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PHYTOCHEMICAL SCREENING AND ANTI-HEMORRHOIDAL POTENTIAL OF LEAVES EXTRACT OF MALVASTRUM COROMANDELIANUM

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ABSTRACT

Hemorrhoid disease accounts for 3.3 million ambulatory care visits annually in the US, making it the fourth commonest outpatient gastrointestinal issues. This research focuses phytochemical and anti-hemorrhoids evaluation of leaves extract of M. *coromandelianum* in experimental animal models. The botanist was verified the authenticity of the fresh *M. coromandelianum* leaves that were harvested in the Meerut area. The leaves are cleaned to remove any dust and then air-dried or dried in the shade and extracted through maceration process using. Croton oil (6%), Pilex and methanol were purchased from local vendor. Animals weighing between 150 and 200g, both male and female Wistar rats were purchased from the Animal House at the Translam Group of Institutions in Rajpura, Mawana Road, Meerut- 250001, UP. The animals are kept in ideal conditions, with a light/dark cycle of 12 hours per day and a room temperature of 25±1°C. Rats were divided in 5 groups i.e., Group 1 administered vehicle; Group 2: rats are given 6% croton oil (10µl, p. o.) + normal saline (p. o.); Group 3: rats are given 6% croton oil (10µl, p. o.) + Pilex (1 tab./kg, p. o.); Group 4: rats are given 6% croton oil (10µl, p. o.) + *M. coromandelianum* (400mg/kg, p. o.) and Group 5: rats are given 6% croton oil (10µl, p. o.) + *M. coromandelianum* (200mg/kg, p. o.). The parameters including inflammatory index, recto-anal coefficient, biochemical analysis and histopathology were evaluated. In results, it was observed that *Malvastrum coromandelianum* significantly decreased the inflammatory score (Grade II). It also facilitated the recto anal coefficient as 0.07 (at higher dose). In biochemical estimation, SOD demonstrated marked decreased in test group, and response was recorded in dose-dependent manner. Histopathology exhibited a significant decline in the inflammatory zone and that might be decreased level of inflammatory mediators in the structures of anal tissues. It concluded that methanolic leaves extract of *Malvastrum coromandelianum* is effective in the management of hemorrhoids in rats when compared with the control group.

KEYWORDS: *Malvastrum coromandelianum*, anti-hemorrhoidal, inflammatory score, recto-anal coefficient, and histopathology.

INTRODUCTION

One of the most prevalent inflammatory diseases, hemorrhoids are defined by changes in the blood vessels, accessory tissues & muscles, and elastic fibers of the anal canal (Everhart & Ruhl, 2009). It is widely known that an imbalance between reactive oxygen species and their metabolites leads to an excess concentration of free radicals (Evans & Halliwell, 2001). Hemorrhoid disease accounts for 3.3 million ambulatory care visits annually in the US, making it the fourth commonest outpatient gastrointestinal issues. In the United States, 4.4% of the population, or 10 million people every year, report experiencing hemorrhoids. The greatest incidence for both sexes occurs between the ages of 45 and 65 (Johanson and Sonnenberg, 1990). Bunch of smooth muscles, vascular tissues and connective tissues line the anal canal on the left side, right side, and right side, respectively, causing the condition known as hemorrhoids (Goenka et al. 1991).

Malvastrum coromandelianum (False mellow), a native of tropical America, is widely dispersed in the tropics and subtropics of the world and is regarded as a highly invasive weed species of the Malvaceae family (Ajibesin et al. 2008). This plant species is particularly abundant on India's penin-sular and disturbed Western Ghats seacoasts (Ghani & Batool, 2012). It is a 60 to 90 cm tall, upright, woody, annual herb or undershrub. The leaf blade has an acute apex and is ovate-lanceolate, petiolate (0.7-3cm), pilose, with lanceolate stipules (5-7mm). The solitary, axillary flower with a pilose pedicel (3 to 5 mm). The calyx is accrescent and cup-shaped (5-7mm).

Yellow and ovate petals. The fruit has 8–12 tricuspidmericarps and is schizocarpic with reniform fruit (Flora of China, 2007).

Fig. 1: Leaves & flower of *M. coromandelianum*.

