

## *In vitro* comparative study of *Bilimbi* and *Star fruit* extracts for Anthelmintic activity

Jyoti Bala Chauhan<sup>1\*</sup>, Wethroe Kapfo<sup>2</sup>, Gulnaz AR<sup>3</sup>

1. Professor and Head, D.O.S Biotechnology, Microbiology and Biochemistry, Pooja Bhagavat Memorial Mahajana Post Graduate Centre, Metagalli, Mysore- 570016, Karnataka, India, Email: drjyotibiotech@gmail.com; Telephone Number:+91 821 2515097/ 2581226/ 4009636; Fax Number:+91 821 2511921
2. Senior Research Fellow, D.O.S. Biotechnology, Microbiology and Biochemistry, Pooja Bhagavat Memorial Mahajana Post Graduate Centre, Metagalli, Mysore- 570016, Karnataka, India; Email: typeakhro@gmail.com
3. Assistant Professor, Dept. of Biochemistry, Farooqia Dental College & Hospital Mysore, Mysore- 570021, Karnataka, India; Email: gulnazar75@gmail.com

\*Corresponding author: D.O.S Biotechnology, Microbiology and Biochemistry, Pooja Bhagavat Memorial Mahajana Post Graduate Centre, Metagalli, Mysore- 570016, Karnataka, India, Email: drjyotibiotech@gmail.com; Telephone Number:+91 821 2515097/ 2581226/ 4009636; Fax Number:+91 821 2511921

### Publication History

Received: 28 October 2014

Accepted: 7 December 2014

Published: 1 January 2015

### Citation

Jyoti Bala Chauhan, Wethroe Kapfo, Gulnaz AR. *In vitro* comparative study of *Bilimbi* and *Star fruit* extracts for Anthelmintic activity. *Discovery Biotechnology*, 2015, 6(15), 5-8

### ABSTRACT

In the present study, aqueous methanolic extract of Bilimbi and aqueous acetone extract of Star fruit were assessed for its anthelmintic activity against adult earthworms (*Pheretima posthuma*). Various concentrations of both extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. Albendazole was used as a reference standard. The result revealed that the bilimbi fruit extract was more effective compared to the standard and star fruit extract with 30.50 and 47 minutes for paralysis and death respectively at the concentration of 100mg/ml, that of standard and star fruit was 34.32 and 48.00 and 40.60 and 57.30 minutes for paralysis and death respectively. The comparative study showed that out of the two, bilimbi fruit extract proves to be a better anthelmintic remedy.

**Keywords:** Bilimbi, Star fruit, Albendazole, Anthelmintic activity.

### 1. INTRODUCTION

Helminthiasis is a macroparasitic disease wherein a part of the body in humans and animals is infested with parasitic worms like roundworms (nematodes), tapeworms (cestodes) or flukes (trematodes) (Rafi et al., 2011). About two billion people throughout the world are affected by parasitic worm infection according to World Health Organization reports. The reason associated being poor management practices and

inadequate control measure (Gaikwad et al., 2011). Indiscriminate use of anthelmintic synthetic drugs has led to the development of resistance as well as chemical residue and toxicity problems (Arshad et al., 2012; Iqbal et al., 2001). Due to these reasons, phytochemical screening for bioactive compounds from various medicinal plants has become a matter of great scientific interest. In the traditional system of medicine and folklore the whole medicinal plant or a part of it is used for the treatment of all types of disease including anthelmintic activity as they are easily available and effective source of medicines to people with a broad spectrum of action like high percentage of cure with single therapeutic dose, cost effective and free from toxicity (Rastogi et al., 2009; Yadav and Singh, 2011).

