# In Vitro and In-Vivo Analysis of the Combined Extract of Tabernaemontanadivaricata and Mangiferaindica Leaves for Enhanced Anti-Inflammatory and Antioxidant Properties

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## Abstract

**Ethnopharmacological Relevance:** *Tabernaemontanadivaricata*(*T. divaricata*) and *Mangiferaindica*(*M. indica*) are plants traditionally used in Ayurvedic medicine for their anti-inflammatory and antioxidant properties. *T. divaricata* is known for its analgesic and wound-healing effects, while *M. indica* is celebrated for its role in managing inflammation and oxidative stress.

**Aim of the Study:** The study aimed to evaluate the synergistic effects of combined hydroalcoholic extracts from *T. divaricata* and *M. indica* leaves, focusing on their anti-inflammatory and antioxidant properties.

**Materials and Methods:** The leaves were collected, authenticated, and subjected to hydroalcoholic extraction. The extracts were then combined in various ratios and analyzed for phytochemical content. The antioxidant activity was evaluated using the DPPH assay, while anti-inflammatory effects were assessed through the carrageenan-induced rat paw edema model.

**Results:** The combined extracts exhibited significantly enhanced antioxidant and anti-inflammatory activities compared to the individual extracts. The most potent combination, a 2:1 ratio of *M. indica* to *T. divaricata*, showed the highest DPPH inhibition (94.37%) and maximum edema reduction in the animal model.

**Conclusion:** The combination of *T. divaricata* and *M. indica* extracts offers a synergistic therapeutic potential, enhancing both antioxidant and anti-inflammatory effects, which supports their traditional use and suggests potential for developing new natural therapeutics.


**Keywords:** Anti-inflammatory, Anti-oxidant,*Tabernaemontana divaricate, Mangiferaindica*

### 1. Introduction

Inflammation and oxidative stress are two pivotal processes implicated in the pathogenesis of various chronic diseases, including cardiovascular disorders, cancer, diabetes, and neurodegenerative conditions(Leyane, Jere, and Houreld 2022). The increasing prevalence of these conditions has heightened the search for natural remedies that can offer effective therapeutic interventions with minimal side effects. In this context, plants and their bioactive compounds have garnered significant attention due to their historical use in traditional medicine and their potential in modern therapeutics(Pandey et al. 2011).

*Tabernaemontanadivaricata*, commonly known as the crepe jasmine(Ghosh, Poddar, and Chatterjee 2021). *Tabernaemontanadivaricata* belongs to the family Apocynaceae. This family is commonly known as the dogbane family, and it includes a wide variety of flowering plants, many of which have medicinal properties.*Tabernaemontanadivaricata* is widely used in Ayurvedic medicine, primarily for its anti-inflammatory, analgesic, and wound-healing properties. The plant is rich in alkaloids, flavonoids, and terpenoids, which contribute to its therapeutic effects.

*Mangiferaindica*, known as the mango tree (Ghosh, Poddar, and Chatterjee 2021) is the plants traditionally recognized for their medicinal properties. *Mangiferaindica* belongs to the family Anacardiaceae, which is known for including several economically important fruit-

bearing trees and plants. *Mangiferaindica*, on the other hand, is renowned not only for its nutritional value but also for its extensive medicinal applications(Ediriweera, Tennekoon, and Samarakoon 2017), particularly in treating inflammation, infections, and oxidative stress-related conditions. The leaves of *Mangiferaindica* are known to contain polyphenols, flavonoids, and other potent antioxidants(Mirza et al. 2021).