Taxonomy

Division	:	Angiosperms
Order	:	Malvales
Family	:	Malvaceae
Genus	:	*Malvastrum*
Species	:	*cormandelianum*

Analyzing M. coromandelianum with gas chromatography-mass spectrometry (GC-MS) revealed 29 bioactive chemicals i.e., tris (2,4-di-tert-butylphenyl) phosphate is an antioxidant, octadecanoic acid is anticancerous, anti-inflammatory, hepatoprotective, anti-arthritic, and antibacterial, 9,12-octadecadienoic acid methyl ester (linoleic acid ester) is antioxidative, antihistaminic, anticholesterolemic, N-methyl-phenyl ethylamine, Dotriacontanol, Dotriacontane, Hypocholesterolemic, ampesterol, -phenyl ethylamine, Stigmasterol and sitosterol (Sanghai et al. 2013).

Based on above ROL, it find-out that anti-hemorrhoids potential of leaves extract of M. *coromandelianum* has not been evaluated yet. This research focuses phytochemical and anti-hemorrhoids evaluation of leaves extract of M. *coromandelianum* in experimental animal models.

MATERIALS AND METHODS

Experimental requirements

M. coromandelianum leaves, methanol, croton oil (6%), Pilex,, water-bath, distilled water, Wistar albino rats, rotatory evaporator and weight machine.

Collection and preparation of extract

The botanist was verified the authenticity of the fresh M. coromandelianum leaves that were harvested in the Meerut area. The leaves are cleaned to remove any dust and then air-dried or dried in the shade. After the leaves have dried, they are ground into a coarse powder and then finer powder. After measuring out 15 days of soaking time in methanol with gentle stirring, the powder is ready to be used. The resultant mixed slurry is dried in a rotary evaporator or water bath at low temperatures and in a partial vacuum. Below is a formula for determining the yield percentage of *M. coromandelianum* leaf extract (Khandelwal, 2002).

$$\text{percent yield} = \frac{\text{actual yield}}{\text{theoretical yield}} \times 100\%$$

Phytochemical Screening

The plant extracts were screened for different phytoconstituents to check their presence. To get better knowledge about the phytochemicals in the obtained extracts qualitative test alkaloids, terpenoids, proteins etc. were performed following standard chemical tests (Bhatt and Dhyani, 2012).

Saponins test

A modest amount of extract and water should be combined in a test tube for the foam test. When saponin is present, vigorous shaking produces foam that lasts for around 10 minutes.

Alkaloids

Chloroform is used to dissolve plant extract in the Dragendroff test. After the chloroform has been acidified and evaporated, add a few drops of Dragendroff's agent. Orange-red precipitate indicates the presence of alkaloids.

Mayer's test

Adding Mayer's reagent to 2–3 ml of filtrate yields ppt.

Wagner's test

When Wagner's reagent is added to 2–3 ml of filtrate, a reddish-brown colour develops. The presence of alkaloids in plant extracts was determined by adding a few drops of Hager's reagent and observing the resulting yellow precipitate.

Carbohydrate analysis

The Fehling's test

Boiling for one minute a millilitre of each of Fehling's A and B solution mixtures. A similar quantity should be added to the solution of test extract. Immerse for about 5-10 minutes maximum. Carbohydrates are present as indicated by the appearance of a precipitate of orange red colour.

Benedict's test

Combine test extract with Benedict's reagent in a test tube at a volume ratio of 1:1. Bring to a boil and let sit for five minutes. The consensus appears to be positive. The test solution's hue will be either yellow or red, depending on the concentration of disaccharide present.

Flavonoid Analysis

Ferric chloride test

Mix a few drops of diluted ferric chloride with the extract's ethanol solution. The colour green represents the presence of flavonoids. Add 5ml of 95% ethanol, a few drops of hydrochloric acid, and 0.5g of magnesium

powder to a dry extract to perform the Shinoda test. There's a hint of pink there. The yellow hue of test solutions is heightened after being treated with sodium hydroxide solution, but it disappears when a weak acid is added. Adding a few drops of lead acetate to the test solution causes a greenish-lemon precipitate to form.

Protein analysis

Biuret test

Add 2 ml of the biuret solution, mix it well, and heat it in a water bath. Red or violet hues indicate the presence of proteins. Mix 3 ml of extract with a few drops of a 15 CuSO4 solution and 4 percent NaOH. It gives off a pink/violated vibe.

