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Original Article

# *Rosa indica* Leaf Contains Molecules with Promising Antioxidant, Antimicrobial and Anticancer Effects *in vitro* with Strong Interaction with Human VEGFR2 *in silico*

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## ABSTRACT

**Background:** Plants-based compounds/biopharmaceuticals are found to be effective against multiple ailments due to their fewer side effects. **Objectives:** In this study, the antioxidant, antibacterial and cytotoxic properties and potential secondary metabolites of *Rosa indica* were evaluated. **Materials and Methods:** The extract of *R. indica* leaf was prepared and tested for in vitro Antioxidant and anti-cancer potential. Using HR LCMS QTOF analysis, a secondary metabolite fingerprint has been identified. The ADME properties of prospective molecules, as well as their molecular docking and molecular dynamics with VEGFR2, were investigated *in silico*. **Results:** The result showed that the extract had profound antioxidant activity and showed cytotoxicity against breast cancer cells (MDA-MB) and also showed significant antimicrobial activity against several human pathogens, especially S.aureus and K. pneumoniae. HR LCMS analysis revealed several secondary metabolites with significant bioactive potential. Among the several compounds identified, a few compounds showed strong ADME properties. Docking studies showed that Maritimetin and Santin had a stronger binding affinity for human VEGFR2 than the standard inhibitor. Santin, maritimetin and vitexin, subjected to MD simulations showed an RMSD value, which consistently remained within 4 Å for both protein and ligands reflecting the stability during drug interaction. **Conclusion:** According to the current research, *R. indica* has several bioactive substances that may be developed into medicinal agents.

**Keywords:** *Rosa indica*, Antibacterial, *in vitro* antioxidant, MDA-MB cells, HR-LCMS.

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## INTRODUCTION

Plant research conducted in recent years throughout the world showed the immense potential of medicinal plants against multiple ailments due to their lower toxic effects and the presence of several pharmacologically active principles and compounds. Uncontrolled generation of free radicals and their inefficient modulation in the cell results in oxidative stress.[1] Oxidative stress is the major process responsible for the pathogenesis of several disorders like cancer, diabetes mellitus, cardiovascular diseases, Alzheimer's and arthritis,[2] though the normal aerobic metabolic processes in the body produce some free radicals which are easily managed.[3] An efficient antioxidant system exists in all cells composed of antioxidant enzymes like catalase, glutathione peroxidase, superoxide dismutase and nonenzymatic antioxidants which include bilirubin, uric acid and lactoferrin among others.[4] Antioxidants are compounds capable of blocking the propagation stage in the oxidative chain.[5] Polyphenols are major plant compounds known to act as antioxidants.[6] They play a vital role against oxidative stress either by directly scavenging free radicals or inhibiting enzymes involved in the production of free radicals.

The primary cause of cancer-related mortality among women, particularly in those between the ages of 20 and 59, is breast cancer.[7] Only by improving our understanding of the biology of breast cancer will we be able to design novel and more effective medicines from natural sources to treat the disease. This will require intensive and sustained study to find an effective therapeutic target. Certain cancer forms, including hepatocellular carcinoma, renal cancer and breast cancer, have elevated expression levels of VEGFR2.[8] Since blocking this receptor, which is largely regarded as a significant cancer drug target with natural or synthetic small molecules decreases the cell proliferation of breast cancer cell lines, MCF-7 and MDA-MB-231, which is mediated by the

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enhancement of Mitochondrial Transcription Factor A (TFAM) expression and mitochondrial biogenesis.[9]

From the *Rosaceae* family, *Rosa indica* is a perennial floral shrub that grows upright or climbs. Various formulations of *R. indica* have been used in Indian traditional medicine as an astringent, a mild laxative and a cure for a variety of conditions, including gallstones, bacterial infections, enlarged tonsils, heart problems and eye disorders.[10,11] Numerous phenolic chemicals found in rosa cultivars have demonstrated a variety of pharmacological actions, including antidepressant, anti-inflammatory, anticancer and antioxidant properties.[12-14]

The current study evaluated the *R. indica* leaf extract's phytochemical, antioxidant, antibacterial and anticancer properties. Also, the probable bioactive components were determined using HRLC MS and assessed how well they interacted with VEGFR2 *in silico*. The combination of solvents used for extraction differ in their polarities (acetone: methanol) and are mixed in equal proportions expecting that phytoconstituents of different polarity will come together and may act synergistically to exhibit more effective pharmacological effect than single solvent extracts reported earlier.

## MATERIALS AND METHODS

### Compiling and preparing the extract

*Rosa indica* was collected from the campus and identified with the help of a botanist. The shade-dried leaves of the plant were cleaned and coarsely ground. The powdered leaves (100 g) were defatted by soaking in petroleum ether (60°C-80°C) and kept at room temperature for a week, with occasional shaking. Following filtration, the residue was treated with a 1:1 methanol: acetone mixture and stored at room temperature for a week. After three successive filtration, the combined filtrate was concentrated in a vacuum until all traces of the solvent were eliminated.

### Preliminary Phytochemical Screening

Preliminary phytochemical screening was performed on the *R. indica* methanol:acetone extract, as follows: Alkaloids (test using picric acid): Dilution HCl was mixed with a little volume of extract and filtered. Following that, a saturated picric acid solution was added to the filtrate. A yellow precipitate was formed, indicating the presence of alkaloids. Tannins (Ferric chloride test): A tiny sample of the extract was treated with 1% ferric chloride. A brownish-green tint indicated the presence of tannins. Flavonoids (Sodium hydroxide test): A few drops of sodium hydroxide solution were mixed into 2-3 mL of extract. The production of a bright yellow tint that turned colorless with the addition of a few drops of weak HCl suggested the presence of flavonoids. Glycosides (Sodium hydroxide test): A small amount of extract in water was mixed with a solution of NaOH. The sight of yellow indicates a positive test. To test for saponins, combine 5.0 mL of distilled water with aqueous crude plant extract in a test tube and violently mix. The foaming was combined with a few drops of olive oil and aggressively stirred and the foam look revealed the presence of saponins. Steroids (Salkowski test): Add 2 mL of chloroform and concentrated $H_2SO_4$ to 5 mL of aqueous plant crude extract. In the lower chloroform layer, a crimson tint developed, indicating the presence of steroids.[15]

### Estimation of total phytocomponents

The colorimetric approach was used to assess the amount of various types of phytochemicals; Using the Folin-Ciocalteau reagent, the total polyphenol content (g/100g of methanol: acetone extract) of the *R. indica* extract was determined.[16] Flavonoids totals using the aluminum chloride method,[17] total tannins by the vanillin method,[18] and the alkaloid content were estimated by the bromocresol green method.[19]

### *In vitro* antioxidant studies

### DPPH radical scavenging effect

Using Lue *et al.*'s approach, the DPPH radical scavenging activity was calculated.[20] Three milliliters of 0.1 mM DPPH (made in ethanol) were combined with one milliliter of *R. indica* extract (containing 0.025-0.5 mg in ethanol) and the mixture was let to stand for 30 min in the dark and at room temperature. At 523 nm, absorbances were then measured. The following formula was used to determine the capacity to scavenge DPPH radical:

$$\text{Scavenging effect (\%)} = [(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}) / \text{Absorbance control}] \times 100.$$

### ABTS cation-free radical-scavenging activity

The extract's free radical scavenging activity was determined by using the ABTS radical cation decolorization test.[21] The reaction of 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1) produced the ABTS•+ cation radical, which was then allowed to stand at room temperature for 12-16 hr in the dark before being employed. The absorbance of 0.700 at 734 nm was then obtained by diluting the ABTS•+ solution with methanol. After 30 min of initial mixing, 5 µL of plant extract was added to 3.995 mL of diluted ABTS•+ solution to test absorbance. Every experiment was carried out using an appropriate solvent blank. This activity was quantified as the proportion of ABTS+ scavenging computed using the following formula: ABTS+ scavenging activity (%)=[(Ac-At)/Ac)×100)], where Ac is the absorbance value of the control and At is the absorbance value of the test samples.

