SCREENING OF POTENTIAL PHYTOCHEMICALS FOR WOUND HEALING

[1]*Dr. B.K. Manjunatha*[*], [2]*Tanusree Chaudhuri*, [3]*B.S. Rithu*, [4]*K Soundarya Vadhulas*, [5]*Harish.M*

[1] *Professor and Head of Department* ,[2]*Assistant professor*, [3,4,5] *Student*

Department of Biotechnology,

The Oxford College of Engineering, Bengaluru, Karnataka-560068, India.

Abstract: Traditional therapies, including the use of dietary components for wound healing and skin regeneration, are very common in Asian countries such as China and India. The increasing evidence of health-protective benefits of phytochemicals, components derived from plants is generating a lot of interest, warranting further scientific evaluation and mechanistic studies. Among different plants showing positive activity towards wound healing, Capparis spinosa-C. *spinosa* has many active constituents such as flavonoids such kaempferal and quercetin . Nature fruits of C. *spinosa*have glucose as 1-H Indole-3-acetonitrile etc, whereas *C. zeylanica* has reported to have fatty acids such as E-octodec-7enynoic acid isolated from chloroform extract of the roots . Extracts of C. decidua stems and flowers showed insecticidal and oviposition inhibitory activities against Bruchus chinensis. the photochemical from these plant extracts were found to possess significant wound healing promoting activity. In the present study, we have identified 12 potent active constituents viz., Capparispine, Glucocapparin, kaempferol, kaempferol-7-rhamnoside, Polyprenols, Proline betaine, Quercetin, Quercetin-3-rutinoside, Rhamnetin, Rutin, Saccharose, Sinigrin for wound healing promoting ability. These active constituents were subjected for docking study using a well established target GSK 3 Beta responsible for wound healing process in human. Our results, indicated that Capparispine, Glucocapparin, kaempferol, Polyprenols, Quercetin, Quercetin-3-rutinoside, Rhamnetin have a potent wound healing property among other compounds. In vivo/ in vitro studies to validate the present finding and to understand the exact mechanism and potential targets of these phytochemicals are under process.

Keywords*:* Wound healing, Ethanol leaf extract, *C. decidua*, *C. spinosa* and *C. Zeylanica*, Antimicrobial activity, Docking.

I. INTRODUCTION

Wound infection has become a major medical distress in recent years. Wound is defined simply as the disruption of the cellular and anatomic continuity of a tissue. Wound may be originated by physical, chemical, thermal, microbial or immunological insult to the tissue. Wound healing is a structured biological process that restores tissue continuity after injury and is a combination of physical, chemical and cellular events that recreate the wounded tissue or replace it with collagen. Wound healing can be divided into three stages, including inflammation, proliferation and re-modelling and maturation phases which includes the interaction of various cells, cytokines and growth factors. The normal healing starts immediately after the injury. When blood spills at the site of injury, the blood platelets interact with collagen and other components of the extracellular matrix. This stimulates the release of clotting factors as well as essential growth factors and cytokines such as platelet-derived growth factor (PDGF) and transforming growth factor beta (TGF-β). The inflammatory phase begins after the migration of neutrophils to the wound site to clean the tissue. The fibroblasts migrate into the tissue to unfold into the proliferative phase and deposit new extracellular matrix. This new collagen matrix gets cross linked and organized.

Capparis is one of the important genus of the family Capparidaceae. Leaves of *Capparis decidua* are used as plaster for boils and swellings, to relieve tooth ache, as antidote to poison, stem bark as laxative, anthelmintic, in treating cough, asthma and inflammation, fruits in cardiac troubles, root and root bark in fever and rheumatism (Chopra, et al., 1956), fruits are known as appetizer, stem bark is used in the treatment of cardiac diseases, whole plant is used in debility, joint pains, pyorrhoea, rheumatism, skin disorders (Keshava Murthy, 1994), fruits and shoots are reported for hypolipidaemic (Purohit and Vyas, 2005), antistress and antidiabetic (Yadav, et al., 1997), anti-inflammatory activity.

Leaves of *C. spinosa* are used as poultice in gout, in nervous disorders, stem bark is used in treating paralysis, rheumatism, tooth ache and tuberculosis (Keshava Murthy, 1994), root bark as tonic, diuretic, expectorant, anthelmintic, analgesic, in rheumatism, paralysis, enlarged spleen and tubercular glands (Chopra, et al., 1956), antihepatitis, anti-inflammatory, antifungal, antidiabetic, in treating chondrocytes (Panico, et al., 2005), as hypolipidaemic (Eddouks, et al., 2005), as antiallergic and antihistaminic (Trombetta, et al., 2005), as antioxidant (Bonina, et al., 2002; Germano, et al.,

2002). The plant contains glucoside, triglucoside, rutin, pentosans, rutic acid, pectic acid and saponin (Chopra, et al., 1956), glucosinolates, fatty acid, sterol and tocopherol (Matthaus and Ozcan, 2005).Leaves of *C. zeylanica* is used in treating cholera, fruits in treating blisters and boils, roots in treating coryza, elephantiasis, hemiplegia, neuralgia, oedema, piles, pneumonia, rheumatism, snakebite, ulcer and vomiting (Keshava Murthy, 1994), as sedative, in treating cholera and stomachic, the plant contains an alkaloid, phytosterols, water soluble acids (Chopra, et al., 1956) and E-octadec-7-en-5-ynoic acid. The tribal groups of Davanagere district, Karnataka state, India use leaves of above mentioned plants in healing septic wound (Fresh leaves were ground with lime juice and mixed with one teaspoon full of honey, a thick paste so obtained is applied to septic wounds) (Manjunatha, 2002).

