

Comparative Evaluation of Anthelmintic and Antibacterial Activities in Leaves and Fruits of *Garcinia cambogia* (Gaertn.) Desr. and *Garcinia indica* (Dupetit-Thouars) Choisy

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ABSTRACT

This study aimed to evaluate the in vitro anthelmintic activity and antibacterial activity of the extracts from the leaves and fruits of *Garcinia indica* (Dupetit-Thouars) Choisy and *Garcinia cambogia* (Gaertn.) Desr. using the Indian earthworm *Pheretima posthuma*. Two concentrations (25 and 50 mg/mL) of various extracts such as petroleum ether, chloroform, ethyl acetate, methanol and water were tested. Albendazole at the concentrations of 25 and 50 mg/mL was used as the standard reference. Significant anthelmintic effects of the fruits and leaves of *G. cambogia* and *G. indica* ($P < 0.05$) were observed and the results were expressed in terms of paralysis and death time. All the extracts showed the dose dependent paralysis and death of earthworms. Among all the extracts used, methanol extract exhibited the highest activity. *G. cambogia* leaf extract (50 mg/mL) had 30% faster paralysis effect on earthworms than the standard reference. Furthermore, the antimicrobial activity of the methanol extracts of the fruits and leaves showed significant ($P < 0.05$) activity against Gram-positive and Gram-negative bacteria. At a concentration of 500 µg/mL, *G. indica* fruit extract presented higher zones of inhibition against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Hence, it could be concluded that the leaves and fruits of *G. indica* and *G. cambogia* contained active anthelmintic and antibacterial phytochemicals, which could find their applications in pharmaceuticals.

Key words: anthelmintic, antibacterial, *Garcinia cambogia*, *Garcinia indica*, Minimal Inhibitory Concentration

INTRODUCTION

Nematode infections cause severe health problems worldwide in humans and animals (Tariq et al. 2009; Das et al. 2011). In animals, these parasites cause frequent economic losses due to mortality (Agrawal and Banerjee 2007). In human beings, they cause mal-absorption, diarrhea, anaemia and other intestinal problems (Kumar et al. 2012). More than two billion people are infected with different intestinal worms worldwide (Somvanshi

et al. 2014). The nematode infections are more prevalent in developing countries due to poor sanitation and hygiene (Dhar et al. 1982). Nematode species such as *Haemonchus contortus* and *Bunostomum trigonocephalum* that feed on the blood were recognized for clinical and sub-clinical indications causing excessive commercial loss in ruminants (Agrawal and Banerjee 2007). The gastrointestinal nematodes are normally treated with chemically synthesized drugs. Control of nematodes with synthetic anthelmintics for

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longer periods has made them drug resistant (Lakshmanan et al. 2011). The other drawbacks of the existing anthelmintic drugs are side effects such as headache, loss of appetite, diarrhoea and vomiting (Goodman and Gilman 2001). In order to overcome these drawbacks, phyto-medicines, which offer greater advantages over synthetic drugs have been developed (Hammond et al. 1997). Since time immemorial, humans have depended on traditional medicines derived from the plants for curing their ailments (Silva et al. 2008). Reports from several parts of the world have shown that plant species could efficiently decrease parasite infections and could be promising alternatives to the conventional anthelmintics (Tariq and Tantry 2012).

The genus *Garcinia* (Clusiaceae family) includes about 200 species throughout the world. Of these, 36 species have been reported from India. *G. indica* and *G. cambogia* species endemic to Western Ghats have been reported to possess anthelmintic activity (Abraham et al. 2006). The compounds derived from the parts of *G. indica* (kokum) have been studied for anti-obesity, anticancer, anti-diabetic, antioxidant and antimicrobial properties (Hemshekhkar et al. 2011). Kokum fruits have been used in the preparation of 'amrutkokum', a drink commonly used to relieve the sunstroke. Kokum has been used for its therapeutic properties as a good appetizer, remedy for flatulence (Dushyantha et al. 2010). Anthelmintic activity of the fruit rind of Kokum was reported by Swapna et al. (2012). There were no reports of the anthelmintic activity of the extracts from the leaves of Kokum tree.