Million's test: A reddish-brown precipitate is formed when a standard solution is added to Million's Reagent and then heated. The xanthoprotein test is boiling the test solution in concentrated nitric acid to create a yellow precipitate.

Ninhydrin test

It involves a reagent called ninhydrin, which gives the test solution a bluish tint.

Glycosides identification

Keller-Kelliani test

Two millilitres of ferric chloride solution and a few drops of glacial acetic acid were combined to make the test solution. As sulfuric acid is dripped into the sides of the test tube, the top layer turns blue-green, and the two layers split to reveal a reddish-brown tint.

Amino acids test

Ninhydrin Test

Bring 3 ml of extract and 3 drops of a 5% Ninhydrin solution to a boil in a water bath, then let it sit for 10 minutes. The sky turns violet or blue. Warm 3 ml of extract and add 3 drops of Million's reagent to conduct the tyrosine test. The answer turns a deep crimson hue when mixed.

Steroid analysis

Add 3 milliliters of extract to 3 milliliters of acetic anhydride to perform the Liebermann reaction. Calm and comfortable Sulfuric acid in the form of a few drops Seems to be a blue colour.

Preparation of animals

Animals weighing between 150 and 200g, both male and female Wistar rats were purchased from the Animal House at the Translam Group of Institutions in Rajpura, Mawana Road, Meerut- 250001, UP. The animals are kept in ideal conditions, with a light/dark cycle of 12 hours per day and a room temperature of 25±1°C. Standard rat pellet meal and water are provided ad libitum, and the relative humidity is kept between 44-56%. Before an experiment, rats had their meal withheld for one hour (Bhajoni et al., 2016).

Experimental design

Rats were divided into 5 groups each having 6 and treated for 15 days (Faujdar et al. 2019) as followings

Group 1: rats are given vehicle.

Group 2: rats are given 6% croton oil (10µl, p. o.) + normal saline (p. o.).

Group 3: rats are given 6% croton oil (10µl, p. o.) + Pilex (400mg/kg, p. o.).

Group 4: rats are given 6% croton oil (10µl, p. o.) + *M. coromandelianum* (400mg/kg, p. o.).

Group 5: rats are given 6% croton oil (10µl, p. o.) + *M. coromandelianum* (200mg/kg, p. o.).

A. Anti-hemorrhoids activity

Inflammatory index

On day five, one hour following treatment, the animals' inflammatory indices were measured. Based on the clinical hemorrhoidal area and the severity index, a scoring system was developed for assessing recto-anal inflammation. On a scale from 1 (mild) to 4 (severe), hemorrhoids were ranked according to their severity. The anal cushions prolapse through the anus on straining but return to their normal position on their own in Grade II cases; in Grade III cases, however, the anal cushions prolapse through the anus on straining or effort and must be replaced manually into the anal canal. The prolapse is permanent and of the fourth degree if it cannot be reduced in size.

Biochemical analysis

Rats had blood drawn from their retroorbital sinuses one hour after treatment on day five (Faujdar et al., 2019) to measure liver enzyme levels. On the fifth day of treatment, after the rats were sacrificed (three hours after treatment), their hemorrhoidal tissue was taken and thoroughly rinsed with Tyrode solution in order to determine several biochemical parameters. Tissue samples were also tested for in vivo antioxidants using the indicated methods, including catalase, superoxide dismutase, and lipid peroxidation.

Recto-anal coefficient

Each animal was exsanguinated while under deep isoflurane anesthesia, and then recto-anal tissue (20 mm in length) was removed and weighed. Tissue containing Evans blue was extracted with 1 ml of formaldehyde and the absorbance at 620 nm was measured with an Elisa Microplate Reader. Evans blue dye standard curve was used to determine concentration.

Histological examination of tissue that had been fixed in 10% neutral buffered formalin revealed results for both the severity score and the recto anal coefficient. The formula was used to calculate the recto anal-coefficient (Azeemuddin et al., 2014).

Rectoanal Coefficient = Weight of rectoanal tissue (mg) / Total body mass (mg)

Histopathology

Histopathological analyses were also performed on hemorrhoidal tissue samples collected on day 5 of therapy. After being washed and blotted dry, the tissues were fixed in 10% formalin and then dehydrated in acetone. Paraffin wax was used to embed the tissue samples before being sectioned at a thickness of 4-6 m using a microtome. Next, haematoxylin and eosin were used to stain tissue sections before they were examined under a microscope.

