# Anthelmintic Efficacy of Mango Seed Extract on Porcine Gastrointestinal Nematodes

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**Research Article**

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# Abstract

**Objective:** The usual control for gastrointestinal parasites is the use of commercial anthelmintics. However, parasites are becoming more resistant due to the frequent and inappropriate use of these anthelmintics. As a result, alternatives for these anthelmintics are becoming increasingly widespread for deworming livestock, particularly those that come from natural sources. Thus, this study aimed to evaluate the efficacy of mango seed extract in reducing the egg per gram counts of common gastrointestinal nematodes in pigs and compare the efficacy with that of levamisole.

**Results:** Naturally-infected pigs served as experimental animals and were given a single dose of the mango seed extract at concentrations of 600, 700, and 800 mg per kg bodyweight. The extracts, regardless of concentration, was able to decrease the epg counts. Within 14 days post-treatment, there was no significant difference in the efficacies in the administration of 800 mg extract per kg bodyweight and levamisole. This comparable efficacies was sustained until 28 days post-treatment. This significant in vivo anthelmintic activity may be attributed to the tannins and flavonoids present in the extract. These results indicate that the mango seed extract is effective in controlling and reducing the gastrointestinal nematodes in pigs and may have the potential to be further developed as an anthelmintic.


# Introduction

The swine industry remains a major industry in livestock production in the Philippines. There is a substantial number of smallholders or backyard pig raisers where it is used for their own consumption or is an important source of household income as a sideline or secondary source of income [1]. Backyard farms are prone to disease outbreaks mainly due to inadequate facilities or poor disease monitoring. Disease prevention and control remains an ongoing problem due to the costs of medicine [2]. This results to parasitism being a common problem in backyard farms.

In particular, parasitic infection due to gastrointestinal nematodes is of economic importance because it affects the appetite, reduces weight gain due to poor feed utilization. If not controlled, heavy infection will result in high mortality [3]. In the Philippines, ascarids, strongyles, trichurids, and hookworms are commonly reported [4, 5]. The main mode of control or treatment remains to be the routine use of different anthelmintic products. However, the repeated and improper use of these anthelmintics particularly in smallholders often result on some degree of anthelmintic resistance [6]. An alternative to these commercial anthelmintics is to explore plants or plant extracts that have the potential to be natural anthelmintics.

Mango (*Mangifera indica*) seed or generally known as stone, is thrown away as a waste product after consuming the fruit. Proximate analysis revealed that it contained polyphenolic compounds, among which tannins and vanillin were in highest amounts. Other compounds in the seed kernel include flavonoids, gallic acid, coumarin, caffeic acid, mangiferin, ferulic acid, and cinnamic acid [7]. These compounds are reported to exert anthelmintic activities against a broad range of nematodes [8]. These

indicate that the mango seed extract could possibly be used as an anthelmintic against common gastrointestinal nematodes in pigs.

Thus, this study aimed to assess the efficacy of mango seed extract against common gastrointestinal nematodes in pigs. Specifically, it aimed to evaluate the efficacy of the mango seed extract in reducing the eggs per gram counts of common gastrointestinal nematodes in pigs in comparison to levamisole, a commercial anthelmintic.

## Methods

# Animals

A preliminary quantitative examination of fecal samples was performed using the modified McMaster counting technique [9] in order to determine the degree of parasitism. Pigs with more than 500 eggs per gram (epg) counts were used as experimental animals identified by tag markings. Fifteen pigs naturally-infected with gastrointestinal nematodes served as experimental animals. The pigs, between ages three to four months regardless of sex and breed, were used to assess the efficacy of different doses of mango seed extract in reducing the epg counts of common gastrointestinal nematodes in pigs. The pigs were from the same backyard farm and the farm's management systems and welfare measures were adapted. All diets were free of anthelmintics and were prepared to meet the nutritional requirements of the pigs. After the study, the experimental animals were released and the backyard farmer was informed of the results for possible interventions.

