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K Venkata Ramana Sai
P.G Scholar, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Tamil Nadu, Veterinary and Animal Sciences University, Orathanadu, Thanjavur, Tamil Nadu, India

V. Ranganathan,
Professor and Head, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Orathanadu, Thanjavur, Tamil Nadu, India

A. Vijayarajan,
Professor and Head, Veterinary Clinical Complex, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Orathanadu, Thanjavur, Tamil Nadu, India

K. Vijayakaran,
Assistant Professor, Veterinary Clinical Complex, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Orathanadu, Thanjavur, Tamil Nadu, India

A. Elamaran
Assistant Professor, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Orathanadu, Thanjavur, Tamil Nadu, India

K. Kannan
Assistant Professor,
Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Orathanadu, Thanjavur, Tamil Nadu, India

M. Pooja
P.G Scholar, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Tamil Nadu Veterinary and Animal Sciences University, Orathanadu, Thanjavur, Tamil Nadu, India

Corresponding Author:
K Venkata Ramana Sai
P.G Scholar, Department of Veterinary Pharmacology and Toxicology, Veterinary College and Research Institute, Tamil Nadu, Veterinary and Animal Sciences University, Orathanadu, Thanjavur, Tamil Nadu, India

Quantitative assessment of gallic acid in *Zingiber officinale* by high-performance thin layer chromatography

K Venkata Ramana Sai, V Ranganathan, A Vijayarajan, K Vijayakaran, A Elamaran, K Kannan and M Pooja

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Abstract
Zingiber officinale, commonly known as ginger, belongs to the family Zingiberaceae. This family is characterized by aromatic rhizomes and includes other notable species such as turmeric (*Curcuma longa*) and cardamom (*Elettaria cardamomum*). Ginger, with its long history in traditional medicine, is widely used for digestive health, alleviating nausea, and stimulating digestion. Its anti-inflammatory compounds, gingerols and shogaols, help reduce pain and swelling, particularly in arthritis. Studies also indicate that ginger may effectively relieve menstrual pain. Gallic acid is a phenolic compound known for its strong antioxidant properties, helping to neutralize free radicals and protect cells from oxidative damage. It also enhances ginger's anti-inflammatory effects, supporting digestive health and potentially alleviating pain associated with conditions like arthritis. Additionally, gallic acid's antimicrobial properties may aid in boosting the immune system and promoting overall wellness. In the present study, plant rhizomes were obtained from the Ethnoveterinary Herbal Garden at the Veterinary College and Research Institute in Orathanadu, Thanjavur, Tamil Nadu, India. Gallic acid was identified and quantified using a straightforward, sensitive, and accurate High-Performance Thin Layer Chromatography technique.

Keywords: Gallic acid, *Zingiber officinale*, phenol, quantification, HPTLC

1. Introduction
Zingiber officinale, or ginger, holds significant cultural and culinary importance in India. In Indian cuisine, ginger is a fundamental ingredient used in a variety of dishes, it is also valued for its medicinal properties, commonly used to alleviate digestive issues, colds, and inflammation, reflecting its integral role in traditional Ayurvedic practices. *Zingiber officinale* is a flowering plant belonging to the family Zingiberaceae, native to Southeast Asia. This rhizomatous plant has been widely utilized in traditional medicine across various cultures for centuries, particularly in Ayurveda and Traditional Chinese Medicine, where it is celebrated for its numerous health benefits. The rhizome of *Zingiber officinale* is rich in phytochemicals, including gingerols, shogaols, and zingerone, which contribute to its anti-inflammatory, antioxidant, and antimicrobial properties, playing a significant role in promoting overall health and well-being. Moreover, phytochemical screening of ethanolic and methanolic extracts of *Zingiber officinale* revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and terpenoids, while steroids were absent, supporting the diverse phytochemical profile of this plant (Bhargava *et al.*, 2012) [2].
Ginger has been demonstrated to reduce lipid peroxidation by preserving the activity of key antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase. Additionally, ginger exhibits a range of beneficial effects, including antiemetic properties, molluscicidal and antischistosomal activities, cardiovascular benefits, relief from migraines, and antirheumatic properties (Mbaveng and Kuete., 2017) [7].
Gallic acid, a powerful natural phenolic compound is found in diverse natural product with different pharmacological and biological properties, including anti-inflammatory, antimutagenic, antioxidative, and anticarcinogenic effects (Harsh *et al*., 2023) [6].

