activity**

Selection of ligands

Most of the 3D (3 Dimensions) structures of drug molecules identified in methanol extract of *Randia spinosa* were downloaded from PubChem Compound section of National Center for Biotechnology Information (NCBI). Ligands during this process also being checked for Torsion count to detect currently active bonds with default settings. Importantly, amide bonds were checked and treated as non-rotatable. Ligands were also utilized to merge non-polar hydrogens. The 3D structures of 09 ligands (3-eicosyne, isopropyl linoleate, methyl isohexadecanoate, n-butyl myristate, 16-octadeconic acid-methyl ester, heptadecanic acid-9-methyl-methyl ester, 9, 12, 15-octadetrienoic acid-ethyl ester, benzoic acid, pyranol [4,3] benzopyran) were obtained from methanol extract of Penicillium species. The 3D structures of these 03 ligands were elucidated from https://www.mn-am.com/online_demos/corina_demo.

Selection of receptors

The receptors were chosen in light of their capacity in the pathway of oxidant, inflammatory, bacterial activity. The 3D structure of oxidant (68) proteins viz., 1acj,1ad4,1aj0, 1b6c, 1bwc, 1cb4, 1cle,1dgb, 1di8, 1em1, 1f4j, 1f6w, 1hwk, 1i9b, 1ik3, 1iyt, 1jnk, 1jvi, 1kvo, 1mfm, 1oaf, 1pau, 1poi, 1puo, 1pxx, 1qmm, 1spd, 1tnr, 1xu7, 1zj3, 2aw1, 2beg, 2cag, 2fba, 2he3, 2i81, 2p31, 2tgi, 2xjk, 2xzb, 2y9x, 2zj3, 3c5k, 3c10, 3dpk, 3g60, 3hsw, 3k35, 3kij, 3lii, 3lhr, 3nle, 3qkg, 4b0o, 4bb6, 4b3e, 4eyz, 4nos, 4y14, 5d9t, 5dyw, 5elt, 5hf5, 5lkr, 10gs, 18gs 1mfm, 1spd, 2fba, 2he3, 1qqw, 1xu7, 2zj3, 4y14, 3k35, 1tnr, 3g60, 2y9x, 4bb6, 5hf5, 1i9b, 5dyw, 5lkr and inflammatory (07) proteins viz., 1cuv, 1k71, 1kvo, 2hwq, 3ntg, 3v8v, 6cox. The 12 different bacterial proteins/receptors are selected for study as follows 1qd4, 1ajo, 1ai9, 1ea1, 1hnj, 1kzn, 1xff, 2vf5, 3b6o, 3gbg, 3u2d, 4g3n. The 3D structures of these receptors were accessible in their local shape in PDB database. The 3D directions of the receptors were obtained from PDB database. To verify the capacity of the model in reproducing experimental observation with new ligand, all these structures were analyzed again at the binding site.

Docking simulations

IGEMDOCKV2.1 was employed for binding affinity measurement between selected ligands and targeted proteins of oxidant, inflammatory and bacterial. The content of configure file was determined as position of receptor file and ligand file.

Adme Test

ADME/Toxicity parameters compliance was evaluated by screening through ADME-SAR, a commercial tool. The ADME-SAR is system pharmacology or system chemical biology and toxicology platform designed for the assessment of would be therapeutic indications, off leaf target effects and potential toxic end points of natural products. In the studied work this database/tool was used to predict and evaluate the human metabolism compliance, toxicity risk assessment and mode of action by using standard experimental data.

RESULTS AND DISCUSSION

The methanol extract of *Randia spinosa* have exhibited the glycosides, phytosterol, alkaloids, flavonoids, phenols, tannins and anthraquinones (Table 1). The anthraquionones was detected in hexane and chloroform.[8] from same plant but we have obtained from methanol extract. The methanol leaf extract of *Randia spinosa* yielded nine different phytochemicals (3-eicosyne, isopropyl linoleate, methyl isohexadecanoate, n-butyl myristate, 16-octadeconic acid-methyl ester, heptadecanic acid-9-methyl-methyl ester, 9,12,15-octadetrienoic acid-ethyl ester, benzoic acid, pyranol [4,3] benzopyran) and they identified based on Retention Time (RT) by using NIST library. Mahabaleswara et al.[8] have reported similar results from leaf extract of Randia species.

Table 1: FRAP assay values for *Randia spinosa* extract.

Species	Extract
Randia spinosa extract	1344.27±0.08
Ascorbic acid	1648±0.08

***In vitro* antioxidant activity**

The data of *Randia spinosa* leaf extract on DPPH radical scavenging activity along with standard natural antioxidant BHT have shown in Figure 1. The methanol extract showed significant antioxidant activity with IC_{50} value of 121.63 µg/ml. In FRAP, the reducing capacity of oxidant molecules was high with *Randia spinosa* leaf extract and it was comparable with standard antioxidant Ascorbic acid (1344.27±0.08) (Table 1). Similar results were reported by Chipiti et al.[20], Bakari et al.[21] Durairaj et al.[22] using plant extracts.

