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Short Communication

Phytochemical evaluation and anti-hemorrhoidal activity of bark of *Acacia ferruginea* DC

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ABSTRACT

The present study has been carried out to evaluate antihemorrhoidal activity of bark of Acacia ferruginea DC. The total phenolic, total flavonoid and saponins were determined. Anti-hemorrhoidal potential of bark extract was determined by levels of inflammatory cytokines such as TNF-α, IL-6, PGE2 and recto-anal coefficient (RAC). The histopathological examination was done to evaluate the severity score in the treated and untreated groups. The results of phytochemical screening of the hydroalcoholic extract of A. ferruginea revealed the presence of alkaloids, flavonoids, triterpenoid, saponins, tannins and phenolic compounds The total phenolic, flavonoid and saponin contents were found to be 438.8mg/g GAE, 66.6mg/gRE and 34%w/w respectively. Hydroalcoholic extract of bark of A. ferruginea significantly reduced the inflammatory cytokines {TNF-α(8.40±0.188), IL-6(3.95±0.181), PGE2(53.27±2.956) and RAC(0.998±0.094)}as compared to positive control group{TNF-α(13.36±0.141), IL-6(7.25±0.161), PGE2(82.34±3.395) and RAC(1.131 ± 0.008)}. Noticeably the results were comparable to that of standard pilex granules {TNF-α (7.12±0.166), IL-6(3.01±0.156), PGE2(42.51±2.157) and RAC (0.968±0.084)}. Molecular docking and structure based pharmacophore mapping further confirmed the anti-inflammatory mediated antihemorrhoidal activity of the hydroalcoholic extract. The antihemorrhoidal activity of hydroalcoholic extract of A. ferruginea may attribute to the flavonoids.

1. Introduction

Hemorrhoid is one of the most common inflammatory disease which can be characterized by alteration in vasculature of the anal canal including blood vessels, supporting tissues, muscles and elastic fibers. According to the report of National Centre for Health Statistics, the prevalence of hemorrhoid is 3.82% in United States[1] and 0.36% in India that frequently appears in the age of 45–65 years.[2] Free radical generation is the primary reason for initiation of many physiological and pathological disorders like hemorrhoids. Involvement of free radicals in the precipitation of hemorrhoids is well documented in the literature. It is well known fact that excess concentration of free radicals is a consequence of improper balance between reactive oxygen species and their metabolites.[3] Antioxidants neutralize free radicals and thus play major role in the eradication of these free radicals and hence are involved in the management of hemorrhoids. Unavoidable and serious adverse effects associated with commercial or synthetic antioxidants are becoming a major concern for the researchers to develop natural antioxidants. Herbal extracts rich in phytoantioxidants like polyphenols, flavonoids, tannins and other related compounds are known to possess positive health effects and eventually reduces the incidence of diseases.[4] Therefore much attention has been focused on the use of natural antioxidants that can provide more significant health benefits with minimal toxicities.[5]

Acacia ferruginea DC. a drought resistant, deciduous tree belonging to family Mimosoideae is native to Pennisular India from Gujrat to Gunjam in the east.[6] The bark of the plant is bitter and

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traditionally used as astringent, cure itching, leucoderma, ulcers, stomatitis and diseases of blood. Traditionally, the extract of leaves has been in use as astringent and in the treatment of dysentery, gonorrhea, urinary tract disorders and is also useful in the diseases of eye and liver. Bark decoction of *A. ferruginea* is one of active ingredient of gargle preparation. Moreover, ethanolic extract of *A. ferruginea* leaves reported to have hepatoprotective,[7] larvicidal,[8] antiulcer,[9] antitumor[10] activities.

In addition to this, different species of this genus has been reported to have antimalarial, antifungal, antibacterial, antidiarrhoeal, anti-oxidant, antiviral, hepatoprotective and antispasmodic activity. Earlier phytochemical studies indicated that this species act as a rich source of tannins (catechin, epigallocatechin), terpenoids, polyphenolics (gallic acid) and saponins.

