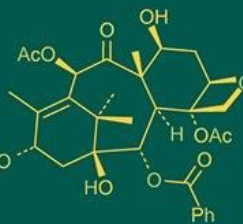
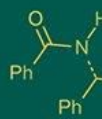
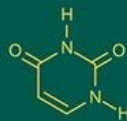


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2025; SP-9(1): 1064-1067
www.biochemjournal.com
Received: 08-11-2024
Accepted: 11-12-2024

R Balamurugan

Assistant Professor,
Department of Animal
Nutrition, Veterinary College
and Research Institute,
TANUVAS, Udumalpet, Tamil
Nadu, India

C Kathirvelan

Professor & Head, Department
of Animal Nutrition,
Veterinary College and
Research Institute,
TANUVAS, Udumalpet, Tamil
Nadu, India

A Abinaya

Assistant Professor,
Department of Animal
Nutrition, Veterinary College
and Research Institute,
TANUVAS, Udumalpet, Tamil
Nadu, India

In-vitro analysis of chemical composition, phytochemicals, and methane reduction potential of guava (*Psidium guajava*) leaves

R Balamurugan, C Kathirvelan and A Abinaya

DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i1Sn.4038>

Abstract

Herbs play a vital role in reducing methane emissions from livestock. This study explores the potential of guava leaves (*Psidium guajava*) in mitigating methane emissions through *in vitro* analysis. The research examines the nutritional composition, phytochemical properties, and methane reduction potential of guava leaves. Nutritional analysis of *Psidium guajava* leaves revealed a dry matter content of 24.05%, crude protein at 19.17%, crude fiber at 12.81%, ether extract at 1.86%, total ash at 5.49%, and nitrogen-free extract at 60.67%. Phytochemical screening was conducted using water, methanol, and ethanol extracts to identify various bioactive compounds. The qualitative analysis confirmed the presence of alkaloids, flavonoids, tannins, phenols, saponins, carbohydrates, proteins, amino acids, phytosterols, and terpenoids across all three extracts. To assess the methane reduction potential, different concentrations of guava leaves (20, 40, 60, 80, and 100 mg) were tested *in vitro*. The findings indicated that higher concentrations, particularly 80 mg and 100 mg, significantly ($p < 0.05$) reduced methane production, yielding values of 4.97 ml and 5.40 ml per 100 mg of truly digested substrate, respectively. These results highlight the potential of guava leaves as a natural strategy for mitigating methane emissions in livestock production systems.

Keywords: Nutritional composition, methane reduction, phytochemical screening, guava leaves, livestock emissions

Introduction

Guava (*Psidium guajava*) is a widely recognized plant known for its numerous medicinal and nutritional benefits. It has been extensively used across different cultures for treating various health conditions, with scientific research validating its antidiabetic, antinociceptive, antimutagenic, antispasmodic, and antimicrobial properties. Traditionally, guava has played a significant role in folk medicine, being utilized in the treatment of fevers, typhoid, and malaria. Its leaves and bark have long been valued for their therapeutic applications, making it a widely used natural remedy. The composition of *Psidium guajava* is highly diverse, encompassing a broad spectrum of bioactive compounds. These include polysaccharides, essential nutrients, volatile oils, minerals, enzymes, proteins, and triterpenoid acids. Additionally, guava is rich in antioxidants and vital nutrients, particularly lutein, which contributes to its various health benefits. The phytochemicals found in guava leaves exhibit different solubility properties, with some being water-soluble while others require specific extraction methods to be effectively utilized.