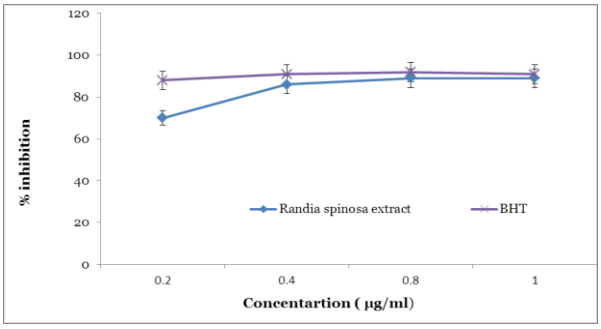


Figure 1: DPPH scavenging activities of the *Randia spinosa* methanol leaf extract.

The *Randia spinosa* extract have showed high membrane stabilization (81.92±0.06) and protein inhibition (86.48±0.04) activity in in vitro anti-inflammatory activity compared to standard aspirin. The albumin denaturation (84.49±0.06) was also high with same extract and it was comparable with standard aspirin (75.89±0.06) (Figure 2). The study of RBCs membrane stabilization is the mechanism of anti-inflammatory action. The *Randia spinosa* extract effectively inhibits the heat induced hemolysis and it is the evidence for membrane stabilization as a mechanism of anti-inflammatory effect. The extract may inhibit the release of lysosomal contact of neutrophils at the site of inflammation. The extract inhibited the heat induced hemolysis of RBCs at different concentration and the results are similar to standard drug diclofenac (Figure 3). The highest activity (86.11) was observed at the concentration of 500 µg/ml of the extract). If the extract increases the activity was also increased. The BSA protein denaturation by extract was 83% at the concentration of 200 µg/ml. Denaturation of proteins is well documented cause of inflammation and rheumatoid arthritis. All the inflammatory drugs ability to inhibit thermally induced protein denaturation.[23] After heat the BSA undergoes denaturation and expresses antigens associated type III hypersensitive reaction which is related to various diseases. The experiment is used to detect the extract stabilize the protein from denaturation process or not.[24] The results are confirmatory with the finding of Govindappa et al.[25]

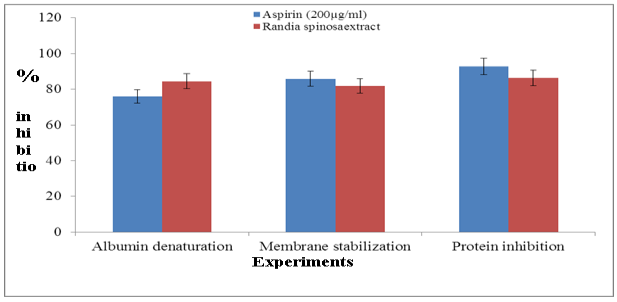


Figure 2: Effect of methanol extract of *Randia spinosa* on albumin denaturation, membrane stabilization and proteinase inhibitory activity percentage inhibition.

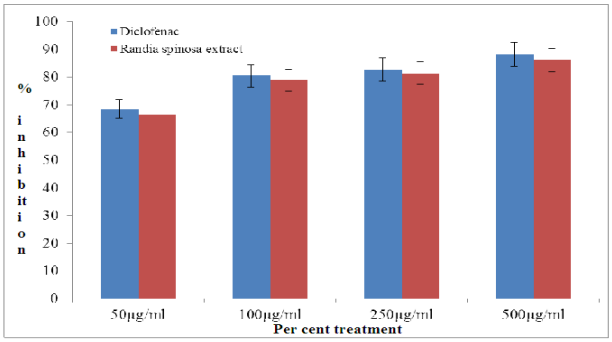


Figure 3: HRBC membrane stabilization of leaf extract of *Randia spinosa* extract and standard drug diclofenac.

The *Randia spinosa* extract inhibited the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* significantly compared to other tested bacteria (Table 2). The similar results were reported by Mostafa et al.[26]

Table 2: Antibacterial activity of *Randia spinosa* methanol extract (in mm).

Organism	Streptomycin	*Randia spinosa* extract
Staphylococcus aureus	13	0.8
Pseudomonas aeruginosa	11	0.8
Shewenella putrefaciens	08	0.6
Shigella dysenteriae	11	0.4
Salmonella species	11	0.6

Based on ADMET-SAR studies, chosen only two phytochemicals (benzoic acid, 2-[2-methoxyethoxy)-5(2,2-dimethylpropanolamine) and Pyranol [4,3] benzopyron-1, 9-dione, 5a-methoxy-9a-methyl-3-(1-propenyl) perhydro-) for *In silico* antioxidant, anti-inflammatory and antibacterial activity based on their non-carcinogens, non-AMES toxicity and readily biodegradable (Table 3).

Table 3: ADMET Predicted profile of the potent phytochemicals of *Randia spinosa* benzoic acid, 2-[2-methoxyethoxy)-5(2,2-dimethylpropanolamine) and Pyranol[4,3]benzopyron-1,9-dione, 5a-methoxy-9a-methyl-3-(1-propenyl) perhydro.