Chemical constituents of *A. ferruginea* include flavonoids, phenols, alkaloids, terpenoids, anthraquinones and tannins. Glycosides and saponins are also present in trace amounts.[11]

On the basis of the ethnopharmacological claims made by tribal community and phytochemical contents particularly secondary metabolites we have attempted to evaluate antioxidant and anti-hemorrhoidal activity of *A. ferruginea*.

2. Materials and methods

2.1. Plant material

Bark of *A. ferruginea* plant was collected from Raholi village of district Tonk, Rajasthan, India in the month of August, 2012 and was authenticated at Department of Botany (Ref. RUBL21147), University of Rajasthan, Rajasthan, India. A specimen was submitted to the Department of Botany, Rajasthan University for further reference. The Bark was shade dried, coarsely powdered and stored in an air tight container for further use.

2.2. Drugs and chemicals

L-ascorbic acid, Rutin and Evans blue were procured from Hi-Media Research Laboratories Pvt. Ltd., Mumbai, India. Croton oil was procured from Sigma Aldrich, St. Louis, USA. Pyridine, Diethyl ether and isoflurane were procured from Merck Specialities Pvt. Ltd., Mumbai, India. Rat TNF-α Elisa kit and Rat IL-6 Elisa kit were purchased from Raybiotech Inc., Norcross, United States. Rat PGE_2 Elisa kit was purchased from Cloud Clone Corp., Houston, USA. All analytical readings were taken on UV–Visible spectrophotometer (UV-1800, Shimadzu), and Elisa microplate reader (Erba Lisa Scan II, Mannheim). Microtome used for histological sections was procured from Scientech Inst., New Delhi, India. Rotary evaporator used for concentrating the extract was procured from Heidolph, Schwabach, Germany.

2.3. Extraction of plant material

Coarsely powdered bark was extracted successively with petroleum ether and 70% methanol using soxhlet apparatus. The extracts were filtered using Whatman filter paper (No. 1) and concentrated using rotary evaporator (Heidolph, Schwabach, Germany). Each time before extracting with next solvent the marc was dried in hot air oven below 40 °C. . Finally, aqueous extract was prepared from dried marc using maceration technique and water extract was filtered. Yields of petroleum ether, hydroalcoholic and aqueous extracts were calculated. In view of maximum yield, hydroalcoholic extract of bark of *A. ferruginea* was used for quantitative estimation of phytoconstituents and assessment of antioxidant and anti-hemorrhoidal activity.

2.4. Phytochemical screening

Hydroalcoholic extract of bark was observed for the presence of alkaloids, carbohydrates, flavonoids, gum and mucilages, tannins, terpenoids, steroids and saponins.[12]

2.5. Quantification of phytoconstituents

Hydroalcoholic extract of bark was evaluated for the estimation of total phenolic content,[13] total flavonoids[14] and saponins.[15]

2.6. Anti-hemorrhoidal studies

2.6.1. Animals

Wistar albino rats (190–230 g) were kept in polypropylene cages at an ambient temperature of 25±2 °C and 55–65% relative humidity. A 12 h light/dark cycle was maintained in the animal house. The rats had free access to water and fed with *ad libitum*. The approved protocol (BU/BT/627/14-15) of animal study was carried out as per the guidelines of IAEC and CPCSEA.

2.6.2. Acute toxicity studies

Acute toxicity of bark extract was performed as per the OECD-420 guidelines. Wistar albino rats of either sex weighing between 180 and 230 g were divided into different groups comprising six animals each. The control group received normal saline (2 ml/kg, p.o.). The other groups received 100–2000 mg/kg of the test extract respectively. Post dosing, the animals were observed continuously for the first 4 h for any behavioral changes. Thereafter, they were then kept under observation up to 14 days after drug administration to find out the mortality if any.