### Ferric-Reducing/Antioxidant Power (FRAP) assay

With a small modification, the FRAP assay was performed using the methodology outlined previously.[22] In summary, 180 µL of newly generated FRAP reagent (prepared by mixing 300 mM acetate buffer pH-3.6, 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in a volume ratio of 10:1:1) was combined with

20 µL of extract. After allowing the mixture to stand at room temperature for 6 min, the absorbance at 595 nm was determined. Ascorbic acid and $FeSO_4$ were taken along with the appropriate extract blanks as standards. The FRAP activity was determined using Ferrous Equivalents (FE), which are the amount of extracts or ascorbic acid that produced an absorbance value equivalent to 1 mM $FeSO_4$.

## Nitric Oxide (NO) radical scavenging assay

To 2.0 mL of extract (1:200 dilutions), 3.0 mL of sodium nitroprusside in phosphate buffer (10 mM) was added. After that, the resultant solution was incubated for 60 min at 25°C. The absorbance of the chromophore generated was measured at 540 nm after 5.0 mL of Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylene diamine dihydrochloride in 2% $H_3PO_3$) was added to 5.0 mL of the incubated sample.[23] The unit of measurement for NO radical scavenging activity was mg/g, or ascorbic acid equivalent.

## Antimicrobial assay

The antibacterial activity was assessed using the disc diffusion method. Negative controls included vehicle-loaded discs and distilled water. Prior to treatment, each disc was completely dried on a bacterial lawn. The strains used were, *E. coli, B. cereus* (MTCC 430), *B. subtilis* (ATCC 6051), *S. aureus* (ATCC 25923), *S.aureus* (ATCC 29213), *S. aureus* (MTCC 96), *K.pneumoniae, E.faecalis, P.aeroginosa, C.parapsilosis* and *C.albicans.* The antibacterial potential of the extract was evaluated at doses ranging from 500 and 1 mg/mL. PBS was used as the negative control and antibiotic discs containing streptomycin, ampicillin and penicillin as the positive control. Zones of inhibition were manually assessed after 12 hr of incubation at 37°C on inoculated plates (Control percentage,%). Antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the leaf extract.[24]

## Cytotoxicity assay

MDA-MB cells were seeded into a 96-well plate at a density of $0.8\times10^5$ cells/mL. The cells were treated with 25 and 50 µg of *R. indica* extract for 24 hr after being adhered to overnight following the incubation. Each well received 20 µL of MTT solution, which was then mixed. After 4 hr, the supernatants were removed and 100 µL of DMSO was added to each well to dissolve the precipitate. By measuring absorbance at 570 nm, the vitality of the cells was evaluated. The percentage of cell vitality was determined by multiplying the absorbance ratio of the treated cell culture by 100. This yielded the cell viability (percentage of control).[25]

## HRLCMS-QTOF analysis

HRLCMS-QTOF analysis was performed to ascertain the active compounds present in the extract (HRLCMS-QTOF-Agilent Technologies, USA). Agilent ZORBAX Eclipse Plus -C18 150x2.1 MM column was utilized, along with a solvent system consisting of acetonitrile for solvent B and 0.1% formic acid in Milli-Q water for solvent A. Agilent MAss Hunter was used for data collecting, while Agilent MAss Hunter Qualitative Analysis B.06 was used for data processing.

## Computational studies

### *In silico* ADME Analysis

Swiss ADME software (http://www.swissadme.ch/) was used to assess the *in silico* prediction of ADME parameters, pharmacokinetic features, druglike nature and medicinal chemistry friendliness of the most potent compounds discovered using HRLC-MS QTof. The parameters used were Mol Wgt, $H_2O$ solubility, Number of H-bonds, number of H-bond acceptors, Lipophilicity, GI tract absorption and Drug-likeness (Lipinski rule of 5).

## Molecular docking

Molecular docking studies were carried out to predict the binding affinity and possible modes of interaction between the protein and ligands using the Schrodinger suit (Maestro version 11.4). The Crystal structure of the VEGFR2 kinase domain in complex with inhibitor (PDB ID: 2XIR) was downloaded from the protein data bank. The protein-ligand complex was pre-processed and water molecules within a 5 Å radius from the ligand were eliminated using the protein preparation wizard of Schrodinger suit. Then, missing hydrogen and loops were incorporated, followed by restrained energy minimization using the OPLS3 force field.[26] (1). A receptor grid was created in the energy-minimized structure by keeping the bound ligand in the crystal structure as the center of the grid box. The dimensions of the box were set as 12x12x12 $Å^3$. The 3D structure of the ligands was downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Before the screening process, the ligands were processed for structural optimization at approximately neutral pH (7±1). All possible tautomers and stereoisomers were generated and protonation states were assigned accordingly. Subsequently, the ligands were subjected to energy minimization utilizing the OPLS3 force field *via* the Ligprep module of the Schrodinger suit. Finally, the binding affinity of the prepared ligands against the protein was assessed by docking using the Glide module of the Schrodinger suit.

## Molecular Dynamics

Molecular Dynamics (MD) simulations using the Schrodinger Desmond module were utilized to evaluate the stability of the interaction between the chosen molecules and the VEGFR2 kinase domain in an explicit solvent system.[27] The protein-ligand complexes' docked postures were used as input structures and the Desmond module's system setup option was used to prepare each complex. Initially, the complexes were solvated with the TIP3P water model and the resulting solvated systems were neutralized

by the addition of $Na^+/Cl^-$ ions. Following system generation, minimization and relaxation of the protein-ligand complexes under NPT conditions were performed using the default protocol of the Desmond module. Molecular Dynamics (MD) simulations were subsequently conducted employing periodic boundary conditions within the NPT ensemble, utilizing the OPLS3 force field. The temperature and pressure were maintained at 300 K and 1 atmosphere, respectively, through Nose-Hoover temperature coupling and isotropic scaling. This procedure was followed by a 100 ns NPT production run. Finally, the binding energy of each complex was evaluated post-simulation by extracting specific frames from the MD trajectory and employing the MM-GBSA method.

### Statistical Analysis

Every sample was evaluated three times and displayed as mean +/- standard error of mean. SPSS software was used to examine the data.

## RESULTS

### Phytochemicals composition

The total yield of the methanol: acetone extract of *R. indica* (100 g) was found to be 11.71 g. Preliminary phytochemical analysis showed the presence of several components under the class of alkaloids, tannins, cardiac glycosides, flavonoids and polyphenols. Quantitative analysis of the extract showed a higher amount of alkaloids (4.4 mg), flavonoids (11.41 mg) and polyphenols (16.66), while the level of tannins (0.72) was found to be much less compared to other constituents (Table 1).