*Averrhoa bilimbi* L. (Oxalidaceae), a native of Malaysia and Indonesia, is a widely cultivated tree in southern India, particularly in Mangalore and Udupi. Commonly known as bilimbi, the oblong very sour fruits are eaten fresh and also used in production of vinegar, wine, pickles, jams and jellies. Bilimbi has been widely used as traditional medicine to treat cough, cold, itches, boils, rheumatism, syphilis, diabetes, whooping cough. Experimental pharmacological studies have shown that the fruit alleviates hypertension (Goh et al., 1995). Aqueous extract of fresh bilimbi have shown to exhibit low antioxidant activities and low nitric oxide inhibition activity (Abas et al., 2004). Fresh bilimbi fruit extracts expressed significant antioxidant and metal chelating activity (Chauhan and Kapfo, 2013). Star fruit (*Averrhoa carambola* L.), a tree fruit with a distinctive star shape in cross section, also known as carambola, is another such edible fruit of the Oxalidaceae family available locally in various parts of India. In local regions where the tree grows, the five ridged tropical fruit is predominantly eaten fresh and is also processed to products like pickles, jam or jelly. The unripe fruit is also eaten as a vegetable. The powdered seed concoction of fruit is also traditionally used for its medicinal properties to treat hemorrhoids, fever, eczema, diarrhoea and asthma. Physical and chemical studies of fruits during maturation and storage have been reported for fruits grown in United States, Brazil and Western Part of India (Weller et al., 1997; Narain et al., 2001; Patil et al., 2010). Star fruit available in Singapore market have been reported to be good source of strong free radical scavengers (Leong and Shui, 2002). Similarly, the carotenoid content of star fruits available in Indonesia have been reported (Setiawan et al., 2002). Though available locally in the southern regions of India, this variety of star fruit has not been studied for its antioxidant and antimicrobial properties. Thus in the knowledge of the wide application of Bilimbi and Star fruit it was observed that no concrete scientific report on the anthelmintic activity of bilimbi and star fruit has been made. For this reason the anthelmintic activity of aqueous methanolic extract of Bilimbi and aqueous acetone extract of Star fruit have been investigated and reported for the first time.

## 2. MATERIALS AND METHODS

### 2.1. Plant material collection

Fresh fruits of Bilimbi were obtained from Mangalore and Udupi, India, during the fruiting season (July- December). Care was taken that the fruits, which were whitish- green in colour and approximately 5 - 7.0 cm in size, were not overripe, spoilt or damaged. Fresh Star fruit samples were collected from the local fruit markets in Mysore, India during the months between March and September. The ripe fruits which were yellowish in colour were chosen. Care was taken that the fruits were not overripe, spoilt or damaged. The fruits of approximately 10.0 cm size were chosen for study.

### 2.2. Extraction

Bilimbi fruit extracts were prepared according to the conditions described by Chauhan and Kapfo (2013). 500g of finely blended edible fresh fruit were subject to continuous agitation in 5000 ml of 60% aqueous methanol respectively in a shaking incubator for 3 hours at 40°C. The filtrates were collected using a muslin cloth and concentrated making them solvent free in a rotary vacuum evaporator (Buchi, Germany). The aqueous portion was dried in hot air oven at 40°C and used to analyse the total antioxidant activity. The extracts were prepared in triplicates. The edible portion of fresh Star fruits were chopped into small pieces and used for extraction. The extraction procedure was followed according to the conditions described by Yap et al. (2009) with slight modification of the temperature. 2000g of the fresh fruit were soaked in 5000ml of 60% aqueous acetone for 3 hours under continuous agitation at room temperature (approximately 30°C). The filtrates were collected using a muslin cloth and subjected to concentration and made solvent free in a rotary vacuum evaporator (Buchi, Germany). The aqueous portion was dried in a hot air- oven at 50°C. The dried residue of both the fruit extracts was collected and used for antioxidant assays.

### 2.3. Evaluation of Anthelmintic Activity

#### 2.3.1. Selection of worms

Indian adult earthworms (*Pheretima posthuma*) were used to carry out the anthelmintic evaluation due to their ready availability and anatomical and physiological resemblance of *Pheretima posthuma* (Qureshi et al., 2010). The earthworms were collected from the moist soil of fields near Mysore; Worms were washed with saline water to remove the fecal matter and were authenticated from the zoologist. Worms of about 8-9 cm length and 0.2 to 0.3 cm wide were selected for the experiment.