2.6.3. Experimental design

Wistar rats were divided into four groups, Group I served as normal control and received only distilled water. Group II served as positive control and received 6% croton oil (10 μl). Group III was served as treated group and was administered with hydroalcoholic extract of bark (400 mg/kg, b.wt, PO) and 6% croton oil (10 μl). Group IV was served as standard and was administered with Pilex granules (400 mg/kg, b.wt, PO) and 6% croton oil (10 μl). After overnight fasting, Evans blue (30 mg/kg i.v.) was injected in tail of animals of all the groups. After 30 min, hemorrhoids were induced in all groups except normal control group by applying croton-oil preparation (deionized water, pyridine, diethyl ether, and 6% croton oil in diethyl ether in the ratio of 1: 4: 5: 10). Sterile cotton swab (4 mm in diameter) soaked in cotton oil preparation (100 μl) was inserted into the anus (about 22 mm diameter) and kept for 10 s.

A linear development of oedema was observed within 7–8 h of induction of croton oil. After 24 h of induction, relevant treatment was given to all the groups for five days. On fifth day, 1 h after the treatment, blood was collected from the retro-orbital sinus. Inflammatory cytokines such as PG, TNF-α and IL-6 was estimated in blood by using Elisa Microplate Reader (Erba Lisa Scan II, Mannheim). All animals were euthanized by exsanguinations under deep isoflurane anesthesia and rectoanal tissue (20 mm in length) was isolated and weighed. Evans blue present in the tissue was extracted by 1 ml formaldehyde and absorbance was taken at 620 nm using Elisa Microplate Reader (Erba Lisa Scan II, Mannheim). Concentration was quantified using standard curve of evans blue dye.

For histological examination, same tissue was examined for severity score and rectoanal-coefficient by fixing the tissue in 10% neutral buffered formalin. The rectoanal-coefficient (RAC) was calculated from the formula

Rectoanal Coefficient = Weight of rectoanal tissue (mg) / Body weight (mg)

Histological observation was performed for inflammation, congestion, hemorrhage, vasodilation and necrosis.[16]

2.6.4. Statistical analysis

The results were expressed as Mean ± SEM and analyzed by one-way ANOVA followed by Bonferroni *t*-test and $P < 0.001$ was considered to be statistically significant.

2.7. Structure based pharmacophore mapping and molecular docking

Accelery's Discovery Studio (DS) was used for structure based pharmacophore mapping and molecular docking studies. The structures of oxaprozin and *A. ferruginea* isolate (5-(3,4-dimethylphenyl)-4-phenylisoxazole) were drawn using Chem3D Ultra and structures were exported to the discovery studio. Crystal structure of COX was downloaded from the protein databank (PDB entry: 4rrw). The water molecules were removed from the protein structure, valency was monitored and hydrogen atoms were added to the protein. The active site was defined for the prepared protein structure that comes within radius of 9 A^0, so that the important protein residues involved in binding interaction with ligands was included. The ready protein structure was used for structure based pharmacophore mapping and molecular docking studies.

For structure based pharmacophore mapping a six featured pharmacophore model which includes 2 hydrogen bond acceptor, 2 hydrogen bond donor and 2 hydrophobe was generated for ready COX structure. The prepared structures of isolate and oxaprozin were mapped over the developed pharmacophore model and the results were analyzed on the basis of fit value.

For molecular docking study LibDocker module of discovery studio was used. The prepared structures of isolate and oxaprozin were docked into the defined active site of COX structure. Different poses for isolate and oxaprozin were generated and analyzed on the basis of docking score.

3. Results and discussion

The results of phytochemical screening of the hydroalcoholic extract of *A. ferruginea* revealed the presence of alkaloids, flavonoids, triterpenoid, saponins, tannins and phenolic compounds. Total flavonoids and phenolic content present in hydroalcoholic extract of bark were found to be 66.6 mg/g RE and 438.8 mg/g GAE. Total saponins content estimated in bark extract was 34% w/w.