Gallic acid derivatives have been found in a number of phytomedicines with diverse pharmacological activities, such as reactive oxygen species scavenging, interfering the cell signaling pathways, and apoptosis of cancer cells (Bharti *et al*., 2015) [3]. In analytical research, gallic acid is used as a standard for determining the phenol content of various analytes (Damiani *et al*., 2014) [5]. Curcumin was quantified using HPTLC method due to its simplicity, precision, specificity, sensitivity, and accuracy. Gallic acid was detected and quantified in the methanol extract of *Homonoia riparia* using HPTLC (Xavier *et al*., 2015) [10]. Bhuvaneswari and Sivasubramanian (2023) [4] studied gallic acid, quercetin, and rutin in *Amorphophallus paeoniifolius* (Dennst.) using chloroform, ethyl acetate, methanol, and formic acid (88:2:2:8 v/v/v/v) in mobile phase. This method is suitable for routine quality control of both raw materials and formulations containing these phytocompounds (Thakker *et al.*, 2011) [9].

The Rf value of gallic acid was consistently recorded at 0.56 across all samples and the reference standard when examined under UV light at both 254 nm and 366 nm. The plate was developed using a solvent system comprising toluene, ethyl acetate, and formic acid in a ratio of 3:3.5:0.5 v/v, and then dried (Meena *et al.*, 2018) [8]. Polyherbal formulations for psoriasis study aimed to formulate psoriasis tablets and standardize them using HPTLC, with gallic acid, curcumin, and quercetin as biomarkers. Chromatography was conducted on silica gel 60F254 plates using a solvent system of toluene, ethyl acetate, and formic acid (4.5:3.0:0.2 v/v), revealing Rf values of 0.40 for gallic acid (Thakker *et al.*, 2011) [9].

In the present study, a sensitive, simple, and reliable HPTLC method was developed to identify and quantify the gallic acid in *Zingiber officinale* rhizome ethanolic extract of *Zingiber officinale* rhizome samples cultivated at Ethno Veterinary Herbal Product Research and Development Centre is located in Orathanadu, Thanjavur District, Tamil Nadu.

2. Materials and Methods

2.1 Plant Source

Zingiber officinale rhizomeswere procured from the Herbal Garden, Ethno Veterinary Herbal Product Research and Development Centre, Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Orathanadu, Thanjavur District, Tamil Nadu. The collection followed cleaning, shade-drying, and pulverization for subsequent analytical studies.

2.2 Preparation of ethanolic extract of *Zingiber officinale*

A cold maceration technique was employed to extract bioactive compounds from *Zingiber officinale* rhizome. A total of 151 g of finely powdered rhizome was mixed with 70% ethanol in a 1:5 ratio and subjected to maceration for seven days at -20 °C, with periodic agitation to enhance extraction efficiency. Subsequent filtration through Whatman No. 1 filter paper yielded a clarified extract. The filtrate was then concentrated with a rotary evaporator to completely remove/condense the solvent, yielding a phytochemical-rich concentrated extract. Cold maceration, a gentle extraction method, mitigates thermal degradation of heat-sensitive phytochemicals, yielding high-quality extracts from medicinal plants. This technique effectively preserves the structural integrity of bioactive compounds, including flavonoids and phenolics, crucial for the medicinal efficacy of *Zingiber officinale*.