#### 2.3.2. Anthelmintic activity

The anthelmintic assay was carried as per the method of Gulnaz and Savitha (2013) with slight modifications. Briefly, test samples of the extract and the standard drug Albendazole was prepared at the concentration of 5, 25, 50 and 100 mg/ml in Tween 20 (1%) solution. Four groups of *Pheretima posthuma* (consisting of three earth worms each in triplicate) were released in to 25ml of experimental formulation, first group serves as normal control which is treated only with normal saline, group two is the standard reference which received standard drug Albendazole at different concentrations, group three and four was treated with different concentrations of aqueous methanolic extract of Bilimbi and aqueous acetone extract of Star fruit. All the test solutions and standard solutions were prepared freshly before starting the

Jyoti Bala Chauhan,

*In vitro* comparative study of Bilimbi and Star fruit extracts for Anthelmintic activity,

Discovery Biotechnology, 2015, 6(15), 5-8,

[www.discovery.org.in/db.htm](http://www.discovery.org.in/db.htm)

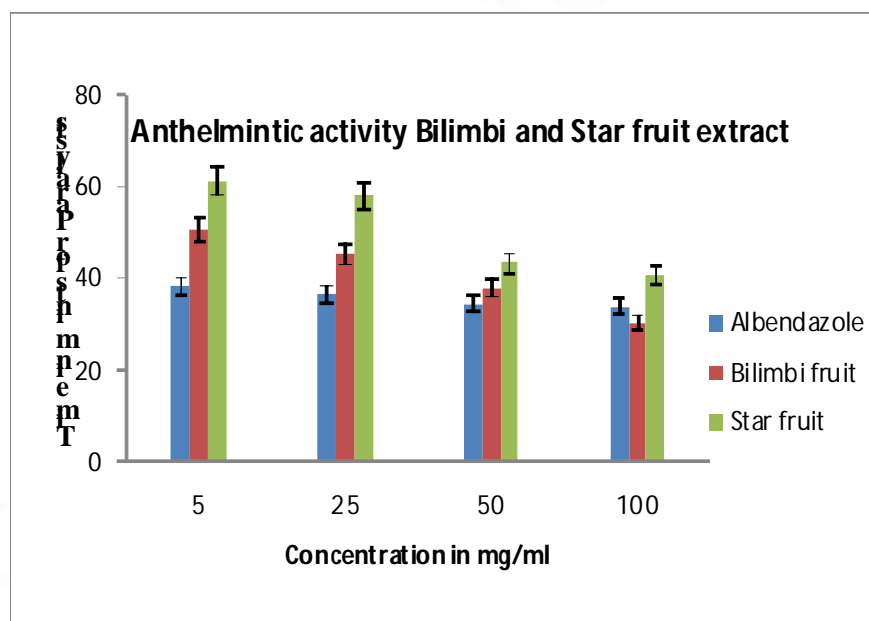
experiment. The mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli by putting motionless worms in 50° C warm water. No movement of worms confirms death. (Dey et al., 2012). Death was concluded when the worms lost their motility followed with white secretion and fading away of their body colors (Karale et al., 2010). The test results were compared with Reference compound Albendazole treated samples.

