



Hemorrhoids: A Review on Herbal Treatments and Models for Pharmacological Evaluation

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ABSTRACT

Hemorrhoids are the most common anorectal disease which is characterized by alteration in vasculature of the anal canal including blood vessels, supporting tissues, muscles and elastic fibers. The aim of this review is to determine the importance of herbal drug in the treatment of hemorrhoids and pharmacological evaluation by using some models and in vitro studies. The role of *Acacia ferruginea*, *Allium iranicum*, *Balanites aegyptiaca*, *Euphorbia prostrata*, *Malva sylvestris*, *Myrtus communis*, *Phlomis grandiflora*, *Polygonum cognatum*, *Portulaca oleracea*, *Wendlandia heynei* in hemorrhoid treatment are discussed. According to this review it is found that above mentioned herbal plants decreased the level of inflammatory mediators and contributed to healing of hemorrhoidal edema.

Keywords: Hemorrhoid, Inflammatory mediators, Flavonoid, croton oil, Jatropha oil.

INTRODUCTION

Hemorrhoids are the most common anorectal disease¹ characterized by alteration in vasculature of the anal canal including blood vessels supporting tissues, muscles and elastic fibres.² There is a network of small veins within the inner lining of the anus and lower rectum. These veins occasionally become wider and engorged with more blood than usual. These engorged veins and the overlying tissue may then develop into one or more small areas of swelling called hemorrhoids.³

The symptoms of hemorrhoids are discomfort, itching, pain, inflammation, bleeding.⁴ Various factors are responsible for hemorrhoids like constipation, life style, pregnancy, low fiber diet, obesity and other environmental factors.⁵ There are three types of Hemorrhoids: Internal Hemorrhoids, External Hemorrhoids, Mixed Hemorrhoids.

Internal Hemorrhoids begin above the dentate line which are covered by mucosa, typically bleed or prolapsed but do not cause pain. It is divided into four categories depending on the grade of prolapsed.

- I. Grade 1: Protrudes in the anal canal but does not prolapse
- II. Grade 2: Prolapses but reduces spontaneously.
- III. Grade 3: Prolapses and requires manual reduction.
- IV. Grade 4: Irreducible prolapsed.

External hemorrhoids originate below the dentate line which cause pain and itching.

Mixed hemorrhoids indicate lesions that arise at the dentate line or the term can be used to describe the presences of both internal and external hemorrhoids.⁶

The exact pathophysiology of hemorrhoids is poorly understood. There are four theories of the

pathophysiology of hemorrhoids. First, the vericose vein theory⁷ which suggested that hemorrhoids are caused by vericose veins in the anal canal, has been shown to be faulty, because hemorrhoids and anorectal varices are proven to be different.⁸ Second, the theory of vascular hyperplasia that hemorrhoids resemble penile erectile tissue and third, internal anal sphincter hypertonia, but both are not completely accepted.⁹ The pathological changes occur like abnormal venous dilatation, vascular thrombosis, degenerative process in the collagen fibers and fibroelastic tissues, distoration and rupture of the anal subepithelial muscle along with this, changes a severe inflammatory reaction, mucosal ulceration, ischemia and thrombosis.¹⁰

Plant Sources Used in Treatment of Hemorrhoids

1. *Acacia ferruginea* DC.

It is deciduous tree belonging to family *Mimosoideae*.¹¹ The bark of the plant is bitter and used as astringent, cure itching, leukoderma, ulcers, stomatitis and blood related diseases. The *A. ferruginea* is a rich source of tannins (catechin, epigallocatechin), terpenoids, polyphenolics (gallic acid) and saponins. Also, the flavonoids, phenols, alkaloids, terpenoids, anthraquinones are chemical constituents of *A. ferruginea*. Glycosides and saponins are also present in trace amounts.¹²