Critical review of the literature revealed that the wound healing potency of these plants has not been subjected to clinical evaluationIn the present study, we have identified 12 potent active constituents viz., Capparispine, Glucocapparin, kaempferol, kaempferol-7-rhamnoside, Polyprenols, Proline betaine, Quercetin, Quercetin-3-rutinoside, Rhamnetin, Rutin, Saccharose, Sinigrin for wound healing promoting ability. These active constituents were subjected for docking study using a well established target GSK 3 Beta responsible for wound healing process in human. Our results, indicated that Capparispine, Glucocapparin, kaempferol, Polyprenols, Quercetin, Quercetin-3-rutinoside, Rhamnetin have a potent wound healing property among other compounds. In vivo/ in vitro studies to validate the present finding and to understand the exact mechanism and potential targets of these phytochemicals are under process.

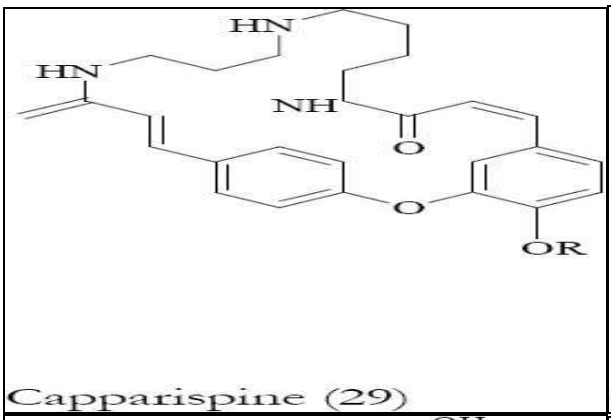
II. MATERIALS AND METHODS

1. **Target Identification:** Glycogen synthase kinase-3 (GSK-3) is a widely expressed and highly conserved serine/threonine protein kinase encoded by 2 genes, GSK3A and GSK3B. Both Gsk3b-CKO mice and fibroblasts showed elevated expression and production of endothelin-1 (ET-1) compared with control mice and cells. Antagonizing ET-1 reversed the phenotype of Gsk3b-CKO fibroblasts and mice. Thus, GSK-3beta appears to control the progression of wound healing and fibrosis by modulating ET-1 levels. These results suggest that targeting the GSK-3beta pathway or ET-1 may be of benefit in controlling tissue repair and fibrogenic responses in vivo. So, we performed Automated docking was used to determine theorientation of inhibitors bound in the active site ofGSK3-b. The protein structure file 1Q5K was taken fromPDB (www.rcsb.org/pdb) was edited by removing the hetero atoms, adding C terminal oxygen (Binkowskiet al., 2003).
2. **Ligand Identification:** 12 ligands namely Capparispine, Glucocapparin, kaempferol, kaempferol-7-rhamnoside, Polyprenols, Proline betaine, Quercetin, Quercetin-3-rutinoside, Rhamnetin, Rutin, Saccharose, Sinigrin was identified through literature, which have potentional wound healing activity belonging to *Capparis spp.*All ligands were searched for the three dimensional structure in the pubchem database, and nonpolar hydrogen atoms were merged in the corresponding three dimensional structure.
3. **Docking**:
    1. **Target preparation:**
       The following steps were followed to prepare the protein file: First the water molecules were removed from protein. Next, we need to add hydrogen because X-ray crystallography usually does not locate hydrogen; hence most PDB files do not include them. Later pdbqt file of protein was generated.
    2. **Ligand preparation:** to generate the ligand pdbqt file, the following steps were followed. In Autodock Tools, torsion tree and no of torsions were selected. And finally it was saved Save as .pdbqt
    3. **Autodock vina working protocol:** Docking studies were carried out using AUTODOCK software. AUTODOCK Vina is a suite of automated docking tools. Autodock Vina was performed in the cigwin command terminal using the following command.
       "C:\Program Files (x86)\The Scripps Research Institute\Vina\vina.exe" -- config conf.txt -- log log.txt.
4. **Visualization of docking results**: PyMOL and DISCOVERY STUDIO are the software used for visualization of interactions between targets and ligands. These help in finding in different types of interactions between our ligand and the target molecule.

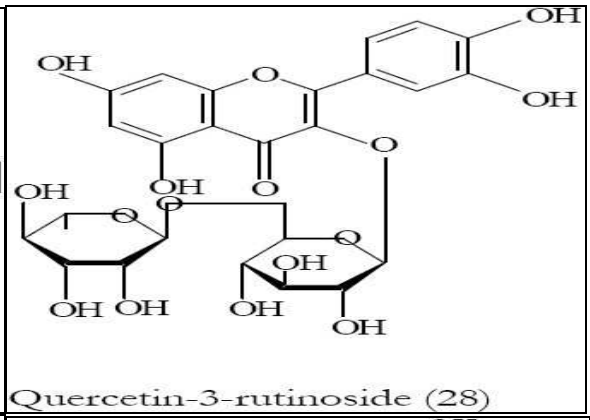
III. RESULTS AND DISCUSSION:

1. 12 ligands namely Capparispine, Glucocapparin, kaempferol, kaempferol-7-rhamnoside, Polyprenols, Proline betaine, Quercetin, Quercetin-3-rutinoside, Rhamnetin, Rutin, Saccharose, and Sinigrin was identified through

