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ORIGINAL ARTICLE

Arbortristoside-A: A Promising Bioactive Moiety from *Nyctanthes arbortristis* Linn. against Hemorrhoid

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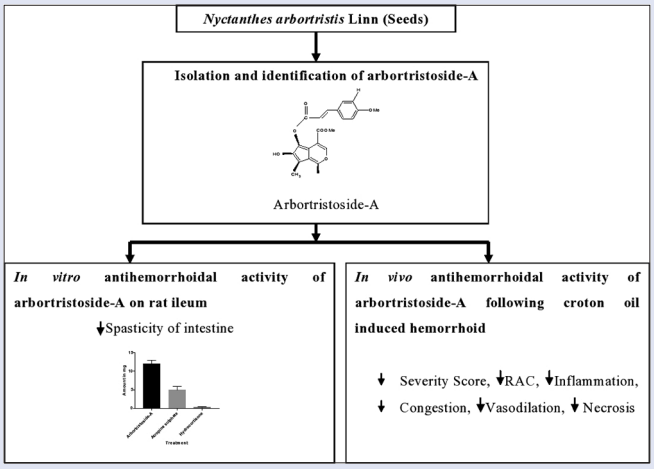
ABSTRACT

Background: Hemorrhoid disease is becoming a common problem in modernized society and its treatment is less developed. Although there are many plants used traditionally, proper scientific evidence is still lacking. *Nyctanthes arbortristis* Linn. is scientifically proved to have pharmacological activities. In this research work, an approach has been made to establish the potentiality of arbortristoside-A from *N. arbortristis* L. seeds against hemorrhoid. **Materials and Methods:** After characterization of arbortristoside-A from *N. arbortristis* L seeds, it was subjected to explore its effectiveness against hemorrhoid using rat ileum and following croton oil-induced hemorrhoid screening models. **Results:** Arbortristoside-A observed to have a significant reduction in inflammation due to hemorrhoid at 50 and 100 mg/kg (1.25 ± 0.06 and 0.76 ± 0.05, respectively). Reduction of severity scores was observed in animals due to arbortristoside-A mainly at its higher dose 100 mg/kg. It was also found to decrease the rectoanal coefficient significantly. **Conclusion:** Oral administration of arbortristoside-A was proved to have significant anti-hemorrhoid effect which was well compared to that of the standard drug. In the course of time, this research work will be helpful in providing new entities to the field of medicines and pharmaceutical sciences for the treatment of hemorrhoid.

Key words: Anti-hemorrhoid , arbortristoside-A, hemorrhoid, *in vitro, in vivo, Nyctanthes arbortristis*

SUMMARY

- The isolated bioactive moiety; arbortristoside-A from *Nyctanthes arbortristis* L seeds was found to possess potential anti-hemorrhoidal activity both *in vitro* and *in vivo* experimental models. It has been observed that arbortristoside-A has showed its significant response *in vitro* by decreasing the spasticity of rat ileum and *in vivo* by decreasing the level of rectoanal coefficient, severity score, inflammation, congestion, vasodilation, and necrosis of rectoanal region of the experimental animals.



Abbreviations used: *N. arbortristis* L.: *Nyctanthes arbortristis* Linn.; UV: Ultraviolet; IR: Infrared; NMR: Nuclear magnetic resonance; mp: Melting point; DMSO: Dimethyl sulfoxide; RAC: Recto anal coefficient; ACh: Acetylcholine.

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INTRODUCTION

In the present era, due to modernized food habits and life style, the occurrence of hemorrhoid is prominent at early stages of life and it becomes prominent in the elderly. It is gradually arising problem medically and socio-economically. Proper treatment and prevention of hemorrhoids in modern medicine are still less improved.[1] In chronic bleeding hemorrhoid, its treatment leads to surgical involvement.[2,3] Hemorrhoid treatment is not yet specific, for which natural source is getting explored by broad research approaches for the treatment of hemorrhoid. As existing use of synthetic anti-hemorrhoidal causes numerous side effects, the present study was targeted to provide an alternate agent against hemorrhoid from natural source with fewer toxicity.