In addition to guava, a variety of other herbal extracts, including neem (*Azadirachta indica*), garlic (*Allium sativum*), ginger (*Zingiber officinale*), kadukka (*Terminalia chebula*), and amla (*Emblica officinalis*), are known to contain a diverse range of bioactive compounds. These compounds, such as lignins, tannins, terpenoids, volatile essential oils, and alkaloids, exhibit various biological properties that contribute to their antimicrobial, antioxidant, and anti-inflammatory effects. Due to these properties, these herbal extracts have been widely explored for their potential applications in animal nutrition, methane mitigation, and overall livestock health management. These natural metabolites possess antimicrobial properties and are known to influence microbial activity within the rumen. By selectively inhibiting specific microbes involved in methanogenesis, these compounds help regulate rumen fermentation,

Corresponding Author:**R Balamurugan**

Assistant Professor,
Department of Animal
Nutrition, Veterinary College
and Research Institute,
TANUVAS, Udumalpet, Tamil
Nadu, India

leading to a potential reduction in methane emissions from livestock. Methane production from ruminants is a significant concern due to its impact on global warming. Research has increasingly focused on natural feed additives that can improve digestion while mitigating methane emissions. The present study aims to assess the nutritional composition, phytochemical profile, and methane reduction potential of guava leaves through *in vitro* experiments. By analyzing its effects on methane production, this study explores the feasibility of using guava leaves as a natural dietary supplement for sustainable livestock management. If proven effective, guava leaves could serve as an eco-friendly alternative for reducing greenhouse gas emissions from ruminant animals while also offering additional health benefits to the animals. Given the increasing demand for sustainable livestock practices, exploring the role of plant-based feed additives is essential. The use of guava leaves in animal nutrition could provide a dual advantage by enhancing rumen function and reducing methane emissions, ultimately contributing to environmental sustainability and improved livestock productivity. This study seeks to build on existing knowledge by providing scientific validation for the potential application of guava leaves in methane mitigation strategies.

Experimental Procedures

The composition analysis of guava leaves (*Psidium guajava*) was carried out to assess crude protein, ether extract, crude fiber, nitrogen-free extract, and total ash content. The study followed standardized methodologies as outlined by the Association of Official Analytical Chemists (AOAC, 2019) [4]. For the phytochemical analysis, guava leaf extracts were prepared using four different solvents: distilled water, 95%

ethanol, and 98% methanol. A total of 20 grams of guava leaves was combined with 100 millilitres of each solvent. The identification of bioactive compounds—including alkaloids, flavonoids, tannins, phenols, saponins, carbohydrates, proteins, amino acids, phytosterols, and terpenoids—was conducted following the methods described by Patra *et al.* (2006) [11] and verified using the protocol from Tiwari *et al.* (2011) [16].

The methane reduction capability of guava leaves was evaluated through *in vitro* gas production trials, employing the Hohenheim gas production technique, as described by Menke and Steingass (1988) [10]. Methane concentration was assessed at varying guava leaf doses (20, 40, 60, 80, and 100 mg) alongside a control group. The analysis of methane emissions was performed using gas chromatography (Perkin Elmer, Clarus 500 model), which was equipped with a Flame Ionization Detector (FID) and a capillary column measuring 30 meters in length and 250 micrometres in diameter. This approach adhered to the guidelines established by Sitaula *et al.* (1992) [13].

The *in vitro* true dry matter digestibility (IVTDM) of the fermented feed was determined using the procedure outlined by Van Soest and Robertson (1988) [18]. Digestibility was quantified by recording the initial weight of the incubated sample and subtracting the weight of the undigested residue after treatment with a neutral detergent solution (NDS). Methane production per 100 mg of truly digested substrate was estimated based on both methane yield and the *in vitro* dry matter digestibility of the incubated material. This research highlights the potential of guava leaves as a natural feed supplement for enhancing livestock digestion and mitigating methane emissions, thereby supporting environmentally sustainable livestock management.

$$\text{Methane production per 100 mg} = \frac{\text{Methane emission (ml)}}{\text{Degradability (\%)}} \times 0.1 \text{ gm truly digested substrate}$$

Data Analysis

The *in vitro* experimental data were subjected to statistical analysis using analysis of variance (ANOVA). IBM SPSS Statistics software (version 20.0 for Windows) was employed for the analysis, following the guidelines described by Snedecor and Cochran (1994) [14]. To assess significant differences among treatment groups, Duncan's Multiple Range Test (DMRT) was utilized. This statistical method provided a robust comparison of results and facilitated the evaluation of various guava leaf concentrations on methane reduction and related parameters.