Garcinia cambogia, also called as Malabar tamarind or Kodampuli is famous for its anti-obesity property of the fruit rind (Jena et al. 2002). Malabar tamarind has been known to have different secondary metabolites such as benzophenones, flavonoids and xanthenes (Iinuma et al. 1998; Koshy et al. 2001; Masullo et al. 2008). The biological activities of these secondary metabolites include antimalarial, antiviral, cytotoxic, antioxidant and anticancer properties (Ito et al. 1998; Hay et al. 2004; Matsumoto et al. 2005; Wang et al. 2011). The fruits, leaves and roots of this plant have been explored (Tharachand et al. 2013). The preliminary *in vitro* anthelmintic activity of *G. cambogia* leaves and fruits have been reported by Mathew et al. (2011) and Rajendran et al. (2011). Rajendran et al. (2011) reported the anthelmintic activity of the

fresh juice and the ethanol extract of the fruit of *G. cambogia*. Mathew et al. (2011) reported the anthelmintic activity exhibited by three different extracts, namely petroleum ether, chloroform and alcohol from the leaves of *G. cambogia*.

Antimicrobial activity of the preserved (salted and sun dried) fruit rind of Kokum against the bacterial strains has been reported. It has reported that the presence of furfural in kokum extract is responsible for its antimicrobial activity (Sutar et al. 2012). Shivakumar et al. (2011) reported the *in vitro* antibacterial activity of the fruit rind extracts of *G. cambogia* using hexane, ethyl acetate and ethanol as the solvents for extraction. Evidently, To the best of knowledge, there have been only a few studies reports on the anthelmintic and antimicrobial activity of the fruit and leaf extracts of *G. cambogia* and *G. indica*. Therefore, the aim of this study was to evaluate the anthelmintic activity of various extracts from the fruits and leaves of *G. cambogia* and *G. indica*. The antibacterial activity of the methanol extract of the leaves and fruits of these plants was also evaluated.

MATERIAL AND METHODS

Plant material

The authentic samples of the fruits and leaves of *G. cambogia* and *G. indica* were collected from the National Bureau of Plant Genetic Resources (NBPGR) regional station, Thrissur, India. The collected leaves and fruits were shade dried at room temperature, homogenized to powder and stored in air-tight containers for further extraction.

Chemicals and reagents

All the chemicals such as solvents used in this study were of analytical grade purchased from Merck India, Mumbai, India. The media for antibacterial activity was purchased from Hi-media Laboratories Ltd, Mumbai, India.

Preparation of the extract

Different extracts of the leaves and fruits of *G. cambogia* and *G. indica* were prepared using Soxhlet apparatus (Borosil Glass works Ltd.), where petroleum ether, chloroform, ethyl acetate, methanol and water (successive extraction) were used as solvents. The extraction time was 6 h with the solvent maintained to continuously reflux over the sample. All the extracts were cooled to the room temperature and filtered. The solvents from

the filtrates (except aqueous extract) were evaporated using a rotary evaporator (Rotavapor R210, Buchi, Switzerland). The dried extracts were stored for further studies (Raaman 2006).

Organisms for the study

Anthelmintic activity was carried out on the Indian adult earthworm (*Pheretima posthuma*), collected from the moist soil of the garden fields at VIT University. Adult earthworms (4 – 8 cm in length) were selected for the experiment. Antibacterial activity of the crude extracts of leaves and fruits of *G.cambogia* and *G.indica* were tested against human pathogenic bacterial strains, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. Pure bacterial strains used in this study were obtained from the Microbiology Laboratory, VIT University, Vellore.