Property	Benzoic acid, 2-[2-methoxyethoxy)-5(2,2-dimethylpropanolamine)		Pyranol[4,3]benzopyron-1,9-dione, 5a-methoxy-9a-methyl-3-(1-propenyl) perhydro-	
	Value	Probability	Value	Probability
Blood Brain Barrier	BBB+	0.7021	BBB+	0.8897
Human Intestinal absorption	HIA+	0.5731	HIA+	0.9575
Caco-2-permeable	CaCo2-	0.5478	CaCo2+	0.5910
P-glycoprotein-substrate	Substrate	0.5854	Substrate	0.5275
P-glycoprotein-inhibitor I	Non-inhibitor	0.8927	Non-inhibitor	0.5621
	Non-inhibitor	0.7883	Non-inhibitor	0.6981
Renal organic cation transporter	Non-inhibitor	0.9042	Non-inhibitor	0.6463
Distribution				
Subcellular localization	Mitochondria	0.8282	Mitochondria	0.8214
Metabolism				
CYP450 2C9 Substrate	Non-substrate	0.8069	Non-substrate	0.7967
CYP450 2D6 Substrate	Non-substrate	0.8265	Non-substrate	0.8416
CYP450 3A4 Substrate	Substrate	0.6472	Non-substrate	0.5450
CYP450 1A2 Substrate	Non-inhibitor	0.8062	Inhibitor	0.8844
CYP450 2C9 Inhibitor	Non-inhibitor	0.7522	Inhibitor	0.8336
CYP450 2D6 Inhibitor	Non-inhibitor	0.9257	Non-inhibitor	0.5849
CYP450 2C19 Inhibitor	Non-inhibitor	0.8328	Inhibitor	0.8688
CYP450 3A4 Inhibitor	Non-inhibitor	0.9328	Non-inhibitor	0.8102
CYP Inhibitory Promiscuity	Low CYP inhibitory	0.9588	Low CYP inhibitory	0.5230
Excretion-Toxicity				
Human Ether-a-go-Related Gene Inhibition	Weak inhibitor	0.9900	Weak inhibitory	0.8174
	Non-inhibitor	0.9208	Non-inhibitor	0.9082
AMES Test	Non- AMES toxic	0.7897	Non AMES toxic	0.5945
Carcinogens	Non-carcinogens	0.8589	Non-carcinogens	0.9621
Fish Toxicity	High FHMT	0.7392	High FHMT	0.7849
Tetrahumena Pyriformis Toxicity	High TPT	0.9465	High TPT	0.9926
Honey Bee Toxicity	Low HBT	0.7177	High HBT	0.6782
Biodegradation	Not ready biodegradable	0.8515	Ready biodegradable	0.6107
Acute Oral Toxicity	III	0.7342	III	0.3842
Carcinogenicity (Three class)	Non-required	0.6397	Non-required	0.3985
Absorption				

Aqueous solubility	-2.8000	LogS	-3.0921	LogS
CaCo2 Permability	0.8447	LogPapp, cm/s	0.9762	LogPapp, cm/s
Distribution, Metabolism, Excretion, Toxicity				
Rat acute toxicity	2.2431	LD50, mol/kg	2.8092	LD50, mol/kg
Fish Toxicity	0.7385	pLC50, mg/L	-0.0592	pLC50, mg/L
Tetrahymena pyriformis Toxicity	0.6198	pIGC50, µg/L	0.9222	pIGC50, µg/L

Table 4: *In silico* antioxidant activity of *Randia spinosa* benzoic acid, 2-[2-methoxyethoxy)-5(2,2-dimethylpropanolamine).