The flavonoids in *A. ferruginea* reduce the concentration of PGE_{2e}, PGE_{2α} and others inflammatory mediator. It also increases the vascular tone and reduces the vascular fragility and resistance. The antioxidant and ant hemorrhoidal activity are due to presence of flavonoids in the bark of *A. ferruginea*.²

2. *Aesculus hippocastunum*

Aesculus hippocastunum belonging to family *Sapindaceae* is a large deciduous tree known as horse-chestnut or



conker tree. It produces large chestnut like seeds that are dried for medicinal use. The seeds consist of complex mixture of triterpene saponins (escins).¹³ It also contains flavonoids and tannins.¹⁴ It is native to southeast Europe. It is used in treatment of Dysentery, bronchitis, haemorrhoids and venuoles problem.¹⁵

In vitro studies showed horse chest nut inhibit activity of elastase and hyaluronidase enzymes responsible for degradation of proteoglycan, degradation of the capillary endothelium and extra vascular matrix.¹⁶

Horse chest nut seed extract reported to possibly reduce capillary permeability and oedema and inhibit enzymes involved in proteoglycan degradation which warrants its use in haemorrhoid treatment.¹⁷

3. *Allium iranicum*

Leek (*Allium iranicum*) belonging to family *Aliaceae* is one of the herbs used for haemorrhoids as oral and topical medication.¹⁸

Leek is a common edible vegetable in Persian people's diet.¹⁹ Leek is traditional Persian medicine as a hot and dry temperament vegetable which is useful in control of bleeding in different conditions such as epistaxis, menorrhagia and haemorrhoids.²⁰

From leek, steroidal saponin such as spirostane type saponin, furostane type saponins and spirostane type saponin which shows the anti-inflammatory and antiulcerogenic effect.^{21,22} Also, flavonoid and phenolic component from the leaves of *Allium iranicum* having the antioxidant activity.²³ Another indication of leek is pain control in painful conditions like headache.²⁰

4. *Balanites aegyptiaca*

The seed kernel of *Balanites aegyptiaca* belongs to family *Zygophyllaceae* are rough and hard. This tree is native to much of Africa and parts of the middle East. In folk medicine, bark, fruits, seeds, seed oil and leaves of this plant are widely used.²⁴ This seed possess many activities like antioxidant, antimicrobial, anticancer, diuretic, antiviral, wound healing, hypocholesterolemic, antidiabetic, hepatoprotective, anti-inflammatory and analgesic, mosquito larvicidal, antivenin, anthelmintic, cardio-protective cum antioxidant activity and antinociceptive properties.²⁵

The seed kernel of *Balanites aegyptiaca* consists of flavonoids and saponins and play important role in oxidative stress, inflammation and hemorrhoid. The flavonoids reduce the concentration of PGE_{2e} and PGE_{2a} and other inflammatory mediators. The flavonoids increase the vascular fragility and resistance.²⁶

5. *Euphorbia prostrata*

Euphorbia prostrata is an annual herb, which belongs to family *Euphorbeaceae* and is abundantly found in India and Africa. Traditionally, it has been used in several digestive system disorders.²⁷ The main constituents of *Euphorbia*

prostrata are flavonoids, phenolic acid and tannins. The flavonoids and phenolic acid have been used as anti-inflammatory, analgesic, antioxidant, haemostatic, antithrombotic and vasoprotective agent.²⁸ Beneficial effect of the *Euphorbia prostrata* in haemorrhoids have multiple mechanism that are due to its active principle flavonoids, tannins and phenolic acid.²⁹

The chemical analysis of *Euphorbia prostrata* revealed that it contains phenolic compounds like Gallic acid which activates Hageman factor which causes hypercoagulability and ellagic acid which suppress histamine release. Tannins have astringent and haemostatic properties.³⁰

Beneficial effect of *Euphorbia prostrata* in haemorrhoids have multiple mechanisms³¹ like improvement of venous tone, increased lymphatic drainage, protection of capillary bed microcirculation, inhibition of inflammatory reactions and reduced capillary permeability.