Nyctanthes arbortristis Linn. (Family: Oleaceae) is scientifically proved to have vast medicinal and pharmacological activities. It is commonly known as Night Jasmin and Harsingar. Its wide geographical finding is in sub-Himalayan regions and southwards to Godavari and so in Indian gardens.[4,5] The *N. arbortristis* L. is found to be used traditionally by the people of India for the management of various ailments according to the Sidha, Unani, and Ayurveda. Many researchers have explored scientifically the presence of important phytoconstituents such as

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nyctanthoside, nyctanthic acid, β-sitosterol, 6 β-hydroxyloganin, polysaccharides, arbortristoside-A and–B.[6-8] Literature showed antiplasmodial,[8] leishmanicidal,[9] antispermatogenic, and antiallergic activities[10] of arbortristoside-A and-B. Anti-inflammatory activity[11-13] and analgesic activity[14] of *N. arbortristis* L. are well documented. The folklore people of India have been using the leaves of *N. arbortristis* L. for curing hemorrhoid.[15] There is not any scientific evidence of the effect of its isolated compound arbortristoside-A against hemorrhoid. With the advanced understanding on ethnopharmacology, study on the interaction of arbortristoside-A with hemorrhoid was explored. Our preliminary studies have already reported the presence of arbortristoside-A with anti-inflammatory activity[16] and antispasmodic effect in ethanol extract of *N. arbortristis* L.[17] Based on the effect of iridoid glycosides against inflammation and abdominal spasm, the study is focused to provide a potent active constituent arbortristoside-A from the ethanol extract of *N. arobotristis* L. seeds as an potent agent against hemorrhoid.

MATERIALS AND METHODS

Materials

In the present research, most of the reagents, chemicals, and solvents such as petroleum ether, chloroform, ethanol (90%), diethyl ether, acetone, ethyl acetate, chloroform, methanol, 2N HCl propylene glycol, silica gel-G, croton oil, n-butanol, pentobarbital sodium, and isoflurane were of analytical grade and were obtained from Merck Ltd., Mumbai. Hydrocortisone was purchased from Sigma Aldrich, Bangalore, India. Acetylcholine (Ach) chloride was obtained from Loba Chemie Pvt. Ltd., Laboratory reagents and Fine Chemicals, Mumbai, and atropine sulfate was purchased from S. D. Fine Chemical Ltd, Mumbai. Rest of the solvents and chemicals were used of analytical grade.

Preparation of extract

In the month of December to February 2005, *N. arbortristis* L. seeds were procured from rural area of Odisha. The authentication of the herbarium of *N. arbortristis* L. (family: Oleaceae) (CHN/I/I (20)/2005-Tech-II/254). The shade dried seeds were coarsely powdered which were further extracted with petroleum ether, chloroform, and ethanol successively.[9,12] The percentage yield of the dark gummy masses of the ethanol extract of the seeds was 26.5% (w/w).

Isolation of arbortristoside-A

The fractionation of the seeds extract was executed with diethyl ether, ethyl acetate, and n-butanol. The yield of dried n-butanol fraction was observed to be 50.32% (w/w), which was then further triturated with acetone and a solid mass was obtained. After washing with 2N hydrochloric acid and then with hot water, it was crystallized from chloroform-methanol at a ratio of 1:1.[6,7] The yield value of the light-colored crystalline powder was found to be 5.25% (w/w), which phytochemical analysis was carried out[18,19] and spectral analysis.[18,20] The isolated constituent was evidently recognized as arbortristoside-A, mp 225°C–228°C (MeOH); [α]D 25°–92° (MeOH). The isolated constituent; arbortristoside-A was eventually verified by spectral data which are mentioned below. Ultraviolet (UV) (MeOH): 206, 227, 300, and 308 nm; IR bands (KBr, MeOH): 3424, 2950, 1719, 1662, 1633, 1514, 1450, 1377, 1216, 1177, 1050, 877, 835, 750, and 591 cm^{-1}; ^{1}H NMR and ^{13}C NMR (400 MHz, DMSO*d*6) data: Table 1, Ms (*m*/z): 371, 226, 200, 178, 161, 139. After dissolving in 10% DMSO, the isolated phytoconstituent was further undergone for pharmacological screening of anti-hemorrhoidal activity.