# Collection and Preparation of Extract

Mango fruits were purchased from the market of Kabacan, Cotabato, Philippines. The seeds were washed and the kernels were removed manually. The kernels were cut into smaller pieces and sundried. A mechanical grinder was used to produce a powder from the dried kernels. For extraction, the ground mango kernel (400 g) was soaked in 95% ethanol (2.40 L) for 48 hours and filtered using an ashless filter paper. The solvent was evaporated in vacuo at 40°C and 200 rpm. The collected extracts were stored in a sterile container.

# Administration of Extract

The fifteen naturally-infected pigs were divided into five homogeneous groups of three pigs each using randomized complete block design. This small sample size was because the extract was evaluated in vivo for the first time in this study. The identified experimental pigs were marked according to their group and were weighed using a calibrated weighing scale to determine the live weight. The treatments, extracts and controls, were administered orally early in the morning. Pigs in Groups A-C were treated with a single dose of 600, 700, and 800 mg extract per kg bodyweight. Group D was given a single dose of levamisole, 8.5 mg per kg bodyweight, as the positive control while Group E was not subjected to any treatment as the negative control. There were no adverse effects observed after the administration of the treatments.

# Parasitological Examination

Fecal samples (3–5 g) were collected pre-treatment (day 0) and 7, 14, 21, and 28 days post-treatment by restraining the pig using a snare loop and inserting the fingers with gloves directly into the rectum. Fecal samples were placed in labeled empty plastic cups with cover prior to analysis. The animal care staff was unaware of the treatments administered to the pigs during fecal collection. Fecal examination was done using the modified McMaster technique [9] to determine the fecal egg per gram counts. The efficacy was calculated as the difference between the post-treatment epg and pre-treatment epg, divided by the pre-treatment epg multiplied by 100.

# Statistical Analysis

Results are expressed as means ± standard errors of the means. One-way Analysis of Variance (ANOVA) was used to assess the significant differences ($p < 0.05$) in mean efficacy among the groups. In addition, post-hoc multiple mean concentration using the Tukey honestly significant difference (HSD) test was used when a significant difference was present and to test the consistency of the effects for treatments and control.

## Results

The epg reduction regardless of the dosage of mango seed extract is evident from the data in Table 1. Among the extract concentrations, the highest reduction was seen using 800 mg of mango seed extract per kg bodyweight of pigs. Statistical analysis revealed that there was a significant difference among the epg counts 7, 14, 21, and 28 days post-treatment. Within 14 days post-treatment, the epg counts administered with 800 mg mango seed extract per kg bodyweight was comparable to that of the commercial anthelmintic levamisole. It should be noted that the epg counts started to increase for all groups within 21 days post-treatment, which continued until day 28.

Table 1
The mean fecal egg per gram of naturally-infected pigs treated with mango seed extract.

| Group | Days post-treatment | | | | |
|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 |
| A (600 mg/kg) | $642 \pm 33^{a}$ | $158 \pm 8^{a}$ | $175 \pm 14_{a}$ | $225 \pm 14^{a}$ | $267 \pm 22^{a}$ |
| B (700 mg/kg) | $550 \pm 14^{a}$ | $108 \pm 8^{ab}$ | $83 \pm 8^{b}$ | $125 \pm 14^{ab}$ | $167 \pm 8^{b}$ |
| C (800 mg/kg) | $583 \pm 44^{a}$ | $75 \pm 0^{b}$ | $67 \pm 8^{bc}$ | $100 \pm 14^{ab}$ | $133 \pm 30^{b}$ |
| D (levamisole) | $633 \pm 36^{a}$ | $8 \pm 8^{c}$ | $8 \pm 8^{c}$ | $58 \pm 8^{b}$ | $108 \pm 8^{b}$ |
| E (untreated) | $550 \pm 14^{a}$ | $583 \pm 22^{d}$ | $600 \pm 29^{d}$ | $683 \pm 46^{c}$ | $767 \pm 22^{c}$ |

*Mean values in the same column followed by the same superscript are not significantly different ($p < 0.05$).