2.3 High-Performance Thin-Layer Chromatography (HPTLC) Analysis

Gallic acid standards were obtained from Sigma Aldrich (USA). HPTLC analysis was performed using silica gel 60 F254 TLC plates (Merck). Standard gallic acid solutions (1 mg/mL) and *Zingiber officinale* crude extract samples (100 mg/mL) were applied in varying volumes (1, 2, 3, 4, 5, 6, 7, and 8 µL and 2, 4, 6, 8, 10, 12, and 14 µL, respectively) using a CAMAG Linomat 5 sample applicator. The plate was developed at room temperature in a CAMAG twin-trough vertical development chamber saturated with toluene, ethyl acetate, and formic acid (14:4:2 v/v) for 20 minutes. The solvent front position was set at 70 mm. The plate was analyzed using a CAMAG visualizer2 with a tungsten light source (366 nm) and Vision CATS software (version 2.5.18262.1). Gallic acid detection in *Zingiber officinale* crude extracts was confirmed by comparing Retardation factor (Rf) values with those of the standard. The Rf value, representing the ratio of the distance travelled by the compound to that of the solvent, was measured for both standard and sample spots.

2.4 Assessment of gallic acid levels in *Zingiber officinale* crude extract

The area calibration for the chemical gallic acid was performed at 366 nm with a CAMAG scanner (S/N: 250410). Eight reference standard samples and seven *Zingiber officinale* crude extract samples were tested to produce a standard curve for determining the gallic acid concentration of the *Zingiber officinale* crude extract samples.

3. Results and Discussion

Table 1 presents the Rf values obtained from High-Performance Thin Layer Chromatography (HPTLC) analyses, indicating the presence of gallic acid in the *Zingiber officinale* crude extract samples. Figures 1, 2, and 3 illustrate the development of the TLC plate with fluorescence at 366 nm and confirm the identification of gallic acid in the *Zingiber officinale* crude extract, along with its corresponding Rf value. The observed Rf value of 0.56 for gallic acid in *Zingiber officinale* samples aligns with previous studies (Thakker *et al*., 2011; Meena *et al*., 2018) [9, 8], validating the HPTLC method's reliability. The calibration curve's linearity (R = 0.997657) and low CV (2.92%) demonstrate the accuracy and precision of gallic acid quantification. These findings suggest *Zingiber officinale* potential as a rich source of gallic acid for pharmaceutical applications, particularly in polyherbal formulations for psoriasis treatment. Previous studies using CAMAG HPTLC relied on win CATS software, while this study utilized the more advanced vision CATS software. Additionally, there are no existing reports on the evaluation of gallic acid from *Zingiber officinale* grown in the Thanjavur district of Tamil Nadu.

Table 1: HPTLC –Gallic acid profile of ethanolic extract of *Zingiber officinale* samples (Gallic acid -1mg/ml; *Zingiber officinale* samples-100 mg/ml)

Track	Rf	Height	Area	Assigned substance
Gallic acid standard	0.408	0.0288	0.00162	Gallic acid
Gallic acid standard	0.411	0.0421	0.00254	Gallic acid
Gallic acid standard	0.411	0.0746	0.00511	Gallic acid
Gallic acid standard	0.413	0.0818	0.00567	Gallic acid
Gallic acid standard	0.413	0.0862	0.00614	Gallic acid
Zingiber officinale sample	0.439	0.0211	0.00077	Gallic acid
Zingiber officinale sample	0.44	0.0407	0.00137	Gallic acid
Zingiber officinale sample	0.437	0.054	0.00178	Gallic acid
Zingiber officinale sample	0.437	0.0625	0.00215	Gallic acid
Zingiber officinale sample	0.352	0.0145	0.00054	Gallic acid
Zingiber officinale sample	0.352	0.017	0.00067	Gallic acid
Zingiber officinale sample	0.353	0.017	0.00069	Gallic acid

Fig 1: HPTLC Plate developed showing bands of standards and samples before visualization