Evaluation of anthelmintic activity

In vitro anthelmintic activity of all the extracts was evaluated as per Patil et al. (2015). The earthworms were divided into individual groups (each group containing five organisms) for each treatment at two different concentrations. The standard drug albendazole at two different concentrations of 25 and 50 mg/mL was used for comparing the activity of the extracts with saline (0.89% NaCl w/v) as a control. Similarly, all the extracts were prepared at concentrations of 25 and 50 mg/mL as test. A volume of 10mL of each extract of both the concentrations were taken in Petri dishes. Five earthworms were added to each Petri dish. Earthworms were observed for their movements. The time taken for the worm to lose its movement (except when external stimulus was given) was considered for paralysis time and the time taken to lose its motility even in the presence of external stimulus (when dipped in warm water at 55°C) and faded body color was considered for death time. Paralysis time and death time of each earthworm in the group was recorded.

Evaluation of antibacterial activity

The methanol extracts of the fruits and leaves of both the plants showed good anthelmintic activity compared to the standard drug albendazole. In order to study the antibacterial activity of these extracts, four food borne pathogenic bacteria (two Gram-positive and two Gram-negative) were selected. The antibacterial activity was carried out by agar-well diffusion method (Das et al. 2014;

Naine et al. 2015). Gram-positive bacteria used in the study were *Staphylococcus aureus* and *Bacillus cereus* and Gram-negative bacteria were *Pseudomonas aeruginosa* and *Escherichia coli* (food borne pathogens). Standard antibiotic used was ampicillin at a concentration of 100 µg/mL. All the four methanol extracts were prepared at two different concentrations of 500 and 1000 µg/mL. The zones of growth inhibition were measured (in mm) after 24 h of incubation at 37°C. The test was assayed in triplicates. Further, the Minimal Inhibitory Concentration (MIC) values of the methanol extracts were determined by micro dilution technique in Luria–Bertani (LB) broth (Hughes et al. 2013). The stock solutions of the extracts were prepared in their respective solvents. Serial dilutions of the methanol extracts were prepared in LB medium ranging from 10 to 500 µg/mL to the final volumes of 100 µL in 96-well microtiter plate. To the wells of the microtiterplate containing diluted methanol extracts, 100 µL of the bacterial suspension was added and incubated overnight at 37°C. Bacteria cultured in LB broth without the extracts were used as control.

Statistical analysis

The data for anthelmintic activity was represented as mean ± SD of five earthworms in each group (n=5). The data for antibacterial activity was represented as mean ± SD of three replicates (n=3). Statistical analysis was performed using Statistical Package for Social Sciences version 19.0 (SPSS Inc., Chicago, IL, USA). All the data was submitted to the Analysis of Variance (ANOVA), followed by the post-hoc analysis of Duncan's Multiple Range Test (DMRT) in order to find the significant differences between the means of the groups. Values of $P < 0.05$ were considered for significant differences.

RESULTS

Anthelmintic activity

The anthelmintic activity of the extracts was confirmed by observing the paralysis and death of earthworms. Among the fruit extracts of *G.cambogia* (Fig. 1), the methanol extract (50 mg/mL) paralyzed the earthworms earlier (15.6 min) than other extracts and albendazole (18.2 min) at the given concentration. The paralyzed earthworms in the methanol extract of fruit

survived for less time (25.4 min) when compared to albendazole (27.8 min). The duration for the paralysis of the earthworms was longer in case of petroleum ether extract (25mg/mL), whereas the duration for the death of the earthworms was longer in case of ethyl acetate extract (25mg/mL).