### Promising antioxidant activity

The extract showed promising antioxidant activity as ecident from the DPPH, ABTS, FRAP, reducing power and NO scavenging assays. The extract showed more than 50% scavenging of DPPH radical at low concentration (83 µg/mL), the activity was comparable to that of vit C. The extract also had strong inhibitory action on ABTS radicals, as 100% inhibition was achieved with 99 µg/mL. Similarly, the extract showed comparable FRAP and reducing power activity as that of Vit C. Lower concentration of both Vit C and the extract showed lower NO scavenging activity. At 666 and 1998 µg of the extract showed far superior NO scavenging activity compared to that of Vit C (Figure 1). Promising bioactivity was demonstrated by the results in terms of antibacterial, cytotoxic and antioxidant properties. Additional HPLC MS QTOF analysis revealed the presence of various classes of chemicals with a variety of medicinal activities, such as fatty acids, alkaloids, flavones and tannins. Through the reduction of DPPH radicals, nitric oxide, ABTS cation free radical and FRAP, the extract demonstrated notable *in vitro* antioxidant activity.

### Effective against human pathogens

The extract showed strong antimicrobial activity against all tested microbes. The extract (1mg/disk) showed strong activity against *S.aureus* (MTCC 96), *S.aureus* (ATCC 29213, *P.aeroginosa and E.faecalis*. The extract also showed activity against *C.parapsilosis* and *C.albicans* as well (Table 2 and Supplementary Figure 1).

### Cytotoxic to breast cancer cells

Treatment of MDA-MB cells with 25 and 50 µg of the *R. indica* extract showed significant cytotoxicity as evidenced by the 35 and 65% cell death. Microscopic analysis also showed that The 50µg extract altered the shape of the cells and stopped them from adhering, which resulted in cell death. Furthermore, the extract demonstrated almost 70% cell death in MDA-MB breast cancer cells, demonstrating the its ability to limit the cells' growth (Figure 2).

### Contains several other potential bioactive components

The presence of various classes of chemicals with a variety of medicinal activities was revealed in HRLCMS QTOF analysis in both +ve and -ve modes (Supplementary Figure 2). The results were in accordance with the preliminary phytochemical analysis. Phenolic compounds like Quercetin-3-O-(2′′-O-galloyl)-β-D-glucopyranoside, 6-C-galactosyl-luteolin, stingin, alkaloids, gamma chaconine and retronecine, Tannins like punicacortein B and Sanguiin H-11 (SH-11) was also seen to be present. Other compounds include myricitrin, a flavones deacetyl ganoderic acid, Triterpenoid, euphornin macrocyclic diterpenoid and isoterchebin, a gallo tannin as well as a fatty acid corchorifatty acids A with significant biological activities are also present in the extract. (Supplementary Tables 1 and 2).

### Few compounds showed significant druggability according to ADME analysis

Among the several compounds identified, only 11 compounds showed a significant drug-likeness as per the ADME analysis *in silico*. Vitexin and ellagic acid were found to be soluble, while other compounds are either poorly soluble or partially soluble in water. vitexin, N-(2,14-eicosadienoyl) piperidine, ganoderic acid F and euphornin were predicted to have poor Gastrointestinal (GI) absorption, while all other compounds showed to have very high GI absorption. All compounds showed no violation as per Lipinski rule of 5 except N-(2,14-eicosadienoyl) piperidine, γ-chaconine, irinotecan, ganoderic acid F and euphornin which has one violation in terms of their higher molecular weight than stipulated. Lipophilicity of all compounds was within the limit except N-(2,14-Eicosadienoyl) Piperidine which has a Log $P_{o/w}$ value of 6.06 (Table 3). But in the case of natural bioactive compounds, lipophilicity may not directly contribute to its physicochemical profile violating the rule of five.[28]

## Docking: Maritimetin and Santin showed strong binding affinity towards human VEGFR2

The docking analysis looks at the molecular interactions between the compounds potential poses and the surrounding environment of active site residues of the VEGFR2 kinase domain and ranks them based on their binding scores. In Schrodinger, the glide score (g score) represents the binding affinity of protein with corresponding ligands. Among the compounds, Maritimetin and Santin showed strong binding affinity, higher than the standard inhibitor. The observed glide score was -11.384 and -11.336 Kcal/mol respectively. The binding of Santin was mainly stabilized by Hydrogen bonds with the active site residues, Leu 840, Lys 868 and Cys 919. Maritimetin interacted with the kinase domain through hydrogen bonds with Leu 840, Cys 919, Asp 1046 and

**Table 1: Qualitative and Quantitative phytochemical analysis of *R. indica* leaf extract.**

| Compound | Presence | Quantity (mg/g sample)* |
|---|---|---|
| Alkaloids | + | 4.4 |
| Tannins | + | 0.72 |
| Flavonoids | + | 11.41 |
| Cardiac Glycosides | + | ND |
| Saponins | - | 0 |
| Steroids | - | 0 |
| Polyphenols | + | 16.66 |

*Values are represented as the average of three independent experiments.ND-Not Detected.

**Table 2: Antimicrobial activity of the methanol: acetone extract of *R. indica*.**

| Bacterial Pathogen | 1 mg/disc | 500 µg/disc |
|---|---|---|
| *S. aureus* (ATCC 29213) | 16 mm | 13 mm |
| *S. aureus* (ATCC 25923) | 13 mm | 11 mm |
| *S. aureus* (MTCC 96) | 20 mm | 13 mm |
| *E. faecalis* | 15 mm | 12 mm |
| *K. pneumoniae* | 15 mm | 12 mm |
| *E. coli* | 17 mm | 14 mm |
| *B. cereus* | 11 mm | 10 mm |
| *P. aeroginosa* | 15 mm | 13 mm |
| *C. parapsilosis* | 13 mm | 11 mm |
| *C. albicans* | 10 mm | <10 mm |

Values are the mean of three independent experiments.

