Proceeding of the Public Health and Well-being Conference, Vol. 2, Issue 2, 2021, pp. 43-50
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ISSN 2659-2096
DOI: https:/doi.org/10.32789/publichealth.2021.1011

# Comparative Evaluation of *in - vitro* Anthelmintic Activity of Sap, Fruit bulb, and Root of *Borassus flabellifer*

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# COMPARATIVE EVALUATION OF *IN - VITRO* ANTHELMINTIC ACTIVITY OF SAP, FRUIT BULB, AND ROOT OF *Borassus flabellifer*

**Abstract:** Helminth infections are major health problems, especially in developing countries with warm and humid environments. Limited synthetic drugs are available for treating helminth infections, and some of them showed resistance to helminths. Phytoconstituents can be used to develop cheap and effective anthelminthic drugs. *Borassus flabellifer* has been used in traditional medicine, in addition to consuming as food. This study was designed to evaluate the comparative *in vitro* anthelmintic activities of aqueous and methanol extracts of root, fruit pulp, and sap of *Borassus flabellifer* using two different concentrations (50 and 100 mg/mL). Adult earthworms, *Eisenia fetida* was used to evaluate the in-vitro anthelmintic activity. In-vitro anthelmintic activity was evaluated by determining times of paralysis and the death of worms. The anthelminthic activity of extracts was compared with mebendazole, and earthworm saline was used as a control. ANOVA followed by Dunnett's multiple comparison test was used to compare the activities of extracts with mebendazole. All extracts showed concentrations dependent activity. All extracts except aqueous extract of roots of *Borassus flabellifer* showed statistically significant in-vitro anthelminthic activity. Further studies are needed to isolate and evaluate the active components from the extracts of *Borassus flabellifer.*

**Keywords**: *Borassus flabellifer*, Sap, Root, Fruit bulb, Extracts, Anthelmintic activity

## Introduction

In developing countries, parasitic intestinal infections are a major health problem. More than one billion people are estimated to be infected with helminth infection in developing countries. (Williams, A.R *et al*., 2014) An estimated 4.7 billion people worldwide live in regions with a high potential for exposure to soil-transmitted helminths (Salmon, M *et al*., 2018).

Helminth infections cause several clinical infestations such as malnutrition, intestinal and biliary obstruction, dysentery, and anemia. Risk of populations by helminth infections include preschool and school-age children and women of reproductive age (WHO, 2010). An area that has poor sanitation is also more at risk for soil-transmitted helminth infections. Children of school age of 5 to 14 years in low-income countries' helminth infections account for 12% of the total disease burden (Awasthi S *et al*., 2013).

Limited drugs are available in the eradication of helminth infections. The repeated occurrence of these helminth infections results in frequent usage of anthelmintic drugs. It results in drug resistance (Fouche G *et al*., 2016a) and increased cost of treatment. Searching for new drugs for helminth infections is important to control the disease as it is widely distributed in the human population (Fouche G *et al*., 2016b). In the last three decades, no new anthelmintic drugs have been introduced for human usage (Panic *et al*., 2014).

According to WHO, 80% of the world's population depends on traditional medicines for their healthcare needs. Traditionally many herbals are used in helminth infections. Some compounds isolated from medicinal plants have shown anthelminthic activity (Liu, Maoxuan, *et al*., 2020). Herbals and their secondary metabolites may be used effectively for parasite control. Screening of traditionally used anthelmintic plants' pharmacological activity will lead to new drug candidates with lesser side effects (Rafi KP *et al.,* 2011). These plants can be used as phytotherapeutics or food supplements in the management of helminth infections. Consuming plants with anthelmintic activity as food supplements may reduce the incidence of helminth infections.

