In-vitro, in-vivo and*in-silico* studies on the phytoconstituents of the plants *Rubiacordifolia* and *Pimentadioica*

MadhuKini, Nida S Khan, Tapasya B Singh

Students of Final Year
Department of Biotechnology,
[1]The Oxford College of Engineering, Bangalore, India

***Abstract*:**The present work aims at providing scientific validation forethno-medicinal claims of *Rubiacordifolia* and *Pimentadioica*. Theethno-medicinal claims for the above mentioned plants reveals that,these plants process potent anti-inflammatory, rejuvenators andanti-cancer property.. In the presence study crude drug is prepared using soxhlet process, the anti-inflammatory studies were done through albumin denaturation and protein inhibition studies and cytotoxicity through MTT assay using MCF-7 cell line study.The study reviled that the plant *P.dioica and R.cordifolia*process potent albumin denaturation ability(0.5mg/ml and 1mg/ml) and protein inhibitory activity(0.5mg/ml and 1.5mg/ml) .MTT assay on MCF-7 cell lines reveals that both the plants possess anti-proliferative property at the range of 100µg/ml with% IC_{50}value 34.13191of *R.cordifolia* showed highest anti-proliferative activity against MCF-7 cell line. In order to screen affinity characteristics of anti-inflammatory, wound healing, antibacterial activity (gram positive and negative) of *R.cordifolia* and *P.dioicab* natural product. To understand the molecular mechanism, Insilco studies were done and the study reviled that they have good affinity for the target.Out of 8 ligands, 3 had good affinity score towards gram +ve(of *S.aureus*)), garam –ve (*E.c*oli) bacteriaand GSK 3 β wound healing target(Homo sapiens). The ligands such as BetacaryophylllenePurpurinQuercetinhaave shown good affinity scoreas well as better ADMET properties. by using ADMET SAR software .

Key words: *Rubia cordifolia* ,*Pimenta dioica* , anti-inflammatory properties, anti-cancer, ADMET SAR software.

I. INTRODUCTION

Rubia cordifolia, also known as, Manjishtha, Indian madder, distributed throughout India [7]. It is found throughout the hilly districts of India from northwest Himalayas eastwards, ascending to 8000ft and southwards to Ceylon. The roots of this plant are of high medicinal value and are recognized as official [8]. *Rubia cordifolia* is an important medicinal plant which is used for treatment of various ailments in the Ayurvedic system of medicine. The biological investigations have shown that many of the medicinal properties claimed for the plant in the historical texts do, indeed, have sound scientific basis .It has a variety of uses such as blood purifier, immune-modulator[11], anti-inflammatory [12] and antioxidant [13]. It is helpful in treating skin diseases,

Pimenta dioica Allspice is derived from the plant Pimento dioica , and is called allspice because of its unique flavor, which is a combination of cinnamon, cloves, ginger, and nutmeg [2]. Allspice is also obtained from another plant closely related to P.dioica called *Pimenta racemose*, mostly found in Central America [3]. Allspice is predominantly found in Jamaica, Mediterranean area and Asia. Some of the secondary metabolites include terpenoids, alkaloids, polyphenols, and glycosides [4]. They are also inhibitors of enzymatic activities in living organisms such as inflammatory enzymes like cyclooxygenase-2 [5].Eugenol is a phenyl propene predominantly found in clove oil, cinnamon, ginger, nutmeg and has anti-bactericidal, and ant- inflammatory effect [6]. Review of the literature reveals that, these plants are less studied for the claimed activity. In this context, in the present study an attempt is made to provide scientific validation for the ethnomedicnal claims of the mentioned plant genetic resources.

Methods and materials

1. **Collection and processing of the plaant samples:**The leaves of *P diocia*, and bark of Rcordifoliea were collected near the western ghats of Karnataka near Sringeri region .These plants leaves and tree barkwas washed, shade dried and powdered. The powder was preserved in air sealed polythene bag for further evaluation.

