WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.453
Volume 15, Issue 3, 1605-1615. Research Article ISSN 2277–7105

HPTLC PROFILING AND QUANTIFICATION OF ANTIOXIDANT MARKER COMPOUNDS IN *TRAGIA INVOLUCRATA LINN (STEM)*

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Article Received on 05 Jan. 2026,
Article Revised on 25 Jan. 2026,
Article Published on 01 Feb. 2026,

https://doi.org/10.5281/zenodo.18480487

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How to cite this Article: Mohithraj RP, Yogaraj P., Sivadharsini S., Vishva K., Sasitharan P., Rajesh M., Rajasekaran A., Arivukkarasu R.* (2026). Hptlc Profiling And Quantification of Antioxidant Marker Compounds In Tragia Involucrata Linn (Stem). World Journal of Pharmaceutical Research, 15(3), 1605–1615.

ABSTRACT

Tragia involucrata Linn. (Euphorbiaceae), a well-known medicinal plant used in Ayurveda and Siddha systems, is an important ingredient of formulations such as Kabasura Kudineer. The present study aimed to standardize and quantify key antioxidant marker compounds in *T. involucrata* using a validated HPTLC method. Methanolic extracts of the plant were analyzed using toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4) as the mobile phase, with densitometric detection at 254 nm. The method showed good resolution and reproducibility, enabling effective phytochemical fingerprinting. Quantitative analysis revealed the presence of gallic acid (0.03%), rosmarinic acid (0.12%), luteolin (0.37%), and kaempferol (0.93%). These bioactive markers are known to exert antioxidant and anti-inflammatory effects through mechanisms such as ROS scavenging, Nrf2/HO-1 activation, and NF-κB/MAPK pathway inhibition. The findings scientifically validate the traditional use of *T. involucrata* and support its therapeutic relevance in polyherbal formulations for managing oxidative stress and inflammatory conditions.

KEYWORDS: *Tragia involucrata*; HPTLC; gallic acid; rosmarinic acid; kaempferol; herbal drug standardization.

INTRODUCTION

Tragia involucrata Linn., (Syn Indian Stinging Nettle, or Kalli) is a valuable medicinal plant of the family Euphorbiaceae, and a commonly used plant in Ayurveda, Siddha, Unani, and in traditional folk medicine of India, Sri Lanka and Southeast Asia.[1,2,3] The plant has had extensive medicinal use especially in the treatment of skin diseases, urinary tract illness, inflammatory diseases, intestinal parasites, fever and wound infections.[4] Historically, preparations like decoctions, pastes, powders, and total-plant extracts are applied to treat the disorders of dermatitis, eczema, dysuria, nephritis, abdominal colic, diarrhoea, rheumatism and other microbial infections. These pharmacological actions which have anti-inflammatory, antimicrobial, diuretic, analgesic, antioxidant, anthelmintic, wound-healing anti-inociceptive and sedative effects prove the medicinal value of *T. involucrate*.[5,6] Phytochemical research has found flavonoids, alkaloids, tannins, phenolics, sterols, triterpenoids, saponins, essential oils and bioactive hydrocarbons like shellsol, most of which have strong anti-bacterial and anti-inflammatory activities. Its wound-healing and antimicrobial properties have been demonstrated in its ability to counteract pathogens such as *Staphylococcus aureus* and *Proteus vulgaris*, thus contributing to its relevance in ethnomedicine.[7] *T. involucrata* is a perennial climbing herb botanically, with stinging hairs on the stems, leaves, and is typically found in the wet tropics, as a decision-maker in the moist deciduous forests, scrub jungles, grasslands, hedges, and hilly slopes to a maximum of 1,200 m. Due to its widespread traditional uses, the phytochemical richness and scientifically proven therapeutic value, the plant has become an interesting target to Pharmacognostic standardization and chromatographic profiling, which has earned it an attractive option to create herbal formulations to treat inflammatory, microbial, and urinary diseases. Among the ingredients of Kabasura Kudineer Chooranam, *Tragia involucrata* L., belonging to the family Euphorbiaceae, is present at a concentration of 6.66%, whereas it is present in Vatha sura Kudineer Chooranam (4.76%), as documented in the siddha formulary of India, part 1. The inclusion of *Tragia involucrata* in Kabasura Kudineer and related siddha formulation indicates its therapeutic relevance in the management of respiratory and inflammatory condition. Therefore, the present study was undertaken on *Tragia involucrata* to estimate and quantify the marker compounds responsible for its therapeutic activity.[8] Gallic acid is main ingredient and responsible for pharmacological mechanisms in the pathophysiological process of the oxidative damage diseases, such as cancer, cardiovascular, degenerative and metabolic diseases.[9,10] Rosmarinic acid is a key antioxidant phenolic compound identified in *Tragia involucrata*. HPTLC analysis of methanolic extracts detected rosmarinic acid at about