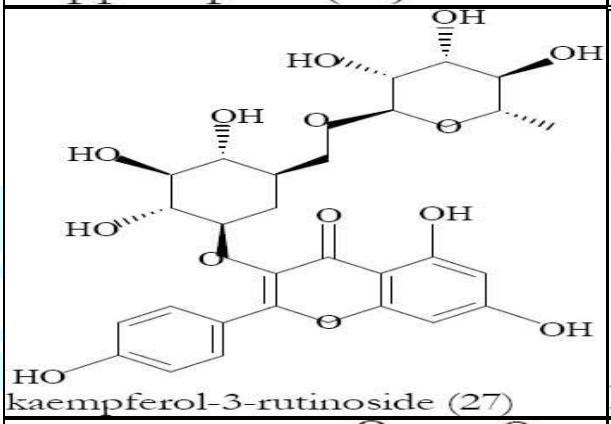
literature, which have potentional wound healing activity belonging to *Capparis spp.* The structure of all the 12 ligands are as follows:



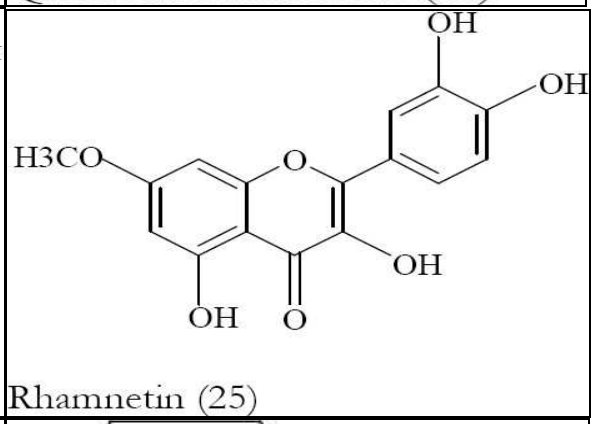
Capparispine (29)



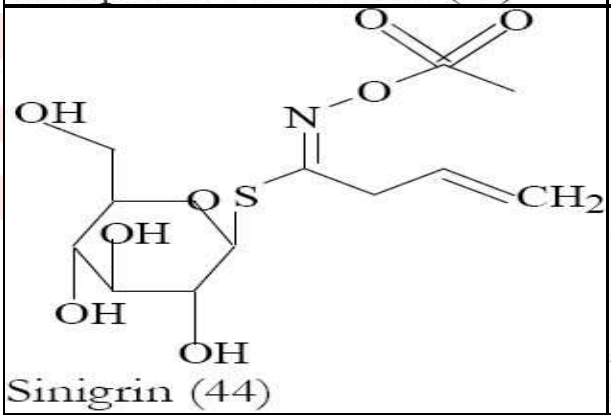
Quercetin-3-rutinoside (28)



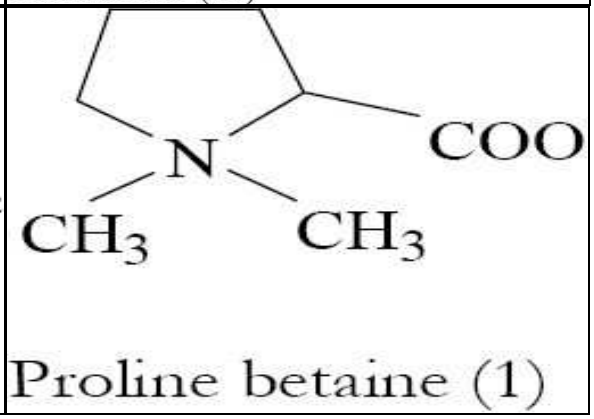
kaempferol-3-rutinoside (27)



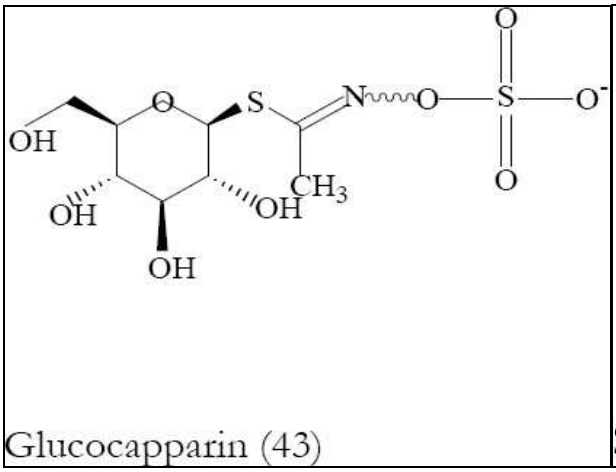
Rhamnetin (25)



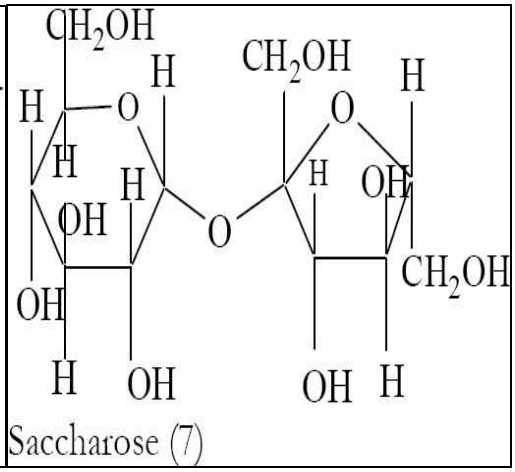
Sinigrin (44)