Experimental animals

Wistar strain of albino rats weighing 150–180 g was obtained from the animal facility, Noida Institute of Engineering and Technology (Pharmacy Institute), Greater Noida. The animals were housed with proper pellet diet and palatable water under regular control of humidity and temperature. Random distribution of animals was done between control, standard, and test groups keeping six animals in each group. The experimental protocol for the present research with animals was approved by the Institutional Animals Ethics Committee (Reg. no. 1845/PO/Re/S/19/CPCSEA).

In vitro Anti-hemorrhoidals activity of arbortristoside-A

Rats, which were fasted overnight, were dissected just before the experiment after anesthesia with pentobarbital sodium (80 mg/kg; intraperitoneally). Its ileum was prepared along with continuous aeration in tyrode salt solution after systematic dissection. About 2–3 cm long ileum piece was mounted in the organ bath containing tyrode salt solution at pH 7.4 and maintained at 37°C and continuous carbonated aeration. The concentration-dependent contractions due to ACh were recorded in the absence and presence of 0.1 ml of arbortristoside-A and atropine sulfate. The responses were observed by tracing the concentration-response curve with the increasing concentrations of the arbortristoside-A and atropine sulfate when the concentration of ACh constant (0.2 ml) was kept constant.[17,21,22]

In vivo anti-hemorrhoidal activity of arbortristoside-A

Chemical-induced model (croton oil) was followed using Wistar albino rats. The rats were administered with croton oil by using cotton swab through rectoanal region at 10 mm depth for 10 s for 7 days once daily to induce hemorrhoid. Propylene glycol was utilized as vehicle to administer arbortristoside-A and the standard drug. In the present research, hydrocortisone was used as the standard antihemorrhoidal agent at the dose of 20 mg/kg body weight through rectoanal route. The experimental animals were randomly distributed between six groups having six animals in each group. The present study comprised of Group I was served as the normal control group where animals were administered with normal saline orally; Group II was the diseased control group where the animals were instilled with croton oil through rectoanal route; Group III was considered as the standard group where the animals were treated with was served as standard group where rats were treated with standard drug (hydrocortisone; recto anal route); Groups IV, V, and VI were three test groups where animals were administered with arbortristoside-A at varying doses of 25 mg/kg, 50 mg/kg, and 100 mg/kg between (based on previous toxicity study[16]), respectively. All the animals were induced with hemorrhoid by instillation of croton oil at rectoanal route for 7 days, except normal control group. The animals of test and standard group were administered with arbortristoside-A and hydrocortisone, respectively, for 5 days 24 h after the induction of hemorrhoid. On the 5th day, after 1 h, all the animals were euthanized by exsanguination overdose of isoflurane (maintaining the flow rate at 3%–5% concentration) by inhalation route, rectoanal tissues (20 mm long) were separated. They were assessed for the seriousness score, weighed, and settled in 10% unbiased supported formalin solution for histopathological examination. The rectoanal coefficient (RAC) was estimated utilizing the recipe.[23,24]

$$RAC = \frac{\text{Weight of recto anal tissue (mg)}}{\text{Body weight (g)}}$$

Table 1: Nuclear magnetic resonance data of isolated compound (dimethyl sulfoxide-*D6*)