PDB	benzoic acid, 2-[2-methoxyethoxy)-5 (2,2-dimethylpropanolamine)			Pyranol [4,3] benzopyron-1,9-dione, 5a-methoxy-9a-methyl-3-(1-propenyl) perhydro-		
	Binding energy (kcal/mol)	VWD	H-bond	Binding energy(kcal/mol)	VWD	H-bond
1acj	-71.57	-63.31	-8.25	-109.03	-96.97	-12.07
1ad4	-81.08	-57.25	-4.4	-78.59	-76.78	-1.82
1aj0	-88.24	-55.29	-26.52	-102.71	-83.33	-19.39
1b6c	-73.18	-68.26	-4.91	-111.18	-105.8	-6
1bwc	-88.83	-63.94	-24.58	-98.36	-76.54	-21.82
1cb4	-86.69	-67.84	-18.85	-95.6	-62.69	-32.91
1cle	-77.11	-52.68	-21.92	-91.15	-73.25	-17.9
1dgb	-82.28	-80.77	-11.8	-113.34	-102.95	-10.39
1di8	-88.41	-65.83	-22.59	-91.71	-76.14	-15.58
1em1	-85.58	-69.42	-15.91	-94.59	-70.65	-23.94
1f4j	-95.14	-83.58	-12.33	-112.73	-106.83	-5.9
1f6w	-83.51	-53.25	-30.42	-85.39	-61.76	-23.64
1hwk	-84.18	-62.07	-21.37	-97.25	-88.85	-8.4
1i9b	-82.73	-77.73	-5	-96.91	-80.35	-16.56
1ik3	-82.99	-69.24	-13.66	-101.26	-87.86	-13.4
1iyt	-324.55	-289.3	-35.24	-67.66	-60.42	-7.24
1jnk	-91.62	-72.45	-17.5	-87.07	-76.21	-10.86
1jvi	-90.43	-63.72	-23.18	-81.08	-62.16	-18.92
1kvo	-92.05	-69.67	-19.56	-105.13	-89.94	-15.18
1mfm	-79.99	-68.61	-11.38	-82.65	-69.34	-13.31
1oaf	-100.42	-84.2	-13.22	-100.98	-84.48	-6.5
1pau	-83.77	-73	-10.68	-97.86	-92.69	5.18
1poi	-87.83	-75.66	-12.29	-99.51	-76.45	-23.06
1puo	-86.99	-69.15	-17.84	-103.45	-91.67	-11.78
1pxx	-85.14	-7383	-11.31	-114.22	-104.11	-10.11
1qmn	-74.59	-61.97	-12.63	-99.65	-79.75	-19.9
1qqw	-96.46	-82.89	-11.89	-106.11	-78.11	-28.09
1spd	-88.43	-55.04	-26.07	-86.23	-72.93	-13.3
1tnr	-81.08	-64.96	-13.6	-84.84	-76.19	-8.65
1xu7	-83.92	-70.76	-12.75	-96.37	-80.73	-15.65
1zj3	-80.87	-62.59	-16.09	-83.64	-77.2	-6.44
2aw1	-77.93	-66.88	-7	-99.63	-89.88	-9.75
2beg	-87.2	-80.38	-6.82	-91.07	-71.8	-19.27
2cag	-81.93	-62.67	-16.11	-108.58	-99.01	-9.57
2fba	-83.65	-66.95	-15.02	-79.89	-63.47	-16.43
2he3	-78.86	-57.49	-21.94	-75.14	-68.14	-7
2i81	-78.41	-72.12	-6.29	-89.36	-76.78	-12.58
2p31	-73.95	-53.14	-21.03	-90.11	-65.71	-24.4
2tgi	-61.45	-48.72	-12.73	-76.94	-63.09	-13.85
2xjk	-70.38	-56.58	-13.8	-81.7	-48.43	-33.27
2xzb	-81.63	-75.25	-4.31	-107.42	-94.24	-13.18
2y9x	-76.8	-62.47	-13.3	-92.06	-80.09	-11.97
2zj3	-83.44	-63.23	-17.86	-87.13	-69.41	-17.72
3c5k	-87.96	-74.7	-13.26	-97.76	-72.22	-25.54

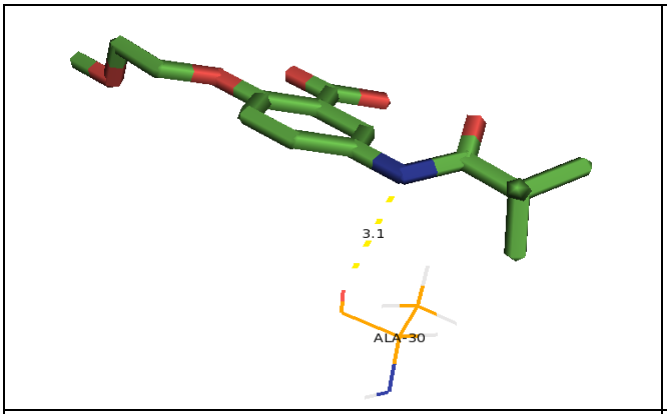
3c10	-79.96	-53.5	-27.2	-85.13	-57.33	-27.8
3dpk	-91.4	-75.59	-13.4	-103.81	-97	-6.81
3g60	-87.36	-51.17	-30.46	-89.42	-69.41	-20
3hsw	-92.74	-81.12	-11.54	-101.63	-91.45	-10.17
3k35	-92.11	-81.15	-9.55	-97.36	-82.25	-15.11
3kij	-83.68	-57.74	-24.14	-90.05	-62.53	-27.52
3lii	-84.89	-74.31	-11.41	-101.69	-90.13	-11.55
3lhr	-86.21	-61.18	-21.04	-92.62	-70.3	-22.32
3nle	-92.51	-90.01	-2.5	-94.76	-91.26	-3.5
3qkg	-75.63	-57.96	-14.62	-81.49	-71.56	-9.92
4bOo	-82.53	-58.43	-21.7	-99.15	-89.36	-9.79
4bb6	-89.5	-67.83	-20.85	-96.41	-72.1	-24.32
4b3e	-80.83	-66.64	-14	-98.43	-72.49	-25.94
4eyz	-73.74	-63.24	-10.51	-96.29	-75.1	-21.18
4nos	-95.77	-86.11	-7	-101.37	-94.3	-7.08
4y14	-96.77	-79.82	-15.73	-100.75	-69.35	-31.4
5d9t	-82.43	-54.38	-26.88	-88.49	-69.61	-18.88
5dyw	-82.12	-58.27	-19.89	-100.65	-87.25	-13.4
5e1t	-82.66	-74.88	-7.42	-86.02	-80.88	-5.14
5hf5	-88.81	-70.85	-17.99	-97.19	-85.59	-11.59
5lkr	-87.06	-72.17	-13.74	-96.78	-92.24	-4.54
10gs	-83.17	-68.29	-18.24	-102.67	-79.13	-23.54
18gs	-80.21	-63.71	-16.76	-85.67	-79.69	-5.98