0.12% w/w using the mobile phase toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4) with detection at 254 nm. It exhibits strong antioxidant and anti-inflammatory activity by scavenging free radicals and activating the Nrf2/HO-1 pathway, supporting the therapeutic relevance of *T. involucrata* in formulations like Kabasura Kudineer.[11,12] Kaempferol is a mjor flavonol detected in *Tragia involucrata*. HPTLC analysis showed kaempferol at about 0.93% w/w using toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4) with detection at 254 nm. It exhibits strong antioxidant and anti-inflammatory activity through ROS scavenging and NF-κB/MAPK inhibition, supporting the plant's therapeutic value.[13,14] Luteolin is a flavonoid detected in *Tragia involucrata* at about 0.37% w/w by HPTC using toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4) at 254 nm. It contributes antioxidant and anti-inflammatory activity via inhibition of NF-κB/MAPK pathways.[15,16] Catechin is a flavan-3-ol, a type of natural phenol and antioxidant Catechins have been shown to demonstrate a variety of antimicrobial properties. Consumption of green tea has been shown to distribute catechin compounds and/or their metabolites throughout the body, which allows for not only the possibility of treatment of infections but also the prevention of infections[17,18] Based on the above findings, we plan to detect and estimate the antioxidant marker in *Tragia involucrata*.

MATERIALS AND METHODS

Collection of raw materials for HPTLC screening

The raw materials were received as gift sample from Traditional siddha medical practitioner Mr. Cinnathambi, Boomidi, Dharmapuri district and confirmed the raw materials with Dr. S. Mutheeswaran Scientist, Centre for Biodiversity and Biotechnology, Xavier Research Foundation, St Xavier's college, Tirunelveli, Tamilnadu, India.

Instruments

A CAMAG HPTLC system comprising of a Linomat-V applicator and CAMAG TLC Scanner-3 and single pan balance of Shimadzu model were used, for weighing the herbal raw material for preparation of extracts.

Preparation of standards and extracts

One gram of dried powdered material was taken and sonicated with 10 ml of methanol. Filtered and the filtrate solution was used for HPTLC analysis. Methanol was used to create standard marker chemicals at a concentration of 1 mg/1 ml each of Gallic acid, Rosmarinic acid, Kaempferol, Luteoline, and Catechin.

Application of sample

The herbal formulations solutions were spotted in the form of bands of width 4 mm with a Hamilton 100µl syringe on recoated plate 60 F254 (10 cm × 10 cm with 0.2 mm m thickness, E. Merck) using a Camag Linomat V applicator. The slit dimension was kept 6mm × 0.45 mm. Eight µl of each herbal raw material extract and five µl of standard solutions were applied on to the plate. The migration distance was 80 mm. TLC plates were dried with air dryer. Densitometric scanning was performed using Camag TLC Scanner-3 at 254 nm operated by a wincat software.

Development

The chromatogram was developed in CAMAG glass twin-through chamber (10-10 cm) previously saturated with the mobile phase toluene: ethyl acetate: formic acid: methanol [3:6:1.6:0.4] for 10 min (temperature 25°C, relative humidity 40%). The development was done 8 cm from bottom.

Detection

The plate was scanned at UV 254 using CAMAG TLC Scanner-3 and LINOMAT-V. Rf value of each compound which were separated on plate and data of peak area of each band was recorded.