Results and Discussion

Nutritional Composition of Guava Leaves

The chemical composition of guava leaves, as shown in Table 1, includes 19.17% crude protein, 12.81% crude fiber, 1.86% ether extract, 5.49% total ash, and 60.67% nitrogen-free extract on a dry matter basis. These values are closely comparable to those reported by Anamika *et al.* (2017) [3], who recorded crude protein, ether extract, and total ash levels of 5.28%, 0.56%, and 4.38%, respectively. However, the present study found relatively higher proportions of crude protein, ether extract, and total ash compared to the previous findings.

Phytochemical Screening and Methane Mitigation Potential of Guava Leaves

Table 2 summarizes the presence of alkaloids, flavonoids, tannins, phenols, saponins, carbohydrates, proteins, amino acids, and phytosterols in aqueous, methanol, and ethanol extracts. The phytochemical analysis of guava leaves confirmed the presence of alkaloids, flavonoids, tannins, phenols, saponins, carbohydrates, proteins, amino acids, and phytosterols in aqueous, methanol, and ethanol extracts. These results align with previous studies by Todkar *et al.* (2010) [17] and Xavier Varghese Raja, who also identified alkaloids, flavonoids, tannins, phytosterols, and saponins in guava leaf extracts. Likewise, Komal Khanpara *et al.* (2012) [8] detected saponins, alkaloids, sugars, and flavonoids. However, their study did not report the presence of tannins.

Effect of Guava Leaves on Gas Production and Methane Emission

The results of the *in vitro* evaluation, including total gas production, methane production, methane percentage in total gas, true dry matter digestibility, and methane production per 100 mg of truly digested substrate, are summarized in Table 3. This evaluation provides insights into the potential of guava leaves in mitigating methane emissions during ruminal fermentation. total gas production was measured *in vitro* for different guava leaf concentrations: 20 mg, 40 mg,

60 mg, 80 mg, and 100 mg, yielding values of 45.17 ml, 44.33 ml, 44.83 ml, 40.50 ml, and 43.17 ml, respectively. In contrast, the control group exhibited a significantly higher total gas production of 58.33 ml, indicating that guava leaf supplementation may influence fermentation dynamics. However, no prior studies were available for direct comparison of gas production across different guava leaf concentrations, highlighting the novelty of this research.

Methane production at varying guava leaf concentrations was recorded as 8.46 ml (20 mg), 8.50 ml (40 mg), 8.33 ml (60 mg), 7.16 ml (80 mg), and 7.00 ml (100 mg), whereas the control group exhibited significantly higher methane production at 12.54 ml. Statistical analysis confirmed a significant reduction in methane production ($p < 0.05$) in the

guava leaf-treated groups compared to the control. This suggests that bioactive compounds present in guava leaves may play a role in suppressing methanogenesis. These findings align with the study by Sirohi *et al.* (2009) [12], which demonstrated that acetone extracts of shikakai pods (*Acacia concinna*) significantly reduced methane production (28.15 ml per gram DM) compared to the control group (37.55 ml per gram DM). The reduction in methane emissions observed in the present study suggests that guava leaves could serve as a natural feed additive to mitigate enteric methane production. Further research is needed to elucidate the specific mechanisms responsible for this reduction and to explore the potential long-term effects of guava leaf supplementation in ruminant diets.