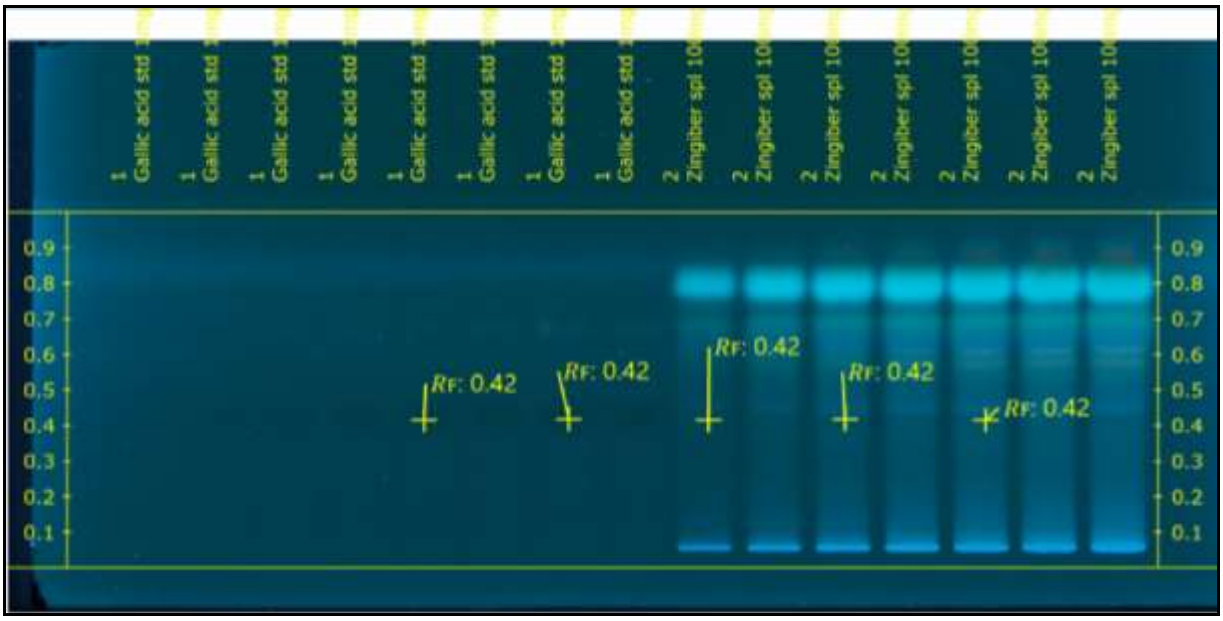

Fig 2: HPTLC Plate developed under visible remission (366 nm) showing bands of standards and samples

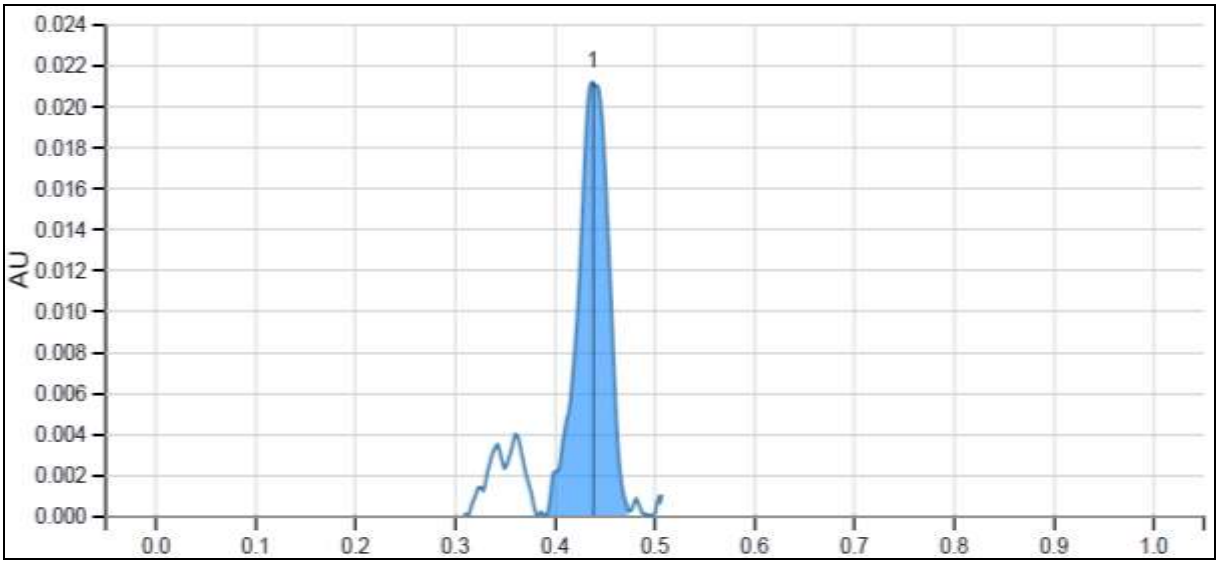

Fig 3: HPTLC plate track peak for identification of gallic acid in *Zingiber officinale* samples at 0.439