RESULTS AND DISCUSSION

The different solvent compositions were tried for monitor the elution of components in herbal extracts.[19,20] Ethyl acetate: glacial acetic acid formic acid: water (100:3:3:28), Ethyl Acetate: Methanol: Water Toluene (100:13:10:13), Chloroform: ethyl acetate: methanol (6:4:0.3), Toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4), Toluene: ethyl acetate (93:7). Among the 5 mobile phases attempted, Toluene: ethyl acetate: formic acid: methanol in the ratio of 3:6:1.6:0.4 gave better elution for all the extracts tested and hence it was used as mobile for detection of constituents in herbal extracts.[21,22,23] The optimized chamber saturation time for mobile phase was 10 min at room temperature (25 ± 1°C). The densitometric analysis was performed at 254 nm in reflectance mode. The Rf values of the marker compounds were in the range of 0.08 to 0.91. The detection and quantity of marker in herbal raw material extracts were given in Table 1. In *Tragia involucrata*, rosmarinic acid (0.12%), gallic acid (0.03%), luteolin (0.37%), and kaempferol (0.93%) were detected.[25,26,28,31] Gallic acid exhibits antioxidant effects[27]; rosmarinic acid activates Nrf2/HO-1 pathway, mitigating oxidative stress.[26,34] Luteolin regulates multiple inflammatory pathways;[32] gallic acid and kaempferol contribute via ROS scavenging and NF-κB/MAPK inhibition.[33] The HPTLC profiles and quantified antioxidant markers correlate with reported pharmacological effects, supporting the therapeutic relevance of *Tragia involucrata* in formulations such as Kabasura Kudineer.[24,35] The higher abundance of luteolin and kaempferol in *Tragia involucrata* underscores their contribution to antioxidant and anti-inflammatory activity.[29,30] Based on these findings and the documented mechanisms of action of the antioxidant markers quercetin, gallic acid, rosmarinic acid, kaempferol, luteoloin, and catechin present in *Tragia involucrata*, one of the key ingredients of Kabasura Kudineer, these compounds may contribute to the therapeutic potential of Kabasura Kudineer in the management of COVID-19-related symptoms.[36,37] Notably, the approximately fivefold higher levels of rosmarinic acid and kaempferol compared with other antioxidant markers may further enhance the therapeutic efficacy of Kabasura Kudineer in managing COVID-19-related symptoms.[26,38] The findings scientifically validate the traditional use of *T. involucrata* and support its therapeutic relevance in polyherbal formulations for managing oxidative stress and inflammatory conditions.

CONCLUSION

The present study successfully optimized an HPTLC method for the qualitative and quantitative evaluation of antioxidant marker compounds in *Tragia involucrata*. Among the various mobile phases tested, the combination of toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4) provided superior resolution and reproducible separation of phytoconstituents. The method enabled effective densitometric analysis at 254 nm, with well-defined Rf values covering a wide range, confirming its suitability for herbal fingerprinting and standardization. Quantitative analysis revealed the presence of key antioxidant and anti-inflammatory markers such as catechin, rosmarinic acid, gallic acid, luteolin, and kaempferol, with luteolin and kaempferol occurring in relatively higher concentrations. The identified phytochemicals are known to exert their biological effects through established mechanisms including free radical scavenging, activation of antioxidant defense pathways, and inhibition of pro-inflammatory signaling cascades. The correlation between the HPTLC profiles, quantified marker compounds, and reported pharmacological activities supports the therapeutic relevance of *Tragia involucrata*. As a key ingredient of Kabasura Kudineer, the presence and abundance of these antioxidant markers may collectively contribute to its potential efficacy in managing oxidative stress and inflammation associated with COVID-19-related symptoms. Overall, the findings provide scientific validation for the traditional use of

Tragia involucrata and highlight the importance of HPTLC-based standardization in ensuring the quality, consistency, and therapeutic reliability of polyherbal formulations.