Hydroalcoholic extract of bark was evaluated for acute toxicity in rats and it was observed that the bark extract is safe even at the higher concentration of 2000 mg/kg b.wt, and no mortality was observed after 14 days of extract administration.

Croton oil application in the rectoanal region caused significant increase in RAC ($P < 0.001$), PGE_2 ($P < 0.001$), TNF-α ($P < 0.001$) and IL-6 ($P < 0.001$), it also resulted in increased exudation (3.850 ± 0.9181, $P < 0.001$) of evans blue dye as compared to normal control group animals (0.316 ± 0.1602). Treatment with bark extract (400 mg/kg, $P < 0.001$) has maintained the RAC of 0.998 ± 0.0944 and IL-6 of 3.95 ± 0.181 nearly same as normal control groups animals (0.912 ± 0.0617, 2.15 ± 0.141) whereas, treatment with pilex granules (400 mg/kg, b.wt, PO) showed the RAC of 0.968 ± 0.0842 and IL-6 of 3.01 ± 0.156. Moreover, the effect of bark extract (400 mg/kg, $P < 0.001$) was statistically significant against elevated levels of TNF-α (8.40 ± 0.188) as compared to normal control group (4.70 ± 0.329) as given in Table 1. Treatment with pilex granules has also significantly reduced the elevated levels of TNF-α (7.12 ± 0.166). Bark extract and pilex granules (400 mg/kg, $P < 0.001$) have also offered significant protection against increased evans blue dye exudation on application of croton oil.

Histopathological examination revealed that there was a significant difference in RAC, severity score in normal control, positive control and treated groups. Normal group animals showed normal cytoarchitecture of the rectoanal region. The RAC of normal control and positive control group was found to be 0.912 ± 0.0167 and 1.131 ± 0.0084 ($P < 0.001$), respectively. Positive control group showed 1.24 times higher RAC as compared to normal control. Treatment with bark extract and pilex granules (400 mg/kg, $P < 0.001$) showed decrease in RAC (0.998 ± 0.0944, 0.968 ± 0.0842) when compared to control group.

Additionally, the isolated tissue was observed for severity. Results revealed no severity in normal control group and the severity score of positive control group was found to be 1.33 ± 0.516 respectively, which is statistically significant when compared to normal group. Interestingly, treatment with bark extract (400 mg/kg) has remarkably improved (0.50 ± 0.547) the rectoanal damage caused by croton oil as compared to the positive control group. Treatment with pilex granules (400 mg/kg) has also reduced the (0.38 ± 0.468) rectoanal damage. Histopathological examination of treated group (bark extract, 400 mg/kg) and pilex granules (400 mg/kg) revealed that there was marked reduction in the RAC severity score, vasodilation extent, hemorrhagic and necrosis level. It is noteworthy that treatment with the bark extract at 400 mg/kg restored almost normal architecture of rectoanal region.

It is a well known fact that herbal drugs have been in use since many decades, most probably owing to their lesser side effects and high safety profile. In view of traditional use of bark of *A. ferruginea* as an anti-inflammatory and anti-hemorrhoid regimen, we have attempted to evaluate its anti-hemorrhoidal activity through well established scientific methods. As a starting point the hydroalcoholic extract of bark of *A. ferruginea* has been screened for the presence of phytoconstituents and the results confirm the presence of flavonoids (66.6 mg/g RE) and phenolic compounds (438.8 mg/g GAE). Since antioxidant activity of hydroalcoholic extract of bark of *A. ferruginea* has been already reported by Sowndhararajan et al.,[17] and Sowndhararajan et al.,[18] it was subjected to anti-hemorrhoidal studies. We have also performed antioxidant activity of bark of *A. ferruginea* but not stated in the present manuscript.

Hemorrhoids are the pathological condition, often characterized by vasodilatation and inflammation in the rectoanal region, which results in increased vascular permeability and extravasation of

Table 1
Parameters of anti-hemorrhoidal activity.