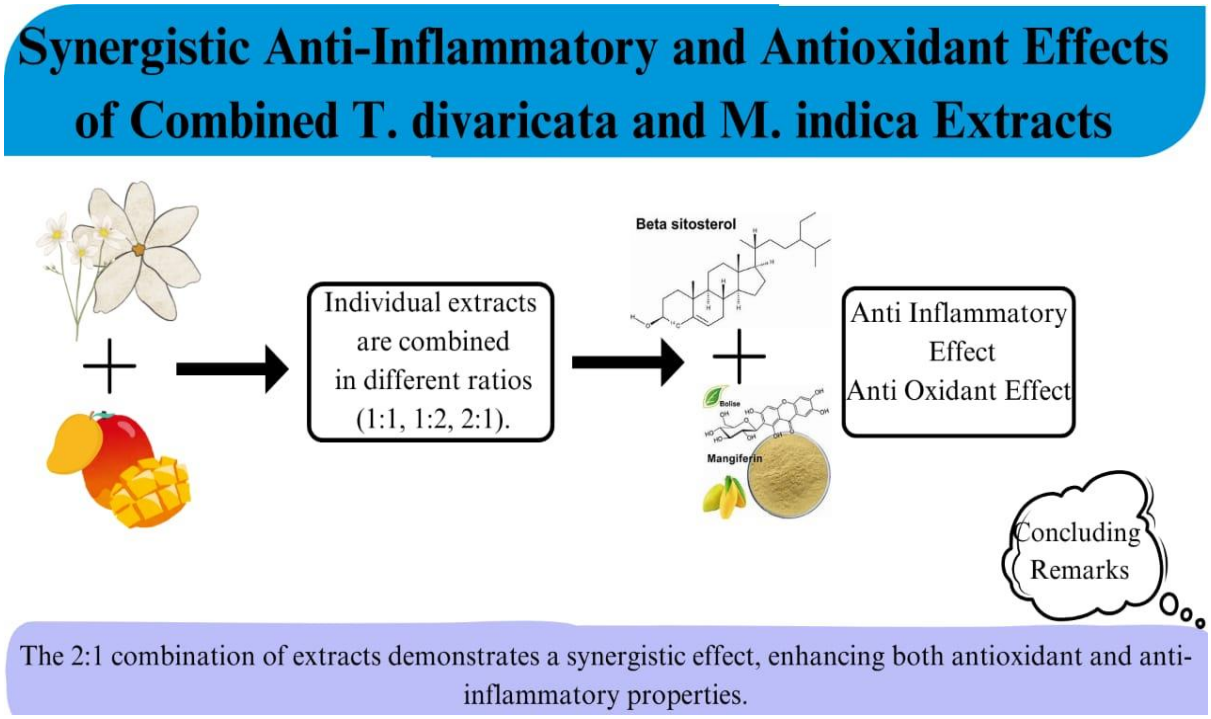
Given the individual therapeutic properties of these plants, combining the extracts of *Tabernaemontanadivaricata* and *Mangiferaindica* leaves presents a promising approach to enhancing their anti-inflammatory and antioxidant effects. The rationale behind this combination is to leverage the synergistic effects of the bioactive compounds present in both plants, which may result in a more potent therapeutic outcome than the individual extracts.

This study aims to explore the anti-inflammatory and antioxidant properties of the combined extract of *Tabernaemontanadivaricata* and *Mangiferaindica* leaves. By evaluating these effects through various in vitro and in vivo models, the research seeks to establish a scientific basis for the traditional use of these plants and potentially contribute to the development of new natural therapeutics for managing inflammatory and oxidative stress-related conditions.

## 2. Material and methods

### 2.1 Collection and identification of plant material

*Tabernaemontanadivaricata* and *Mangiferaindica* leaves were gathered from the surrounding area of Bhopal, Madhya Pradesh, and verified at RB Science, Bhopal. leaves were gathered from a nearby farm in Bhopal, Madhya Pradesh, and verified at RB Science, Bhopal.



### 2.2 Chemicals and reagents

Analytical grade compounds were all that were used. Reagents are chemicals used to prepare buffers, analytical solutions, and other things for experiments. The leaves of *Tabernaemontanadivaricata*and *Mangiferaindica* were freshly collected from the local area, providing the primary plant materials for the study. Several chemicals were sourced from well-known suppliers: ethanol was procured from LobaChemie (P) Ltd, Mumbai, while gallic acid and vanillin were obtained from CDH, New Delhi. Folin-Ciocalteu reagent was sourced from Avra, Hyderabad, and sodium carbonate and glacial acetic acid were acquired from Oxford Fine Chemicals, Mumbai. Methanol was provided by S.D. Fine Chemicals, Mumbai, and petroleum ether and chloroform were purchased from Rankem, Mumbai. Additionally, sulfuric acid was also supplied by Rankem, Mumbai. For experiments requiring water, freshly distilled water was prepared in the laboratory to ensure purity.