H	^{1}H NMR data	C	^{13}C NMR data
1	5.32 d, *J*=7.8 Hz	1	95.4
3	7.53 s	3	152.5
5	3.1 m	4	110.0
6	4.65 m	5	40.7
7	4.1 t, *J*=4.2 Hz	6	75.9
8	2.1 m	7	70.0
9	2.1 m	8	35.5
10	1.03 s	9	44.0
12	3.65 s	10	15.2
α	6.49 AB q, *J*=16 Hz	11	169.5
β	7.53 AB q, *J*=16 Hz	12	50.8
Aromatic protons	6.97 d, *J*=10 Hz	1'	100.8
	7.66 d, *J*=10 Hz	2'	75.0
	3.8 s (-OCH_3)	3'	78.2
Sugar protons	3.6-4.65 m	4'	70.0
		5'	74.9
		6'	62.8
		1"	127.5
		2"	132.0
		3"	114.0
		4"	162.2
		5"	114.0
		α	144.5
		β	115.0
		-CO	168.8
		-OCH_3	56.3

NMR: Nuclear magnetic resonance

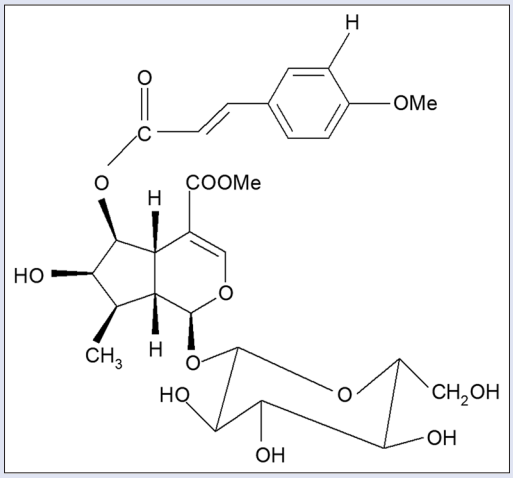


Figure 1: Arbortristoside-A

RESULTS AND DISCUSSION

Isolation of arbortristoside-A

The isolate compound; arbortristoside-A, $C_{27}H_{34}O_{13}$, mp 225°C–228°C, [α]d 25-92° (MeOH) was confirmed as an iridoid glycoside from the phytochemical studies. From UV spectral analysis, it was observed to have–O-C = C-CO_2CH_3, phenyl group, and *p*-substituted benzene ring. Infrared spectral analysis showed the existence of monosubstituted aromatic system (750), esters (1177, 1216),-CH_3 (1377),-C = C-(1662), and–OH (3434) group. From its NMR spectral analysis, it was also revealed the presence of trans-olefinic protons (δ 7.5), aromatic protons (δ 6.95),-C = C-H group (δ 4.7), esters (δ 4.1 and δ3.7),-OH (δ 2.7), and–CH_3 (δ 1.03) groups. Mass spectral data confirmed the existence of vital fragments such as $[c]^+$ (371), $[a]^+$ (226), $[a\text{-}H_2O]^+$ (200), *p*-methoxycinnamic acid (178), *p*-methoxycinnamoyl moiety (161), $[b]^+$ (139). The mass spectral analysis revealed the molecular weight of the isolated compound to be 548.0, which was then corroborated with the molecular structure of the phytoconstituent [Table 2].

The isolated constituent was confirmed to be arbortristoside-A [Figure 1] as revealed previously.[6,7,25]

In vitro anti-hemorrhoidal activity of arbortristoside-A

In the presence of 0.1 ml of the arbortristoside-A (10 mg/ml), the concentration-dependent responses of ACh were found to be reduced. When 0.2 ml of ACh (1 µg/ml) was kept constant in both the cases, the concentrations of the arbortristoside-A and atropine sulfate were increased in respective experiments. The response due to 0.2 ml (0.0002 mg) of ACh comes to the baseline at the dose of 12 mg of arbortristoside-A. Whereas in case of atropine sulfate at a dose of 0.3 mg inhibited the contractile response of 0.2 ml (0.0002 mg) of ACh by bringing down to the baseline [Figure 2].

