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Effect of Ankenda (*Acronychia pedunculata*) Extract as a Fermentation Inhibitor in Tapped Inflorescence Sap of Kithul (*Caryota urens*) in Sri Lanka

R.A.G.G. Tharuka, W.S.P. Wagasenevi and K.H.I.K.Hewavitharana

Department of Biosystems Technology, Faculty of Technological Studies, Uva Wellassa University of Sri Lanka.
*indika.h@uwu.ac.lk

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Abstract: The production of jaggery and treacle from fermented *Caryota* inflorescence sap presents significant challenges. This study aimed to evaluate the effectiveness of extracts from the leaves, bark, and flowers of *Acronychia pedunculata* in inhibiting the fermentation of *Caryota urens* inflorescence sap and to determine the most effective part and concentration for this purpose. Essential oils were extracted from fresh plant parts (leaves, bark, and flowers) using hydro-distillation. These oils were applied to non-fermented *Caryota* inflorescence sap at four different concentrations to assess their effects on turbidity, pH, Brix value, alcohol content, and microbial growth. The results showed that the bark of *Acronychia pedunculata* yielded significantly more oil (13.67%) compared to the leaves (1.61%) and flowers (4.31%). Application of 2-3 ml of bark oil resulted in higher pH values in the *Caryota* sap. The highest mean alcohol content (2.09) and Brix value (8.87) were observed with 3 ml of bark oil, indicating effective fermentation inhibition. The lowest mean turbidity value (785.4) was also recorded with 3 ml of bark oil. Additionally, FTIR analysis suggested that the oils contained polyphenols (such as flavonoids and tannins) and terpenes. Polyphenols were indicated by aromatic peaks, suggesting their role in antimicrobial activity, while terpenes were indicated by alkenes and C=C bending peaks, known for their broad-spectrum antimicrobial effects. This study recommends *Acronychia pedunculata* bark oil as an effective fermentation inhibitor for *Caryota urens* inflorescence sap, providing valuable insights for enhancing the production of jaggery and treacle in Sri Lanka.

Keywords: *Acronychia pedunculata*, *Caryota urens*, Inhibition of alcohol content in sap fermentation, Indigenous plants for fermentation control, Jaggery production, Natural Fermentation inhibition, Natural preservatives for Sap, Treacle production

1. INTRODUCTION

Caryota urens, commonly known as the fishtail palm or toddy palm, is a species of palm native to Southeast Asia and the Indian subcontinent. In Sri Lanka, it is referred to as Kithul. This palm is typically found in lowland rainforests such as Sinharaja, wetlands, and moist environments in regions including Kandy, Matale, and Badulla. It belongs to the *Arecaceae* family. The *Caryota urens* tree features a single, tall, slender trunk that can reach heights of 15 to 25 meters. The trunk is characterized by extensive ring-like leaf scars left by fallen old leaves. The leaves are large and fishtail-shaped, which is reflected in its common name. They can grow to 6 to 9 meters in length and are divided into numerous segments and smaller leaflets, giving them a feathery appearance. After flowering, the tree produces

small, round fruits, 1.5 to 2 centimeters in diameter, which are dark purple or black when mature and contain a single seed. The secretions from these fruits can cause staining and severe itching if rubbed on the skin. The inflorescence, a large branching structure with tiny cream-colored blooms, is where the sap is harvested. [1-3]

The inflorescence sap of *Caryota urens* is economically significant in Sri Lanka. It is harvested twice daily from the tree for a period of 3 to 6 months, yielding products such as jaggery, treacle, toddy, and vinegar. The sap is rich in nutrients, primarily composed of sucrose, glucose, and fructose. The production of treacle and jaggery involves evaporating the inflorescence sap. However, natural yeast cultures can convert the sap into toddy, making it unsuitable for producing treacle or jaggery. This fermentation reduces treacle yield and affects the quality of jaggery, causing it to become waxy and deteriorate quickly. Consequently, consumer acceptance of Kithul treacle and jaggery decreases. To combat fermentation, various techniques are employed in Sri Lanka, such as lining collection pots with fresh lime, adding Hal (*Vateria copallifera*) bark, kakata (*Careya arborea*) bark, or Kohomba (*Azadirachta indica*) leaves. [1-3]

Acronychia is a genus in the *Rutaceae* family, with species such as *Acronychia pedunculata* being widely distributed in Sri Lanka. This small evergreen tree has smooth, light grey bark and simple leaves ranging from elliptic to slightly obovate. Its green-white flowers yield edible fruits that turn brown as they dry, with a sweet acidic flavor. *Acronychia* plants are traditionally used as fermentation inhibitors due to their antifungal and antibacterial properties. The essential oil of *Acronychia pedunculata* contains key components like alpha-pinene (57.4%) and beta-caryophyllene (13.6%). Studies have shown its broad-spectrum antibacterial activity against pathogens such as *Salmonella enterica* and *Staphylococcus epidermidis*. This study aims to evaluate the effectiveness of different parts of *Acronychia pedunculata*—including leaves, bark, and flowers—and their concentrations in inhibiting the fermentation of *Caryota urens* sap. The objective is to identify the most effective part and concentration for use as a fermentation inhibitor. [1-7]

2 METHODOLOGY

Experimental Location

The experiment was conducted at the Biosystems Technology Laboratory, Department of Biosystems Technology, Faculty of Technological Studies, Uva Wellassa University, Sri Lanka.