Flavonoids in *Euphorbia* inhibit the prostaglandin E₂ (PGE₂) and thromboxane A₂(TXA₂) and inhibit the leukocyte activation, migration and adhesion. It is non-toxic and may be given orally without loss of efficiency.³²

6. *Malva sylvestris*

Leaves of *Malva sylvestris*, belonging to family *Malvaceae*, have been utilized as a folk medicine for the treatment of haemorrhoids. It is known as mallow in English, 'Ebegummei' in Turkish.³³

In traditional medicine, leaves and flowers of plant are useful in many diseases like haemorrhoids, inflammatory and respiratory problems, dermatological and urologic symptoms and gastrointestinal diseases such as heartburn, diarrhoea, constipation and stomach-ache.³⁴

The *Malva sylvestris* rich in mucilage and flavonoids. The flavonoids reduce the concentration of inflammatory mediators and contributed to healing of hemorrhoidal oedema, vasoconstriction of vessels and inflammation.³⁴

7. *Myrtus communis*

Myrtus communis is an important plant in the treatment of many different diseases. *Myrtus communis* is known as Murt or Murd in traditional books. It is a cold temper, astringent and decisive temper.³⁵

It is used topically, orally, and in inhaled form in the treatment of pain and inflammation of testitis, hedaque, arthritis, otitis, chronic eye diseases, gingivitis and haemorrhoids.³⁶

Inflammation has a key role in the pathogenesis of chronic venous insufficiency conditions such as hemorrhoids.³⁷ 1,8- cineole is a main constituent of *Myrtus communis* essential oil which reduce the inflammatory cytokines, mediators (TNF- α AND IL-6) and inhibit the migration of neutrophils to areas with inflammation.³⁸ Other constituents of *Myrtus communis* such as α - pinene and 1,8-cineole shows anti-inflammatory and anesthetizing effects respectively.³⁵



8. *Phlomis grandiflora*

Phlomis grandiflora, belonging to the family *Lamiaceae*, are widespread in Asia, Europe and North Africa has been utilizing traditionally for the treatment of wound, inflammation, ulcer, diabetes and haemorrhoid.³⁹

Phlomis grandiflora known as ballokotu, cauba or sauba in Anatolia has been used in a tea form due to its tonic appetizer, carminative and stimulant effects.⁴⁰

The plant includes mainly phenylethanoid, phenylpropanoid, irridanoid, glycosides and essential oils.^{41,42} The phenolics such as flavonoids or iridoids glycosides of are responsible for anti-hemorrhoidal activity.⁴²

The phenolic/ flavonoid composition of plant has the anti-hemorrhoidal activity through inducing capillary permeability.⁴³

9. *Polygonum cognatum*

Polygonum cognatum Meissn called 'Madimark' is an endemic plant belonging to family *Polygonaceae*. It is widely consumed in Turkey. *Polygonum cognatum* used as anti-inflammatory and antioxidant. The chemical constituents present in *Polygonum cognatum* Meissn are berbamine, Berberine, berberrubine alkaloids, tannins, saponins, sterols, triterpenes, carbohydrate, polyuronides and many other compounds which has antibacterial, antioxidant and antitumoral effects.⁴⁴ Tannins are compound of *Polygonum cognatum* have antiradical activities and antioxidant.⁴⁵ Tannins are considered to be components "promoting and development of health" in plant derived beverages and foods.⁴⁶ It is also able to chelate metal ions and results in delaying oxidation. Saponins are effective as antimicrobial, anti-inflammatory, antitumoral, antiviral, antioxidant.⁴⁷ *Polygonum cognatum* reduce the concentration of inflammatory mediators and used in haemorrhoid treatment.⁴⁴

10. *Portulaca oleracea*

Portulaca oleracea L. belonging to the *Portulacaceae* family is a cold in nature, sour in taste and is used to cool the blood, stanch bleeding, clear heat and resolve toxins.⁴⁸ *Portulaca oleracea* has been used as a folk medicine in many countries acting as a febrifuge, antiseptic vermifuge and so forth.⁴⁹