2. **Preparation of plant extract**
   The plant parts were shade dried, powdered mechanically and material was defatted with petroleum ether (30-50°C) by hot extraction method in a soxhlet apparatus. The defatted powder materials were further extracted with methanol and concentrated methanol extracts were used for the analysis. The extracts were concentrated using Rotavapor at 40° C under reduced pressure and stored in dessicator for furtherr use.

3. Biological Screening:

3.1Anti-inflammatory assay:anti-inflammatory assay for crude drug, fraction and isolated compounds were studied using

- **Inhibition of albumin denaturation**: The reaction mixture (1ml) was consisting of test extract at different concentrations and 1% aqueous solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of 1N HCl. The samples were incubated at 37°C for 20 minutes and then heated for 57°C for 20 minutes, After cooling the samples, the turbidity was measured spectrophotometrically at 660nm [1]. The experiment was performed in triplicate. Percentage of protein denaturation was calculated as follows :

$$Percentage\ inhibition = 1 - \frac{Asample}{Acontrol} * 100$$

- **Proteinase inhibitory action**: The test was performed according to the modified method of Oyedepo. The reaction mixture (2ml) was containing 0.06mg trypsin, 1ml 20 mMTrisHCl buffer (pH 7.4) and 1ml test sample of different concentrations. The mixture was incubated at 37°C for 5 minutes and then 1ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 minutes. 2ml of 70% per-chloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210nm against buffer as blank[1]. The experiment was performed in triplicate. The percentage inhibition of Proteinase inhibitory activity was calculated.

$$Percentage\ inhibition\ (\%) = 1 - \frac{OD\ of\ sample}{OD\ of\ control} * 100$$

3.2 Anti-cancer activity:

Cytotoxicity study using MTT assay on MCF7 cell line

MTT assay is an well established method to study the cytotoxicity, the reduction of tetrazolium salts is widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. The assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability. The assay was done following the method mentioned in Benencia, et al., 2009 with modification, in brief.

1. The cells were trypsinized and aspirated into a 50ml centrifuge tube. Cell pellet was obtained by centrifugation at 300 x g. The cell count was adjusted, using DMEM Low Glucose medium containing 10% FBS, such that 200µl of suspension contained approximately 15,000 cells.
2. To each well of the 96 well microtitre plate, 200µl of the cell suspension was added and the plate was incubated at 37^{o}C and 5% CO_2 atmosphere for 24 h.
3. After 24 h, the spent medium was aspirated. 200µl of different test concentrations of test drugs were added to the respective wells. The plate was then incubated at 37^{o}C and 5% CO_2 atmosphere for 24 h.
4. The plate was removed from the incubator and the drug containing media was aspirated. 200µl of medium containing 10% MTT reagent was then added to each well to get a final concentration of 0.5mg/ml and the plate was incubated at 37^{o}C and 5% CO_2 atmosphere for 3 hr
5. The culture medium was removed completely without disturbing the crystals formed. Then 100µl of solubilisation solution (DMSO) was added and the plate was gently shaken in a gyratory shaker to solubilize the formed formazan.
6. The absorbance was measured using a microplate reader at a wavelength of 570 nm and also at 630 nm. The percentage growth inhibition was calculated, after subtracting the background and the blank, and concentration of test drug needed to inhibit cell growth by 50% (IC_{50}) was generated from the dose-response curve for the cell line.

4. IN SILICO MOLECULAR DOCKING:

The receptor molecule of *S.aureus*, *E.coli*and *Homo sapiens* origin were retrieved from PDB (protine database).the ligand molecule for each plant was retrieved from the PubCem.The ligands selected wereBeta-caryophyllene, Purpurin and Quercetin For the plant *Rubiacordifolia* and *Pimentadioica* respectively.The receptor and ligand molecule were selected and their fee availability and feasibility to convert the fail format of the ligand to one that is compatible with the docking software .the ligand and the receptor were uploaded in the autodoc software and result were viewed and photographed using PyMOL viewer.