3.1 Quantification of Gallic Acid in *Zingiber officinale* via HPTLC

A calibration curve was generated using the CAMAG scanner, employing five reference standards and five *Zingiber officinale* samples. The calibration curve exhibited a high degree of linearity (R = 0.997657) and precision (CV = 2.92%). This enabled accurate quantification of gallic acid in *Zingiber officinale*. The calibration curve revealed a gallic acid content of 184.7 µg per 100 mg *of Zingiber officinale* sample. The linear dynamic range for gallic acid quantification in *Zingiber officinale* samples was determined to be 184.7 µg/mL.

The high correlation coefficient (R = 0.997657) and low coefficient of variation (CV = 2.92%) demonstrate the reliability and reproducibility of the HPTLC method for gallic acid quantification. Figure 4 illustrates the calibration curve for relative quantification of gallic acid in *Zingiber officinale* samples.

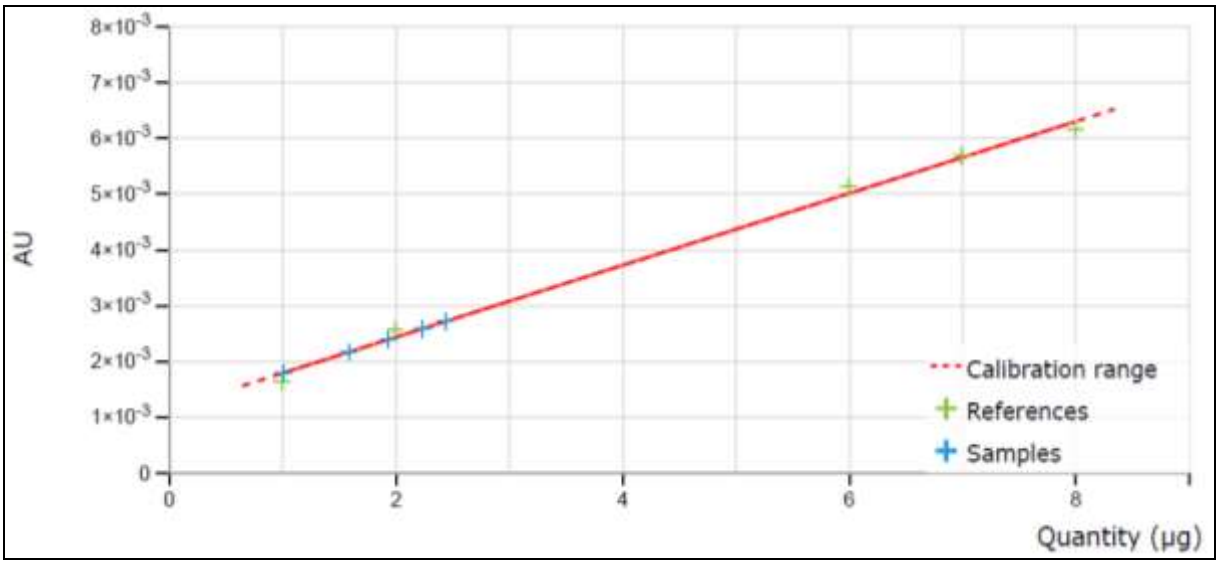

Fig 4: HPTLC - calibration curve for relative quantification of gallic acid in the *Zingiber officinale* samples

4. Conclusion

The present study demonstrates the presence of gallic acid in the rhizomes *of Zingiber officinale* cultivated in the herbal garden of the Ethno Veterinary Herbal Product Research and Development Centre, Veterinary College and Research Institute, Orathanadu. This finding suggests potential therapeutic applications of *Zingiber officinale* extracts in healthcare, leveraging gallic acid's well-documented bioactive properties.

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