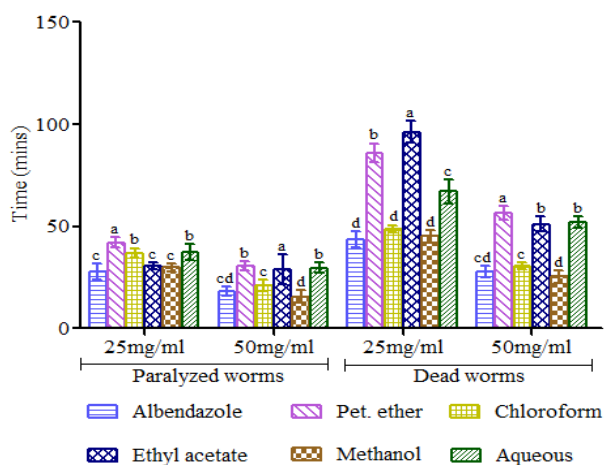


Figure 1 - Anthelmintic effect of *Garcinia cambogia* fruit extracts. Each value represents means \pm SD. Means followed by different superscripts within a group are significantly different at $P < 0.05$ according to Duncan's Multiple Range Test (DMRT).

Among all the extracts of *G. cambogia* leaves (Fig. 2), the methanol extract at 50 mg/mL concentration took the least time (12 min) to paralyze the earthworms and those worms survive up to 16.6 min. The leaf extracts of *G. cambogia* showed better anthelmintic activity when compared to the corresponding extracts of the *G. cambogia* fruit. Furthermore, it was observed that the lower concentration of leaf extract (25 mg/mL) paralyzed the earthworms in virtually the same time taken by the fruit extract at higher concentration (50 mg/mL).

In *G. indica*, the methanol extract of the fruit (Fig. 3) at a concentration of 50 mg/mL paralyzed the earthworms in 15.6 min (10% faster effect than albendazole), whereas albendazole took 17.2 min. Even at the lower concentration of 25 mg/mL, the methanol extract showed 10% faster paralysis effect than albendazole. Similarly, the death time of the methanol extracts at both the concentrations corresponded with the standard reference. No significant difference was observed in paralysis time and death time between the two groups. Aqueous extract showed slower paralysis effect

than other extracts at both the concentrations (25 and 50 mg/mL). At higher concentration (50 mg/mL), the pet ether and ethyl acetate extracts exhibited same effect on the death of earthworms.

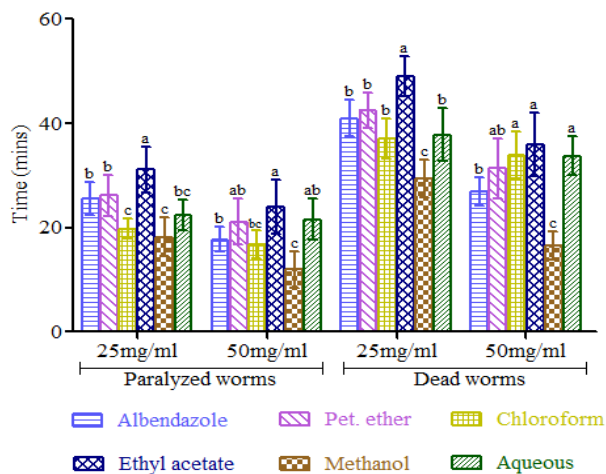


Figure 2 - Anthelmintic effect of *Garcinia cambogia* leaf extracts. Each value represents means \pm SD. Means followed by different superscripts within a group are significantly different at $P < 0.05$ according to Duncan's Multiple Range Test (DMRT).

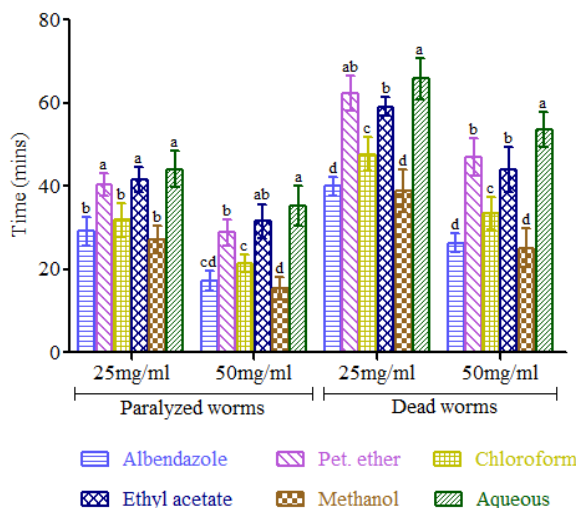


Figure 3 - Anthelmintic effect of *Garcinia indica* fruit extracts. Each value represents means \pm SD. Means followed by different superscripts within a group are significantly different at $P < 0.05$ according to Duncan's Multiple Range Test (DMRT).