It exhibits a wide range of pharmacological effects like antibacterial, antiulcerogenic, anti-inflammatory, antioxidant and wound healing properties. It is listed by WHO as one of the most used medicinal plants and it has been given the term "Global Panacea"

It is described as "vegetable for long life" and it has been used for thousands of years in traditional Chinese medicine. One of the most effective constituents present in Chinese Herbal medicines are flavonoids which have wide range of pharmacological properties. The flavonoids of *Portulaca oleracea* reduce the concentration of PGE_{2e} and PGE_{2a} and other inflammatory mediators. The butanol

fraction reduced the level of inflammatory markers like prostaglandins, leukotrienes, interleukins.⁴⁸

11. *Wendlandia heynei*

Wendlandia heynei, a flowering plant, belonging to family *Rubiaceae* natively identified as Ukan and Pansara.⁵⁰ The folklore use of this plant is in ulcers, wound, swelling, dysentery diarrhoea, urinary ailments, colds and coughs, skin diseases, fever and body aches.^{51,52}

The phytoconstituents of *Wendlandia heynei* like alkaloids and terpenoids shows anti-inflammatory activities.⁵³ The phenolics and flavonoids compounds also have the anti-inflammatory activity. Rutin, gallic acid, catechin and myricetin are secondary metabolite of plants with admirable therapeutic role in inflammation disorders.⁵⁴

Catheic acid imparted both in vitro and in vivo anti-inflammatory properties through modulation of iNOS expression or others inflammatory mediator [55]. The phenolics and flavonoids of *Wendlandia heynei* exert the anti-inflammatory activity because of inhibition of cytokines (TNF α and IL-6) and nitric acid.⁵⁰



Figure 1 - *Aesculus hippocastnum*



Figure 2 - *Phlomis grandiflora*



Figure 3 – *Malva sylvestris*

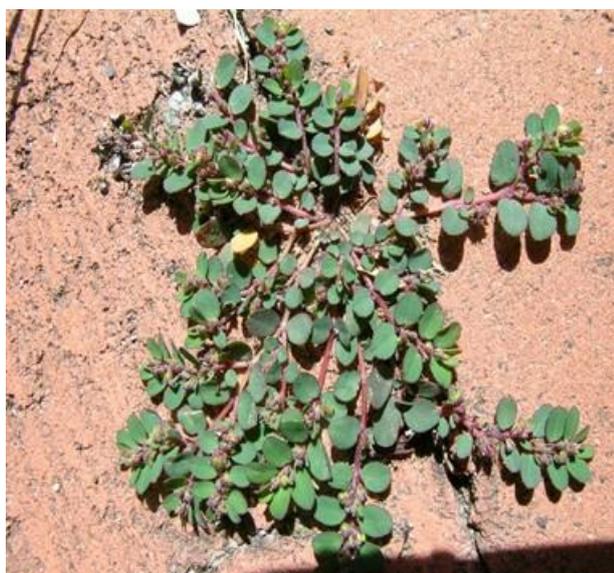


Figure 4 – *Euphorbia prostrata*

Pharmacological Evaluation of Herbal Drugs by Using Induction Models for the Treatment of Haemorrhoids

There are two models for induction of hemorrhoids.

1. Croton oil induction model
2. Jatropha oil induction model

1. Croton oil induction model

A Hemorrhoid model prepared by applying croton oil preparation on to recto-anus of animal [56]. Hemorrhoids induce by croton oil solution (containing deionized water, pyridine, diethyl ether, and 6% croton oil in diethyl ether in the ratio of 1:4:5:10). Sterile cotton swabs (4 mm diameter) soaked in 100 μ l of croton oil preparation insert into the anus (rectoanal portion, 20mm from anal opening) of the study animals followed by an overnight fasting and kept for 10 seconds. The edema will develop linearly until 7 to 8 hours after croton oil application.⁵ This procedure repeats for three days once a daily.