S. No.	Groups	Severity score	Rectoanal Coefficient	Evans Blue	TNF-α	IL-6	PGE_2
1.	Normal control (Only vehicle)	0 ± 0	0.912 ± 0.016	0.316 ± 0.160	4.70 ± 0.329	2.15 ± 0.141	36.44 ± 2.876
2.	Positive control (Vehicle + Inducer)	1.33 ± 0.516	1.131 ± 0.008	3.850 ± 0.918	13.36 ± 0.141	7.25 ± 0.161	82.34 ± 3.395
3.	Bark extract (400 mg/kg)+ Inducer	0.50 ± 0.547	0.998 ± 0.094	1.333 ± 0.739	8.40 ± 0.188	3.95 ± 0.181	53.27 ± 2.956
4.	Pilex granules (400 mg/kg)+ Inducer	0.38 ± 0.468	0.968 ± 0.084	1.102 ± 0.731	7.12 ± 0.166	3.01 ± 0.156	42.51 ± 2.157

$P < 0.001$ i.e., There is statistically significant difference among the treated groups.

inflammatory cytokines in the interstitial spaces. In the present study, croton oil has been used as phlogistic agent for experimental induction of hemorrhoids. Croton oil causes inflammation due to release of inflammatory lipid metabolites such as prostaglandins, leukotrienes, TNF-α, nitric acid and bradykinins. These factors alone or in combination regulates the activation of fibroblasts, endothelial cells, macrophages and newly recruited monocytes, lymphocytes, neutrophils and eosinophils which leads to severe inflammation.

In present studies, extravasation of evans blue dye has been used for estimation of inflammatory cytokines. The positive control group showed severe exudation of evans blue dye and increased concentration of pro-inflammatory cytokines {TNF- α (13.36 ± 0.141), IL-6 (7.25 ± 0.161), PGE_2 (82.34 ± 3.395) and rectoanal-coefficient (1.131 ± 0.008)}. These changes were further supported by the histopathological changes in the rectoanal region such as severe vasodilatation, infiltration of inflammatory cytokines and hemorrhagic spots. On the other hand, as expected the extract showed good anti-hemorrhoidal activity as reflected by reduced levels of inflammatory cytokines such as TNF-α (8.40 ± 0.188), IL-6 (3.95 ± 0.181), PGE_2 (53.27 ± 2.956) and rectoanal-coefficient (0.998 ± 0.094).

The earlier reports have established the role of flavonoids in oxidative stress, inflammation and hemorrhoid in both clinical and pre-clinical settings. It is well known fact that flavonoids reduce the concentration of PGE_{2e} and $PGE_{2\alpha}$ and other inflammatory mediators. Moreover, it is also well documented that flavonoids increases the vascular tone and reduces the vascular fragility and resistance. In view of aforementioned facts, the observed antioxidant and anti-hemorrhoidal activity can be attributed to presence of flavonoids in the bark of *A. ferruginea*.

It is noticeable that extract has exhibited equipotent activity to that of standard therapy of pilex granules with reduced levels of inflammatory cytokines (TNF- α (7.12 ± 0.166), IL-6 (3.01 ± 0.156), PGE_2 (42.51 ± 2.157) and rectoanal-coefficient (0.968 ± 0.084). The anti-hemorrhoidal activity of pilex granules is again a consequence of presence of flavonoid containing plant parts viz. *Commiphora wightii, Emblica officinalis, Cassia fistula and Bauhinia variegata*. Even the histological studies of rectoanal tissues has revealed that the animals treated with extract of bark of *A. ferruginea* has normal cytoarchitecture [Fig. 1(c)] of the rectoanal region similar to that of pilex granules treated group [Fig. 1(d)] and normal control group [Fig. 1(a)].