### 2.3 Extraction Process of leaves of *Taebermontanadivaricata* and *Mangiferaindica*

Powdered leaves (91 g, *Taebermontanadivaricata*; 102 g *Mangiferaindica*) wereseparately sealed in the Soxhlet apparatus's extractor and defatted with petroleum ether utilizing heated continuous extraction process until colorless siphoning solution is obtained (5-6 h). The extraction After the solvent was eliminated, the marc was dried, and then extracted with a blend of ethanol-water (70:30) as the solvent. The extractswere filtered hot to remove impurities andthe solvent was evaporated using rotary vacuum evaporator. To eliminate the oleo-resinous extract, it was gathered and put in a water bath remaining solvent and finally placed in desiccatormaximum drying**.** The dried/semidried extracts were stored in desiccator for further experimental procedures.

### 2.4 Qualitative Phytochemical Screening

Both thehydro-alcoholic extracts were assessed using phytochemical qualitative responses to determine whether typical plant secondary metabolites are present or absent. Many classes, including triterpenes/steroids, alkaloids, glycosides, flavonoids, saponins, tannins, and phenolics, were screened for. As analytical reactions to these tests, the color intensity or the precipitate formation were employed(Banu and Cathrine 2015).

#### 2.4.1 Test forAlkaloids

- **Mayer’s test:** To a few ml of plant sample extract, two drops of Mayer’s reagent was added along the sides of test tube.
- **Wagner's test:**Test tube walls were lined with a few drops of Wagner's reagent mixed with a few milliliters of plant extract.
- **Hager's test:** In the test tube, a small amount of plant extract and a few drops of Hager's reagent are applied along the sides.
- **Dragendroff’s Test:**Each extract was mixed with one milliliter (ml) and a few drops of Dragendroff's solution.

### 2.4.2 Test for Glycosides

- **Froth test:**A test tube containing 1ml of the extract in water was filled and given a good shaking.
- **Borntrager's test:**The extract and 1.0 milliliter of diluted sulfuric acid were combined in a test tube and brought to a boil for five minutes. Following the chilling and shaking of the filtrate with an equivalent volume of dichloromethane, the bottom layer (dichloromethane) was separated and shaken with half its volume of diluted ammonia.
- **Kedde's test:**After extracting the material using hloroform, evaporation dries it out. One drop of 90% alcohol and two drops of 2% 3, 5-dinitro benzoic acid (3, 5-dinitro benzene carboxylic acid, Kedde's reagent) in 90% alcohol should be added to the previously indicated residue. The solution becomes alkaline when 20% sodium hydroxide solution is added.
- **Keller killiani test (Test for deoxy sugars):**Following a chloroform extraction, the extract is evaporated until it is completely dry. 0.4 milliliters of glacial acetic acid with a tiny amount of ferric chloride solution were added to the residual. After moving the mixture into a test tube, 0.5 ml of concentrated sulfuric acid was applied along the test tube's wall.

### 2.4.3 Test forTannins and phenolic compounds

- **Gelatin test:** To the extract, a 1% gelatin solution containing 10% sodium chloride was added.
- **Ferric chloride test:** Iron chloride solution that had been newly made was added to the extract.
- **Vanillin hydrochloride test**: A few drops of vanillin hydrochloride reagent were added to the extract test solution for treatment.
- **Alkaline reagent test:** Sodium hydroxide solution was used to treat the extract test solution.

### 2.4.4 Test for flavonoids

- **Shinoda test:** To Conc. hydrochloric acid was added dropwise to the extract test solution along with a few pieces of magnesium ribbon.
- **Zinc hydrochloride reduction test:**A mixture of concentrated hydrochloric acid and zinc dust was added to the test solution.
- **Alkaline reagent test:**The test solution received a few drops of sodium hydroxide solution added to it. A small amount of strong hydrochloric acid was added. Later, if color showed.