RESULTS AND DISCUSSION

Percentage yield

The percentage yield of *M. coromandelianum* extract was calculated as 68.24% when weight of practical yield was compared with theoretical yield.

5.2 Phytochemicals

Foam test for foam test was found positive that indicated for its saponins. Moreover, all the tests for alkaloids i.e., Mayer's test etc. were found positive when observed. Tests for carbohydrates i.e., Fehling's test and Benedict's test were found positive that proved for its carbohydrates content. The *M. coromandelianum* showed absence of tannins when examined through its tests. Tests for proteins i.e., Biuret test exhibited the presence of proteins. The positive sign of Keller-killiani test confirmed for its glycoside content.

Terpenes were found absent; however, flavonoids were found presence when observed in lead acetate test.

Table 1: Phytochemical tests of *M. coromandelianum* extract.

phytochemical al test	Observation			
	n-hexane	Chloroform	ethyl acetoacetate	Methanol
Saponins	++	-	+	+
Alkaloids	++	++	++	++
Tannins	+	-	+	_
Proteins	++	+	++	++
Glycosides	++	++	+	++
Amino acids	+	+	-	+
Steroids	-	+	+	+

(+) = moderate presence, (++) abundant presence, (-) = absent

Whole plant exhibited a rich source of above-mentioned chemical constituents.

Evaluation of anti-hemorrhoidal activity

Inflammatory score

The inflammatory score was recorded in terms of severity of inflammation in the anus. Vehicle treated group showed Grade I in the rats when observed while croton oil+ normal saline treated group exhibited as Grade III. 6% croton oil (10µl, p. o.) + *M. coromandelianum* (200mg/kg, *p. o.*) treated rats showed negligible decline in the severity of hemorrhoids and observed as Grade III. Highest decline in severity was estimated in the rats treated with 6% croton oil (10µl, p. o.) + *M. coromandelianum* (400mg/kg, *p. o.*) and 6% croton oil (10µl, *p. o.*) + Pilex (1 tab./kg, *p. o.*) as Grade II.

Table 2: Inflammatory score.

Treatment	Inflammatory score			
	Grade I	Grade II	Grade III	Grade IV
Vehicle	✓	_	_	_
6% croton oil (10µl, *p. o.*) + normal saline (*p. o.*)	_	_	✓	_
6% croton oil (10µl, *p. o.*) + Pilex (1 tab./kg, *p. o.*)	_	✓	_	_
6% croton oil (10µl, p. o.) + *M. coromandelianum* (200mg/kg, *p. o.*)	–	–	✓	–
6% croton oil (10µl, p. o.) + *M. coromandelianum* (400mg/kg, *p. o.*)	–	✓	–	–

Recto anal coefficient

The disease control group treated with 6% croton oil (10µl, *p. o.*) + normal saline (*p. o.*) demonstrated the recto anal coefficient as 0.12 that was maximum among all. Standard group administered 6% croton oil (10µl, *p. o.*) + Pilex (1 tab./kg, *p. o.*) showed the recto anal coefficient as 0.05 that was almost similar (0.07) to group 5 treated with 6% croton oil (10µl, p. o.) + *M. coromandelianum* (400mg/kg, *p. o.*).

Thus, anti-hemorrhoidal action was observed in dose-dependent way.

Table 3: Recto anal coefficient.

Treatment	Recto anal coefficient
Vehicle	0.03
6% croton oil (10µl, *p. o.*) + normal saline (*p. o.*)	0.12
6% croton oil (10µl, *p. o.*) + Pilex (1 tab./kg, *p. o.*)	0.05
6% croton oil (10µl, p. o.) + *M. coromandelianum* (200mg/kg, *p. o.*)	0.09
6% croton oil (10µl, p. o.) + *M. coromandelianum* (400mg/kg, *p. o.*)	0.07

Biochemical analysis

In terms of biochemical analysis, different parameters were evaluated including SOD, CAT, AST, and ALT. The SOD level was observed as 0.17±0.02***(U/mg of protein) and 0.13±0.06**(U/mg of protein) in 6% croton oil (10µl, p. o.) + *M. coromandelianum* (400mg/kg, *p. o.*) and 6% croton oil (10µl, *p. o.*) + Pilex (1 tab./kg, *p. o.*), respectively.