*Borassus flabellifer* belongs to Arecaceae, is growing widely in tropical countries. It is cultivated in many tropical countries like India, Bangladesh, Burma, Sri Lanka, Malaysia, and tropical Africa (Aval A *et al.,* 1995). Different parts of plants are used medicinally as well as food supplements. Saps obtained from inflorescence were used as a refreshing drink. Some traditional practitioners used sap for the treatment of intestinal worm infections in Sri Lanka. Fruits are sweet and consumed by people in Asian countries. The root of palmyrah also was consumed by people, and it is powdered to make different foods. In the present study, different solvent extracts of sap, root, and fruit bulb are screened for *in vitro* anthelmintic activity.

**Materials**

Drug: Mebendazole

Chemicals: Methanol, Distilled water, Sodium chloride (NaCl), Potassium chloride (KCl), Calcium chloride ($CaCl_2$), Sodium bicarbonate ($NaHCO_3$), Dimethyl sulfoxide (DMSO)

**Methods**

***Preparation of Extracts***

Aqueous and Methanol extracts were prepared using sun-dried fruit pulp (Pinnatu), roots (Odial / Kottakilangu), and sap (toddy palm). The roots and "Pinnatu" were collected and air-dried in the shade at room temperature. Methanol and aqueous extracts of fruit bulb and root were prepared using the soxhlet apparatus and concentrated using a rotary evaporator. Methanol extract of toddy was prepared by evaporating it using a rotary evaporator, and the resultant residue was dissolved in methanol and filtered to get methanol extract of toddy.

***Experimental worms***

The adult earthworms, *Eisenia fetida*, known under various common names such as redworm, brandling worm, panfish worm, trout worm, tiger worm, red wiggler worm, red Californian earthworm, etc., resemble the intestinal roundworms parasites of humans in their anatomical and physiological characters were used for this study (Kainsa S. *et al.,*2012). The above worms were collected from "Green Visva Lanka Farm Developers, Thoddilady, Villavily, Chankanai." The collected worms were washed initially with tap water followed by earthworm saline to remove the soil particles and debris.

***Sample preparation***

Samples of aqueous extracts were prepared by dissolving 500 mg and 1000 mg of crude extract in 1ml of DMSO and make up the volume with saline up to 10 mL with saline solution. Final concentration of extracts was obtained as 50mg/mL and 100mg/mL respectively. Earthworm saline was used as a control, and mebendazole was used as the standard drug for this study.

***Evaluation of anthelmintic activity***

The anthelmintic activity was performed on the adult Indian earthworm *Eisenia fetida*. The in-vitro studies were performed according to the previous studies (Prasad Govind Rao Jamkhande *et al*., 2014). The worms were divided into 24 groups containing two earthworms of approximately equal size and weight. Worms were placed in Petri dishes containing different concentrations of the extracts separately. 10 mL suspensions of aqueous and methanolic extracts were used as test samples. Mebendazole (20 mg/mL) with 2% Gum Acacia in saline was used as a reference, and the earthworm saline was used as the control group.

Two worms were placed in each group, and they were observed for their spontaneous motility and evoked responses. Time of death was noted when the worms showed zero response to the stimuli even after performing the prick test and when dropped in warm water (50ºC) followed with fading away of their body color (Ajaiyeoba E O *et al*., 2001). All experiments were done in triplicate.

***Statistical analysis***

Statistical analysis was conducted with SPSS version 25. All values were presented as mean with standard deviation. Comparison of anthelmintic activity of extracts with reference drug in terms of paralyzed and death times were assessed by one-way- ANOVA followed by Dunnett test. The values of P less than 0.05 were considered a statistically significant difference.

**Results and Discussion**

The effects of different concentrations of aqueous and methanolic extracts of the products derived from *Borassus flabellifer* plant and mebendazole on adult earthworms (*Eisenia fetida*) were depicted in Table 1.

Extracts of sap, root, and fruit bulb of *Borassus flabellifer* showed anthelmintic activity. A dose-dependent onset of paralysis and mortality was observed in the earthworms treated with the extracts. They were compared with mebendazole as the reference drug.