Results and discussion

1. Anti-inflammatory assay

Inhibition of albumin denaturation: Protein denaturation has been employed as an in-vitro screening method for anti-phlogistic agents. Drug binding to plasma albumin may inhibit thermal denaturation of albumin which perhaps block ø-NH2 groups in case

of histidine decarboxylase or may displace urate from albumin. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of extracts, protein denaturation was studied. The methanolic extracts showed the higher percentage inhibition at(0.5mg/mland 1mg/ml) for both *P.diocia and R.cordifolia*. Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of extracts, protein denaturation was studied. The methanolic extracts showed the higher percentage inhibition (%). The study revealed that*P.dioica*the maximum inhibition at 0.5mg/ml and *R.cordifolia* at 1mg/ml of concentration

a) **Proteinase inhibitory action**: Proteinases have been implicated in the arthritic reactions. Neutrophils are known to be a rich source of proteinase which carries in their lysosomal granules many serine proteinases.It was previously reported that leukocyte proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitory. The study was conducted for the methanolic extracts. The methanolic extracts showed higherihiniton activity at(1.5mg/ml and 0.5mg/ml)of extract *Rubiacordifolia* and *Pimentadioica*. It was previously reported that leukocyte proteinase play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitory. The study reveals that, *P.diocea* has highest activity at o.5mg/ml and *R.cordifolia* at 1.5mg/ml.

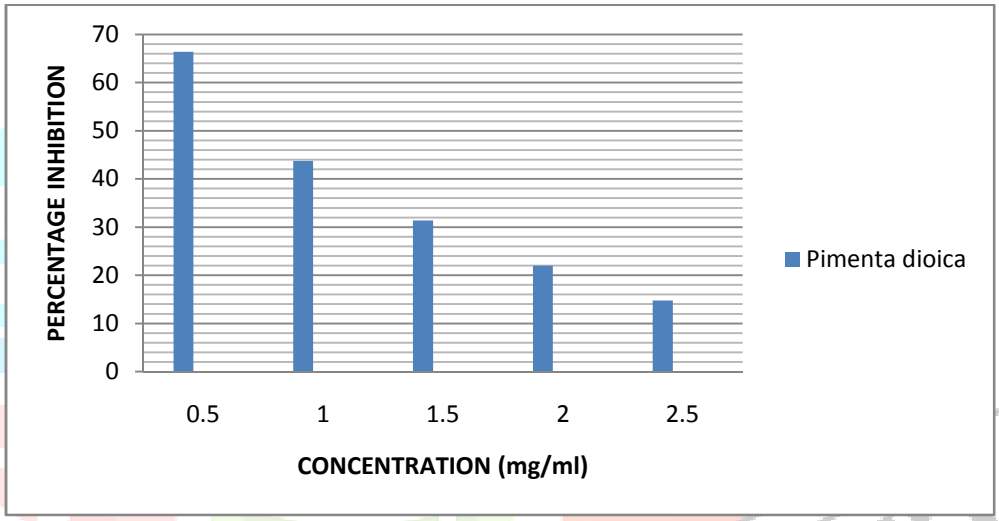
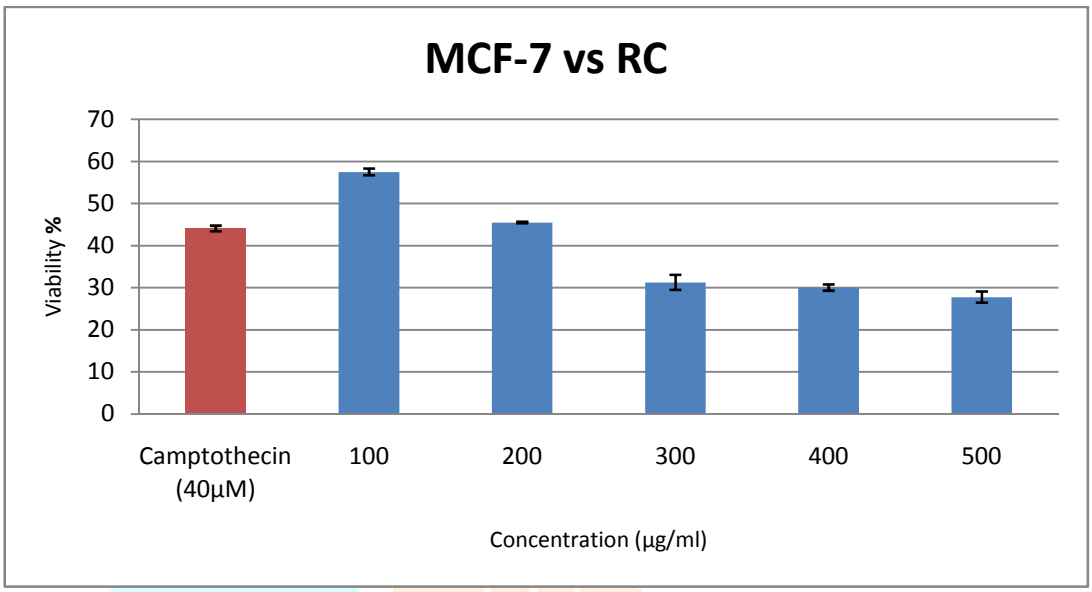