In comparison of control group, 6% croton oil (10µl, p. o.) + *M. coromandelianum* (400mg/kg, *p. o.*) treated group showed a significant anti-hemorrhoidal activity as 13.47±0.26*** (U/min/mg of protein).

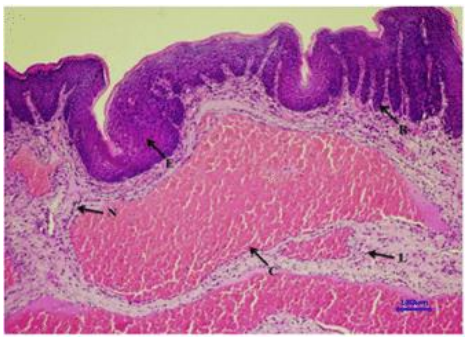
Table 4. Biochemical analysis.

Treatment	SOD (U/mg of protein)	CAT (U/min/mg of protein)	AST (U/ml)	ALT (U/ml)
Vehicle	0.21±0.02	13.13±.19	259.36±12.36	72.34±24.15
6% croton oil (10µl, *p. o.*) + normal saline (*p. o.*)	0.29±0.04	21.53±0.67	360.42±13.53	119.23±13.25
6% croton oil (10µl, *p. o.*) + Pilex (1 tab./kg, *p. o.*)	0.13±0.06**	8.21±.48***	309.32±10.21	101.18±21.37
6% croton oil (10µl, p. o.) + *M. coromandelianum* (200mg/kg, *p. o.*)	0.21±0.05***	17.47±0.26***	337.21±12.11	117.34±18.23
6% croton oil (10µl, p. o.) + *M. coromandelianum* (400mg/kg, *p. o.*)	0.17±0.02***	13.47±0.26***	319.43±17.42	110.32±12.26

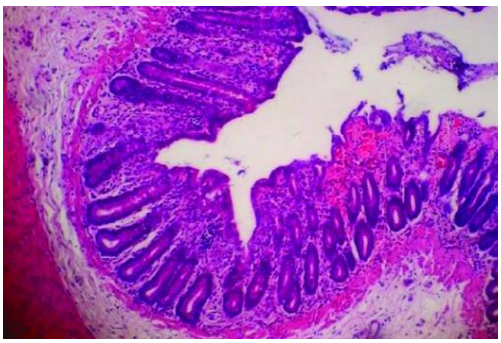
Histopathology

The histopathological examinations for all the 5 groups are shown as below. Group 1 treated with vehicle has shown plain structures while disease control group have demonstrated inflammation that might be due to infiltration of leukotrienes, cytokines, interferons.

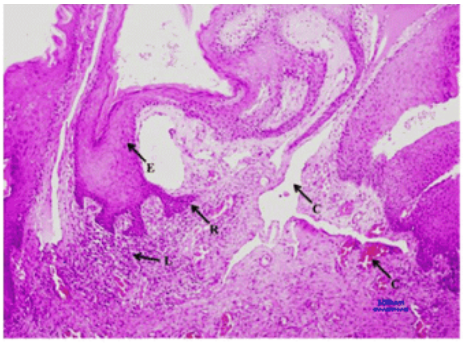
The 6% croton oil (10µl, p. o.) + *M. coromandelianum* (400mg/kg, *p. o.*) treated groups were exhibited significantly decreased inflammatory zones in anal tissues of rats.



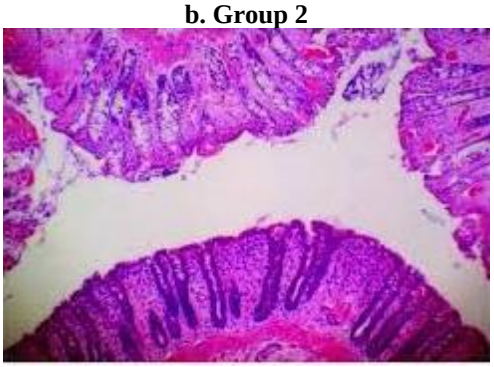
a.