Anthelmintic effect of the leaves of *G. indica* with different extracts is shown in Figure 4. The leaf extracts of *G. indica* showed faster paralysis effect

than their corresponding fruit extracts. In this case, except aqueous extract, all other extracts at a concentration of 50 mg/mL showed equivalent paralysis effect. The time taken by the paralyzed earthworms to die was almost the same in case of methanol extract and albendazole at both the concentrations. All the extracts showed dose dependent paralysis and death of the earthworms. It was found that the higher concentration (50 mg/mL) of all the extracts had a faster effect than the lower concentration (25 mg/mL).

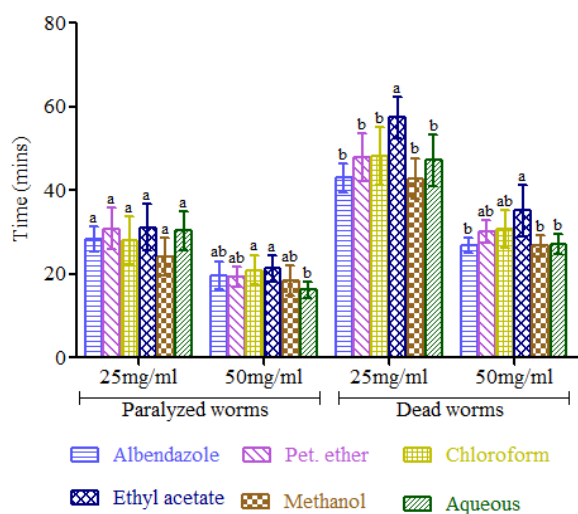


Figure 4 - Anthelmintic effect of *Garcinia indica* leaf extracts. Each value represents means \pm SD. Means followed by different superscripts within a group differ significantly with each other at $P < 0.05$ according to Duncan's Multiple Range Test (DMRT).

Antibacterial activity

Antibacterial activity of methanol extracts of the fruits and leaves of *G. cambogia* and *G. indica* are depicted in Figure 5. The antibacterial activity was carried out through agar-well diffusion method. The zones of inhibition were considered for the effectiveness of the extract against the tested bacterial pathogens. All the extracts showed inhibitory effect against the test organisms. *G. cambogia* fruit extract (500 $\mu\text{g/mL}$) was more effective on Gram-positive bacteria, *S. aureus* (zone of inhibition being 15.6 mm) and *B. cereus* (15.3 mm). *B. cereus* (17 mm) was more susceptible to *G. cambogia* leaf extract than other tested bacterial strains. Fruit extract of *G. indica* showed highest antibacterial activity on both *P. aeruginosa* and *S. aureus* (19 mm). *S. aureus*

(17.3 mm) was also highly susceptible to *G. indica* leaf extract. Among all the methanol extracts of *G. cambogia* and *G. indica* at a concentration of 500 $\mu\text{g/mL}$, *P. aeruginosa* and *S. aureus* were the most susceptible bacterial strains among the tested pathogens. At 1000 $\mu\text{g/mL}$ concentration, all the extracts showed similar activity against all the bacterial strains (nearly equal range of inhibition zones). At this concentration, the effect shown by different extracts towards the tested bacterial strains was very low (ranged between 17 mm and 19 mm).

