



# Neuronal nitric oxide synthase enzyme activation and calcium channel-dependent relaxation of the internal anal sphincter by *Tridax procumbens*

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## Abstract

**Objective:** To investigate the role of neuronal nitric oxide synthase (nNOS) enzyme activation and calcium channel-dependent mechanisms in mediating the relaxation of the internal anal sphincter (IAS), with specific reference to the pharmacological effects of *Tridax procumbens*. **Data sources:** Relevant literature was retrieved from PubMed/MEDLINE, and Google Scholar encompassing published review articles, pharmacological studies, phytochemical investigations, and clinical research related to *T. procumbens*. **Study selection:** Published literature describing the phytochemistry, pharmacological activities, therapeutic uses, and formulations of *T. procumbens*, along with studies on nNOS activation, calcium channel-dependent relaxation, and IAS physiology were included in this review. Only peer-reviewed, English-language articles were considered. Studies with insufficient data, duplicates, and non-peer-reviewed sources were excluded. **Summary of contents of the article:** Anal fissure results from internal anal sphincter hypertonicity causing ischaemia, spasm, and pain. *T. procumbens* shows consistent smooth muscle relaxant effects via calcium channel inhibition and nitric oxide release across preclinical studies. A recent randomized controlled trial confirmed its clinical efficacy, matching standard Lignocaine-Nifedipine cream in healing, spasm, and pain relief, with fewer side effects. **Conclusion:** *T. procumbens*, acting as a safe chemical sphincterotomy through nitric oxide and calcium-mediated sphincter relaxation, warrants consideration as a first-line treatment for anal fissure.

**Keywords:** Calcium Channels L-Type, Fissure in ano, Internal Anal Sphincter, Neuronal Nitric Oxide Synthase, *Tridax procumbens*

## Introduction

The internal anal sphincter (IAS) and external anal sphincter (EAS) are two adjacent muscles that surround the anal canal. The IAS is a band of smooth muscle, involuntary in nature, that contributes to resting anal pressure; it is the continuation of the involuntary circular muscle of the rectum into the distal anal canal. The IAS contributes between 50% and 85% of resting anal tone, with the remainder supplied by the vascular anal cushions. The EAS is voluntary in nature, while the IAS plays the crucial role in maintaining continence. Structural injury or functional weakness of these muscles results in passive incontinence of feces and flatus.<sup>1</sup> This has created a need, within surgery, for newer and safer drug therapy alternatives to reduce IAS spasm. IAS spasm is considered a primary cause of anal fissure, and surgical procedures for fissure treatment are therefore directed at the IAS rather than the EAS.<sup>2</sup> The exact etiology of anal fissure remains uncertain, but recent studies point to nitric oxide deficiency in the internal anal sphincter of patients with chronic anal fissure. An abnormal failure of internal sphincter

relaxation is thought to underlie chronic fissure development, caused by an intrinsic lack of neuronal nitric oxide synthase (nNOS) in the IAS; this deficiency in nNOS is linked to reduced local nitric oxide availability. Nitric oxide has been identified as a key biological messenger mediating the anorectal inhibitory reflex in humans.<sup>3</sup> Anal fissure presents as a linear tear in the anoderm, primarily affecting the posterior midline of the anus. The condition causes pain during defecation, bleeding, and sphincter spasm, significantly affecting quality of life.<sup>4</sup> Conventional management of anal fissure includes dietary modification, sitz baths, and topical agents such as nitroglycerin or calcium channel blockers.<sup>5</sup> Nitroglycerin, a nitric oxide donor, has an established therapeutic role in fissure treatment,<sup>6</sup> while calcium channel blockers aid healing by reducing sphincter pressure and increasing local blood supply.<sup>7</sup> Both approaches aim to reduce sphincter hypertonicity and promote healing. However, side effects headache in the case of nitroglycerin, and local irritation or dermatitis with diltiazem and nifedipine limit their clinical use.<sup>8</sup> Surgical options, including lateral internal

sphincterotomy, achieve high success rates but carry a risk of postoperative fecal incontinence<sup>9</sup>, underscoring the need for effective non-invasive alternatives. India is a leading global consumer of herbal medicine, and government support has helped expand research into herbal therapeutics.<sup>10</sup> *T. procumbens* is an herbaceous plant with a long history in ethno-herbal medicine, traditionally used for its wound-healing, antimicrobial, and anti-inflammatory properties.<sup>11</sup> This review investigates a single herbal candidate, *T. procumbens*, for its potential to activate nNOS and produce calcium channel-dependent relaxation of the IAS.