Table 5: *In silico* anti-inflammatory and antibacterial activity of *Randia spinosa* two phytochemicals.

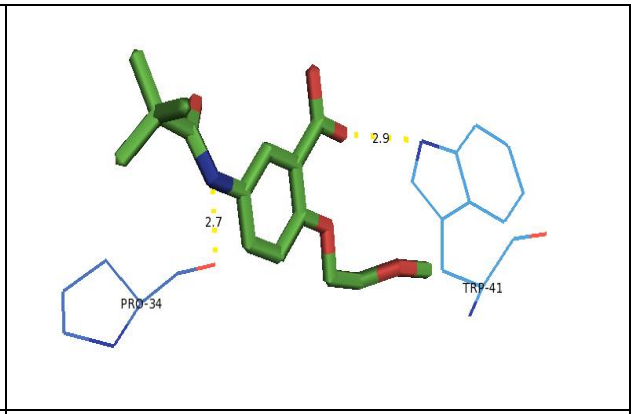
PDB	benzoic acid, 2-[2-methoxyethoxy)-5(2,2-dimethylpropanolamine)			Pyranol[4,3]benzopyron-1,9-dione, 5a-methoxy-9a-methyl-3-(1-propenyl) perhydro-		
	Binding energy(kcal/mol)	VWD	H-bond	Binding energy(kcal/mol)	VWD	H-bond
Anti-inflammatory						
1cuv	-90.19	-81.32	-8.87	-106.65	-99.75	-6.9
1k7l	-91.65	-73.96	-17.69	-102.69	-86.47	-16.23
1kvo	-101.99	-70.43	-28.28	-104.61	-87.04	-17.57
2hwq	-87.66	-68.99	-18.68	-96.3	-87.51	-8.79
3ntg	-97.33	-82.18	-10.29	-99.53	-80.66	-18.87
3v8v	-84.65	-71.9	-10.31	-102.94	-81.13	-21.81
6cox	-93.39	-72.71	-18.96	-100.3	-88.36	-11.94
Antibacterial/ fungal						
1ad4	-78.17	-59.33	-16.52	-93.88	-73.99	-19.89
1ajo	-86.83	-74.59	-12.25	-84.32	-69.73	-14.6
1ai9	-97.8	-74.11	-21.81	-98.29	-84.97	-13.31
1ea1	-72.38	-67.38	-5	-96.03	-71.97	-24.06
1hnj	-76.56	-67.33	-9.23	-81.24	-64.79	-16.45
1kzn	-90.14	-81.06	-9.09	-101.96	-93.36	-8.61
1xff	-79.5	-47.44	-27.94	-88.63	-72.29	-16.34
2vf5	-69.65	-66.04	-5	-77.23	-66.98	-10.25
3b6o	-91.44	-63.09	-26.22	-90.78	-77.41	-13.36
3gbg	-88.83	-63.18	-24.19	-83.23	-82	-1.22
3u2d	-88.18	-74.63	-18.72	-102.79	-88.15	-14.63
4g3n	-97.99	-80.73	-15.04	-84.9	-63.91	-20.99

The Pyranol[4,3]benzopyron-1,9-dione, 5a-methoxy-9a-methyl-3-(1-propenyl) perhydro-) have showed highest interaction with oxidant proteins viz., 1acj (-109.03), 1aj0 (-102.71), 1b6c (-111.18), 1bwc (-98.36), 1cb4 (-95.6), 1cle (-91.15), 1dgb (-113.34), 1di8 (-91.71), 1em1 (-94.59), 1f4j (-112.73), 1hwk (-97.25), 1i9b (-96.91), 1ik3 (-101.26), 1kvo (-105.13), 1oaf (100.98), 1pau (-97.86), 1poi (-99.51), 1puo (-103.45), 1pxx (-114.22), 1qmm (-99.65), 1qqw (-106.11), 1xu7 (-96.37), 2aw1 (-99.63), 2beg (-91.07), 2cag (-108.58), 2p31 (90.11), 2xzb (-107.42), 2y9x (-92.06), 3c5k (-97.76), 3dpk (-103.81), 3hsw (-101.63), 3k35 (-97.36), 3kij (-90.05), 3lii (101.69), 3ljr (-92.62), 3nle (-94.76), 4b0o (-99.15), 4bb6 (-96.41), 4b3e (-98.43), 4eyz (-96.29), 4nos (-101.37), 4y14 (-100.75), 5dyw (-100.65), 5hf5 (-97.19), 5lkr (-96.78)10gs (-102.67). The benzoic acid, 2-[2-