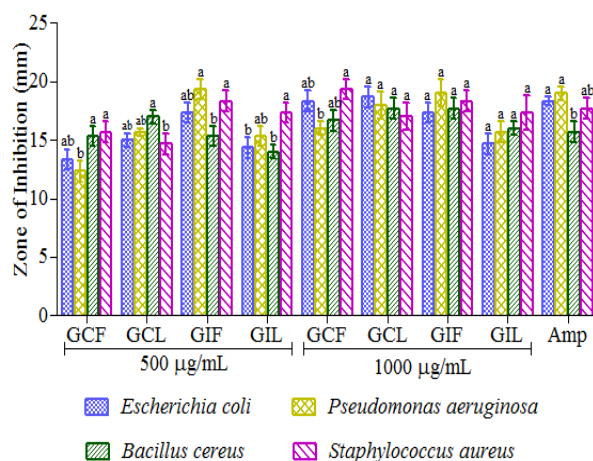


Figure 5 - Antibacterial activity of *Garcinia cambogia* and *G. indica* fruit and leaf extracts. Each value represents means \pm SEM in each group. Means followed by different superscript within a group differ significantly with each other at $P < 0.05$ according to Duncan's Multiple Range Test (DMRT). GCF - *G. cambogia* Fruit extract, GCL - *G. cambogia* Leaf extract, GIF - *G. indica* Fruit extract and GIL - *G. indica* Leaf extract.

Minimal inhibitory concentration (MIC) of the methanol extracts towards Gram-positive and Gram-negative bacteria are represented in Table 1. Among all the methanol extracts used in this study, *G. indica* fruit extract inhibited *P. aeruginosa* at a lower concentration (25 $\mu\text{g/mL}$). *G. indica* fruit extract showed significant difference in the inhibition between the Gram-positive and Gram-negative bacteria. *B. cereus* was the most resistant among all the tested strains (MIC value of 79 ± 7 towards *G. cambogia* fruit extract). The other extracts did not show any considerable differences between the Gram-positive and Gram-negative bacteria. All the

extracts inhibited the tested bacterial strains within a concentration of 80 µg/mL.

Table 1 - Minimal inhibitory concentration (MIC) of methanol extracts of the leaves and fruits of *G.indica* and *G.cambogia* against the test pathogens.

Methanol extract	MIC (µg/mL)			
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>B. cereus</i>	<i>S. aureus</i>
GCF	58 ± 9	62 ± 13	79 ± 7	67 ± 4
GCL	71 ± 16	49 ± 3	55 ± 9	61 ± 12
GIF	30 ± 7	25 ± 6	68 ± 4	73 ± 9
GIL	72 ± 8	38 ± 11	40 ± 15	49 ± 2

Values represented as mean ± standard deviations of triplicate experiments; GCF – *G.cambogia* fruit extract, GCL – *G.cambogia* leaf extract, GIF – *G.indica* fruit extract and GIL – *G.indica* leaf extract.

DISCUSSION

Plants produce many phytochemicals to protect themselves from the microbial infections and other biological toxicities. Hence, plant materials can serve as the good sources of herbal medicines (Kim et al. 2013). The antimicrobial and anthelmintic properties of plants have been explored throughout the world. The inhibitory effect of the plant extracts are attributed to the phytochemicals present in the plant parts.

The objectives of this study were to evaluate the *in vitro* anthelmintic activity of different extracts of *G. cambogia* and *G.indica* leaves and fruits in comparison to the reference drug albendazole. The extracts showing better anthelmintic activity were tested for their antibacterial activity. The *in vitro* anthelmintic effect of different extracts was carried out on Indian adult earthworm (*Pheretima posthuma*) due to its physiological and structural similarity with intestinal parasites such as *Ascaris lumbricoides* (Gogoi et al. 2014). The results of this study revealed the anthelmintic activity of all the extracts of *G.indica* and *G.cambogia* leaves and fruits using solvents such as petroleum ether, chloroform, ethyl acetate, methanol and water. The methanol extract of the leaves of *G.cambogia* took the least time to paralyze the earthworms. The efficiency of the plant material was determined based on the loss of movement of the worm and death of the worm in *in vitro* studies. Of all the extracts used in this study, methanol extract of both the fruits and leaves of *G.cambogia* and *G.indica* exhibited maximum effect. The anthelmintic effect (paralysis and death of earthworms) shown by the methanol extract was almost equal to the effect shown by the reference drug, albendazole in all the studied cases, with a maximum effect compared to other extracts, which could be ascribed to its polarity. Methanol being a mid-polar solvent was capable of extracting both