## Methods

A systematic literature review was conducted across multiple databases, including PubMed, and Google Scholar. Keywords used included “fissure in ano”, “anal fissure”, “*Tridax procumbens*”, “wound healing”, “spasm reduction”, “relaxant”, “nitric oxide”, “hypotensive”, “antihypertensive”, “internal anal sphincter”. The inclusion criteria focused on studies with nNOS and calcium channel-dependent relaxation of the IAS by *T. procumbens*. Studies without available abstracts or those published in languages other than English were excluded.

## Discussion

### Etiology of Anal Fissure: Current Research

The etiology of anal fissure remains uncertain, though spasm of the IAS is a constant feature. Nitric oxide (NO) is the major inhibitory neurotransmitter of the IAS, and, as in other spasmodic conditions of the gastrointestinal tract, a deficiency of normal nitric oxide synthase (NOS) activity has been reported.<sup>12</sup>

### Available Conservative Treatment Options

Anal fissures are a frequent and painful condition linked to excessive tone in the internal anal sphincter (IAS). Lowering this heightened muscle tone helps restore blood flow to the area and supports healing of the fissure. While surgical sphincterotomy remains highly effective for achieving healing, it carries the risks and recovery burden that come with any surgical procedure.<sup>13</sup> Because of this, several non-surgical approaches have been developed to reduce sphincter tone without operative intervention. One important consideration with conservative treatment is that patients must also follow dietary measures aimed at softening stool consistency throughout the treatment period, as this supports the healing process alongside the medication's effects.<sup>14</sup>

Botulinum toxin is a substance produced by the bacterium *Clostridium botulinum*. It works by blocking the release of acetylcholine at nerve endings, effectively interrupting the signal that would otherwise trigger muscle contraction. When injected locally, it relaxes both the external and internal anal sphincters, with effects that can persist for as long as three months.<sup>15</sup> Calcium channel blockers work by limiting calcium entry into smooth muscle cells, which in turn reduces the contractile tone of the internal anal sphincter. Two commonly used agents in this class- nifedipine (applied

as a 0.2–0.5% gel) and diltiazem (used as a 2% cream) have demonstrated the ability to lower resting anal pressure and support fissure healing.<sup>16</sup> Nitrate compounds such as glyceryl trinitrate (GTN) are converted within cells into nitric oxide, a molecule that acts as a relaxing signal for the internal anal sphincter. Applying GTN topically at a low concentration (0.2%) has been shown to reduce resting pressure in the anal canal.<sup>17</sup>

### New Phyto-therapeutic Agent for Chemical Sphincterotomy

*T. procumbens* is a perennial plant originally native to Central America, though it has since spread and become naturalized throughout tropical and subtropical regions worldwide. Reaching a height of only 15–40 cm, it grows as a low-lying herb with creeping stems that arise from a woody base and take root at the nodes along their length.<sup>18</sup>

Methyl 14-oxooctadecanoate, methyl 14-oxononacosanoate, 3-methylnonadecylbenzene, heptacosanyl cyclohexane carboxylate, 1(2,2-dimethyl-3-hydroxypropyl)-2-isobutyl phthalate, 12-hydroxytetracosan-15-one, 32-methyl-30-oxotetratriacont-31-en-1-ol, and 30-methyl-28-oxodotriacont-29-en-1-oic acid have been identified in *T. procumbens* by spectral data and chemical studies. Also, the following nine molecules have been identified as dotriacontanol,  $\beta$ -amyrone,  $\Delta$ 12-dehydrolupen-3-one,  $\beta$ -amyrin, lupeol, fucosterol, 9-oxoheptadecane, 10-oxononadecane, and sitosterol.<sup>19</sup> Two water-soluble polysaccharide fractions, WSTP-IA (l-arabino-d-galactan with a  $\beta$ -(1  $\rightarrow$  6)-d-galactan) and WSTP-IB ( $\beta$ -(1  $\rightarrow$  6)-d-galactan) purified from the leaves of *T. procumbens*. This is the first report of polysaccharides containing a  $\beta$ -1(1  $\rightarrow$  6)-d-galactan main chain isolated from plant sources.<sup>20</sup> B-sitosterol 3-O- $\beta$ -D-xylopyranoside, characterized as a steroidal saponin, has been isolated from the flowers of *T. procumbens*.<sup>21</sup> The metabolite found in higher plants, oleanolic acid, has also been isolated from *T. procumbens*.<sup>22</sup>