Though the bark extract of *A. ferruginea* has showed anti-hemorrhoidal activity equal to standard pilex granules, but it has to be noted that the standard regimen (pilex granules) comprises of many plant parts whereas the extract under investigation is single plant part. Undoubtedly this provides a logical basis for consideration of higher potency of the bark extract of *A. ferruginea* over currently used therapy of pilex granules.

In order to elucidate the possible mode of action of the anti-hemorrhoidal activity of the extract of *A. ferruginea*, a through literature survey was performed and one of the previous study revealed the presence of 5-(3,4-dimethylphenyl)-4-phenylisoxazole in *A. ferruginea* extract.[11] A close inspection of this isolate revealed structural resemblance to oxaprozin, a known NSAID. Since no anti-inflammatory and anti-hemorrhoidal activity has been reported for *A. ferruginea*, we thought to compare binding mode of oxaprozin and this isolate to the active site of cyclo-oxygenase (COX) enzyme which is responsible for catalysis of inflammatory mediators. Structure based pharmacophore exhibited specific interfeature distances required for binding of inhibitor to the active site of COX. The structures of oxaprozin and earlier reported *A. ferruginea* isolate (5-(3,4-dimethylphenyl)-4-phenylisoxazole) were mapped over developed pharmacophore. Oxaprozin exhibited two features mapping with a fit value of 1.783. Surprisingly, compound 5-(3,4-dimethylphenyl)-4-phenylisoxazole also showed two feature mapping with a

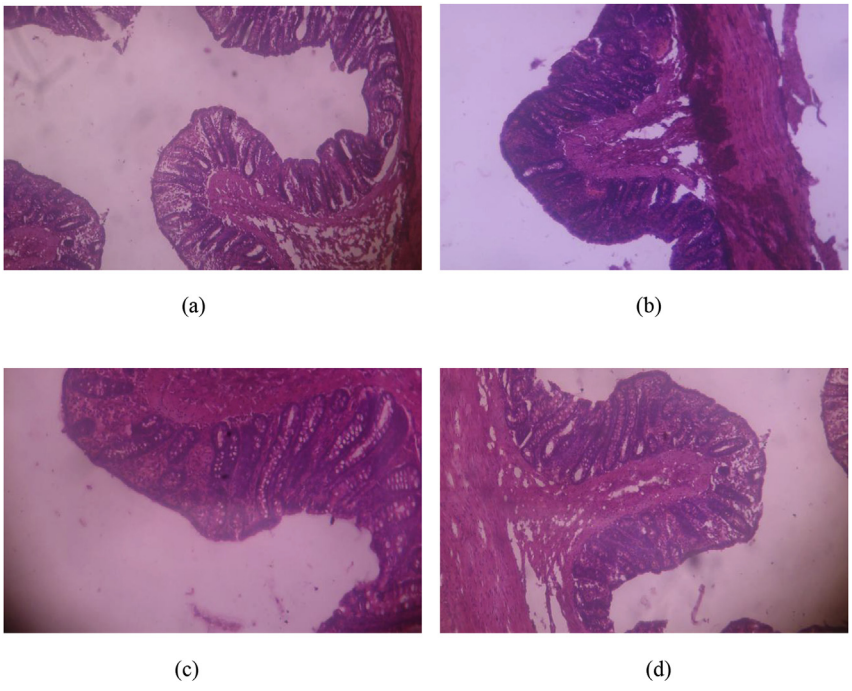
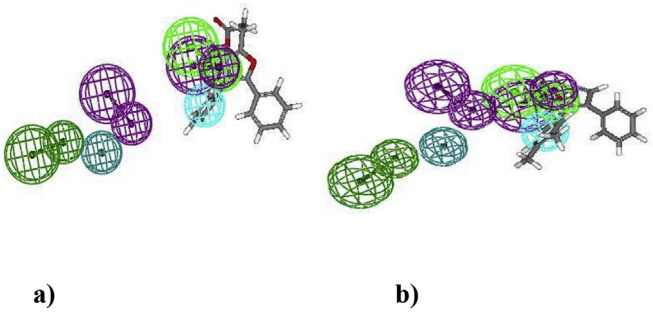
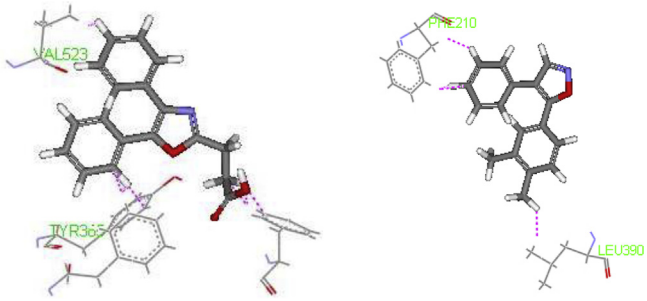