2. Jatropha oil induction model

Hemorrhoids induce by applying jatropha oil preparation on to recto-anus of study animal. Sterile cotton swabs (4 mm diameter) soaked in jatropha oil preparation insert into the anus (rectoanal portion, 20mm from anal opening) of the study animals followed by an overnight fasting and kept for 10 seconds This application repeat for five days.⁵⁷

PHARMACOLOGICAL EVALUATION

For the pharmacological evaluation of herbal drugs following methods are used.

1. Anorectal coefficient
2. Biochemical parameter
3. Histopathological observation
4. In vitro tests

1. Anorectal coefficient

The animals and their isolated recto- tissues (2cm in length) weigh to calculate the anorectal coefficient. ARC calculated by following formula:

ARC= Anorectal tissue weight (milligram)/ Animal weight (gram).³³

2. Biochemical parameter

ELISA kit (YL Biont), Rat Vascular Endothelial cell Growth Factor (VEGF), Rat tumor necrosis factor- α (TNF- α), superoxide dismutase (SOD) Assay KIT 500 test (Sigma Aldrich) are used for evaluating serum TNF- α , VEGF and SOD levels. TNF- α and VEGF levels to interpret the degree of inflammation. SOD is associated with the antioxidant capacity of the tissue.³³

3. Histopathological observation

Recto-anal tissue samples were fixed in 10% neutral buffered formalin before processing and embedding in paraffin blocks. Sections of the samples were prepared at 4 μ m thickness using a rotary microtome and were stained with haematoxylin and eosin (H&E) stain; then they are observed under a microscope using a digital camera system to note the appearance of inflammatory cells, congestion, haemorrhage, vasodilatation, and medium to high degrees of necrosis.⁵⁸

4. In vitro tests

Following are the in vitro tests for evaluating the anti-inflammatory activity of herbal plants.

- Inhibition of protein denaturation assay
- Membrane stabilization method
 - A] Hypotonic solution induced haemolysis
 - B] Heat induced haemolysis
- Assay of cyclooxygenase and 5-lipoxygenase inhibition
 - A] Anti- cyclooxygenase activity

B] Anti-lipoxygenase activity

- Assay of proteinase inhibition
- Hyaluronidase inhibition assay

1. Inhibition of protein denaturation assay

In this assay either egg albumin or bovine serum albumin (BSA) are used as protein. Denaturation of protein is induced by keeping the reaction mixture at 70°C in a water bath for 10 minutes.

A reaction mixture consists of various concentrations of plant extract 1000 µL (100-500 µg/ml), 200 µL of egg albumin or 450 µL (5% w/v aqueous solution) bovine serum albumin, 1400 µL of phosphate buffered saline. Distilled water instead of extracts with above mixture is used as a negative control. Afterward, the mixtures incubated at 37 °C for 15 min and then heated at 70°C for 5 min. After cooling under running tap water, their absorbances are measured at 660 nm. Acetyl salicylic acid or diclofenac sodium or ibuprofen or indomethacin is taken as a positive control. The experiment is carried out in triplicates and percent inhibition for protein denaturation is calculated using following equations:

$$\% \text{ Inhibition of denaturation} = (1-D/C) \times 100$$

Where D is the absorbance of test sample and C is the absorbance of negative control (without the test sample or reference drug).⁵⁹

2. Membrane stabilization method

The membrane stabilizing activity of the extracts can be determined through heat induced haemolysis and hypotonic solution induced haemolysis using human erythrocytes, rat erythrocytes or mice erythrocytes. The erythrocyte membrane analogues to lysosomal membrane therefore the effect of extracts on the stabilization of erythrocyte applied to the stabilization of lysosomal membrane.