*T. procumbens*'s rich phytochemistry is associated with a wide range of pharmacological properties. In vivo and in vitro studies have demonstrated its pharmacological properties. These properties include anti-inflammatory<sup>23,24</sup>, immunomodulatory effects<sup>25</sup>, antioxidants<sup>26</sup>, anti-diabetic<sup>27</sup>, anti-hypertensive<sup>28</sup>, anti-arthritis<sup>29</sup>, antileishmanial<sup>30</sup>, and wound healing<sup>31</sup>. *T. procumbens* extract has shown no signs of toxicity in normal rats at therapeutic doses, indicating its safety.<sup>32</sup>

### Probable Mode of Action of *T. procumbens* on Internal Anal Sphincter Spasm

#### The Internal Anal Sphincter

The internal anal sphincter (IAS) is a ring of smooth, involuntary muscle encircling the upper portion of the anal canal — roughly the top two-thirds. Structurally, it's a continuation of the rectum's circular muscle layer, generally spanning 2.5–4 cm in length with a thickness of about 2–3 mm.<sup>33</sup> Unlike most muscles, the IAS doesn't rest in a relaxed state — it sits in a condition of ongoing,

near-maximal contraction. This baseline tension, known as resting tone, accounts for roughly 70–85% of the anus's overall resting pressure and is the primary mechanism preventing involuntary leakage of stool or gas between bowel movements.<sup>34</sup> Autonomic control of the IAS works in opposition- sympathetic input sustains its default contracted state, while parasympathetic activation triggers relaxation, as occurs during defecation. When stool distends the rectal wall, signals from local (intramural) nerve networks prompt the sphincter to relax momentarily, permitting passage of rectal contents. Once this occurs, the muscle quickly returns to its contracted baseline.<sup>35</sup>

### Sphincter Spasm and Pain

An anal spasm occurs when the IAS contracts abruptly and forcefully, well beyond its normal resting tension. Because this contraction is so intense, it compromises local blood supply to the densely packed muscle fibers of the canal, producing ischemia. This oxygen deprivation is what generates the characteristic sharp, stabbing pain associated with fissures.<sup>36</sup> Pain sensitivity in this region is heightened by anatomy; the anal canal below the dentate (pectinate) line is densely supplied with somatic sensory nerve fibers, unlike the visceral innervation above it. As the spasming muscle cramps and the canal distends, this pressure is transmitted directly onto highly pain-sensitive tissue, intensifying the discomfort. This creates a self-perpetuating cycle in anal fissures: a tear in the anal lining-frequently extending down into the IAS itself-provokes a reflexive, protective spasm of the sphincter. Ironically, this defensive spasm restricts blood flow further, impairs healing, and can deepen or prolong the original tear, sustaining the pain-spasm-ischemia loop.<sup>37</sup>

### *T. procumbens* as a Smooth Muscle Relaxant: Relevance to Anal Fissure Pathophysiology

The persistence of anal fissures is now understood to be driven less by the initiating trauma itself than by what follows it: a hypertonic IAS that compresses local arterioles, starves the wound bed of oxygen, and locks the tissue into a self-sustaining cycle of pain, spasm, and ischemia.<sup>38</sup> This mechanistic understanding has reoriented fissure pharmacotherapy away from wound care alone and toward agents that can pharmacologically relax smooth muscle and restore perfusion the rationale underlying topical nitrates, and calcium channel blockers as first-line therapies.<sup>39</sup> Nearly two decades of preclinical work show *T. procumbens* repeatedly relaxing the same smooth muscle whose contraction defines the fissure problem and it's this convergence of evidence, not any single study, that stands out.