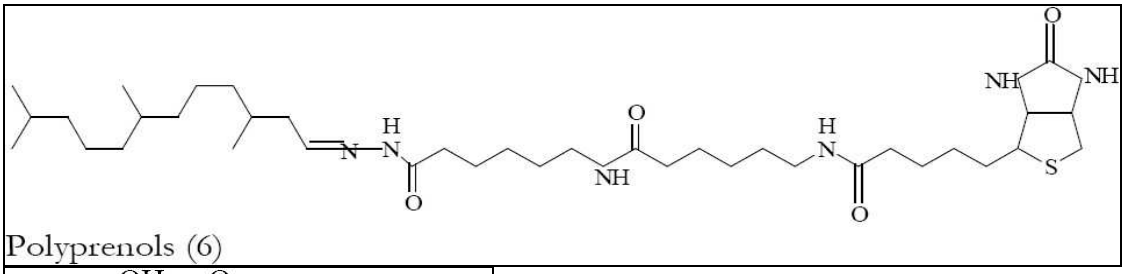
Proline betaine (1)

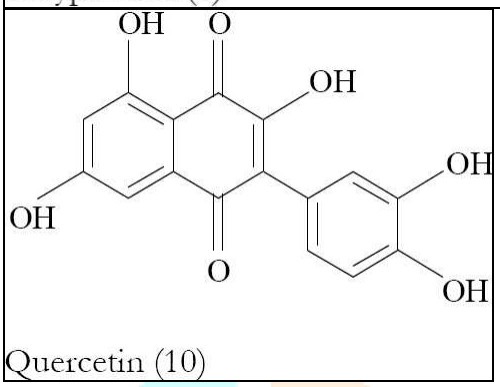


Glucocapparin (43)



Saccharose (7)





The protein structure file 1Q5K was taken fromPDB (www.rcsb.org/pdb) was edited by removing the hetero atoms, adding C terminal oxygen is being given as follows:

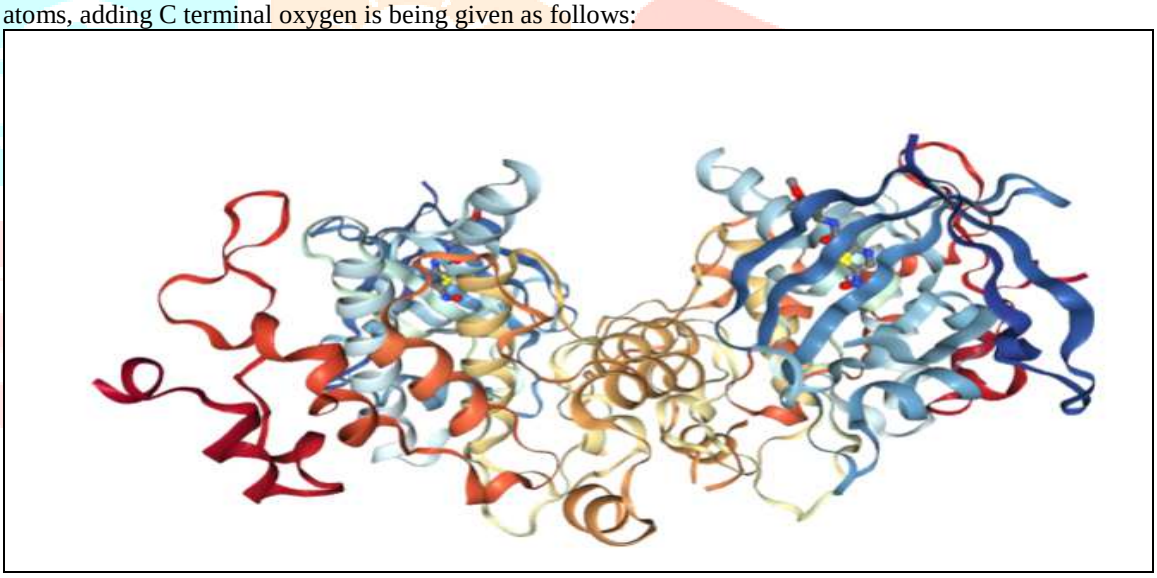


Figure1: Structure of 1Q5K protein

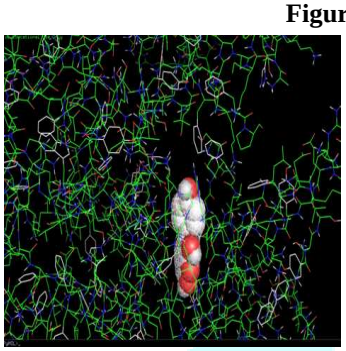
Using Autodock Vina software, docking is done and the results are obtained in the form of dlg files. These files are converted to PDBQT files and then into PDB file. Binding energy score and corresponding PDB structure is obtained for the runs. The run giving the highest negative score for estimated binding energy in kcal/mol is considered for complex file formation.

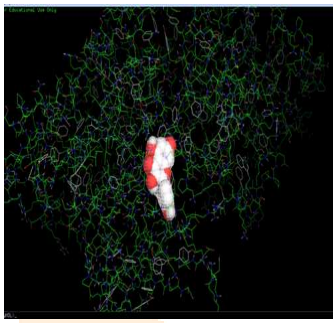
Table-1
Affinity values after Docking studies.