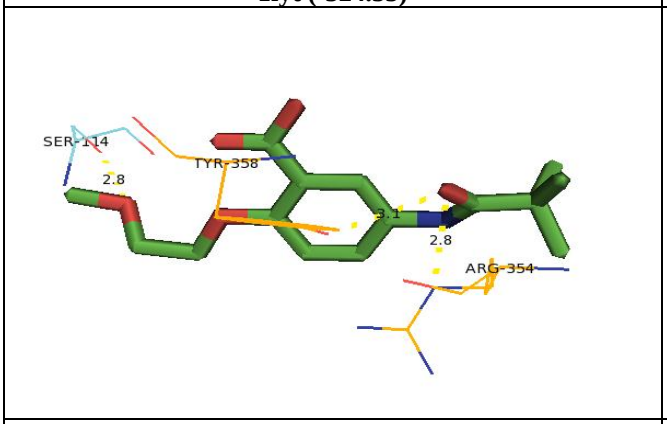
methoxyethoxy)-5(2,2-dimethylpropanolamine have interact with 1f4j (-95.14), 1iyt (-324.55), 1jnk (-91.62), 1jvi (-90.43), 1kvo (-92.05), 1oaf (100.42), 1qqw (-96.46), 3dpk (91.4), 3hsw (-92.74), 3k35 (-92.11), 3nle (-92.51), 4nos (-95.77), 4y14 (-96.77). Both the compounds are able bind with similar oxidant proteins they are 1f4j, 1kvo, 1oaf, 1qqw, 3dpk, 3hsw, 3k35, 3nle, 4nos and 4y14 with high binding energy (Table 4; Figure 4 & 5). The results are confirmatory with the findings of Saturnino et al.[27], Salqueiro et al.[28], Rajalakshmi and Anita.[30], Lakshmiani et al.[31], Rangahanumaiah et al.[32] Daisy et al.[33] and Jorge et al.[34]



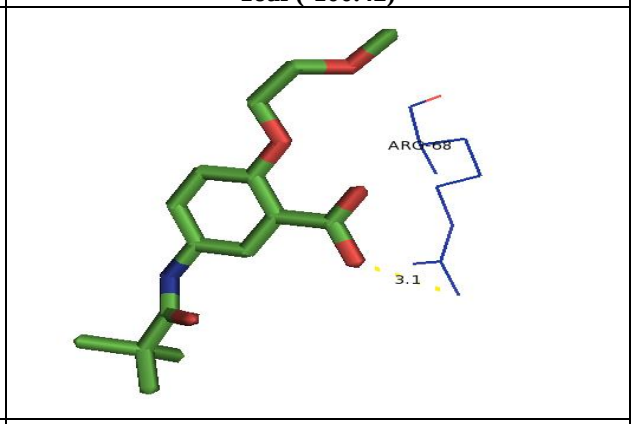
1iyt (-324.55)



1oaf (-100.42)

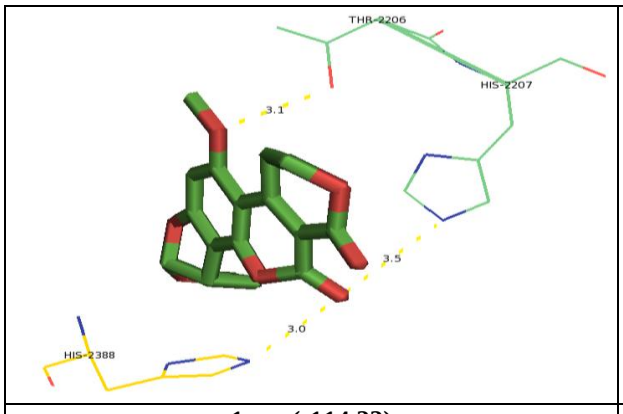


1qqw (-96.46)

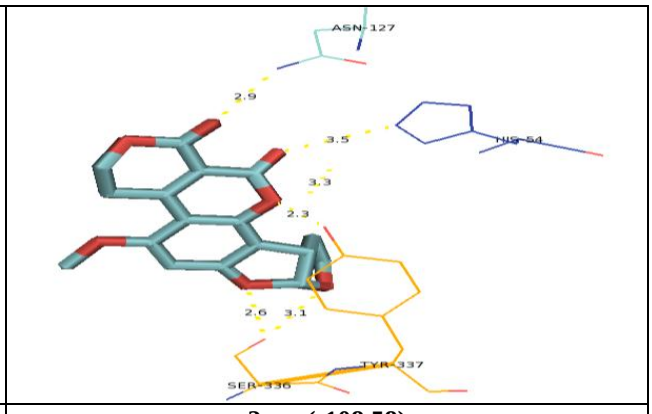


1f4j (-95.14)

Figure 4: *In silico* antioxidant activity of Ranida spinosa Benzoic acid, 2-[2-methoxyethoxy)-5(2,2-dimethylpropanolamine).