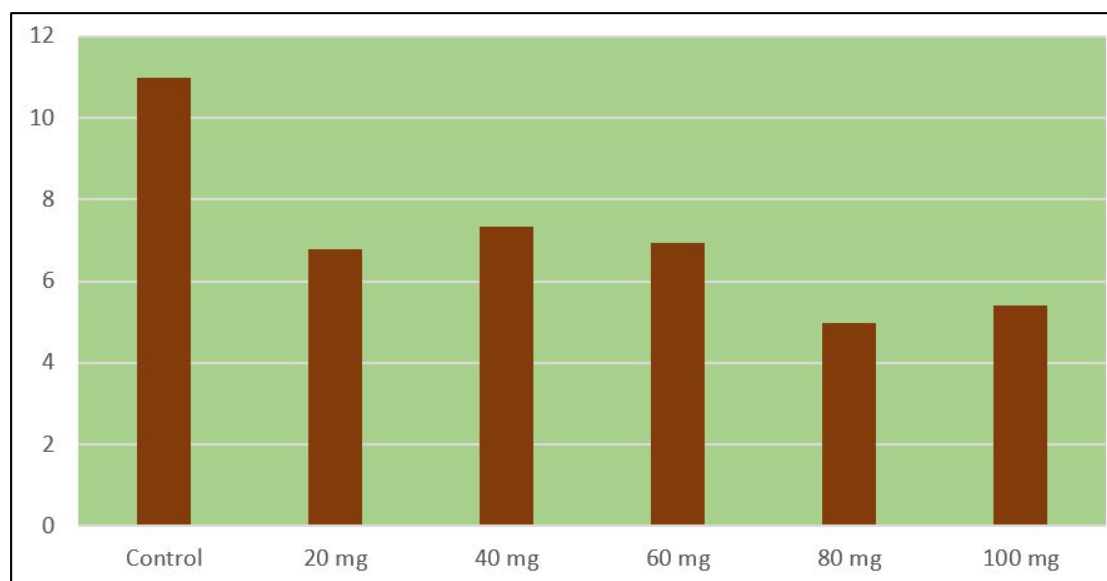


Fig 1: Impact of Various Guava Leaf Levels on Methane Reduction (Methane Output per 100 mg of Truly Digested Substrate in ml)

True Dry Matter Digestibility and Methane Reduction Potential of Guava Leaves

The true dry matter digestibility (TDMD) at varying guava leaf concentrations was recorded as follows: 60.14% (20 mg), 62.74% (40 mg), 59.73% (60 mg), 78.47% (80 mg), and 57.13% (100 mg), compared to 65.25% in the control group. Statistical analysis revealed a significantly higher ($p < 0.05$) TDMD in the control group and at the 80 mg guava leaf dose level. These findings suggest that guava leaves, particularly at optimal concentrations, can enhance digestibility while simultaneously reducing methane emissions.

Patra *et al.* (2006) [11] conducted a study demonstrating that the addition of shikakai pod extracts (water, ethanol, methanol) to wheat straw and concentrate-based diets led to a significant ($p < 0.05$) decrease in *in vitro* organic matter digestibility (IVOMD) compared to the control group. The *in vitro* methane production per 100 mg of truly digested substrate was measured for different guava leaf doses: 10.98 ml (control), 6.80 ml (20 mg), 7.35 ml (40 mg), 6.93 ml (60 mg), 4.97 ml (80 mg), and 5.40 ml (100 mg) is shown in figure 1. A significant ($p < 0.05$) reduction in methane production per 100 mg of truly digested substrate was observed at the 80 mg and 100 mg guava leaf dose levels compared to the control. These results indicate that guava leaves have the potential to serve as a natural feed supplement, enhancing digestion while mitigating methane emissions in livestock.

Summary

The findings of this study indicate that among the different concentrations of guava leaves evaluated *in vitro*, the 80 mg dose exhibited the highest effectiveness in reducing methane production per 100 mg of truly digested substrate. This reduction was significantly greater than that observed with lower concentrations of 20 mg, 40 mg, and 60 mg. The observed decline in methane production suggests that guava leaves contain bioactive compounds capable of influencing ruminal fermentation patterns in a way that suppresses methanogenesis.

Moreover, the potential application of guava leaves as a natural feed additive presents an environmentally sustainable approach to reducing methane emissions from ruminant livestock, a major contributor to global greenhouse gas emissions. By integrating guava leaf supplementation into livestock feeding strategies, farmers and researchers could explore ways to enhance feed efficiency while simultaneously addressing environmental concerns. Further studies, including *in vivo* trials, are necessary to validate these findings and determine the optimal inclusion levels, long-term effects, and potential impacts on animal health and productivity.