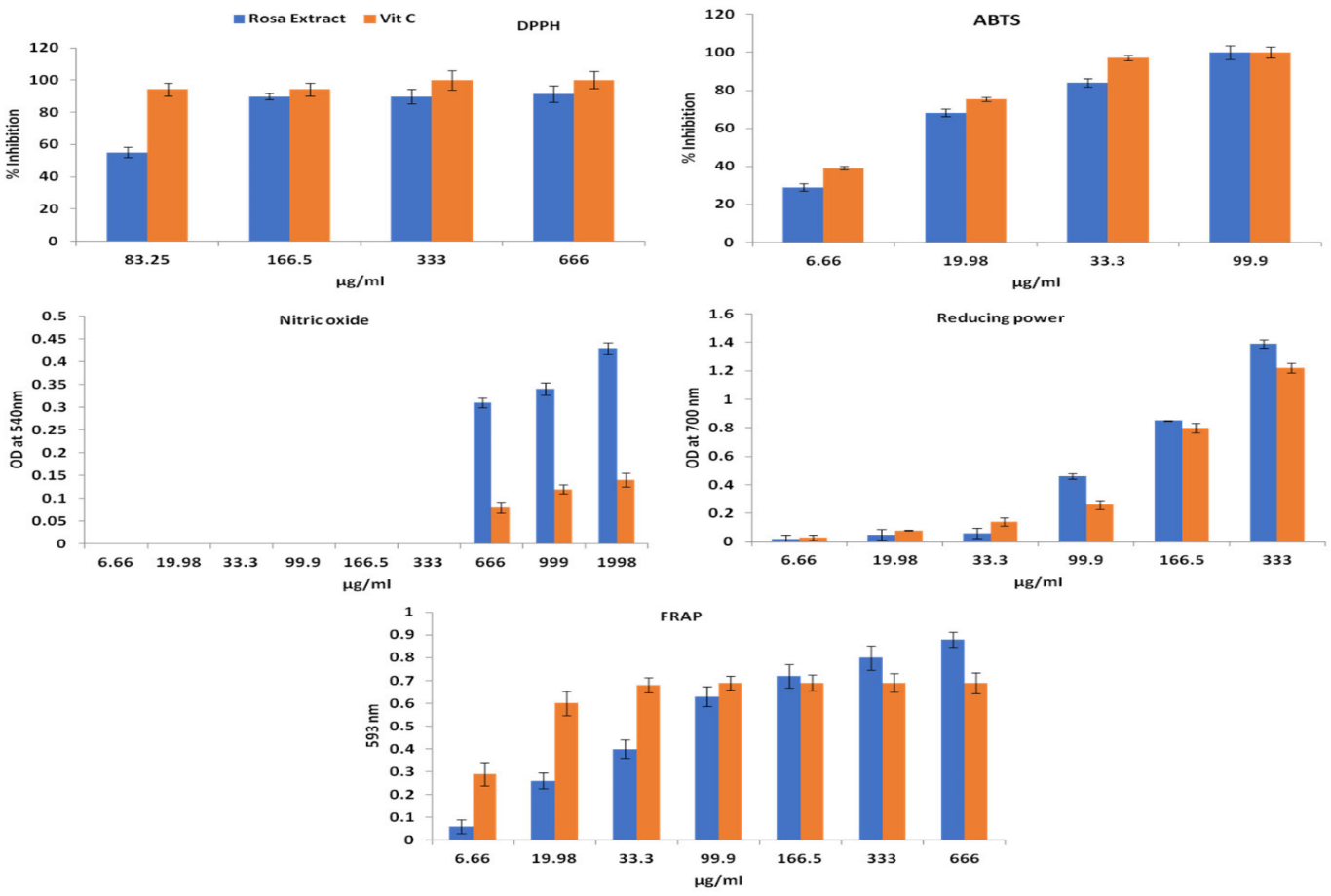


**Figure 1:** *In vitro* antioxidant effect of *R. Indica* extract. The values are expressed as Mean±SEM of three independent experiments.

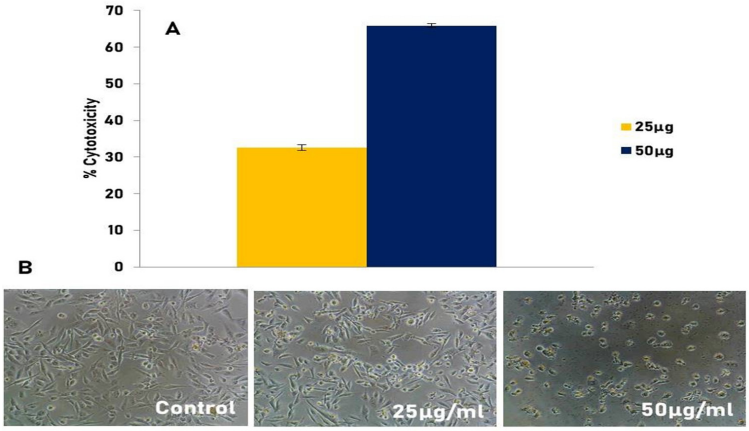


**Figure 2:** *In vitro* cytotoxic effect of *R. indica* leaf extract on MDA-MB breast cancer cells. A: Graph showing the % cytotoxicity of 25 µg and 50 µg of *R. indica* extract. B: shows the morphology of the control and the cancer cells treated with *R. indica* extract (x20). The pictures are the representation of three independent experiments.

a pi-pi stacking interaction with Phe 1047. Another compound, Vitexin showed the same degree of binding affinity, -7.387 Kcal/mol with the standard inhibitor and formed hydrogen bonding with Asn 923, Lys 920 and Glu 850. ellagic acid and euphornin, showed comparatively less, but significant affinity towards the VEGFR2 kinase domain. The binding of these compounds was mainly stabilized by van der Waals forces (Supplementary Figure 3). Table 4 summarizes the binding affinity and major bonds that stabilized the interactions of each compound with the protein.

### Molecular Dynamics: Three complexes, Santin, Maritimetin and Vitexin exhibited protein RMSD deviations below 3 Å

MD simulations were done for the most active hits selected based on the glide score. Changes in the protein's and ligand's conformations during the simulation were quantified using Root Mean Square Deviation (RMSD). For globular proteins, RMSD values within 3-4 Å are typically deemed satisfactory. However, larger deviations suggest substantial conformational changes occurring within the protein during the simulation, indicating potential instability of the protein-ligand bound complex. The three potent molecule, Santin, Maritimetin and Vitexin, subjected to MD simulations exhibited protein RMSD deviations below 3 Å relative to the initial frame. The RMSD for both protein and ligands reflecting the stability during drug interaction, consistently remained within 4 Å (Figure 3).

## DISCUSSION

The present work aimed to evaluate *in vitro* the diverse bioactivities of the methanol:acetone (1:1) extractable fraction *from R. indica* leaf. Promising bioactivity was demonstrated by the results in terms of antibacterial, cytotoxic and antioxidant properties. Additional HPLC MS QTOF analysis revealed the presence of various classes of chemicals with a variety of medicinal activities, such as fatty acids, alkaloids, flavones and tannins. Through the reduction of DPPH radicals, nitric oxide, ABTS cation free radical and FRAP, the extract demonstrated notable *in vitro* antioxidant activity. These results are noteworthy because there is now a movement to switch from synthetic antioxidants to naturally occurring antioxidants found in plants since they are less expensive, safer and more widely available.[1]

The extract contained a number of chemicals that had anti-inflammatory and antioxidant properties. A phenolic molecule, quercetin-3-O-(2″-O-galloyl)-β-D-glucopyranoside has been shown to have strong anti-inflammatory properties and can be used to lessen edema and aortic endothelium-dependent relaxation damage.[29] Flavones C- glycoside, 6-C-galactosyl-luteolin have exhibited antibiosis activity.[30] The results of the *R. indica* extract's strong antimicrobial activity against several human pathogens, including *S. aureus, E. faecalis, K. pneumoniae, E. coli, B. cereus, P. aeroginosa, C. parapsilosis and C. albicans*, revealed the presence of several compounds in the extract. Few components in the extract have been found through experiments to have antimicrobial effects. It is reported that maritimetin had significant antibacterial action against *S. aureus*, a Gram-positive bacterium.[31] By controlling the production of cytokines like IL-10 and IL-12p40 at the protein and mRNA levels, vitexin can reduce the hydrophobicity of the S. aureus surface, which can interfere with aggregation during the biofilm formation phase and subsequent host disease.[32]