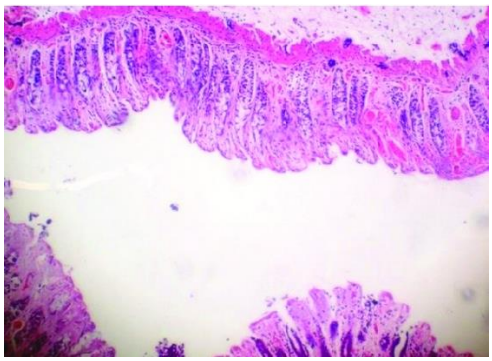
b. Group 2



c. Group 3



d. Group 4



e. Group 5

Fig. 2: Histopathology of hemorrhoids in rodents.

In results, it was observed that *Malvastrum coromandelianum* significantly decreased the inflammatory score (Grade II). It also facilitated the recto anal coefficient as 0.07 (at higher dose). In biochemical estimation, SOD demonstrated marked decreased in test group, and response was recorded in dose-dependent manner. Histopathology exhibited a significant decline in the inflammatory zone and that might be decreased level of inflammatory mediators in the structures of anal tissues.

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CONFLICT OF INTEREST: None.

CONCLUSION

Like other invasive weeds, M. cormandelianum is frequently misused because of the harm it does to native plant life anywhere it grows. Our analysis, however, shows that there is another side to this herb, namely that it can be a useful resource with several applications. Even though weed plants are bad for the environment and many organizations around the world are working on eradication programs, they do have some medicinal value. This is because their genetic constitution is more robust than that of native plants when it comes to the biosynthesis chemical constituents and metabolites of medicinal use, allowing them to thrive in unfavorable conditions.

It concluded that methanolic leaves extract of *Malvastrum coromandelianum* is effective in the management of hemorrhoids in rats when compared with the control group. It significantly shown in every parameter evaluated i.e., inflammatory index, recto-anal coefficient, biochemical analysis and histopathology.

In the future, bioactive phytoconstituents might give a green and safe cure as a substitute for synthetic pharmaceuticals if they were properly identified, authenticated, standardized, and applied in therapeutic procedures.

REFERENCES

1. Everhart J E, Ruhl C E. Burden of digestive diseases in the United States part I: overall and upper gastrointestinal diseases. Gastroenterology, 2009; 136(2): 376–386.
2. Evans P, Halliwell B. Micronutrients: oxidants/antioxidants status. Br J Nutr., 2001; 85(2): S67eS74.
3. Johanson J F, Sonnenberg A. The prevalence of hemorrhoids and chronic constipation. An epidemiologic study. Gastroenterology, 1990; 98(2): 380–386.
4. Goenka M K, Kochhar R, Nagi B, Mehta S K. Rectosigmoid varices and other mucosal changes in patients with portal hypertension. Am J Gastroenterol, 1991; 86(9): 1185–1189.
5. Ajibesin KK, Ekpo BA, Bala DN, Essien EE, Adesanya SA.Ethnobotanical survey of Akwa Ibom state of Nigeria. Journalof Ethnopharmacology 2008; 115(3): 387-408.
6. Ghani A, Batool M. Folk recipes of some medicinal plants used bythe inhabitants of Soon valley Khushab (Pakistan). International Journal of Current Pharmaceutical Research, 2012; 4(1): 60-63.
7. Flora of China. Malvastrum A. Gray in flora of China., 2007; 12: 269-270.
8. Sanghai Dhirendra B., S. Vijaya Kumar, K. K. Srinivasan, H. N. Aswatharam and C. S. Shreedhara. Pharmacognostic and phytochemical investigation of the leaves of Malvastrum coromandelianum (L.) Garcke. Anc Sci Life., Jul-Sep, 2013; 33(1): 39–44.
9. Khandelwal K R. *Practical Pharnacognosy, Nirali Prakashan,* Pune, 9th Edition, 2002; 157-158.
10. Bhatt J. S. and S. Dhyani, Preliminary Phytochemical Screening of Ailanthus Excelsa Roxb., *Int. J. Curr. Pharmaceut. Res.,* 2012; *4*(1): 87-89.
11. Bhajoni et al. Evaluation of the Antiulcer Activity of the Leaves of Azadirachta indica: An Experimental Study. Integrative Medicine International, 2016; 3: 10–16.
12. Faujdar Samriti, Bhawana Sati, Swapnil Sharma, A.K. Pathak, Sarvesh Kumar Paliwal. Phytochemical evaluation and anti-hemorrhoidal activity of bark of Acacia ferruginea DC. ournal of Traditional and Complementary Medicine, 2019; 9: 85e89.