1pxx (-114.22)



2cag (-108.58)

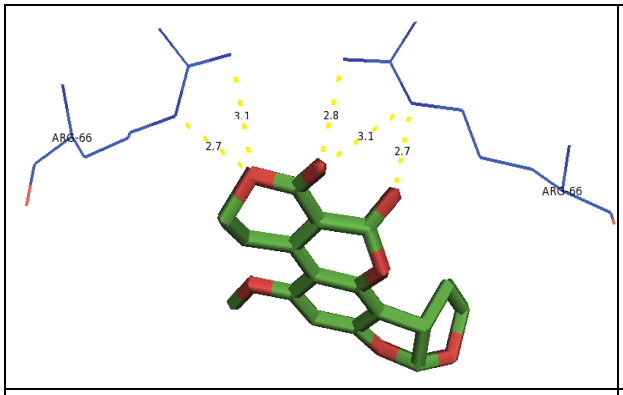
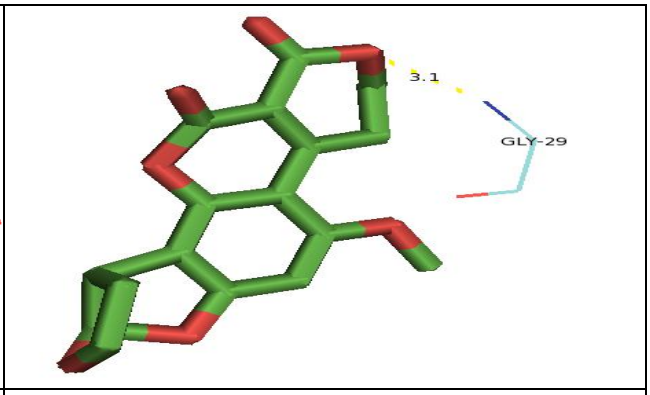
	
1qqw (-106.11)	**1kvo (-105.13)**

Figure 5: *In silico* antioxidant activity of Ranida spinosa Pyranol[4,3]benzopyron-1,9-dione, 5a-methoxy-9a-methyl-3-(1-propenyl) perhydro.