### 2.4.5 Proteins and amino acids

- **Millons test:** The extract's Two milliliters of Millon's reagent (mercuric nitrate in nitric acid with traces of nitrous acid) were mixed with the test solution, and left to react.
- **Ninhydrin test*:*** A 0.2% ninhydrin solution was added to the extract solution and brought to a boil.

### 2.4.6 Sterols and terpenoids

- **Libermann-Burchard test:**After adding a few drops of acetic anhydride to the extract, it was heated and chilled. Concentrated sulfuric acid was poured into the test tube from the sides.

- **Salkowski test:**By dissolving the extract in chloroform, a little amount of concentrated sulfuric acid was included. After giving the mixture a good shake, it was allowed to stand for some time.

**2.5 Chromatographic analysis of the extracts**

The extracts were dissolved in ethanol by sonication and filtered through a 0.45µ nylon syringe filter prior to injecting in the sample loop of HPLC instrument.

Acetonitrile-buffer (pH 2.5) (15:85) was the solvent system used to extract Mangiferaindica; the C18 column (chromosil, 5µ) was used to measure the flow rate, which was set at 1.0 mL per minute. The extract's mangiferin content was found using a 253 wavelength (KUMAR, SRIVASTAVA, and KUMAR[1] 2008). After injecting a 20µL sample into the sample loop, the chromatogram was acquired. Using the aforementioned technique, a chromatogram of standard mangiferin (5µg/mL) was also produced. Acetonitrile-water (pH 2.5) (95:5) was the solvent system used to extract Taebermontanadivaricata. A C8 column (chromosil, 5µ) was used to measure the flow rate, which was set at 1.5 mL per minute. The presence of ß-sitosterol in the extract was determined using a wavelength of 202 (Shah et al. 2010). An example 20µL was injected into the sample loop and the chromatogram was obtained. A chromatogram of standard ß-sitosterol (5µg/mL) was also chromatographed using the above method.

**2.6 FT-IR analysis of the extracts**

The dried extracts were scanned in the range of 400 to 4000 cm-1 using a FT-IR spectrophotometer and the stretching and bending vibrations were observed.

**2.7 Total Phenolic Content**

An adaptation of the procedure described served as the foundation for the extraction of phenolic chemicals. One dried extract (0.1 g) was dissolved in 5 mL of ethanol to determine the total phenolic content. The fixes served as the stock solutions for further studies and were kept in amber-colored bottles at 4°C (Mishra and Jain 2021).

In order to ascertain the overall phenolic content One milliliter (200 µL) of the extract sample was combined with 1.4 milliliters Folin-Ciocalteu reagent (100 µL). After two minutes, add 300 µL of a 20% Na2CO3 aqueous solution, and let the mixture remain for two hours. A UV-Vis spectrophotometer was used to measure the absorbance at 765 nm. To construct the calibration curve, standard solutions containing 10–100 ppm of gallic acid were treated in a similar manner. With the same chemicals and 200 µL of methanol, the control solution was prepared and incubated similarly to the other samples. The results were expressed in milligrams per 100 mg of the dry material using the gallic acid equivalent (GAE).

**2.8 Preparation the combined extracts for the antioxidant and anti-inflammatory effect**

The hydro-alcoholic extracts from *Mangiferaindica* and *Taebermontanadivaricata* were combined in three different ratios (1:1, 1:2, and 2:1), respectively, and the antioxidant and anti-inflammatory effect was assessed using the techniques described in the following sections. The statistical significance of the combined extracts' antioxidant and anti-inflammatory properties was examined by comparing them to those of the individual extracts.