Figure 4: Protein inhibitory action of *Pimenta dioica*

2) MTT Assay:MCF-7 cell line vs *Rubiacordifolia*

The study reveals that, the standerd drug Camptothecin at 40µM showsviability percentage of 44.067% however the test drug *Rubiacordifolia*at 200 µg/ml concentration showed the closest viability percentage compared to the standard drug i.e., 45.48%. with IC_{50} valueIC_{50}144.085µg/ml.as shown in the table…….

MCF-7 cell line vs RC				Concentration Unit: µg/ml				
	BLANK	UNTREATED	Camptothecin (40µM)	100	200	300	400	500
Reading 1	0.006	0.534	0.242	0.305	0.247	0.18	0.16	0.159
Reading 2	0.003	0.537	0.235	0.314	0.245	0.161	0.168	0.145
Mean	0.0045	0.5355	0.2385	0.309727	0.246	0.1705	0.164	0.152
Mean OD-Mean B	NA	0.531	0.234	0.305227	0.2415	0.166	0.1595	0.1475
STANDARD DEVIATION		0.00212132	0.004949747	0.006043	0.001414	0.013435	0.005657	0.009899
STANDARD ERROR		0.001500227	0.003500529	0.004273	0.001	0.009501	0.004001	0.007001
		0.282528541	0.659233262	0.804778	0.188352	1.789347	0.753409	1.318467
Viability %	NA	100	44.06779661	57.4816	45.48023	31.26177	30.03766	27.77778



3.Insilico studieds : The in silico work was carried out for two of the most pathogenic orgsnisum such as E.coli and S. aureus, wound healing target from Homo sapiens and Brest cancer target . LT enterotoxin and ISDH linker domain were the receptors for E.coli and S .aureus respectively. These receptors and ligand were docked using Autodoc vena 4.0 software and fowling result were obtained .It was found that the ligand β-caryophyilinefrom the plant *Pimentadiocia*and Quercetin from the plant*Rubiacordifolia have shown good affinity score towods the active binnding site* .