In vivo anti-hemorrhoidal activity of arbortristoside-A

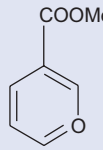
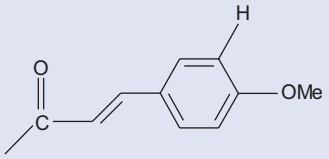
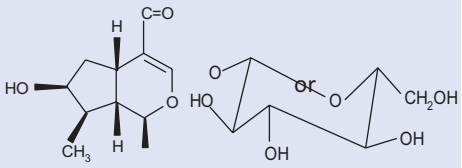
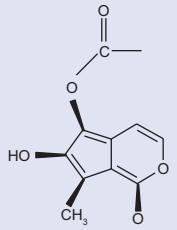
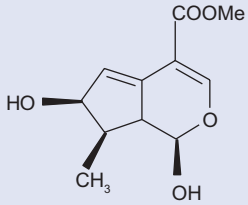
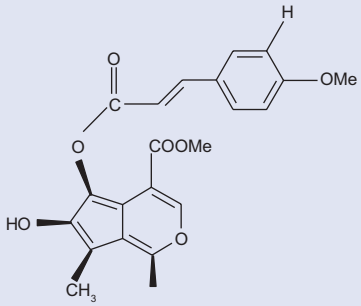
From the previous acute toxicity study on arbortristoside-A, its doses for the evaluation of anti-hemorrhoidal activity were selected to be 25, 50, and 100 mg/kg body weight. In this study, significant deviation in RAC, severity score, and histopathological observations were developed by applying croton oil on the rectoanal potion of the rats. The hemorrhoid-induced groups treated with arbortristoside-A and hydrocortisone were compared with that of the diseased control group for RAC, severity score, and necrosis. The findings of the anti-hemorrhoidal activity of arbortristoside-A are presented in Table 3. In case of RAC, it also showed significant ($P < 0.01$) mainly at its higher dose of 100 mg/kg. It has been observed that arbortristoside-A has showed its significant response at 50 ($P < 0.001$) and 100 mg/kg ($P < 0.001$) against the inflammation (1.25 ± 0.06 and 0.76 ± 0.05, respectively) due to hemorrhoid in the animals. There is well remarkable reduction of severity scores in animals due to arbortristoside-A mainly at its higher dose 100 mg/kg ($P < 0.001$) and it was very negligible at its lowest dose 50 mg/kg ($P < 0.01$). For congestion, which is a major pathophysiology of hemorrhoid, arbortristoside-A showed its best reduction at 100 mg/kg dose ($P < 0.01$) which is well comparable to hydrocortisone. As in hemorrhoid there is anal bleeding due to vasodilatation, it was also examined and found that arbortristoside-A can also be effective to reduce this untoward effect at both 50 and 100 mg/kg doses with $P < 0.01$ (1.41 ± 0.11 and 0.82 ± 0.07, respectively). The histopathological studies revealed a dose-dependent reduction in necrosis by arbortristoside-A-treated groups in a significant ($P < 0.001$) manner. The overall anti-hemorrhoidal activity of arbortristoside-A was observed to be dose-dependent and was well compared to the standard drug hydrocortisone.

DISCUSSION

The phytochemical and spectrophotometric analysis of the isolated compound was conducted to verify it to be arbortristoside-A, which had been isolated from the seeds of *N. arbortristis* L. The isolated constituent was revealed to be arbortristoside-A as reported previously. The reduction in concentration-response curves of ACh due to arbortristoside -A showed the reduced spasticity of intestine which is required for the

treatment of hemorrhoid. It will be beneficial in the management of hemorrhoid. Reduction in the level of RAC by arbortristoside-A and reduction in inflammation of hemorrhoid at its higher doses supported its anti-hemorrhoidal activity. The pathophysiological features such as congestion and necrosis due to hemorrhoid in the experimental animals were observed to be reduced at its higher dose which was also well comparable to that of the standard drug hydrocortisone. Earlier studies on arbortristoside-A revealed its anti-inflammatory, antioxidant, and antispasmodic activities which support the present investigation. The suppression of oxidative free radicals is having a key role for remedy of inflammation and tissue necrosis. The overall correlation and outcome confirmed the potentiality of the bioactive moiety arbortristoside-A from *N. arbortristis* L. in the successful management of hemorrhoid.