**2.9Evaluation of Anti-oxidant Activity**

The steady free radical DPPH was used to gauge the test solution's free radical scavenging activity in terms of its capacity to donate hydrogen or scavenge radicals. The previously described approach was used to determine the DPPH radical scavenging activity(Amreen and Chaurey 2021). A 1 mM DPPH solution and an extract solution (100 µg/mL) were produced separately in ethanol. A 1.5 ml DPPH solution was mixed with 1.5 ml of the test solution. The equivalent blank solution, made with 3 mL of ethanol, was used to measure the absorbance at 517 nm. 3 mL of DPPH was the control sample that was used. Three duplicates of the assay were run. The following formula was used to determine the percentage inhibition of the free radical DPPH based on the control reading.

$$\boldsymbol{DPPH\ scavenged}\ (\%) = \frac{Acom - Atest}{Acom} \times 1000$$

Where, $A_{con}$ - is the absorbance of the control reaction, $A_{test}$ - is the absorbance in the presence of the test solution.

**2.10 Animal**

Adult male Wistar rats weighing 250 - 300g were maintained in the Institute of Biological Science of the Federal University of Rio Grande at 22 ± 2ºC, with a relative humidity of 50-60% under a 12-12 h light-dark cycle with food and water *ad libitum*. The experiments were performed after approval of the protocol by the Institutional Ethics Committee (approval number P021/2013).

**2.11 Experimental protocol**

Animals were distributed in six groups and received the following treatments:

Group I - Control - treated with vehicle (normal saline)

Group II– *Taebermontanadivaricata* extract (200 mg/kg)

Group III – *Mangiferaindica*extract (200 mg/kg)

Group IV – Combined extract 1:1 (100 mg/kg)

Group V – Combined extract 1:2 (100 mg/kg)

Group VI – Combined extract 2:1 (100 mg/kg)

**2.12 Evaluation of anti-inflammatory action**

The anti-inflammatory activity of the extracts was assessed using the rat paw edema technique caused by carrageenan (Kemisetti and Manda 2018).

To induce paw oedema, 0.1 mL (1% solution) of carrageenan was subcutaneously injected into the plantar surface of the rat's right hind paw. Thirty minutes prior to the carrageenan injection, each animal group received a dosage of extracts at a rate of 100 mg/kg. The following groups (n = 6) of animals were formed.

Using a vernier caliper, the Paw diameters were measured immediately prior to the administration of carrageenan, and then at 1, 2, 4, and 6 hours later. The outcomes were contrasted with those of the control group. The following formula was used to determine each group's % suppression of paw inflammation:

$$\%\ \boldsymbol{inhibtion}\ = \frac{C - T}{C} \times 100$$

Where, C= Paw volume (mL) in vehicle treated group (control), T= Paw volume (mL) in drug treated group

## 3. RESULTS AND DISCUSSION

### 3.1 Extraction yield

The defatting of the leaves was done by petroleum ether and the extraction of desired phytoconstituents by ethanol-water (70:30) as the solvent blend (Table 1 and 2).

It was found that the hydro-alcoholic solvent blend was able to extract more contents from *T. divaricata* as compared to *M. indica* while the fatty material in both the plants was almost similar.

### 3.2 Preliminary phytochemical Screening

The extracts included alkaloids, flavonoids, triterpenoids, phenols, tannins, and saponins, as shown by the qualitative phytochemical screening.

### 3.3 Chromatographic and spectral analysis of extracts

The hydro-alcoholic extracts of both the plants were analyzed by HPLC for the presence of specific phytoconstituent (qualitatively). The *M. indica* extract was found to contain Mangiferin with retention time of 2.819 (Fig. 2 and 3), while the *T. divaricata* extract exhibited the presence of ß-sitosterol at retention time 5.82 minutes (Fig. 4 and 5).

### 3.4 FTIR results

A broad peak around 3400-3200 $cm^{-1}$, which may correspond to O-H stretching vibrations, indicating the presence of hydroxyl groups (alcohols, phenols), 1700 $cm^{-1}$ suggests the presence of carbonyl groups, which could indicate the presence of ketones, aldehydes, or carboxylic acids, 2850-3000 $cm^{-1}$ suggest C-H stretching, which is typical in alkanes and other hydrocarbon chains, 1600-1500 $cm^{-1}$, which may indicate C=C stretching in aromatic rings, 1050-1250 $cm^{-1}$ is typical of C-O stretching vibrations, which can be found in alcohols, ethers, esters, and carboxylic acids (Fig. 6).