Figure 1 and 2 shows paralyzed and death times of different extracts of Based on the results, paralyzed and death times is more in the methanol extracts than aqueous extracts except in the sap extracts. This may be due to more number of active phytoconstituents and their higher proportion present in the methanol extracts than aqueous extract. Aqueous extract of sap showed shorter paralysis and death times than the methanol extract. The better activity of sap extracts may be due to alkaloids and steroids present in a higher proportion than other extracts.

*Table 1: Paralyzed and death times of different extracts with P values*

| **Plant parts** | **Extracts** | **Concentration (mg/mL)** | **Paralyzed time (mins)** | **P value** | **Death time (mins)** | **P value** |
|---|---|---|---|---|---|---|
| Sap | Methanol extract | 50 | 19.86 ± 0.65 | 0.00 | 25.26 ± 0.28 | 0.00 |
| | | 100 | 8.9 ± 0.00 | 0.00 | 9.50 ± 0.00 | 0.005 |
| | Aqueous extract | 50 | 11.10 ± 0.16 | 0.00 | 14.00 ± 0.16 | 0.00 |
| | | 100 | 5.43 ± 0.09 | 0.031 | 7.00 ± 0.00 | 0.006 |
| Fruit bulb | Methanol extract | 50 | 35.13 ± 0.04 | 0.00 | 38.70 ± 0.28 | 0.00 |
| | | 100 | 7.36 ± 0.12 | 0.00 | 8.30 ± 0.04 | 0.680 |
| | Aqueous extract | 50 | 81.5±0.57 | 0.00 | 108.3 ± 0.80 | 0.00 |
| | | 100 | 14.50 ± 0.14 | 0.00 | 18.23 ± 0.38 | 0.00 |
| Root | Methanol extract | 50 | 6.56 ± 0.17 | 0.002 | 11.66 ± 0.175 | 0.00 |
| | | 100 | 4.50 ± 0.08 | 0.997 | 5.43 ± 0.09 | 0.00 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Aqueous extract | 50 | _ | - | Survived | - |
| | | 100 | - | - | Survived | - |
| Mebendazole | 20 | 4.33±0.33 | - | 8.33±0.33 | - | |
| Control (Normal Saline) | - | - | | Survived | - | |

Methanol extract of root showed the death for earthworms even in its minimum concentration (50 mg/mL). However, the aqueous root extract did not show any paralysis or death for earthworms even in its maximum concentration (100 mg/mL).

According to a literature survey on phytochemical screenings, the toddy of *Borassus flabellifer* is positive for alkaloids and steroids such as Borassosides and Dioscin (Tribuvan Singh *et al*., 2016). The aqueous and methanolic extracts of raw palmyrah fruit pulp (RPFP) and thermally processed palmyrah fruit pulp (PPFP) of the *Borassus flabellifer* plant are positive for alkaloids, flavonoids, terpenoids, glycosides, saponins, phenolics, tannins, steroids, and sterols (Saranya, P. and Poongodi Vijayakumar, T., 2016). The root of the *Borassus flabellifer* plant is positive for tannins, phenols, steroids, flavonoids, saponins, and alkaloids (Saravanan, C et *al*.,2012) (Chayanika Sahni *et al.,* 2014).