It was discovered that the extract considerably slowed down the proliferation of breast cancer cells. This trait might be explained by the fact that several of the extract's ingredients have been demonstrated to have anti-cancer capabilities. Retronecine, a pyrrole alkaloid, has been found to be toxic and to produce toxic changes in both human liver and lung cancer cells. Gamma chaconine, a glycol alkaloid, inhibits the growth of human colon; HT29 and liver HepG2 cancer cells.[33] The tannin punicacortein B was discovered to be efficacious (>10 µg/mL) against tumor cells linked to ileocecal adenocarcinoma (HCT-8), medulloblastoma (TE-671), lung carcinoma (A-549) and epidermoid carcinoma of the nasopharynx (KB).[34] Another

**Table 3: Prediction of the ADME properties of the potent bioactive compounds *in silico* with significant drug-likeness derived from *R. indica* leaves.**

| Compound | Mol.Wgt (g/mol) | $H_2O$ solubility | H-bond acceptors | H-bond donors | Lipophilicity* (Log $P_{o/w}$) | GI absorption | Drug-likeness** |
|---|---|---|---|---|---|---|---|
| Vitexin | 432.38 | Soluble | 10 | 7 | 1.63 | Low | Yes (1violation: NHorOH>5) |
| Ellagic acid | 302.19 | Soluble | 8 | 4 | 0.79 | High | Yes (0 violation) |
| Asiatic acid | 488.70 | Poorly soluble | 5 | 4 | 3.20 | High | Yes (0 violation) |
| Retronecine | 155.19 | Highly soluble | 3 | 2 | 1.36 | High | Yes (0 violation) |
| Maritimetin | 286.24 | Soluble | 6 | 4 | 1.70 | High | Yes (0 violation) |
| N-(2,14-Eicosadienoyl) Piperidine | 375.63 | Poorly soluble | 1 | 0 | 6.06 | Low | Yes (1violation: MLOGP>4.15) |
| γ-Chaconine | 559.78 | Poorly Soluble | 7 | 4 | 4.47 | High | Yes (1violation: MW>500) |
| Santin | 344.32 | Moderately soluble | 7 | 2 | 3.25 | High | Yes (0 violation) |
| Irinotecan | 586.68 | Moderately soluble | 8 | 1 | 4.95 | High | Yes (1violation: MW>500) |
| Ganoderic acid F | 570.67 | Moderately soluble | 9 | 1 | 2.58 | Low | Yes (1 violation: W>500) |
| Euphornin | 584.70 | Poorly soluble | 9 | I | 4.70 | Low | Yes (1 violation: MW>500) |

*Partition coefficient between n-octanol and water (log $P_{o/w}$); **As per LipinskiRO5.

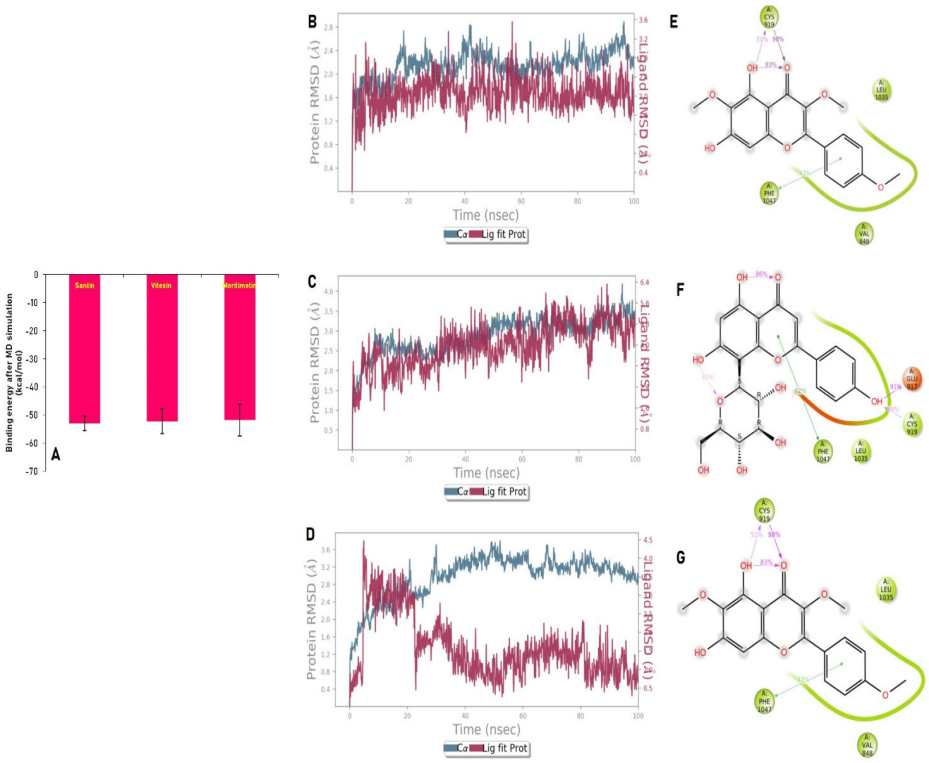


**Figure 3:** Protein and ligand RMSD changes observed during the 100 ns MD run and the characteristics of protein-ligand contacts. A: Binding energy of Santin, maritimetin and Vitexin after MD simulation; B: RMSD changes of Santin Interaction with VEGFR2, C:- RMSD changes of Vitexin Interaction with VEGFR2, D: RMSD changes of Maritimetin Interaction with VEGFR2. E: Santin-VEGFR2 Contacts, F: Vitexin- VEGFR2, G: Maritimetin -VEGFR2Contacts. FPO Pi-Pi stacking Hydrophobic Solvent exposure Water Charged Negative).

**Table 4: Binding score of potential compounds from *R. indica* leaf after docking.**

| Compound | Binding Score (Kcal/mol) | Type of interaction |
|---|---|---|
| Santin | -11.336 | Leu 840, Lys 868, Cys 919 (H-bonding) |
| Maritimetin | -11.384 | Leu 840, Cys 919, Asp 1046 (H-bonding) Phe 1047 (pi-pi stacking) |
| Vitexin | -7.387 | Asn 923, Lys 920, Glu 850 (H-bonding) |
| Ellagic acid | -6.597 | Cys 919 (H-bonding) |
| Euphornin | -5.656 | Van der waals force |
| PF-00337210(known inhibitor)* | -7.387 | Glu 885, Asp 1046 (H-bonding), Lys 868, Phe 1047(pi-pi stacking) |

*Known Inhibitor of VEGFR2.

compound found, stingin, a flavone, was discovered to have a significant cytotoxic effect on colon cancer cells (SW480 and SW620) by inducing TRAIL-mediated apoptosis and causing damage to the mitochondrial membrane.[35]A derivative of camptothecin called irinotecan targets topoisomerase 1 to stop advanced or metastatic solid tumors such as pancreatic, colon and stomach cancers, among others.[36] In HeLa cells, euphornin macrocyclic diterpenoid induced G2/M cell cycle arrest by increasing the quantity of the phospho-CDK1 (Tyr15) protein. Consequently, this increased the rate of death through the caspase and mitochondrial pathways.[37]

There are further compounds that have been found to have strong immunological and neuroprotective properties. By preventing oxidative stress-mediated MAPK activation, Sanguiin H-11 (SH-11), a hydrolyzable ellagitannin, has been demonstrated to have a potent neuroprotective impact against glutamate-mediated apoptotic cell death. Moreover, it prevents nitric oxide synthesis and neutrophil migration.[38] (Supplementary Tables 1 and 2).