The FTIR spectrum of *Tabernaemontanadivaricata* extract presents distinct peaks indicative of various functional groups. A broad peak around 3400-3200 $cm^{-1}$ corresponds to O-H stretching, suggesting the presence of hydroxyl groups commonly found in alcohols and phenols. The peak near 1700 $cm^{-1}$ indicates C=O stretching, pointing to the presence of carbonyl groups, which may be associated with ketones, aldehydes, or carboxylic acids. Peaks around 2850-3000 $cm^{-1}$ represent C-H stretching vibrations typical of aliphatic hydrocarbons. The peak near 1600 $cm^{-1}$ is likely due to C=C stretching, often associated with aromatic rings. Additionally, the region around 1050-1250 $cm^{-1}$ suggests C-O stretching vibrations, indicating the presence of alcohols, ethers, or ester functional groups. This analysis highlights the diverse chemical nature of the *T. divaricata* extract, confirming the presence of multiple functional groups, including hydroxyl, carbonyl, and aromatic structures (Fig. 7).

### 3.5 Total Phenolic Content

As one of the most stable and reasonably priced natural phenols, gallic acid was utilized as the benchmark for determining the total phenolic content. Using the blue color absorption data collected in the concentration range of 10 to 100 ppm, the standard curve for gallic acid was created (Table 4 and Fig. 8). Regression coefficient and Beer's law range were ascertained from this. Gallic acid's linearity equation was determined to be Abs = 0.006x - 0.0042. GAE mg/100 mg was the determined total Phenolic content of the samples

The amount of phenolics present (total) in the *M. indica* and *T. divaricata* extracts were found to be 62.5 GAE mg/100 mg and GAE 55 mg/100 mg respectively.

### 3.6 Pharmacologicalevaluation

The anti-oxidant and anti-inflammatory properties, extracts of *M. indica* and *T. divaricata* were combined in three different ratios. These combined extracts were labelled as C1, C2 and C3 containing 1:1, 1:2 and 2:1 ratio of *M. indica* and *T. divaricata* respectively.

#### 3.6.1 DPPH radical scavenging assay

Using the DPPH radical scavenging assay method, the combined extracts' antioxidant activity was ascertained. When appropriate reducing chemicals are used to react with DPPH radicals, the radicals lose color stoichiometric to the number of electrons consumed, which may be detected using a 517 nmusing spectrophotometer. If DPPH has antioxidant properties, the deep purple color of the molecule becomes less intense. The antioxidant activity was found to be synergistically improved on combining the extracts (Table5 and fig. 9).

It was found from the result that combining the extracts resulted in synergizing the antioxidant activity when compared to the individual extracts. The highest amount of DPPH inhibition was witnessed in C3 (94.37 ± 0.702 %) where *M. indica* and *T. divaricata* were mixed in the ratio 2:1 (Figure 9).

#### 3.6.2 Anti-inflammatory activity

The anti-inflammatory characteristics of the combined extracts were examined using the rat carrageenan-induced paw edema approach. Acute inflammation caused by carrageenan is one of the finest test methods for anti-inflammatory medication evaluation. Having the capacity to extracts and the mixed extracts to inhibit edema on pretreatment was calculated from the paw diameter obtained at different times post treatment (Table 6 and Fig.10).

## 4. Conclusion

The study concludes that the combination of *Mangiferaindica* and *Tabernaemontanadivaricata* leaf extracts significantly enhances their antioxidant and anti-inflammatory properties compared to the individual extracts. The observed synergistic effects, particularly in the 2:1 ratio of *M. indica* to *T. divaricata*, suggest that these plant extracts could be effectively utilized in developing natural therapeutics for managing conditions associated with oxidative stress and inflammation. The presence of bioactive compounds such as flavonoids and phenolics likely contributes to these enhanced activities, reinforcing the potential of these plant combinations in therapeutic applications. Research

results led to the conclusion that combining the extracts greatly enhanced their anti-inflammatory and antioxidant properties. The greatest increase in activity was specifically observed when two parts *M. indica* extract and one-part *T. divaricata* extract were used together.

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