Table 2: Mass spectroscopic data of the isolated compound

M/e	Probable fragments of the molecule
139.2	
161.3	
178.2	
200.2	
226.2	
371.2	

CONCLUSION

In the present research after isolation of the iridoid glycoside, arbortristoside-A has been isolated from the seeds of *N. arbortristis* L, a systematic research protocol was framed to screen the anti-hemorrhoidal activity of arbortristoside-A following both *in vitro* and *in vivo* methodologies. The initiation of the study from the literature showing iridoid glycosides is having anti-hemorrhoidal activity made the research successful in finding a promising novel compound arbortristoside-A against hemorrhoid. Previous work on arbortristoside-A for its antinociceptive and anti-inflammatory activity[16] made the research targeted toward its potentiality against hemorrhoid. Along with this, the previous research on the antispasmodic activity of *N. arbortristis*[17] contributed central idea of the present study for the anti-hemorrhoidal activity of the isolated active principle arbortristoside-A for its potentiality to inhibit the hemorrhoidal pathophysiology with extensive pain. In the present investigation the targeted anti-hemorrhoidal activity was successfully obtained in arbortristoside-A was scientifically proven both by *in vitro* and *in vivo* screening animal models based on previous findings by the coworkers. Furthermore, arbortristoside-A is found to be significantly effective in each pathological complications of

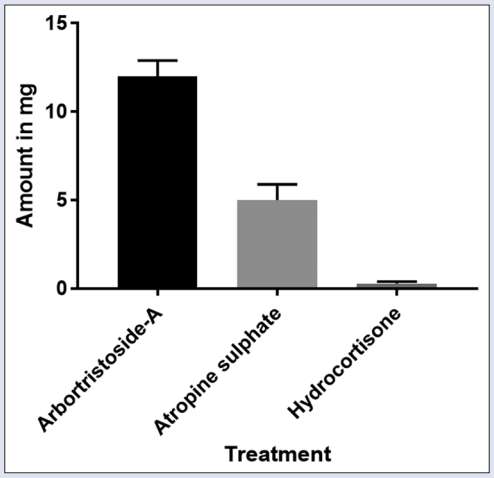


Figure 2: *In vitro* anti-hemorrhoidal activity of arbortristoside-A

Table 3: Anti-hemorrhoidal activity of arbortristoside-A

Groups	Treatment	Dose	Severity score	RAC	Inflammation	Congestion	Vasodilation	Necrosis
I	Normal saline	10 ml/kg	0.17±0.07	0.79±0.03	0.2±0.03	0.0±0.0	0.28±0.05	0.0±0.0
II	Normal saline + croton oil	10 ml/kg	1.45±0.07	1.48±0.03	3.2±0.03	3.01±0.01	2.98±0.05	3.99±0.05
III	Hydrocortisone + croton oil	20 mg/g	0.43±0.04	0.98±0.03**	0.93±0.03	1.01±0.02*	0.51±0.07*	1.49±0.03**
IV	Arbortristoside-A + croton oil	25 mg/kg	1.34±0.05	1.41±0.06	2.21±0.13	2.91±0.05	2.75±0.06	3.76±0.03
V	Arbortristoside-A + croton oil	50 mg/kg	0.58±0.07*	1.33±0.06	1.25±0.06**	2.13±0.11*	1.41±0.11*	2.84±0.07*
VI	Arbortristoside-A + croton oil	100 mg/kg	0.42±0.03**	1.11±0.03*	0.76±0.05**	1.06±0.08*	0.82±0.07*	1.78±0.03**

The observations are expressed in mean±SD, where n=6 and the significance of activity are considered to be at *P<0.1 and **P<0.01. SD: Standard deviation, RAC: Rectoanal coefficient

hemorrhoid.[26] Thus, the present study explored one novel and potential management of hemorrhoid by the bioactive principle arbortristoside-A of *N. arbortristis* which can have potential clinical applications after further clinical trial.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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