Isoterchebin, a gallo tannin, demonstrated concentration-dependent suppression of peroxynitrite-mediated protein tyrosine nitration.[39] It also showed considerable inhibition of BACE1 and ChE, suggesting that it could be useful as a novel multi-targeted molecule for anti-AD therapies. It has been demonstrated that the naturally occurring chemical ellagic acid possesses anti-oxidant, anti-cancer, neuroprotective and anti-aging qualities.[40,41]According to Zhang *et al*, myricitrin, a flavone, has potent anti-oxidative, anti-inflammatory and anti-nociceptive properties that help shield a range of cells from harm both *in vivo* and *in vitro*.[42] Therapeutics containing luteolin-4'-O-glucoside may be created to treat gouty arthritis and hyperuricemia.[43] Lutein-4'-O-glucoside prevents eosinophilia-associated allergic inflammation by acting as an eosinophil chemotactic factor (IL)-5, which further enhances eosinophil proliferation and survival.[44]

In cultured mouse peritoneal macrophages, lipopolysaccharide-induced NO generation was inhibited by corchorifatty acids A, B and C.[45] Astragal acid has shown great potential in treating a variety of neurological disorders, such as Spinal Cord Injury (SCI), cerebral ischemia, epilepsy, Traumatic Brain Injury (TBI), neural tumors, Alzheimer's disease (AD) and Parkinson's disease (PD).[46] A triterpenoid called deacetyl ganoderic acid F (DeGA F) showed impressive anti-inflammatory properties and therapeutic promise for disorders linked to brain inflammation.[28]

The Three complexes, Santin, Maritimetin and Vitexin, subjected to MD showed stability during drug interaction. This suggests that the protein maintains stability while interacting with the compounds within the active site. Among the analyzed compounds, santin showed excellent binding stability, below 2.8 Å RMSD in both protein and ligand deviation. The others, marimetin and vitexin didn't show any significant structural change and RMSD change was recorded as below 3.5. The MD studies in the explicit solvent system revealed the three compounds showed good stability during the entire run in terms of structure and bonding. After the MD run, MM-GBSA was used to calculate the binding energy of particular frames from the MD trajectory. For this experiment, the structure of every frame covering 10 ns of the entire 100 ns journey were used. The obtained binding energies were averaged and their standard deviations calculated. In terms of binding energy, three compounds exhibited higher binding energies during the entire MD run. As Santin was the most effective candidate to bind with the VEGFR2 kinase domain in docking and RMSD analysis; the binding energy was determined to be -53.0 kcal/mol. The others, marimetin and vitexin also showed excellent binding potential during the MD study and the binding energy calculated was -51.87 and -52.28 kcal/mol. The blocking of VEGFR2 with these compounds should be evaluated in detail in *in vivo* systems and can be developed as therapeutic phyto-pharmaceuticals in future.

## CONCLUSION

In conclusion, *R. indica* extract contains several bioactive components with anti-microbial, antioxidant and cytotoxic activity with few showing high levels of druggability properties as evident from the ADME and molecular docking studies. The binding of santin, maritimetin and vitexin to VEGFR2 has not been proved *in vivo* or *in vitro* using cell culture systems as well as the binding kinetics, which is an interesting subject for future research. Separation and functional annotation of these

individual components in detail and adding these compounds/extracts in combination with other drugs may be effective in developing therapeutically beneficial medicine for human and veterinary use.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHOR'S CONTRIBUTIONS

S H, K G N: Conceptualization, Experimental Design, Manuscript first draft writing, reviewing and editing A G: Designed and executed *in silico* analysis and manuscript writing, N J: Antimicrobial studies, quantification of phyto components and manuscript writing.

## ABBREVIATIONS

**HRLCMS-QTOF:** High resolution Liquid Chromatography-Mass spectrometry Quadrupole Time-of-Flight; **ADME:** Absorption, Distribution, Metabolism, Excretion; **VEGFR2:** Vascular endothelial growth factor receptor 2; **MM/GBSA:** Molecular mechanics with generalized Born and surface area solvation; **RMSD:** Root Mean Square Deviation; **TBI:** Traumatic brain injury; **AD:** Alzheimer's disease; **PD:**Parkinson's disease; **BACE1:** Beta-secretase 1; **ChE:** Cholinesterase; **MAPK:** Mitogen-activated protein kinases; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **ABTS:** 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; **FRAP:** Ferric reducing ability of plasma; **SPSS:** Statistical Package for the Social Sciences; **OPLS:** Orthogonal partial least squares; **ATCC:** American Type Culture Collection; **MTCC:** Microbial Type Culture Collection; **NO:** Nitric oxide; **TFAM:** Mitochondrial transcription factor A; **GI**-Gastrointestinal tract.

## SUMMARY

This paper deals with elucidating the biological activity and the potential components in *Rosa indica* leaf extract, through phytochemcal screening, HRLCMS-QTOF, *in vitro* biological and *in silico* assays. The study established that the *R. indica* extract contains several potent molecules with antioxidant, anti-bacterial and anticancer properties. *In silico* analysis showed that some of the molecules present, have a strong binding towards VEGFR2 which could be utilised as a possible target for its anti breast cancer activity after detailed studies in future.