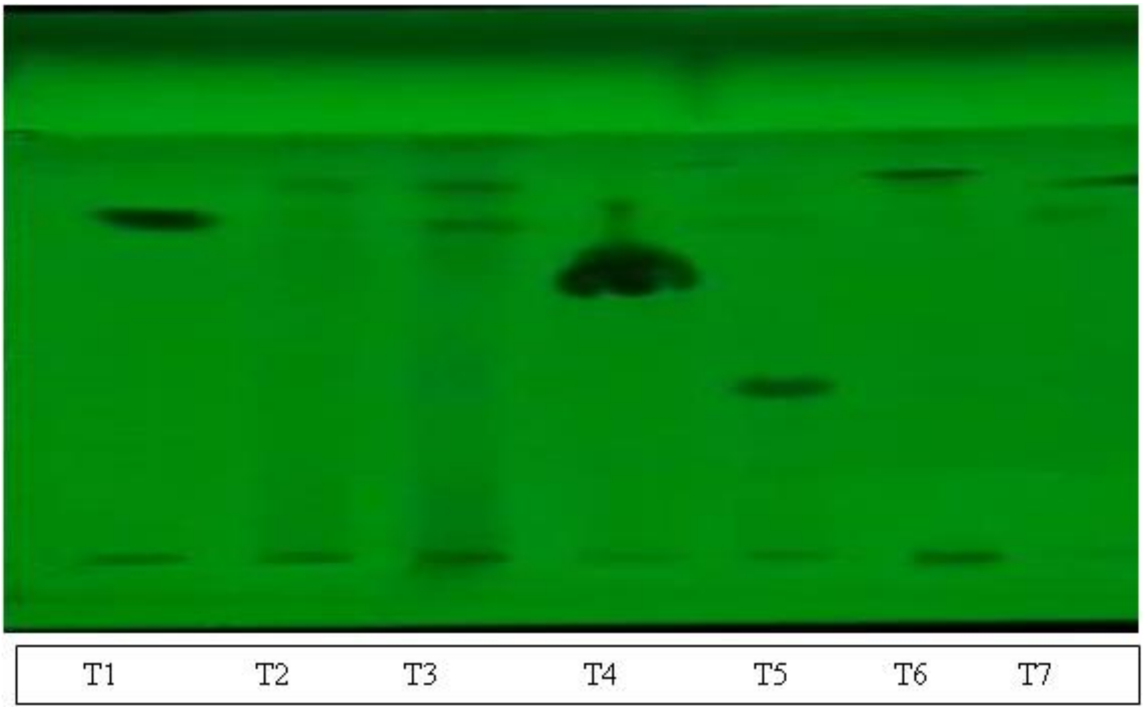

Figure 1: T1-Gallic acid, T2-*Tragia involucrata* (5 µl), T3-*Tragia involucrata* (10 µl), T4-Catechin, T5-Rosmarinic acid, T6-Luteolin, T7-Kaempferol.

Table 1: RF Value of 1-gallic acid, 2-*Tragia involucrata* (5µl) 3-*Tragia involucrata* (10µl) 4-catechin, 5-rosmarinic acid, 6-luteolin, 7-kaempferol.

S.no	Name of sample	Rf values of compound	Rf values of markers in extract	Name of marker in extract	Area of standards	Amount of marker present in µg/ 5 µl and 10 µl of extracts/ 5 µl of standards	Percent Of Marker present In Extracts
1	Gallic acid	0.81	0.81	Gallic acid	17748.5	5.0	100%
2	*Tragia involucrata* (5µl)	Not considered for calculation due to negligile amount were present in 5µl of extract					
	Tragia involucrata (10µl)	0.79	0.79	Gallic acid	547.4	0.154	0.03%
		0.44	0.44	Rosmarinic acid	1621.9	0.615	0.12%
		0.88	0.88	Luteolin	6227.0	1.871	0.37%
		0.88	0.88	kaempferol	6227.0	4.688	0.93%
4	Catechin	0.68	0.68	Catechin	55294.3	5.0	100%
5	Rosmarinic acid	0.40	0.40	Rosmarin acid	13166.7	5.0	100%
6	Luteolin	0.89	0.89	Luteolin	7412.6	5.0	100%