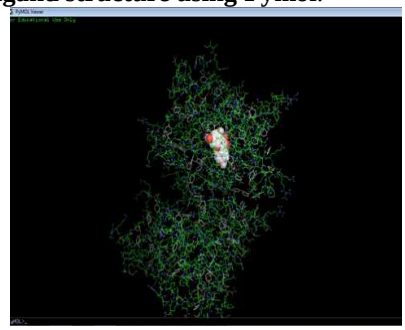
Sl no.	Ligand Compounds	Affinity values
1	Capparispine	-8.4
2	Glucocapparin	-8.4
3	kaempferol	-8.4
4	kaempferol-7-rhamnoside	-7.4
5	Polyprenols	-8.4

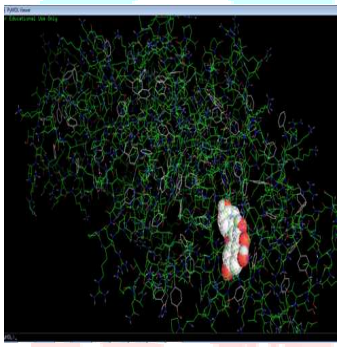
6	Proline betaine	-8.1
7	Quercetin	-8.4
8	Quercetin-3-rutinoside	-8.4
9	Rhamnetin	-8.4
10	Rutin	-9.1
11	Saccharose	-8.4
12	Sinigrin	-8.5

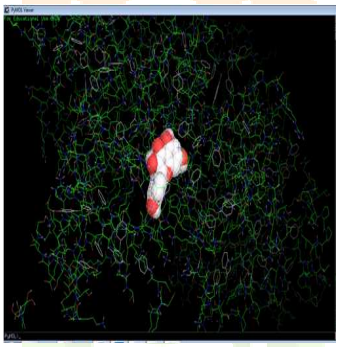
Figure 2: Visualization of protein-ligand structure using Pymol.