### 3. RESULTS AND DISCUSSION

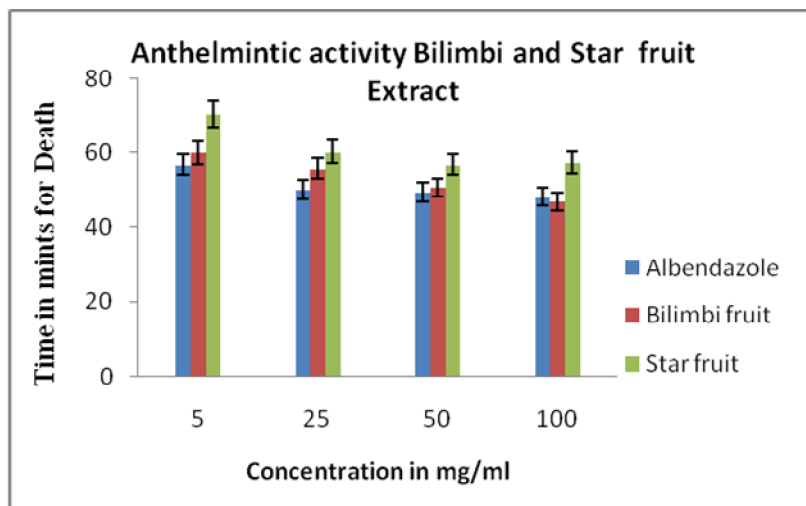
The results of anthelmintic activity of Bilimbi and Star fruit extracts, and the Standard Albendazole are presented in Figure 1 and Figure 2 respectively, wherein, comparative time of paralysis and death is shown. Both the fruit extracts showed significant anthelmintic activity at all tested doses when compared to reference standard as vermifuge and vermicide. Potency of the extract was inversely proportional to time for paralysis and death of worms.

Significant activity was seen in the extract of Bilimbi fruit at 100mg/ml with 30.50 and 47 minutes for paralysis and death respectively followed by extract of star fruit with 40.60 and 57.30 minutes for paralysis and death respectively, compared to standard at 34.32 and 48.00 minutes for paralysis and death. In the present study aqueous methanolic extract of Bilimbi fruit was more potent than the standard reference drug used.

Albendazole exhibits anthelmintic activity by blocking glucose uptake and depletion of glycogen stores in test parasite. The primary phytochemical screening revealed the presence of alkaloids, and tannins in both the plant extract (Hasanuzzaman et al., 2013; Dasagupta and Chakraborty, 2013). Tannins are known to interfere with the energy generation in helminth parasites by uncoupling oxidative phosphorylation (Athnasiadou et al., 2001) and also bind free proteins in the gastrointestinal tract of host animals or glycoprotein on the cuticle of the parasite, leading to death. The tannins present in both the extracts could contribute to the anthelmintic activity. Further study is under progress to isolate the pure bioactive component from the crude extracts of the fruits.



**Figure 1**  
Dose dependent Time for paralysis (minutes) of *Pheretima posthuma* by Albendazole, bilimbi fruit extract and star fruit extract



**Figure 2**  
Dose dependent time for death (minutes) of *Pheretima posthuma* by Albendazole, bilimbi fruit extract and star fruit extract

## ACKNOWLEDGEMENT

The authors thank the University Grants Commission, Delhi, India for their financial support (Project F.No. 37-519 (2009) SR), the Director of Pooja Bhagavat Memorial Mahajana Education Centre, PG Wing of SBRR Mahajan First Grade College and Mahajana Education Society, Mysore, India for providing the infrastructure for conducting the experiments.