7	Kaempferol	0.87	0.87	kaempferol	6640.3	5.0	100%

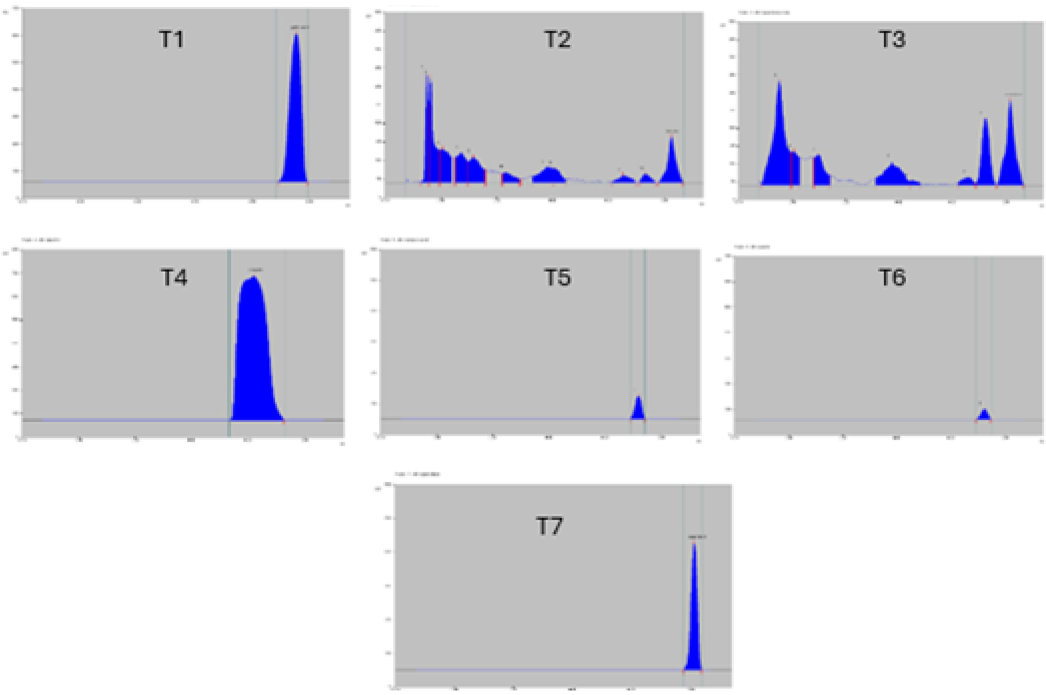

Figure 2: T1-gallic acid, T2-*Tragia involucrata*, T3-*Tragia involucrata*, T4-catechin, T5-rosmarinic acid, T6-luteolin, T7-kaempferol.

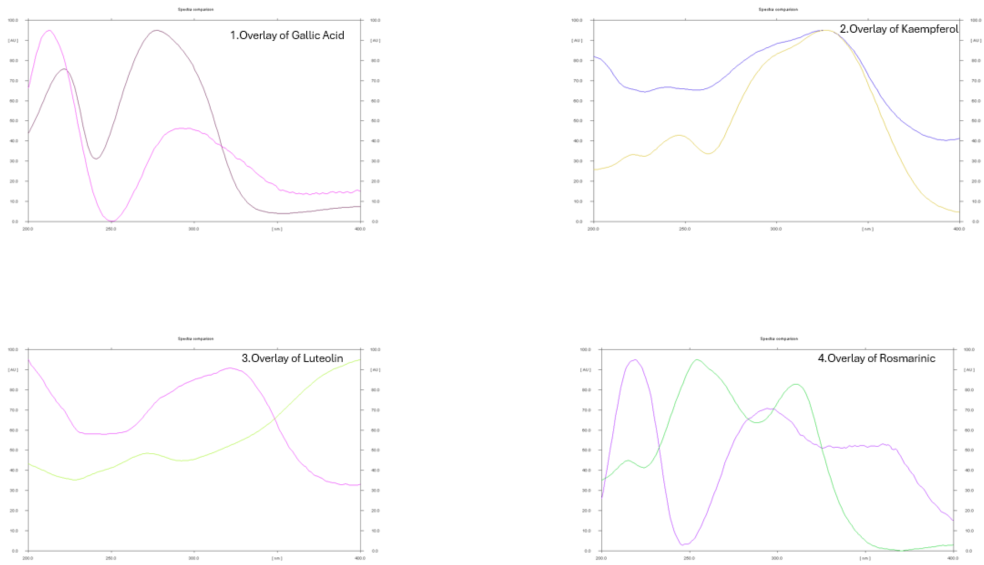

Figure 3: 1. Overlay of gallic Acid, 2. Overlay of Kaempferol, 3. Overlay of Luteolin, 4. Overlay of Rosemarinic.

ACKNOWLEDGMENT

The authors express their sincere gratitude to Chairman, Dr. Nalla G. Palaniswami and Managing Trustee, Dr. Thavamani D. Palaniswami, for providing the necessary infrastructure, laboratory facilities, and instrumental support to carry out this research work. The authors declare that no specific grant was received from public, private, or nonprofit funding organizations for this study.

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