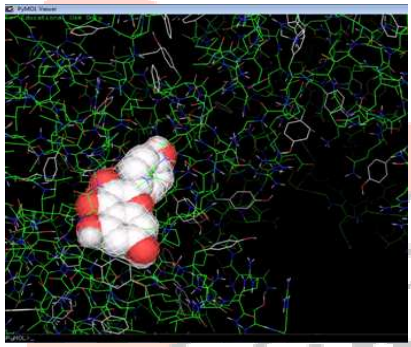

Quercetin

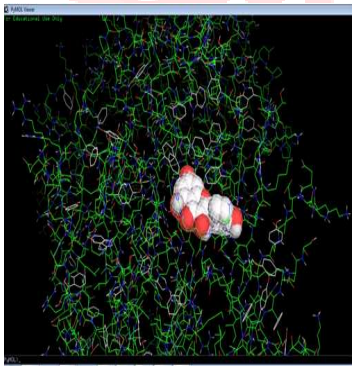

capparispine

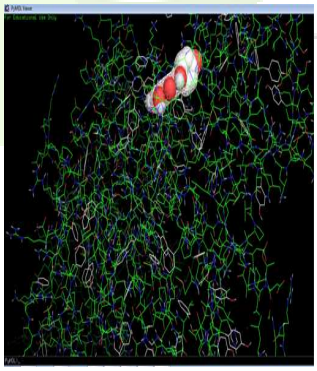

Proline betaine

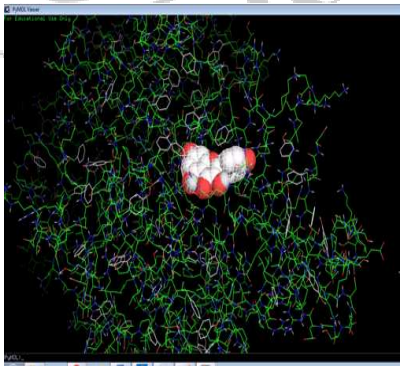

Polyprenols

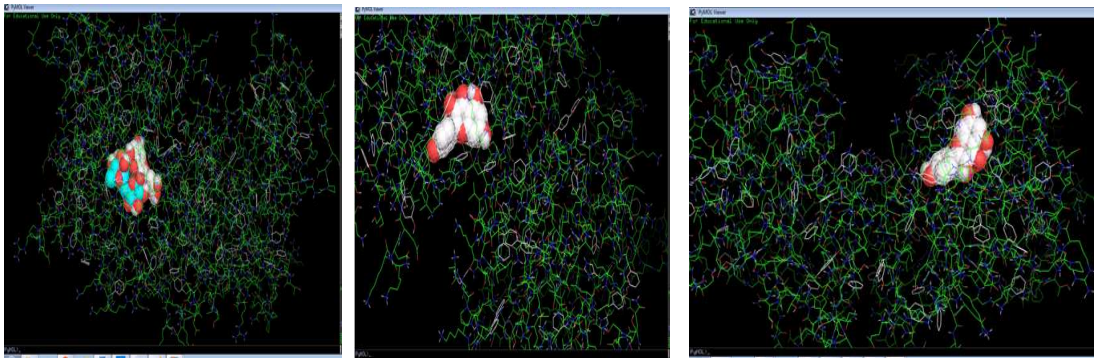

Glucocapparin


kaempferol-7-rhamnoside


Kaempferol


Quercetin-3-rutinoside

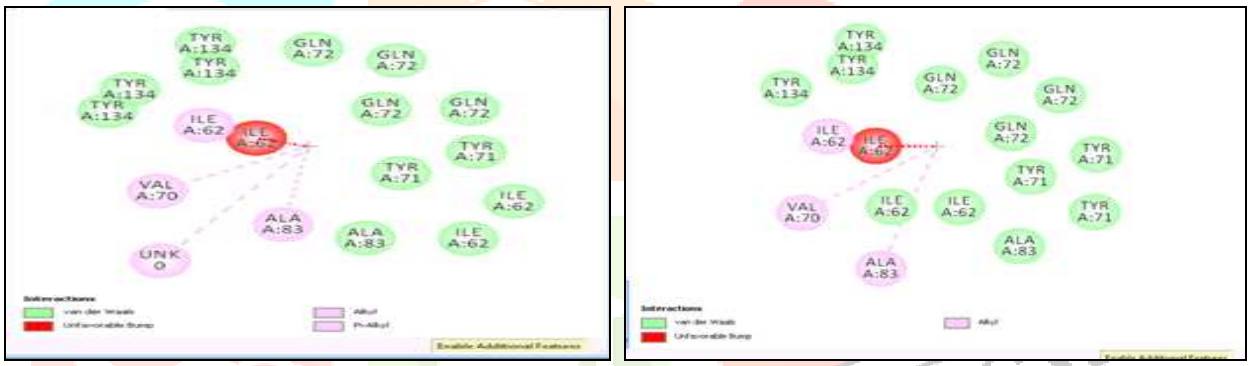

Rhamnetin



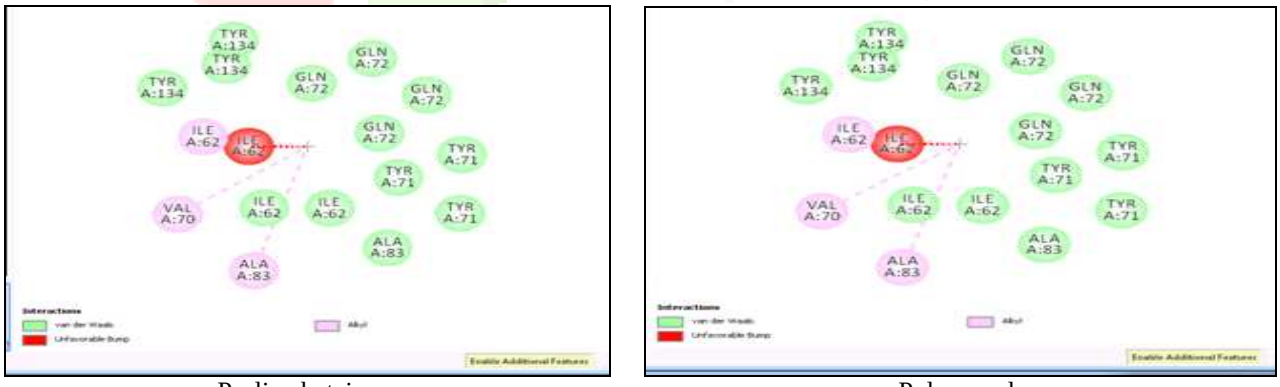
Rutin Saccharose Sinigrin

From the docked complex, pharmacophore is being identified and analyzed though protein ligand 2-D interaction. It is being observed that the Tyr and Gln are the most two important amino acids present in the active site of the target protein, making the favourable interaction with the ligand molecule.

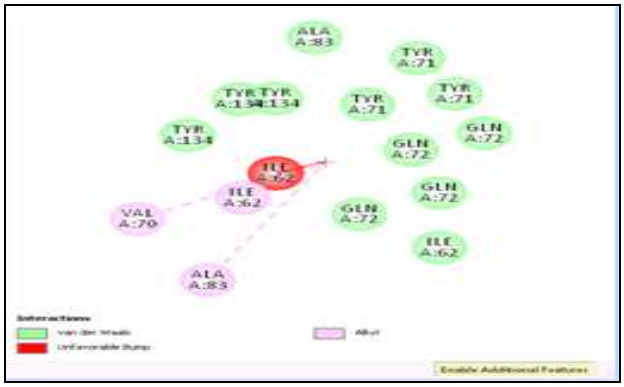
Figure3: Protein ligand 2-D interaction of Pharmacophore