polar as well as non-polar components into it due to which broad range of phyto-constituents were available for the activity (Bae et al. 2012).

The *in vitro* anthelmintic activity of the methanol extracts could be either due to the damage of cellular integrity or neuromuscular coordination. The damage of cellular integrity could be achieved by the inhibition of tubulin polymerization and inhibition of enzymes in the glycolytic pathway. The damage of neuromuscular coordination could be caused in the parasite by hyperpolarizing the nerve membrane and inhibiting the enzyme acetylcholinesterase (Martin, 1997; Guest 2008).

The preliminary antibacterial activity of the crude methanol extract was carried out by agar-well diffusion method at two different concentrations (500 and 1000 µg/mL). At 500 µg/mL concentration, *G.indica* fruit extract showed highest zone of inhibition against *P. aeruginosa* and *S. aureus*. At a concentration of 1000 µg/mL, the fruit extracts of both *G.cambogia* and *G.indica* showed maximum inhibitory effect against *S. aureus*. In addition, *G.indica* fruit extract showed maximum activity against *P. aeruginosa*. The inhibitory effect of *G.indica* fruit methanol extract towards *P. aeruginosa* was supported by previous reports. Sutar et al. (2012) reported that *E.coli* did not show any zone of inhibition when tested with methanol extract of *G.indica* fruit. Contrary to this, in the present study *E. coli* was susceptible to the methanol extract of *G.indica* fruit. In another study, the antibacterial activity of ethanol extract of *G.cambogia* fruit was studied. It is reported that *E. coli*, *P. aeruginosa* and *S. aureus* showed zones of inhibition with ethanol extract (Shivakumar et al. 2013).

In this study, methanol extract showed similar zones of inhibition with the mentioned strains of bacteria. The methanol extracts of *G.cambogia* and *G.indica* fruits and leaves showed significant bactericidal activity against the tested Gram-

positive and Gram-negative bacteria. The antibacterial effect shown by all the extracts was similar on both Gram-negative and Gram-positive bacteria. This similarity could be related to the common solvent used for the extraction of compounds from the plant materials. The inhibitory effect shown by the extracts was due to the phyto-constituents present in the leaves and fruits of *G.cambogia* and *G.indica*. One of the reasons for the bactericidal activity of these phytochemicals could be due to membrane permeability resulting in the leakage of intracellular materials causing cell death (Karsha and Lakshmi 2010).

CONCLUSION

Based on the results of this study, it could be concluded that the fruits and leaves of *G.cambogia* and *G.indica* tested in the form of crude extracts showed significant ($P < 0.05$) *in vitro* anthelmintic activity at two different concentrations tested against Indian earthworm as determined by worm motility inhibition. The findings of this study suggested that *G.cambogia* leaves could become a source of anthelmintics instead of chemically synthesized drugs. However, further *in vivo* studies against different parasites of human and other animals at different doses are needed to determine the potential of *G.cambogia* as an anthelmintic against gastrointestinal worms. The methanol extract of *G.indica* fruit showed significant ($P < 0.05$) MIC value at very low concentration. The results showed that the selected methanol extracts possessed active components capable of inhibiting the bacterial growth. These results presented the basis for selecting the plant species for further exploration of biologically important compounds. Future studies aiming the isolation and structure interpretation of the biologically active compounds present in the methanol extract of the leaves and fruits from *G.cambogia* and *G.indica* should be done.

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