The efficacies of the groups are shown in Table 2. The maximum efficacy for Group A was at 75.35% by day 7, which then continually decreased until day 28. For Group B, the maximum efficacy as observed 14 days post-treatment at 84.90%. Group C consistently elicited the highest mean efficacy among the mango seed extract concentrations for the entire duration of the study. Administering 800 mg extract per kg bodyweight resulted to the highest mean efficacy among extract concentrations at 88.57% within 14 days post-treatment. Statistical analysis revealed that this efficacy is comparable to that of levamisole 98.81%. There was no significant difference between the efficacies Groups C and D, highlighting the potential of the extract to elicit similar anthelmintic activity with that of the commercial anthelmintic. It was further observed that the efficacy increased with increased concentration of the mango seed extract, implying a dose-dependent response.

Table 2
The mean efficacy of mango seed extract in different groups.

| Group | Days post-treatment | | | |
|---|---|---|---|---|
| | 7 | 14 | 21 | 28 |
| A (600 mg/kg) | $75.35 \pm 1.26^{a}$ | $72.79 \pm 1.21^{a}$ | $64.95 \pm 1.08^{a}$ | $58.56 \pm 1.57^{a}$ |
| B (700 mg/kg) | $80.34 \pm 1.07^{a}$ | $84.90 \pm 1.16^{b}$ | $77.31 \pm 2.35^{ab}$ | $69.73 \pm 0.94^{ab}$ |
| C (800 mg/kg) | $86.99 \pm 1.03^{b}$ | $88.65 \pm 0.73^{bc}$ | $83.03 \pm 1.23^{b}$ | $77.59 \pm 4.10^{b}$ |
| D (levamisole) | $98.81 \pm 1.19^{c}$ | $98.81 \pm 1.19^{c}$ | $90.86 \pm 0.81^{b}$ | $82.77 \pm 1.65^{b}$ |
| E (untreated) | $-6.00 \pm 1.35^{d}$ | $-9.11 \pm 4.57^{d}$ | $-24.08 \pm 6.11^{c}$ | $-39.52 \pm 4.57^{c}$ |

*Mean values in the same column followed by the same superscript are not significantly different ($p < 0.05$).

# Discussion

This efficacy of administering increasing dosages of mango seed extract resulting on the reduction of epg counts may be attributed to the anthelmintic components present in the mango seeds. Mango seed kernel extracts have been shown to contain tannins, flavonoids, alkaloids, saponins, and glycosides [10]. Extracts have been reported to exhibit a wide range of activities such as antioxidant, antimicrobial [11], antifungal [12], antidiarrheal [13], antiproliferative against colon cancer cells [14], and tyrosinase inhibition [15].

The mango seed has been considered a good source of polyphenols. For Egyptian mango Hindi cultivar, a significant amount of phenolics and flavonoids are present in mango seed kernel extracts at 17,400 and 3,325 mg per 100 g seed [16]. Similarly, among three Chinese mango cultivars, the kernels contained 15.5 mg of gallotannins per g dry matter [17]. Kernels of the Ubá variety from Brazil contained 82,540 mg per kg dry matter [18]. Across these studies, it was observed that the mango seed kernel contained the highest amounts of phenolics compared to the pulp and peel, making it a promising source of polyphenolics.

Gallates and gallotannins are the major family of compounds present in the mango seed kernel extract. Gallotannins are hydrolyzable tannins that contain gallic acid substituents [19]. When hydrolyzed by acids and under physiological conditions in the gastrointestinal tract, these gallotannins may release gallic acid [20]. Most studies report the role of condensed tannins related to anthelmintic activity. However, hydrolyzable tannins are reported to exhibit similar anthelmintic activity with that of condensed tannins. Extracts containing only hydrolyzable tannins have similar activity with extracts which have both condensed and hydrolyzable tannins and can even have higher anthelmintic activity than extracts containing only condensed tannins [21]. Gallotannins isolated from *Camelia sinensis* have shown strong

anthelmintic activity against *Caenorhabditis elegans* [22]. Moreover, condensed tannins with gallic acid units had higher anthelmintic activity compared to that with no gallic acid units [23].

