Short Communication

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

Samriti Faujdar a,*, Bhawana Sati a, Swapnil Sharma a, A.K. Pathak b, Sarvesh Kumar Paliwal b

a Department of Pharmacy, Banasthali University, Rajasthan, 304022, India
b Barkatullah University, Hosangabad Road, Bhopal, Madhya Pradesh, India

**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, triterpenoids, 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 plexigranules (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.

© 2018 Center for Food and Biomolecules, National Taiwan University. Production and hosting by Elsevier Taiwan LLC. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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 and 0.36% in India that frequently appears in the age of 45–65 years. 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. 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. Therefore much attention has been focused on the use of natural antioxidants that can provide more significant health benefits with minimal toxicities.

*Acacia ferruginea* DC, a drought resistant, deciduous tree belonging to family Mimosoideae is native to Pennsular India from Gujrat to Gunjam in the east. The bark of the plant is bitter and...
traditionally used as astringent, cure itching, leucoderma, ulcers, stomatitis and diseases of blood. Traditionally, the extract of leaves has been in use as an 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, ethanol extract of A. ferruginea leaves reported to have hepatoprotective, anti-ulcer, anti-tumor activities. In addition to this, different species of this genus has been reported to have antimalarial, anti-fungal, antibacterial, anti-diarrhoeal, anti-oxidant, antiviral, hepatoprotective and anti-spasmodic 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. In 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 iso-flurane 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 PGE2 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). Micromote 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.

2.5. Quantification of phytoconstituents

Hydroalcoholic extract of bark was evaluated for the estimation of total phenolic content, total flavonoids and saponins.

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 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

Accelrys 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 database (PDB entry: 4rtw). 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 Å8, 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 LibDock 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 rectoanl region caused significant increase in RAC (P < 0.001), PGE2 (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 group 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 ± 0.0884 (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 hydro-alcoholic 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.,16 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 | PGE2 |
|-------|-----------------------|----------------|-----------------------|------------|-------|------|------|
| 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 | 0.988 ± 0.094         | 1.333 ± 0.739 | 8.40 ± 0.388 | 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 revealed by the histological studies of rectoanal tissues has revealed that the animals treated with extract of bark of A. ferruginea has normal architecture of rectal tissue; (b) Group II (positive control) exhibited severe inflammation, hemorrhage, necrosis, vasodilation and congestion (c) Group III treated with standard therapy of pilex granules 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.

![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.](image-url)
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.

Acknowledgement

The authors are thankful to Department of Pharmacy, Banasthali University for their continuous support. We are thankful to Dr. Manvendra Singh, Research Co-coordinator Pinnacle Biomedical Research Institute (PBRI), Bhopal for carrying out anti-hemorrhoidal studies.

References

1. LeClerc 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 August 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, Pavithra S, Vasanthan 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, Ahnud 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, Muthia A, Kindi R, Jammadass R, Anthony S. Agroforestry Database: A Tree Reference and Selection Guide Version 4.0. Kenya: World Agroforestry Centre; 2009. Available: http://www.worldagroforestry.org/resources/databases/agroforestry.
8. Akare SC, Sahare AV, 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. Valitha 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. Sakhthivel MR, Guruvayoorapppan 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(5):3909–3915.
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 ciocalteau reagent. Meth Enzymol. 1999;299:152–178.
14. Wosicky R, Salatin A. Analysis of propolis: some parameters and procedures for chemical quality control. J Apicult Res. 1998;37:99–105.
15. Raja R. Standardization of Botanicals. New Delhi: Eastern publishers; 2002: 226–227.
16. Azeemuddin M, Viswanatha GL, Raflq M, et al. An improved experimental model of hemorrhoids in rats: evaluation of antimicrobial 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;16:1717–1725.
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):695–691.