Salahdeen et al. first demonstrated that aqueous leaf extract of *T. procumbens* produced concentration-dependent relaxation in noradrenaline-precontracted rat aortic smooth muscle, supporting its traditional use for hypertension.<sup>40</sup> A follow-up study by the same author showed that this vasorelaxant action involves

calcium-related pathways the extract blunted contractile responses to noradrenaline and potassium chloride, and reduced the contractile effect of calcium chloride under calcium-free conditions, pointing to inhibition of both calcium influx and calcium release from intracellular stores.<sup>41</sup>

In corpus cavernosum tissue, ethanolic extract of *T. procumbens* again produced dose-dependent relaxation. Blocking nitric oxide synthase only partially reduced this effect, while the extract enhanced relaxation triggered by acetylcholine and sodium nitroprusside — indicating that part of its action involves endothelial nitric oxide release, with possible relevance to erectile function.<sup>42</sup> A later mechanistic study in rat thoracic aorta found that the extract's vasodilatory effect was significantly reduced when cGMP and cAMP pathways were blocked, suggesting these second messengers help mediate the relaxation.<sup>43</sup>

In an in vivo model, rats made hypertensive with L-NAME and then treated with *T. procumbens* extract showed improvement in several markers of hypertension-related damage, including blood pressure, organ weight changes, lipid abnormalities, liver enzyme elevations, and tissue injury in the liver and heart.<sup>44</sup> Related studies by Salami et al. found that the extract also reduced impairments in reproductive function and corpus cavernosum contractility caused by L-NAME-induced hypertension.<sup>45,46</sup>

Separately, Omedine et al. linked the hypotensive effect of *T. procumbens* to its high potassium content rather than to diuresis or reduced blood volume, proposing that resulting hyperkalemia promotes cardiac hyperpolarization, slowed heart rate, and lowered blood pressure.<sup>47</sup> Further work by Salami et al. identified activation of the Na<sup>+</sup>-K<sup>+</sup> pump and the forward-mode Na<sup>+</sup>-Ca<sup>2+</sup> exchanger as contributors to extract-induced relaxation in mesenteric arteries.<sup>48</sup> Most recently, the same author reported that the extract also relaxes tracheal smooth muscle, largely through blockade of L-type calcium channels combined with increased endothelial nitric oxide release — raising the possibility of applications in respiratory disorders as well.<sup>49</sup>

A recent randomized controlled trial clinically validated *T. procumbens*, showing its topical cream matched standard Lignocaine-Nifedipine cream in improving wound healing, spasm, per rectal bleeding, and pain (p=0.0001), while causing no adverse effects, unlike the standard treatment.<sup>50</sup> This clinical efficacy aligns with its established mechanism: calcium channel inhibition, enhanced nitric oxide release, and modulation of cGMP/cAMP and ion-transport pathways collectively relax smooth muscle. Applied to the IAS, this lower resting sphincter pressure, restores anodermal blood flow, and relieves ischemic pain. Together, the clinical outcomes and preclinical mechanisms support *T. procumbens* as a scientifically grounded, effective candidate for chemical sphincterotomy in anal fissure management.