The anthelmintic activity of hydrolyzable tannins is mainly egg hatching and larval migration inhibition while condensed tannins only exert minor effects on egg hatching. This implies that hydrolyzable tannins have a different mode of action for its anthelmintic activity [24]. It was shown that the size of the hydrolyzable tannins, overall flexibility, type and number of functional groups, and the linkages between the monomeric hydrolyzable tannins had different effects on the activity [25, 26]. Particularly, the molecular size and the number of free hydroxyl groups seem to be crucial elements for anthelmintic activity [8]. This may be due to the difficulty of larger molecules to permeate into target cells or within the parasite. In contrast, smaller molecules will be able to move easily and bind with target molecules.

In addition to hydrolyzable tannins, mango seed kernel also contain a substantial amount of flavonoids, which may have also contributed towards the reduction of epg counts. It is hypothesized that flavonoids have a mode of action similar to that of tannins. This means that flavonoids may interfere with the biology of the nematodes through egg hatching or larval motility inhibition such as the activity shown by sainfoin against *Haemonchus contortus* [27]. Though in smaller amounts, the alkaloids present may also have anthelmintic effects. Alkaloids have been reported to inhibit egg hatching [28] and may have the ability to intercalate with DNA synthesis of parasites [29].

Aside from the mango seed kernel, studies have shown that other parts of the mango have anthelmintic activity. Immature mango fruits have shown high in vivo efficacy yet moderate anthelmintic action against nematodes, particularly *H. contortus*, in sheep [30]. However, it was not reported as to what may have caused the activity nor the decrease in activity when it was tested in vivo. In contrast, this study was able to exhibit a strong anthelmintic action in vivo. This is largely due to the difference of the metabolism between sheep and pig but may also be due to the amount of the active substance in different parts of the mango. This study shows that the mango seed contains significant amounts of bioactive compounds enough to have an efficacy similar to a commercial anthelmintic.

# Conclusion

The results of the study show that the mango seed extract has potential in vivo anthelmintic activity against gastrointestinal nematodes in pigs. Administering 800 mg of the extract per kg bodyweight is able to elicit a similar efficacy with that of the commercial anthelmintic levamisole within 14 days post-treatment. This suggests that the anthelmintic activity of the mango seed extract is enough to warrant the potential to be developed as a naturally-sourced anthelmintic.

# Limitations

The animals used in the study were pigs that are naturally infected with gastrointestinal nematodes and had at least 500 egg per gram counts, limiting the experimental animals. Increasing the sample size of

the experimental animals may give a clearer response to the administration of the extract. Moreover, as previous studies on mango seed extract reported strong *in vitro* yet moderate *in vivo* anthelmintic activity in sheep, this study focused on the *in vivo* anthelmintic activity in pigs. Future studies may be done to target specific gastrointestinal nematodes *in vitro* as this study already supports that there is strong activity *in vivo*.

# Abbreviations

epg: egg per gram

# Declarations


### Acknowledgements

Not applicable.


### Authors' contributions

SRTR, LJAG, and EAG developed the research concepts and design. SRTR conducted the experiments under the supervision and guidance of EAG. LJAG wrote the manuscript draft. All authors read and approved the final manuscript.


### Funding

Not applicable.


### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

### Ethics approval and consent to participate

For the conduct of the study, a verbal consent was obtained from the backyard farmer. The procedures used in the study were approved by the University of Southern Mindanao Institutional Animal Care and Use Committee MC-CVM-001687. The methods for research and animal handling are all in compliance with Republic Act 8485, The Animal Welfare Act.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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