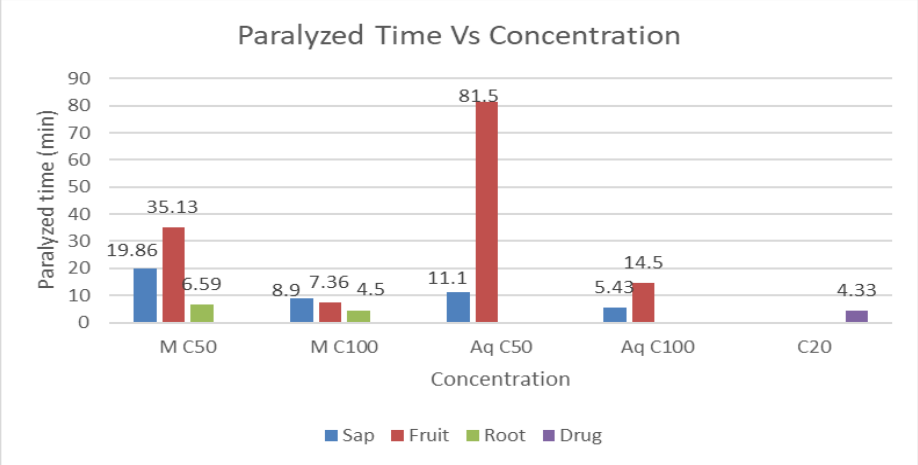


Figure 1: Paralyzed time of different extracts

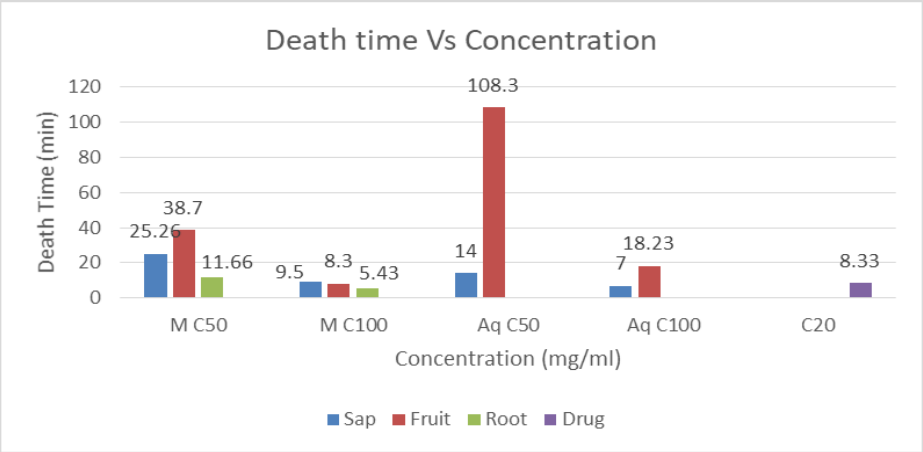


Figure 2: Death time of different extracts

Several phytochemicals have anthelmintic activities. Extracts of the plant contain several phytoconstituents, and the plant's anthelmintic activity may be due to these compounds' synergistic effect. Phytocompounds that showed anthelminthic activity include saponins, alkaloids, polyphenols, tannins, etc. Different phytoconstituents act as anthelmintic agents in different mechanisms (Manke *et al.,* 2014). These compounds are present in *Borassus flabellifer* and could be responsible for the anthelmintic activity.

According to the ANOVA test, all plant extracts at the 50 and 100 mg/mL concentrations showed a statistically significant difference in the anthelminthic activity except for two extracts. Methanol extract of root at 100mg/mL and methanol extract of fruit bulb at 100 mg/mL did not show a statistically significant difference. Extracts such as aqueous sap extract at 100 mg/mL, methanol extract of fruit bulb at 100 mg/mL, and methanol extract of root at 100 mg/mL showed comparable activity to the reference drug.

Further in-vitro studies are needed to confirm the pharmacological activity of these extracts. Phytochemical screening and isolation of actives present in the extracts should be further conducted. Also, the precise mechanism of anthelmintic activity needs to be found out.

**Conclusion**

Extracts of sap, root, and fruit bulb of *Borassus flabellifer* showed good anthelmintic activity against adult earthworms, *Eisenia fetida*. Isolation of active principles and screening of their anthelminthic activity may explore new drug candidates.

**Acknowledgment**

The authors are grateful to all academic and non-academic staff of Faculty of Allied Health Sciences and Faculty of Medicine, University of Jaffna, Sri Lanka for their extended support to complete this study.

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