Fig. 1. Effect of Bark extract and Pilex granules on the histology of rectoanal tissue in croton-oil induced hemorrhoids. Rectoanal sections of rats: Fig. 1 (a) Group I (normal control) has normal architecture of rectal tissue; (b) Group II (positive control) exhibited severe inflammation, hemorrhage, necrosis, vasodilation and congestion (c) Group III treated with hydroalcoholic extract of bark showed minimal inflammation, congestion, hemorrhage, dilatation, degeneration and necrosis (d) Group IV treated with pilex granules (400 mg/kg p.o.) showed nearly normal architecture of the tissue.



a) b)

Fig. 2. Pharmacophore mapping of a) oxaprozin b) 5-(3,4-dimethylphenyl)-4-phenylisoxazole.



a) b)

Fig. 3. Docking pose of a) Oxaprozin b) 5-(3,4-dimethylphenyl)-4-phenylisoxazole.

comparable fit value of 1.885. In case of oxaprozin, phenyl ring mapped over hydrophobic feature and neighbouring nitrogen of oxazole ring mapped over hydrogen bond acceptor feature. Similarly, dimethylphenyl ring of 5-(3,4-dimethylphenyl)-4-phenylisoxazole mapped over hydrophobic feature, whereas oxygen of oxazole mapped over hydrogen bond acceptor feature. From the results, it is very clear that a well known COX-inhibitor like oxaprozin shares common feature to that of 5-(3,4-dimethylphenyl)-4-phenylisoxazole a constituent of *A. ferruginea*. Since our extract showed anti-inflammatory and anti-hemorrhoidal activity, there are fair enough chances that this activity is due to presence of 5-(3,4-dimethylphenyl)-4-phenylisoxazole {Fig. 2 (a,b)}.

In order to further confirm the molecular interaction of oxaprozin and 5-(3,4-dimethylphenyl)-4-phenylisoxazole, the molecular docking studies were performed. Oxaprozin docked very well with a lib dock score of 120.832. The interaction results of molecular docking and pharmacophore mapping appeared to be of similar nature. The specific interactions of oxaprozin and 5-(3,4-dimethylphenyl)-4-phenylisoxazole with catalytic site of amino acid are depicted in Fig. 3 (a, b).

4. Conclusion

In the present studies, phytochemical screening of the hydroalcoholic extract of bark of *A. ferruginea* has shown the presence of alkaloids, flavonoids, saponins, tannins and phenolic compounds. Results clearly shows that bark extract possess anti-inflammatory activity in croton oil induced hemorrhoids which could be attributed to the presence of potent antioxidants in the hydroalcoholic extract of bark of *A. ferruginea.* Moreover, extract had significantly reduced the levels of inflammatory markers like prostaglandins, leukotrienes interleukins. Molecular docking and structure based pharmacophore mapping confirms the potential of *A. ferruginea* as anti-hemorrhoidal agent.