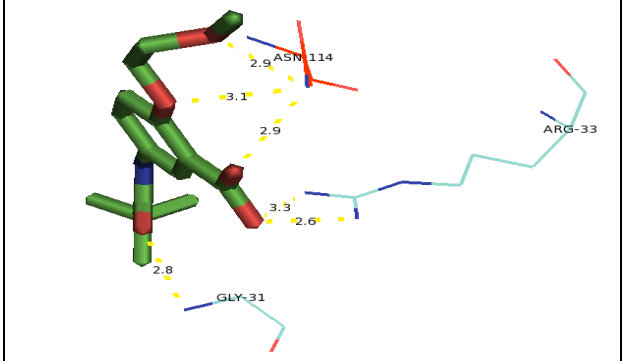
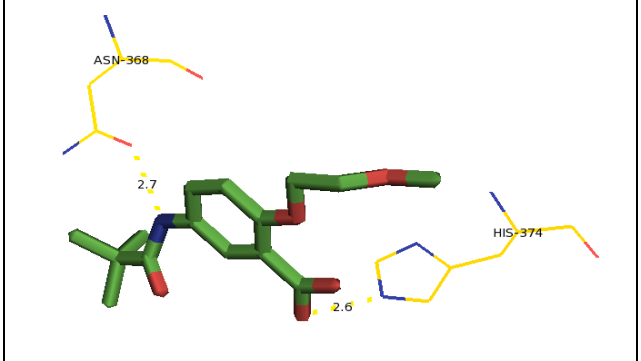
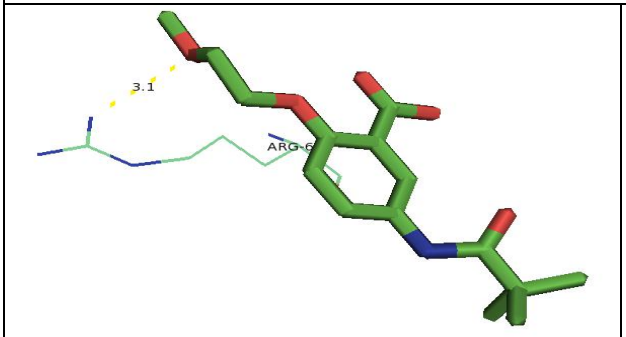
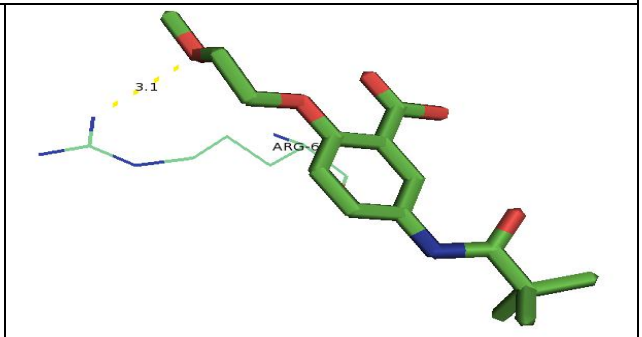
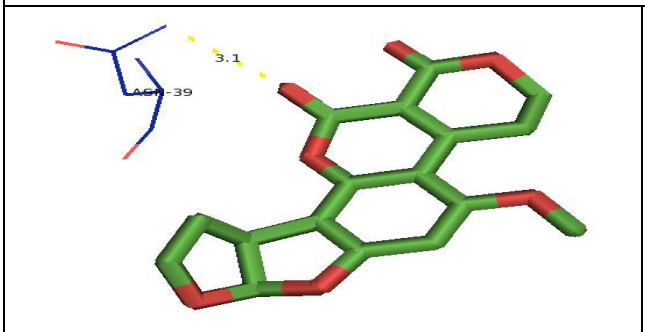
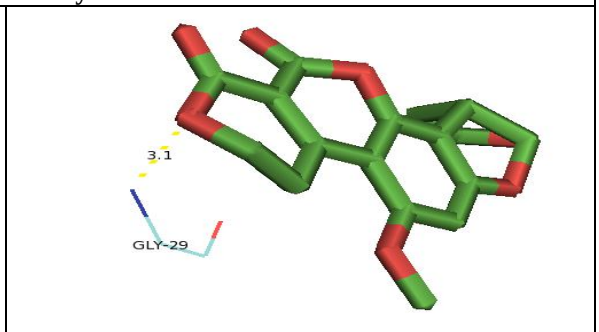
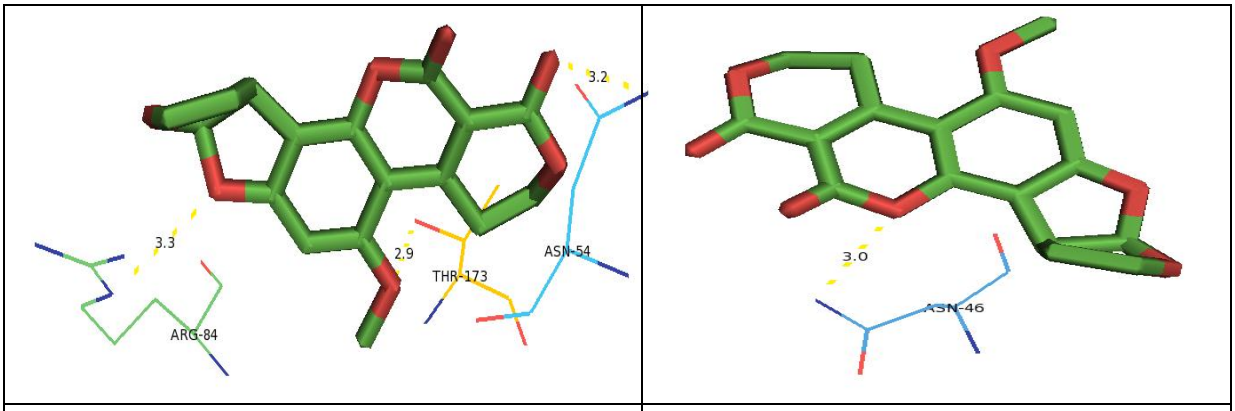
Anti-inflammatory	
	
1kvo (-101.99)	**3ntg (-97.33)**
Anti-bacterial	
	
4g3n (-97.99)	**1ai9 (-97.8)**

Figure 6: *In silico* anti-inflammatory and antibacterial activity of Ranida spinosa Benzoic acid, 2-[2-methoxyethoxy)-5(2,2-dimethylpropanolamine).

Anti-inflammatory	
	
1cuv (-106.65)	**1k71 (-102.69)**
Anti-bacterial	



3u2d (-102.79)	1kzn (-101.96)

Figure 7: *In silico* anti-inflammatory and antibacterial activity of Ranida spinosa Pyranol[4,3]benzopyron-1,9-dione, 5a-methoxy-9a-methyl-3-(1-propenyl) perhydro.

The Pyranol[4,3] benzopyron-1,9-dione, 5a-methoxy-9a-methyl-3-(1-propenyl) perhydro- compound have shown high anti-inflammatory and antibacterial activity in *In silico* method with 1cuv (-106.65), 1kvo (-102.69) and 3u2d (-102.79), 1kzn (-101.96) respectively. The inflammatory proteins 1kvo (-101.99), 3ntg (-97.33) have interacted with benzoic acid, 2-[2-methoxyethoxy)-5(2, 2-dimethylpropanolamine with high binding energy (Table 5; Figure 6 & 7). The same compound have showed more affinity towards bacterial proteins of 4g3n (-97.99) and 1ai9 (-97.8).[31-34]

Briefly, our results demonstrate the methanolic leaf extract of *Randia spinosa* showed biologically important phytochemicals can inhibit oxidant, inflammatory and bacteria effectively in in vitro and *In silico* assays. Future, in vivo studies are conducted to validate our results.

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