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## Supplementary

**Supplementary Table 2: Compounds identified in *R. indica* leaves using HRLCMS -ve mode.**

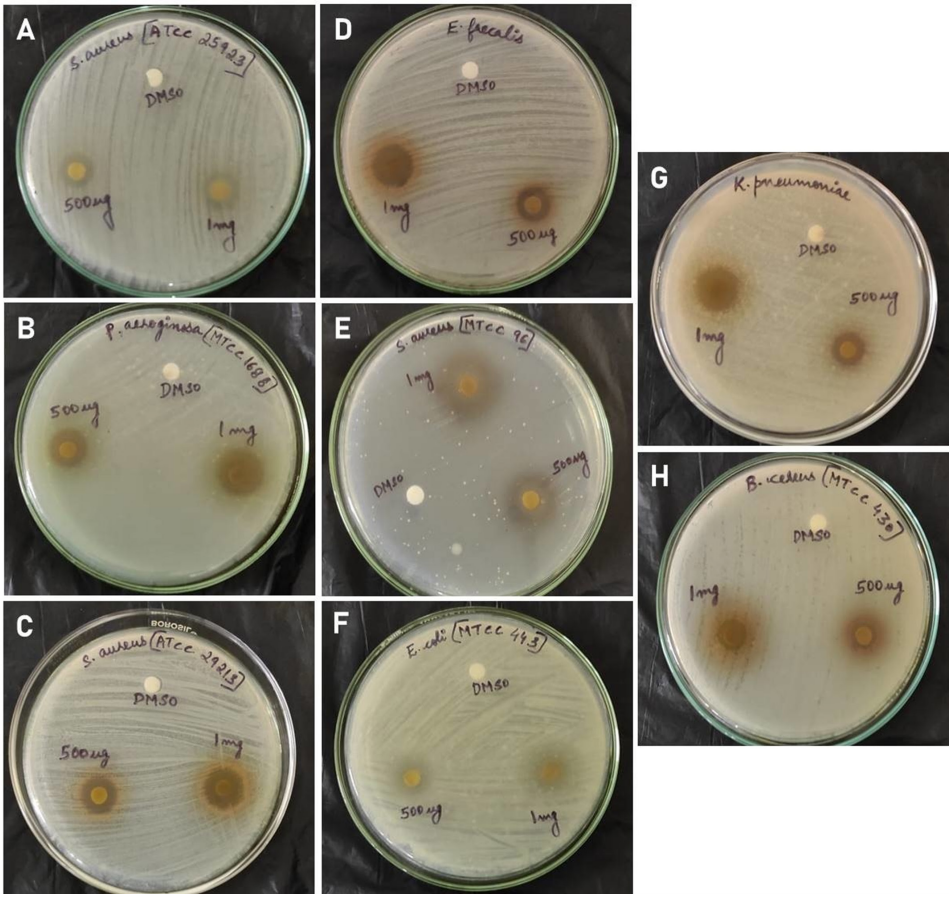
| Sl. No. | RT | Mass | Name | Formula |
|---|---|---|---|---|
| 1 | 1.204 | | | |
| 2 | 3.881 | 952.0827 | Sanguiin H11 | $C_{41}H_{28}O_{27}$ |
| 3 | 4.33 | 786.092 | Heterophylliin A | $C_{34}H_{26}O_{22}$ |
| 4 | 4.595 | 634.0823 | Punicacortein B | $C_{27}H_{22}O_{18}$ |
| 5 | 4.898 | 786.0929 | Heterophylliin A | $C_{34}H_{26}O_{22}$ |
| 6 | 4.981 | 952.0812 | Sanguiin H11 | $C_{41}H_{28}O_{27}$ |
| 7 | 5.162 | 954.0977 | Isoterchebin | $C_{41}H_{30}O_{27}$ |
| 8 | 5.409 | 786.0906 | Heterophylliin A | $C_{34}H_{26}O_{22}$ |
| 9 | 5.688 | 1570.1694 | Heterophylliin F | $C_{68}H_{50}O_{44}$ |
| 10 | 6.136 | 616.1073 | Quercetin 3-(2- galloylglucoside) | $C_{28}H_{24}O_{16}$ |
| 11 | 6.367 | 302.0058 | Ellagic acid | $C_{14}H_{6}O_{8}$ |
| 12 | 6.469 | 610.1537 | Quercetin 3-rhamnoside-7- glucoside | $C_{27}H_{30}O_{16}$ |
| 13 | 6.471 | 464.0967 | Myricitrin | $C_{21}H_{20}O_{12}$ |
| 14 | 6.509 | 616.1071 | Quercetin 3-(2- galloylglucoside) | $C_{28}H_{24}O_{16}$ |
| 15 | 6.733 | 302.0058 | Ellagic acid | $C_{14}H_{6}O_{8}$ |
| 16 | 6.806 | 464.0954 | Myricetin 7-rhamnoside | $C_{21}H_{20}O_{12}$ |
| 17 | 6.943 | 424.1713 | Chitobiose | $C_{16}H_{28}N_{2}O_{11}$ |
| 18 | 7.126 | 448.1023 | Luteolin 4'-O-glucoside | $C_{21}H_{20}O_{11}$ |
| 19 | 7.322 | 302.0073 | Ellagic acid | $C_{14}H_{6}O_{8}$ |
| 20 | 7.461 | 448.1019 | Luteolin 4'-O-glucoside | $C_{21}H_{20}O_{11}$ |
| 21 | 7.84 | 432.1067 | Vitexin | $C_{21}H_{20}O_{10}$ |
| 22 | 10.003 | 328.2241 | Corchorifatty acid F | $C_{18}H_{32}O_{5}$ |
| 23 | 10.34 | 328.2237 | Corchorifatty acid F | $C_{18}H_{32}O_{5}$ |
| 24 | 13.209 | 488.3478 | Asiatic acid | $C_{30}H_{48}O_{5}$ |
| 25 | 13.593 | 488.3479 | Asiatic acid | $C_{30}H_{48}O_{5}$ |

RT Retention time.

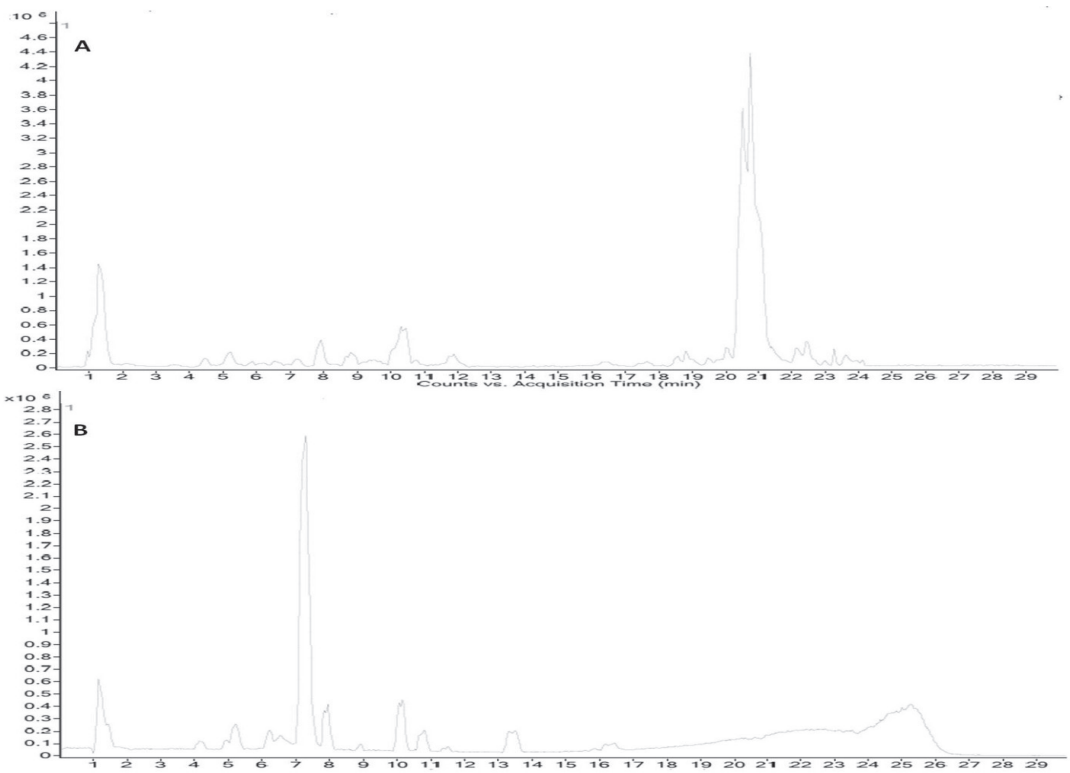
**Supplementary Table 1: Compounds identified in *R. indica* leaves using HRLCMS +ve mode.**