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References

1. LeClere FB, Moss AJ, Everhart JE, Roth HP. Prevalence of major digestive disorders and bowel symptoms. *Adv Data*. 1992;212:1–15.
2. http://www.rightdiagnosis.com/h/hemorrhoids/stats-country.htm (Accessed 23.Augest.2014).
3. Johanson JF, Sonnenberg A. The prevalence of hemorrhoids and chronic constipation: an epidemiologic study. *Gastroenterology*. 1990;98(2):380–386.
4. Evans P, Halliwell B. Micronutrients: oxidants/antioxidants status. *Br J Nutr*. 2001;85(Suppl 2):S67–S74.
5. Gowri SS, Pavitha S, Vasantha K. Free radical scavenging capacity and antioxidant activity of young leaves and barks of *Acacia nilotica* (l.) Del. *Int J Pharm Pharmaceut Sci*. 2011;3(1):160–164.
6. Iqbal P, Ahmed D, Asghar MN. A comparative in vitro antioxidant potential profile of extracts from different parts of Fagonia cretica. *Asian Pac J Trop Med*. 2014;7(Suppl 1):S473–S480.
7. Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S. *Agroforestree Database: A Tree Reference and Selection Guide Version 4.0*. Kenya: World Agroforestry Centre; 2009. Available: http://www.worldagroforestry.org/resources/databases/agroforestree.
8. Akare SC, Sahare AY, Shende MA, Bondre AV, Wanjari AD. Hepatoprotective activity of *Acacia ferruginea* DC. leaves against carbon tetrachloride induced liver damaged in rats. *Int J Pharm Technol Res*. 2009;1(3):962–965.
9. Vahitha R, Venkatachalam MR, Murugan K, Jebanesan A. Larvicidal efficacy of *Pavonia zeylanica* l. and *Acacia ferruginea* DC against *Culex quinquefasciatus* Say. *Bioresour Technol*. 2002;82(2):203–204.
10. Sowndhararajan K, Kang SC. Protective effect of ethyl acetate fraction of *Acacia ferruginea* DC. against ethanol-induced gastric ulcer in rats. *J Ethnopharmacol*. 2013;148(1):175–181.
11. Sakthivel MK, Guruvayoorappan C. *Acacia ferruginea* inhibits tumor progression by regulating inflammatory mediators-(TNF-a, iNOS, COX-2, IL-1β, IL-6, IFN-γ, IL-2, GM-CSF) and pro-angiogenic growth factor-VEGF. *Asian Pac J Cancer Prev APJCP*. 2013;14(6):3909–3919.
12. Harborne JB. *Phytochemical Methods*. third ed. London: Chapman and Hall; 1998.
13. Singleton VL, Orthofer R, Lamuela- raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin ciocalteu reagent. *Meth Enzymol*. 1999;299:152–178.
14. Woisky R, Salatino A. Analysis of propolis: some parameters and procedures for chemical quality control. *J Apicult Res*. 1998;37:99–105.
15. Rajpal V. *Standardization of Botanicals*. New Delhi: Eastern publishers; 2002: 226–227.
16. Azeemuddin M, Viswanatha GL, Rafiq M, et al. An improved experimental model of hemorrhoids in rats: evaluation of antihemorrhoidal activity of an herbal formulation. *ISRN Pharmacol*. 2014:1–7.
17. Sowndhararajan K, Joseph JM, Manian S. Antioxidant and free radical scavenging activities of Indian *Acacias: Acacia leucophloea* (Roxb.) Willd., *Acacia ferruginea* DC., *Acacia dealbata* Link. and *Acacia pennata* (L.) Willd. *Int J Food Prop*. 2013;16:1717–1729.
18. Sowndhararajan K, Hong S, Jhoo JW, Kim S, Chin NL. Effect of acetone extract from stem bark of *Acacia* species (*A. dealbata*, *A. ferruginea* and *A. leucophloea*) on antioxidant enzymes status in hydrogen peroxide-induced HepG2 cells. *Saudi J Biol Sci*. 2015;22(6):685–691.