Plant Material

Fresh leaves, bark, and flowers of *Acronychia pedunculata* were collected from Morankanda, Kandy, Sri Lanka. The Kithul inflorescence sap was obtained from Kailagoda, Badulla, Sri Lanka.

Experimental Procedure

The fresh leaves, bark, and flowers of *Acronychia pedunculata* were washed under running tap water, cut into small pieces with a sharp knife, and blended. For extraction, 300 g of blended plant material (leaves, bark, or flowers) was placed into separate 1000 ml round-bottom flasks. Each flask was filled with 600 ml of distilled water. The flasks were then set on heating mantles, with each apparatus fully sealed. The heating mantles were set to an optimal temperature of 126°C, and steam distillation was carried out for 15-16 hours. After extraction, the mixture was separated using three separation funnels to isolate the oil from the water. The separated oils were transferred to clean 10 ml glass bottles with plastic lids, labeled, and stored in a refrigerator at 4°C. To assess the effect of *Acronychia pedunculata* oils, the collected *Caryota urens* inflorescence sap was used. A total of 500 ml of inflorescence sap was placed

into each of six, 600 ml sterilized glass beakers. *Acronychia pedunculata* leaf oil, bark oil, and flower oil were added to the sap in the following volumes: 0.5 ml, 1 ml, 2 ml, and 3 ml. A control beaker containing inflorescence sap without any added oil was also maintained. Each treatment was replicated three times.[4]

3 RESULTS AND DISCUSSIONS

To evaluate the effectiveness of *Acronychia pedunculata* fresh leaves, bark, and flowers as fermentation inhibitors for *Caryota urens* inflorescence sap, hydro-distillation extraction was performed. The extracted oils were light yellow in color, with the bark oil being lighter in hue compared to the oils extracted from the leaves and flowers. All oils had a very pungent odor. Table 1. and Fig. 1. A, displays the yield of extracted oil obtained through hydro-distillation.

Table 1. Yield of Oil Extracted from *Acronychia pedunculata* Plant Parts by Hydro-Distillation

Acronychia pedunculata plant part	Weight of Plant Material (kg)	Volume of Extracted Oil (ml)	Oil Yield (%)
Fresh Leaves	6.00	9.6	1.61
Fresh Bark	2.10	28.7	13.67
Fresh Flowers	2.18	9.4	4.31