Hypotonic solution induced hemolysis

This experiment carries out with hypotonic solution. A number of different agents can be used as hypotonic solutions, including hypo saline (50mM NaCl in 10mM sodium phosphate buffer saline-pH7.4) and distilled water. Reaction mixture contain erythrocyte suspension, plant extract and hypotonic solutions. Control is prepared by omitting the plant extract. Acetyl salicylic acid or indomethacin or diclofenac can be used as reference standard drug. This mixture is incubated at 56°C for 30 minutes in water bath and centrifuged at 3000rpm for 10 minutes. Finally the hemoglobin content of the supernatant solution is estimated by spectrophotometrically at 560 nm. The percentage of Red blood cell membrane stabilization or protection is calculated by the following equations.

$$\% \text{ protection} = 100 - \frac{\text{Optical density of drug treated sample} \times 100}{\text{Optical density of control}} \times 100$$

Heat induced hemolysis

This method carries out with produce the heat to aliquot by incubation. The reaction mixture (2ml) consist of 1 ml test sample of different concentrations 100 - 600µg/ml or 5ml of isotonic buffer containing 2.0mg/ ml with different extractives and 10% erythrocyte suspension 1 ml or 30 µl, instead of test sample only vehicle is added to the control test tube. 50 -400µg/ml Diclofenac sodium or acetyl salicylic acid (aspirin) is used as a standard drug. This reaction mixture is mixed gently by inversion. All the centrifuge tubes containing reaction mixture is incubated in water bath at 60°C or 56°C or 54°C for 30 minutes or 20 minutes. Some workers have prepared the duplicate of above reaction mixture and the other pair is maintained at 0-5° C in an ice bath. At the end of the incubation the tubes are cooled under running tap water. The reaction mixture was centrifuged at 3000 rpm for 5 min or 2500 rpm for 10 minutes or 1300rpm for 3 minutes and the absorbance of the supernatants is taken at 560 nm. The experiment is performed in triplicates for all the test samples. The percentage inhibition of hemolysis is calculated as follows:

$$\text{Percentage inhibition of hemolysis} = \frac{\text{Absorbance control} - \text{Absorbance test} \times 100}{\text{Absorbance control}}$$

$$\% \text{ Inhibition of hemolysis} = 100 \times [1 - (\text{OD1} - \text{OD2}) / (\text{OD3} - \text{OD1})]$$

Where OD1 = Optical density of unheated test sample

OD2 = Optical density of heated test sample

OD3 = Optical density of heated control sample.⁵⁹

3. Assay of cyclooxygenase and 5-lipoxygenase inhibition

Arachidonic acid (AA) is metabolized in the body through two main metabolic pathways with the enzymes: Cyclooxygenase and 5-LOX. The COX pathway produces prostaglandins and thromboxane whereas 5-LOX pathway produce eicosanoids and leukotrienes.⁶¹ It has been suggested that the inhibition of both pathways prevent the production of prostaglandins and leukotriene thereby it might produce the synergistic effects and achieve optimal anti-inflammatory activity. Hence the dual inhibition of the COX and 5-LOX pathway could have a wider spectrum of anti-inflammatory effects.⁶²

Anti- cyclooxygenase activity

This enzymatic assay is determined by the using colorimetric COX (ovine) inhibitor screening assay kit. The chromagenic assay is based on the oxidation of TMPD during reduction of P G-G2 (prostaglandin-G2) to PG-H2 and the change in colour is measured using a spectrophotometer.