Acknowledgements

We sincerely appreciate the support provided by the Dean of Madras Veterinary College, Chennai-600 007, for facilitating the resources required to carry out this study.

References

- Alabi AA, Luky OO, Kate EI. Phytochemical, proximate and metal content analysis of leaves of *Psidium guajava*. Intl J Health Res. 2010 Apr;3(4):217.
- Adefagha S, Obah G. Water extractable phytochemicals from some Nigerian spices inhibit Fe²⁺ induced lipid peroxidation in rats' brain *in vitro*. J Food Process Tech. 2011 Dec;1(2):2-6.
- Anamika KK, Singhal AK, Singh, Kerketta S. Nutritional evaluation of indigenous plants and quantification of total saponins in plant extracts. Int J Curr Microbiol Appl Sci. 2017 Sep;6(9):1368-1377.
- AOAC. Official methods of analysis. 21st ed. Washington, D.C.: Association of Official Analytical Chemists; 2019.
- Begum S, Hassan SI, Siddiqui BS, Shaeen F, Ghayur MN. Triterpenoids from the fresh leaves of *Psidium guajava*. J Phytochem. 2010 Apr;61(4):399-403.
- Islam M, Dahlan I, Rajion MA, Jelan ZA. Rumen pH and ammonia nitrogen of cattle fed different levels of oil palm (*Elaeis guineensis*) frond-based diet and dry matter degradation of fractions of oil palm frond. Asian-Australas J Anim Sci. 2000 Jul;13(7):941-947.
- Kamra DN, Singh R, Agarwal N, Pathak NN. Soapnut (Reetha) as natural defaunating agent-its effect on rumen fermentation and in sacco degradability of jowar hay in buffaloes. Buffalo J. 2000; 16:99-104.
- Khanpara K, Harisha CR. A detailed investigation on shikakai (*Acacia concinna* Linn.) fruit. J Curr Pharm Res. 2012 Jan;9(1):6-10.
- Makkar HPS, Becker K. Effect of *Quillaja saponaria* in *vitro* rumen fermentation. Adv Exp Med Biol. 1996; 405:387-394.
- Menke KH, Steingass H. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim Res Dev. 1988;28:7-55.
- Patra AK, Kamra DN, Agarwal N. Effect of plant extracts on *in vitro* methanogenesis, enzyme activities, and fermentation of feed in rumen liquor of buffalo. Anim Feed Sci Technol. 2006 Jul;128:276-291.
- Sirohi SK, Pandey N, Goel N, Singh B, Mohini M, Pandey P, *et al.* Microbial activity and ruminal methanogenesis as affected by plant secondary metabolites in different plant extracts. Int J Environ Sci Eng. 2009 Jan;1(1):52-58.
- Sitaula BK, Luo J, Bakkan LR. Rapid analysis of climate gases by wide bore capillary gas chromatography. J Environ Qual. 1992 Aug;21:493-496.
- Snedecor CW, Cochran WG. Statistical methods. 8th ed. Ames, Iowa: Iowa State University Press; 1994.
- Teferedegne B. New perspectives on the use of tropical plants to improve ruminant nutrition. Proc Nutr Soc. 2000 May;59:209-214.
- Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. Int J Pharmaceutica Sci. 2011 Jan;1(1):98-106.
- Todkar SS, Chavan VV, Kulkarni AS. Screening of secondary metabolites and antibacterial activity of *Acacia concinna*. Res J Microbiol. 2010 Oct;5(10):974-979.
- Van Soest PJ, Robertson JD. A laboratory manual for animal science. Ithaca, New York: Cornell University; 1988.
- Woodward SL, Waghorn GC, Ulyatt MJ, Lassey KR. Early indication that feeding lotus will reduce methane emission from ruminants. Proc New Zealand Soc Anim Prod. 2001;61:23-26.
- Vargeese Xavier R, Kavitha S. Phytochemical and biochemical analysis of the plant extract of *Acacia concinna* (wild). Int J Pharmaceut Res Dev. 2012 Dec;3(12):136-139.