TABLE 1:In silico molecular docking result for receptor of *S.aureus:*

PLANT	LIGAND	CID	MOLECULAR WEIGHT (g/wt)	MOLECULAR FORMULA	logp	HBD	HBA	SCORE (1T2P)*S.aureus*
Pimentadiocia	β-caryophyiline	5281515	204,19	$C_{15}H_{24}$	6.044	0	0	-7.5
Rubiacordifolia	Quercetin	*5280343*	*302.04*	$C_{15}H_{10}O_7$	*1.834*	*5*	*7*	*-7.5*
	Purpurin	*6683*	*256.04*	$C_{14}H_8O_5$	*0.976*	*3*	*5*	*-7.1*

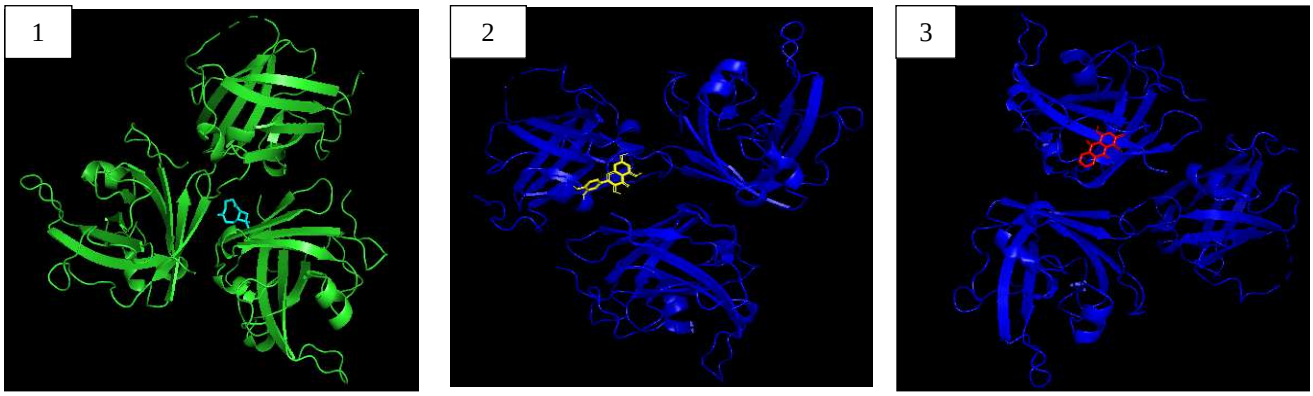


Fig:*G+VEβ-caryophylleneFig: G+VE* purpurinFig: G+VEQuercetin

TABLE 2:In silico molecular docking result for receptor of E.coli

PLANT	LIGAND	CID	MOLECULAR WEIGHT (g/wt)	MOLECULAR FORMULA	logp	HBD	HBA	SCORE E.coli
Pimentadiocia	β-caryophyiline	5281515	204,19	$C_{15}H_{24}$	6.044	0	0	-5.5
Rubiacordifolia	Quercetin	*5280343*	*302.04*	$C_{15}H_{10}O_7$	*1.834*	*5*	*7*	*-7.8*
	Purpurin	*6683*	*256.04*	$C_{14}H_8O_5$	*0.976*	*3*	*5*	*-7.3*