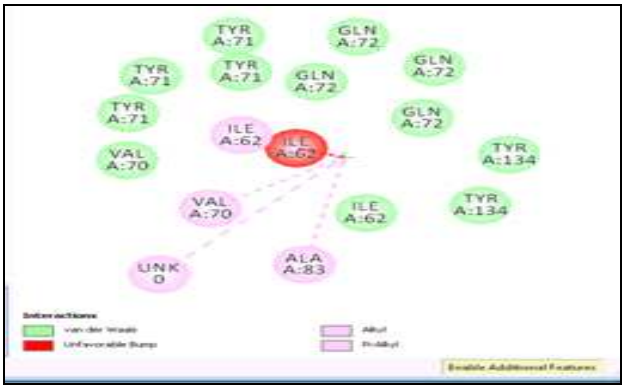
Quercetin Capparispine



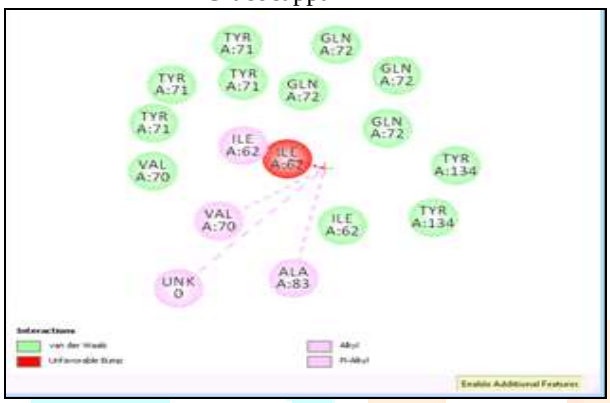
Proline betaine Polyprenols



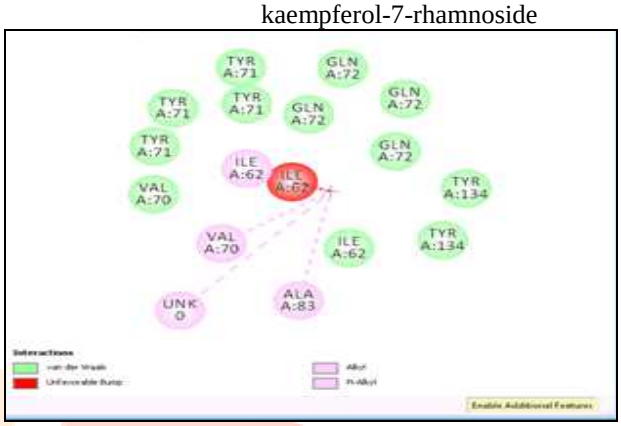
Glucocapparin



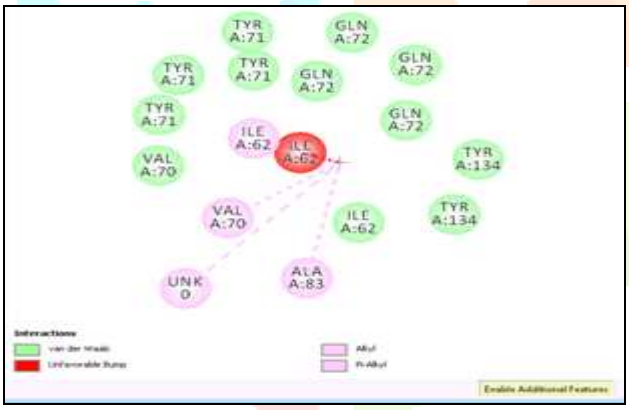
kaempferol-7-rhamnoside



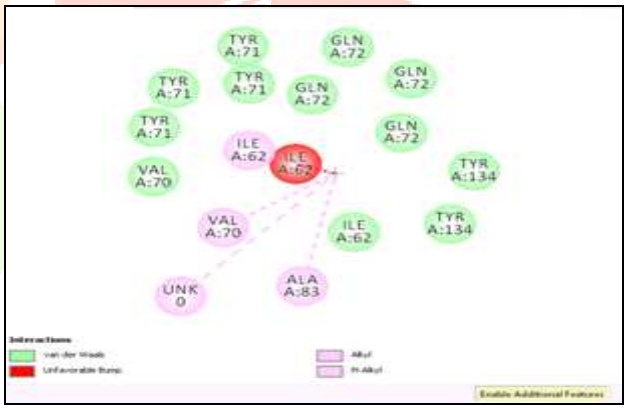
Kaempferol



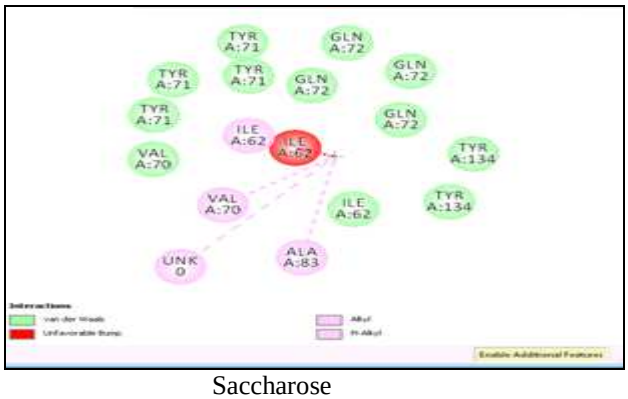
Quercetin-3-rutinoside



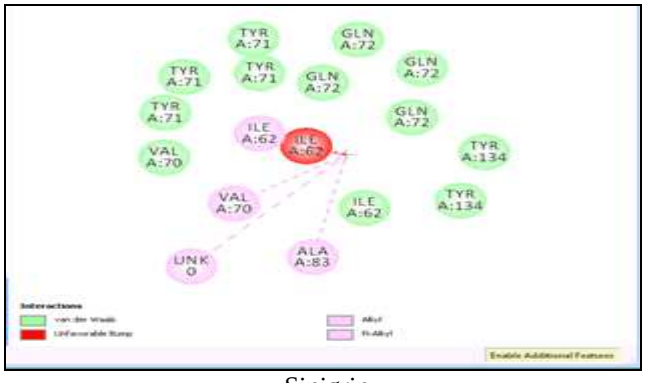
Rhamnetin



Rutin



Saccharose



Sinigrin

Table 2: Analysis of Pharmacophore

Name	environmental definition

Van der walls interaction	Val, Tyr, Gln, Ile
Alkyl, pi-alkyl	Val , Ile and ala

IV. DISCUSSION

In the present investigation preliminary phytochemical analysis revealed the presence of glycosides, triterpenoids, flavonoids, alkaloids, saponins, sterols and tannins. These phytoconstituents are known to inhibit lipid peroxidation and increases the viability of collagen fibrils by increasing the strength of collagen fibers, by increasing the circulation, by preventing the cell damage and by promoting the DNA synthesis (Geite, et al., 2002). Thus wound healing potency of *C. decidua*, *C. spinosa* and *C. zeylanica* may be attributed to the antibacterial and antioxidant property of the phytoconstituents present in them which may be either due to their individual or additive effect which help the process of wound healing. Among the ligands studied for wound healing Capparispine, Glucocapparin, kaempferol, Polyprenols, Quercetin, Quercetin-3-rutinoside, Rhamnetin showed very high potential towards wound healing.