## REFERENCES

- Abas F, Lajis NH, Israf DA, Khozirah S, Kalsom YU. Antioxidant and nitric oxide inhibition activities of selected Malay traditional vegetables. *Food Chem*, 2004, 95, 566- 573.
- Arshad H, Anuj KS, Parwez A, Shadma W. *In vitro* anthelmintic activity of *Oleus aromaticus* root in Indian adult earthworm. *Asian Pac J Trop Dis*, 2012, 1, 425-427.
- Athnasiadou S, Kyriazakis I, Jackson F, Coop RL. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *In vitro* and *In vivo* studies. *Vet Parasitol*, 2001, 99, 205- 219.
- Chauhan JB and Kapfo W. Effect of traditional sun- drying on phenolic antioxidants of *Averrhoa bilimbi* L. *Int J Appl Biol Pharma Tech*, 2013, 4, 26-34.
- Dasgupta P, Chakraborty P, Bala NN. *Averrhoa carambola*: An Updated Review. *Int J of Pharma Sci Res Rev*, 2013, 2, 54- 63.
- Dey P, Debnath P. and Bhakta T. (). Evaluation of anthelmintic activity of *Molinieria recurvata* leaf extracts" *Int Res J Pharma App Sci*. 2012, 2, 16-20
- Gaikwad SA, Kale AA, Jadhav BG, Deshpande NR, Salvekar JP. Anthelmintic activity of *Cassia auriculata* L. extracts – *in vitro* study. *J Nat Prod Plant Res*, 2011, 1, 62-66.
- Goh SH, Chuah CH, Mok JSL, Soepadmo E. Malaysian medicinal plants for the treatment of cardiovascular diseases. Pelanduk Publications (M) Sdn Bhd: Malaysia, 1995
- Gulnaz AR and Savitha G. Evaluation of Anthelmintic Activity of Different Leaf and Stem extract of *Sida cordata* Burma (brossa) burm. F. *Int J Curr Microbiol App Sci*. 2013, 2, 246- 255
- Hasanuzzaman M, Ali MR, Hossain M, Kuri S, Islam M.S.. (). Evaluation of total phenolic content, free radical scavenging activity and phytochemical screening of different extracts of *Averrhoa bilimbi* (fruits). *Int Curr Pharma J*. 2013, 2, 92-96
- Iqbal Z, Nadeem OK, Khan MN, Akhtar MS, Waraich FN. *In vitro* anthelmintic activity of *Allium sativum*, *Zingiber officinale*, *Cucurbita mexicana*, *Ficus religosa*. *Int J Agr Biol*, 2001, 3, 454-457.
- Karale SS, Godad AP, Yashodhan BW and Suhas AS. Evaluation of *in vitro* anthelmintic activity of *Ceratophyllum demersum* Linn. *Indian Drugs*, 2010, 47, 63- 65.
- Narain N, Bora PS, Holschuh HJ, Vasconcelos MA, Da S. (2001). Physical and chemical composition of carambola fruit (*Averrhoa carambola* L.) at three stages of maturity. *Cienc Tecnol Aliment* 2001, 3, 144- 148.
- Patil AG, Patil DA, Phatak AV, Chandra N. Physical and chemical characteristics of carambola (*Averrhoa carambola* L.) fruit at three stages of maturity. *Int J Appl Biol Pharma Tech*, 2010, 1, 624- 629.
- Qureshi S Md, Patel J, Giri CI, Hasan S, Shaik M and Hamed Md. *In vitro* anthelmintic activity of root extract of *Trapa bispinosa* Roxb against *Pheretima posthuma* and *Ascardia gilli*. *Int J Pharma Sci Res*, 2010, 1, 353- 356.
- Rafi KP, Karthikeyan M, Kannan M, Rajasekar S. Anthelmintic activity of *Nerium olender* flower extract in Indian adult earthworm. *J Nat Prod Plant Resour*. 2011, 1, 40- 46.
- Rastogi T, Bhutda V, Moon K, Aswar PB, Khadabad SS. Comparative studies on anthelmintic activity of *Moringa oleifera* and *Vitex negundo*. *Asian J Res Chem*, 2009, 2, 181- 182
- Setiawan B, Sulaeman A, Giraud DW and Driskell JA. Carotenoid content of selected Indonesian fruits. *J Food Comp Anal*, 2001, 14, 169- 176.
- Shui G & Leong LP. Analysis of polyphenolic antioxidants in star fruit using liquid chromatography and mass spectrometry. *J Chrom A*, 2004, 1022, 67- 75.
- Weller A, Sims CA, Matthews RF, Bates RP, Brecht JK. Browning susceptibility and changes in composition during storage of carambola slices. *J Food Sci*, 1997, 62: 256- 260.
- Yadav P, Singh R. A review on anthelmintic drugs and their future scope. *Int J Pharmacol Pharma Sci*, 2011, 3: 17- 21.
- Yap CF, Ho CW, Wan Aida WM, Chan SW, Lee CY and Leong YS. Optimization of extraction conditions of total phenolic compounds from Star Fruit (*Averrhoa carambola* L.) residues. *Sains Malays*, 2009, 38: 511- 520.