| Sl. No. | RT | Mass | Compound | Formula |
|---|---|---|---|---|
| 1 | 1.218 | 155.0945 | Retronecine | $C_8H_{13}NO_2$ |
| 2 | 1.516 | 155.0942 | Retronecine | $C_8H_{13}NO_2$ |
| 3 | 6.15 | 616.1033 | Quercetin 3-(2- galloylglucoside) | $C_{28}H_{24}O_{16}$ |
| 4 | 7.166 | 448.0986 | 6-C-Galactosylluteolin | $C_{21}H_{20}O_{11}$ |
| 5 | 7.74 | 286.0464 | Maritimetin | $C_{15}H_{10}O_6$ |
| 6 | 8.106 | 286.0462 | Maritimetin | $C_{15}H_{10}O_6$ |
| 7 | 8.78 | 375.3505 | N-(2,14-Eicosadienoyl)piperidine | $C_{25}H_{45}NO$ |
| 8 | 8.782 | 559.3846 | gamma-Chaconine | $C_{33}H_{53}NO_6$ |
| 9 | 8.837 | 705.4415 | beta1-Chaconine | $C_{39}H_{63}NO_{10}$ |
| 10 | 11.93 | 290.1866 | (9Z,11E,13E,15Z)-4-Oxo-9,11,13,15-octadecatetraenoic acid | $C_{18}H_{26}O_3$ |
| 11 | 12.235 | 344.0876 | Santin | $C_{18}H_{16}O_7$ |
| 12 | 19.681 | 586.2782 | Irinotecan | $C_{33}H_{38}N_4O_6$ |
| 13 | 19.914 | 602.273 | Oxidized dinoflagellate luciferin | $C_{33}H_{38}N_4O_7$ |
| 14 | 20.022 | 586.2785 | Irinotecan | $C_{33}H_{38}N_4O_6$ |
| 15 | 20.302 | 570.2841 | Ganoderic acid F | $C_{32}H_{42}O_9$ |
| 16 | 20.35 | 586.2784 | Irinotecan | $C_{33}H_{38}N_4O_6$ |
| 17 | 20.616 | 570.2842 | Ganoderic acid F | $C_{32}H_{42}O_9$ |
| 18 | 20.932 | 570.2841 | Ganoderic acid F | $C_{32}H_{42}O_9$ |
| 19 | 21.209 | 570.2841 | Ganoderic acid F | $C_{32}H_{42}O_9$ |
| 20 | 21.558 | 570.2835 | Ganoderic acid F | $C_{32}H_{42}O_9$ |
| 21 | 22.034 | 584.2987 | Euphornin | $C_{33}H_{44}O_9$ |
| 22 | 22.4 | 584.299 | Euphornin | $C_{33}H_{44}O_9$ |
| 23 | 22.781 | 584.2984 | Euphornin | $C_{33}H_{44}O_9$ |
| 24 | 22.939 | 562.4356 | 2-Hexaprenyl-3-methyl-6-methoxy-1,4-benzoquinol | $C_{38}H_{58}O_3$ |
| 25 | 23.263 | 600.4141 | 3'-N-Acetyl-4'-O-(14-methylheptadecanoyl)fusaroch romanone | $C_{35}H_{56}N_2O_6$ |
| 26 | 23.285 | 562.4357 | 2-Hexaprenyl-3-methyl-6-methoxy-1,4-benzoquinol | $C_{38}H_{58}O_3$ |

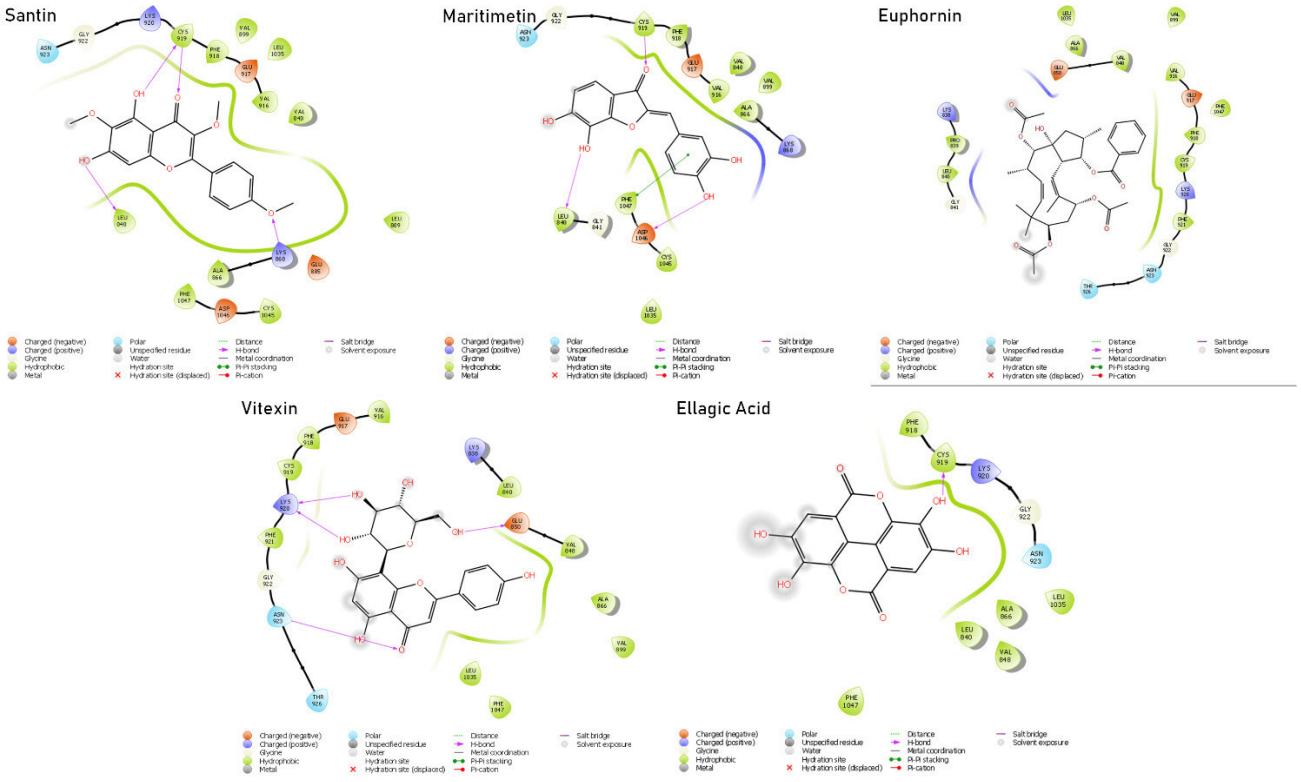
RT Retention time.




**Supplementary Figure 1:** Effect of methanol: acetone extract of R. indica leaf extract against different human pathogens using disk diffusion method. A: S. aureus (ATCC 25923), B: P. aeroginosa, C: S. aureus (ATCC 29213), D: E. faecalis, E: S. aureus (MTCC 96), F: E.coli, G: K. pneumoniae, H: B. cereus.




**Supplementary Figure 2:** HRLC-MS-QTOF analysis of Rosa indica leaf extract.




**Supplementary Figure 3:** Degree of binding affinity of Santin, Maritimetin, vitexin, ellagic acid and Euphornin with VEGFR.