V. ACKNOWLEDGEMENTS

The authors is very grateful to Girimaji N. Raj Gopal, S.V.Thimmaiah and Prof. Darmanada Rao National Educational Society, Shimoga.

VI. REFERENCES

1. Ageel, A.M., Parmar, N.S., Mossa, J.S., Al-Yahya, M.A., Al-Said, M.S. and Tariq, M., *Agents Actions*, 1986, 17(3-4), 383.
2. Azad, S. 2002. Essentials of surgery. PARAS Medical Publishers, Hyderabad, India.
3. Bonina, F., Puglia, C., Ventura, D., Aquino, R., Tortora, S., Sacchi, A., Saija, A., Tomaino, A., Pellegrino, M.L. and de Caprariis, P. 2002. *In vitro* antioxidant and *in vivo* photoprotective effects of a lyophilized extract of *Capparis spinosa* L. buds. J. Cosmet. Sci. 53(6), 321-335.
4. Calis, I., Kuruuzum-Uz, A., Lorenzetto, P.A. and Ruedi, P., *Phytochem.*, 2002, 59(4), 451.
5. Chopra, R.N., Nayar, S.L. and Chopra, I.C. 1956. Glossary of Indian Medicinal Plants. C.S.I.R. Publications, New Delhi.
6. Ehrlich, H.P. and Hunt, T.K. 1968. Effect of cortisone and vitamin A on wound healing. Ann. Surg. 167, 324.
7. Germano, M.P., De Pasquale, R., De Angelo, V., Catania, S., Silvari, V. and Costa, C. 2002. Evaluation of extracts and isolated fraction from *Capparis spinosa* L. buds as an antioxidant source. J. Agric. Food Chem. 50(5), 1168-1171.
8. Getie, M., Gebre Mariam, T., Reitz, R. and Neubert, R.H. 2002. Evaluation of the release profiles of flavonoids from topical formulations of the crude extract of the leaves of *Dodonea viscosa* (Sapindaceae). Pharmazie. 57, 320-322.
9. Haque, M.E., Haque, M., Rahman, M.M., Rahman, M.M., Khondkar, P., Wahed, M.I., Mossadik, M.A., Gray, A.I. and Sarker, S.D., *Fitoter.*, 2004, 75(2), 130.
10. Keshavamurthy, K.R. 1994. Medicinal plants of Karnataka. Karnataka Forest Department (ed.), Bangalore, India.
11. Kokate, C.K., Purohith, A.P. and Gokhale, S.B. 1990. Pharmacognosy. Nirali Prakashan, Pune.
12. Lee, K.H. 1968. Studies on mechanism of action of salicylates, retardation of wound healing by aspirin. J. Pharm. Sci. 57(6), 1042-1043.
13. Manjunatha, B.K. 2002. Floristic composition of davanagere district, Karnataka, India. Ph. D. Thesis, Kuvempu University, Shankaraghatta, Karnataka, India.
14. Matthaus, B. and Ozcan, M. 2005. Glucosinolates and fatty acid, sterol and tocopherol composition of seed oils from *Capparis spinosa* Var. *spinosa* and *Capparis ovata* Desf. Var. canescens (Coss.) Heywood. J. Agric. Food Chem. 53 (18) 7136-7141.
15. Morton, J.P. and Malone, M.H. 1972. Evaluation of vulnerary activity by an open wound procedure in rats. Arch. Int. Pharmacodyn. 196(6), 117-136.
16. Panico, A.M., Cardile, V., Garufi, F., Puglia, C., Bonina, F. and Ronsisvalle, G. 2005. Protective effect of *Capparis spinosa* on chondrocytes. Life Sci. 77 (20) 2479-2488.
17. Patil, M.B., Jalalpure, S.S. and Nagoor, V.S. 2004. Wound healing activity of the roots of *Eclipta alba* Linn. Indian Drugs. 41(1), 40-45.
18. Purohit, A. and Vyas, K.B. 2005. Hypolipidaemic efficacy of *Capparis decidua* fruit and shoot extracts in cholesterol fed rabbits. Indian J. Exp. Biol. 43 (10) 863-866.
19. Trombetta, D., Occhiuto, F., Perri, D., Puglia, C., Santagati, N.A., De Pasquale, A., Saija, A. and Bonina, F. 2005. Antiallergic and antihistaminic effect of two extracts 0of *Capparis spinosa* L. flowering buds. Phytother Res. 19 (1): 29-33.

20. Woessner, J.F. 1963. The determination of hydroxyproline in tissue and protein samples containing small proportions of the iminoacid. Archiv. Biochem. 93, 440-447.
21. Yadav, P., Sarkar, S. and Bhatnagar, D., *Pharmacol. Research*, 1997, 36(3), 221.