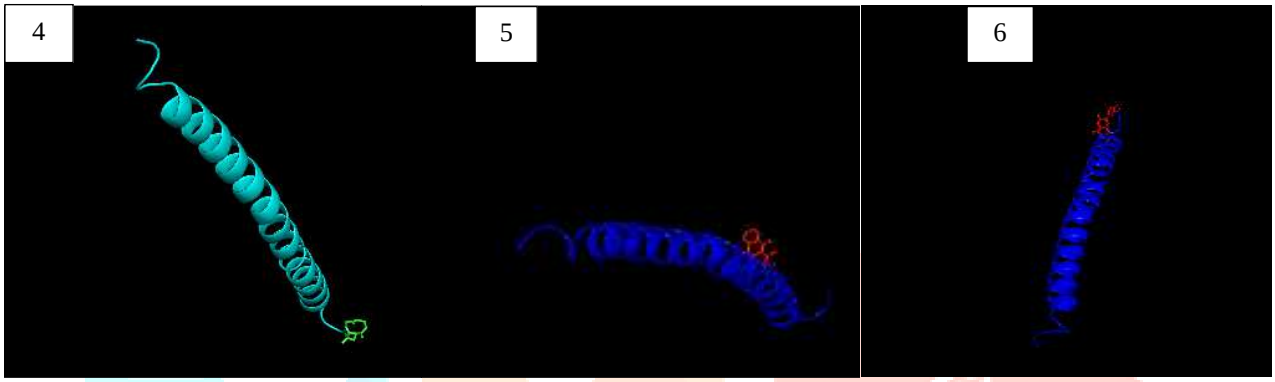


Fig 5:G-ve*ß-caryophyllene* **Fig 6: *G-ve*purpurin** **Fig 7: G-veQuerecetin**

TABLE 3:In silico molecular docking result for receptor of wound healing *:*

PLANT	LIGAND	CID	MOLECULAR WEIGHT (g/wt)	MOLECULAR FORMULA	logp	HBD	HBA	SCORE *Wound healing*
Pimentadiocia	β-caryophyiline	5281515	204,19	$C_{15}H_{24}$	6.044	0	0	-5.6
Rubiacordifolia	Quercetin	*5280343*	*302.04*	$C_{15}H_{10}O_7$	*1.834*	*5*	*7*	-8.2
	Purpurin	*6683*	*256.04*	$C_{14}H_8O_5$	*0.976*	*3*	*5*	-8.5

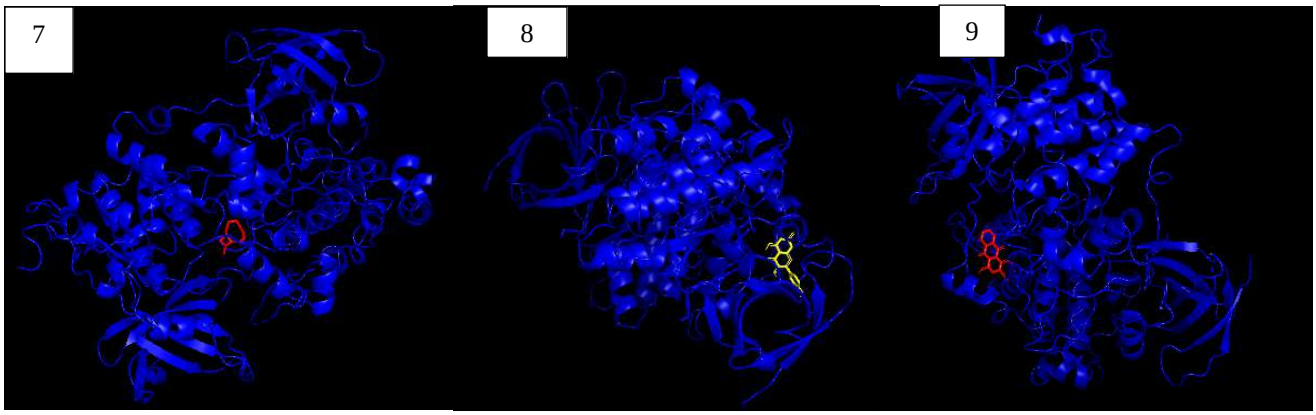


Fig7: Wound healing***ß-caryophyllene*** **Fig 8:**Wound healingpurpurin **Fig 9:** wound healing Querecetin

CONCLUSION:

- From the present study it can be concluded that the medicinal plants tested viz., *Pimenta diocia*and *Rubia cordifolia* possess anti-inflammatory activity found to be higher in the methanol extracts. In vitro cell line based MTT assay using MCF-7 cell lines revealed that, the extract possess potent anticancer activity with IC_{50} values of the test compounds found to be*Rubia cordifolia* (144.085 µg/ml) ,in order to reduse the expenxes of durg In silico studies was conducted to 10 ligands from eachplant*Pimenta diocia*and *Rubia cordifolia*out of 8different plant 3 molecule showed good affinity value towards the target binding pocket .

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