In brief, the assay mix consist of test compounds in different concentrations, either aspirin or indomethacin is used as a reference drug and other chemicals are added according to the manufacturer's instructions. The plate is shaken for few seconds and incubated for 5 minutes at

25°C. The reaction is initiated by the addition of arachidonic acid (20µl) and TMPD (20 µL) to all the wells. The plate is shaken for few seconds and incubated for 5 minutes at 25°C. The absorbance is measured at 590 nm using micro plate reader. All the reactions are carried out in triplicates.⁵⁹

Anti-lipoxygenase activity

Anti-lipoxygenase activity has studied using linoleic acid as substrate and lipoxygenase as enzyme. Soybean lipoxygenase or human recombinant lipoxygenase can be used as enzyme. Here we summarize the method of analyzing this assay. Reaction mixture consist of sodium phosphate buffer (1 mL, 0.1 M, pH 8.8), 10 µl of plant extract, (10 µL, final concentration 8000U/mL of soybean lipoxygenase solution (167 U/ml) are mixed and incubated at 25°C for 10 min. The reaction is initiated by the addition of 10 µl of the substrate in the form of sodium linoleic acid solution. The absorbance is measured at 234 nm over a period of 3 minutes in every minute using UV-vis spectrophotometer. Nordihydroguaiaretic acid (NDGA) or indomethacin or quercetin is used as positive reference drug. Control is prepared by omitting the plant extract/ drug to the above mixture. All the reactions are performed in triplicates. The percentage of inhibition is calculated as:⁶³

$$\% \text{ inhibition} = \frac{\text{Abs control} - \text{Abs extract} \times 100}{\text{Abs control}}$$

4. Assay of proteinase inhibition

It is demonstrated that proteinase implicate the tissue damage during the inflammatory reactions. Proteinases abundantly exist in lysosomal granules of neutrophils. Therefore, proteinase inhibitors provide the significant level of production.

In this assay different enzymes and different protein can be used, enzymes are proteinase or trypsin and casein and bovine serum albumin are used as protein. The reaction mixture (2 ml) contain 0.06 mg proteinase or trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample/ standard drug, Diclofenac sodium, of different concentration 100600 g/ml. The mixture is incubated at 37°C for 5 min and then 1 ml of 0.8% (w/v) casein or 4% (w/v) bovine serum albumin is added. The mixture is incubated for an additional 20 min. 2 ml of 70% perchloric acid or 5% trichloroacetic acid (TCA) is added to terminate the reaction. Cloudy suspension is centrifuged at 3000 rpm for 10 minutes or 2500 rpm for 5 minutes and the absorbance of the supernatant is read at 210 nm or 217 nm against buffer as blank. The experiment is performed in triplicate. The percentage inhibition of proteinase inhibitory activity is calculated using the following equation.

Percentage inhibition = (Abs control – Abs sample) X100/ Abs control.⁶³

5. Hyaluronidase inhibition assay

In this assay, hyaluronic acid is used as the substrate. Plant extract samples (5mg) are dissolved in dimethyl sulphoxide (250 µL). The samples are prepared at various concentrations (100, 200, 300, 400 and 500 µg/mL) by dissolving in sodium phosphate buffer (200 mM, pH 7). Hyaluronidase (4U/mL, 100 µL) is mixed with sample solution (25 µL) incubated for 10 min at 37°C.

After that, the reaction is initiated with the addition of substrate, hyaluronic acid solution (0.03% in 300mM sodium phosphate, pH 5.4, 100 µL) and incubated at 37°C for 45min. The undigested hyaluronic acid is then precipitated with acid albumin solution (bovine serum albumin (0.1%) in sodium acetate (24 mM), pH 3.8, 1 mL). After 10min incubation at room temperature, absorbance is measured at 600nm. The absorbance measurement in the absence of enzyme is used as a control value for maximum inhibition. Either quercetin or indomethacin is used as the positive control to verify the performance of the assay. The assay is performed in triplicate. The percentage of hyaluronidase inhibition is determined using the following formula:

Percentage inhibition = Abs sample/ Abs control X100 .⁵⁹

CONCLUSION

In the present review, it shows that many herbal plants have the anti-hemorrhoidal activity and also anti-inflammatory activity which is effective in the treatment of hemorrhoid. This anti-hemorrhoidal activity is evaluated by using the croton oil induction model and *Jatropha* oil induction model and also In vitro tests.

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