**Table 1: Summary of Preclinical and Clinical Studies on the Smooth Muscle Relaxant Effects of *Tridax procumbens***

| Sr. No. | Author (Year)            | Study Design                                                            | Title                                                                                                                                                                                                                                | Outcome                                                                                                                              |
|---------|--------------------------|-------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|
| 1       | Salahdeen HM (2012) [40] | In vitro, isolated rat aortic smooth muscle                             | Vasorelaxant effects of aqueous leaf extract of <i>Tridax procumbens</i> on aortic smooth muscle isolated from the rat                                                                                                               | Extract produced vasorelaxation of isolated aortic smooth muscle                                                                     |
| 2       | Salahdeen HM (2014) [41] | In vitro, rat aortic ring                                               | Calcium-dependent mechanisms mediate the vasorelaxant effects of <i>Tridax procumbens</i> aqueous leaf extract in rat aortic ring                                                                                                    | Vasorelaxant effect is mediated by calcium-dependent mechanisms                                                                      |
| 3       | Salahdeen HM (2015) [42] | In vitro, rat corpus cavernosum smooth muscle                           | The relaxant actions of ethanolic extract of <i>Tridax procumbens</i> (Linn.) on rat corpus cavernosum smooth muscle contraction                                                                                                     | Ethanolic extract relaxed corpus cavernosum smooth muscle contraction                                                                |
| 4       | Salahdeen HM (2016) [43] | In vitro, rat thoracic aorta                                            | Mechanism of vasorelaxation induced by <i>Tridax procumbens</i> extract in rat thoracic aorta                                                                                                                                        | Identified mechanistic pathway(s) underlying vasorelaxation in thoracic aorta                                                        |
| 5       | Salahdeen HM (2017) [44] | In vivo, L-NAME-induced hypertensive rats                               | Biochemical parameters as indicators of antihypertensive efficacy of leaf aqueous extract of <i>Tridax procumbens</i> (Linn.) in L-NAME-induced hypertensive rats                                                                    | Biochemical markers indicated antihypertensive efficacy of the extract                                                               |
| 6       | Salami SA (2017) [45]    | In vivo, L-NAME-induced hypertensive male rats                          | Oral administration of <i>Tridax procumbens</i> aqueous leaf extract attenuates reproductive function impairments in L-NAME-induced hypertensive male rats                                                                           | Extract attenuated hypertension-related reproductive function impairment                                                             |
| 7       | Salami SA (2018) [46]    | In vivo, N-nitro-L-arginine methyl ester-induced hypertensive male rats | Effects of aqueous leaf extract of <i>Tridax procumbens</i> on contractile activity of corpus cavernosum in N-nitro-L-arginine methyl ester-induced hypertensive male rats                                                           | Extract modulated corpus cavernosum contractile activity under hypertension                                                          |
| 8       | Omedine K (2018) [47]    | In vivo, Wistar rats                                                    | Hypotensive activity of <i>Tridax procumbens</i> hydroethanolic extract: Roles of sodium and potassium transport in Wistar rats                                                                                                      | Extract exhibited hypotensive activity linked to Na <sup>+</sup> /K <sup>+</sup> transport mechanisms                                |
| 9       | Salami SA (2021) [48]    | In vitro, rat mesenteric artery                                         | <i>Tridax procumbens</i> leaf extract elicits relaxation of rat mesenteric artery by activation of Na <sup>+</sup> -K <sup>+</sup> ATPase/Na <sup>+</sup> -Ca <sup>2+</sup> exchanger                                                | Relaxation mediated by activation of Na <sup>+</sup> -K <sup>+</sup> ATPase/Na <sup>+</sup> -Ca <sup>2+</sup> exchanger              |
| 10      | Salami SA (2022) [49]    | In vivo, tracheal smooth muscle, male Wistar rats                       | Preliminary mechanistic study on the trachea smooth muscle relaxant activity of aqueous leaf extract of <i>Tridax procumbens</i> in male Wistar rats                                                                                 | Extract showed relaxant activity on tracheal smooth muscle (preliminary mechanism identified)                                        |
| 11      | Bobade S (2025) [50]     | Single-blind, double-arm Randomized clinical trial, N= 70               | Comparative Evaluation of the Healing Effect of Topical <i>Jayanti</i> ( <i>Tridax procumbens</i> ) Cream versus Topical Lignocaine and Nifedipine Cream in <i>Parikartika</i> (Acute Fissure in Ano): A Randomised Controlled Trial | Topical <i>Jayanti</i> ( <i>T. procumbens</i> ) cream is equally efficacious as Lignocaine-Nifedipine cream in acute fissure in Ano. |

## Conclusion

On the basis of this pharmacological profile, supported by clinical evidence demonstrating efficacy comparable to standard Lignocaine-Nifedipine therapy, *T. procumbens* may be regarded as a form of “chemical sphincterotomy” and warrants consideration as a first-line treatment for anal fissure.

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**Conflict of Interest:** Nil

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