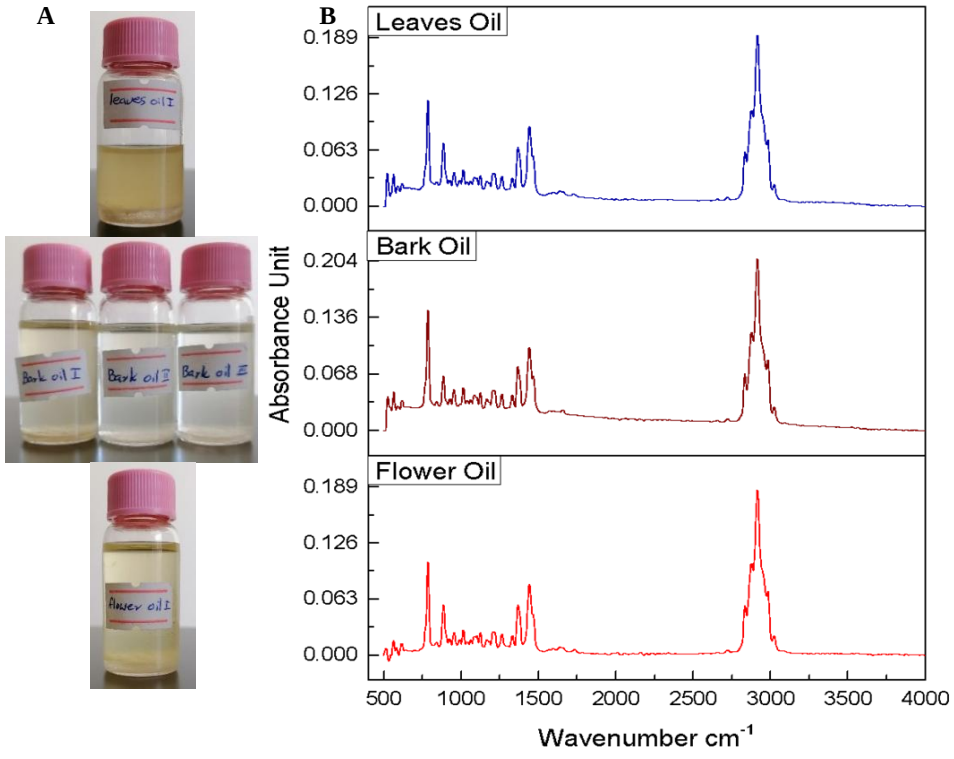

Fig. 1. A) Oils Extracted from *Acronychia pedunculata* Bark, Leaves, and Flowers respectively using Hydro-Distillation. B) FT-IR Spectroscopy Analysis of Essential Oils Extracted from *Acronychia pedunculata* Leaves, Bark, and Flowers.

Figure 1B, presents the FTIR analysis results for oils extracted from various parts of the *Acronychia*

pedunculata tree. The FTIR spectra for the oils from the bark, leaves, and flowers were similar, indicating a consistent chemical composition across these extracts. The analysis revealed several key peaks include: alkenes (C-H Stretch) were observed at 3024.84 cm^{-1}, 3024.94 cm^{-1}, 3024.39 cm^{-1}, 2984.37 cm^{-1}, 2983.48 cm^{-1}, 2983.56 cm^{-1}, 2915.50 cm^{-1}, 2916.03 cm^{-1}, 2915.95 cm^{-1}, 2877.85 cm^{-1}, 2877.37 cm^{-1}, 2877.46 cm^{-1}, 2834.93 cm^{-1}, 2835.46 cm^{-1}, 2835.36 cm^{-1} (within 3000–2830 cm^{-1}). Alkanes (C-H Bend) were noted at 1441.44 cm^{-1}, 1442.05 cm^{-1}, and 1441.99 cm^{-1} (within 1470–1440 cm^{-1}). Alkanes (C-H Rock) appeared at 1367.61 cm^{-1}, 1368.52 cm^{-1}, and 1368.90 cm^{-1} (within 1370–1350 cm^{-1}). Aromatics (C-H Out-of-Plane Bending) were present at 886.04 cm^{-1}, 886.52 cm^{-1}, and 886.70 cm^{-1} (within 900–675 cm^{-1}). and Alkenes (C=C Bending) were detected at 786.79 cm^{-1}, 786.97 cm^{-1}, and 787.00 cm^{-1} (within 840–780 cm^{-1}). These results confirm the presence of similar functional groups across the different *Acronychia pedunculata* oils, highlighting their comparable chemical profiles. Based on the FTIR data, polyphenols (such as flavonoids and tannins) and terpenes are the most likely metabolites present in the oils from *Acronychia pedunculata* that could contribute to yeast inhibition. Polyphenols presence is suggested by the aromatic peaks, indicating their role in antimicrobial activity. Terpenes presence is suggested by the alkenes and C=C bending peaks, known for their broad-spectrum antimicrobial effects. Both classes of compounds are strong candidates for yeast inhibition, with polyphenols likely playing a significant role due to their well-documented antimicrobial properties. [5-9]

Effect of *Acronychia pedunculata* Leaf, Bark, and Flower Oils on the pH Value of *Caryota urens* Inflorescence Sap

In reference to Fig. 2., the mean pH values of *Caryota urens* inflorescence sap treated with *Acronychia pedunculata* leaf oil at concentrations of 0.5 ml, 1 ml, 2 ml, and 3 ml were significantly elevated compared to the control after 4 days. Among these treatments, the 2 ml and 3 ml concentrations resulted in higher pH values compared to the 0.5 ml and 1 ml concentrations. Similarly, the mean pH values of *Caryota urens* inflorescence sap treated with *Acronychia pedunculata* bark oil at concentrations of 0.5 ml, 1 ml, 2 ml, and 3 ml were higher than those of the control after 4 days. Among these, the 1 ml and 3 ml concentrations of bark oil produced the highest pH values. For *Acronychia pedunculata* flower oil, the mean pH values of *Caryota urens* inflorescence sap at 2 ml and 3 ml concentrations were higher than those at 0.5 ml, 1 ml, and the control after 4 days. The 3 ml concentration of flower oil achieved the highest pH value among the flower oil treatments.

Accordingly, *Acronychia pedunculata* leaf and bark oils consistently resulted in significantly higher pH values compared to *Acronychia pedunculata* flower oil and the control. Although the 3 ml concentration of flower oil produced a higher pH value than the lower concentrations and control, it was still lower than the pH values achieved with leaf and bark oils. Therefore, *Acronychia pedunculata* flower oil was less effective in increasing the pH of *Caryota urens* inflorescence sap compared to leaf and bark oils.

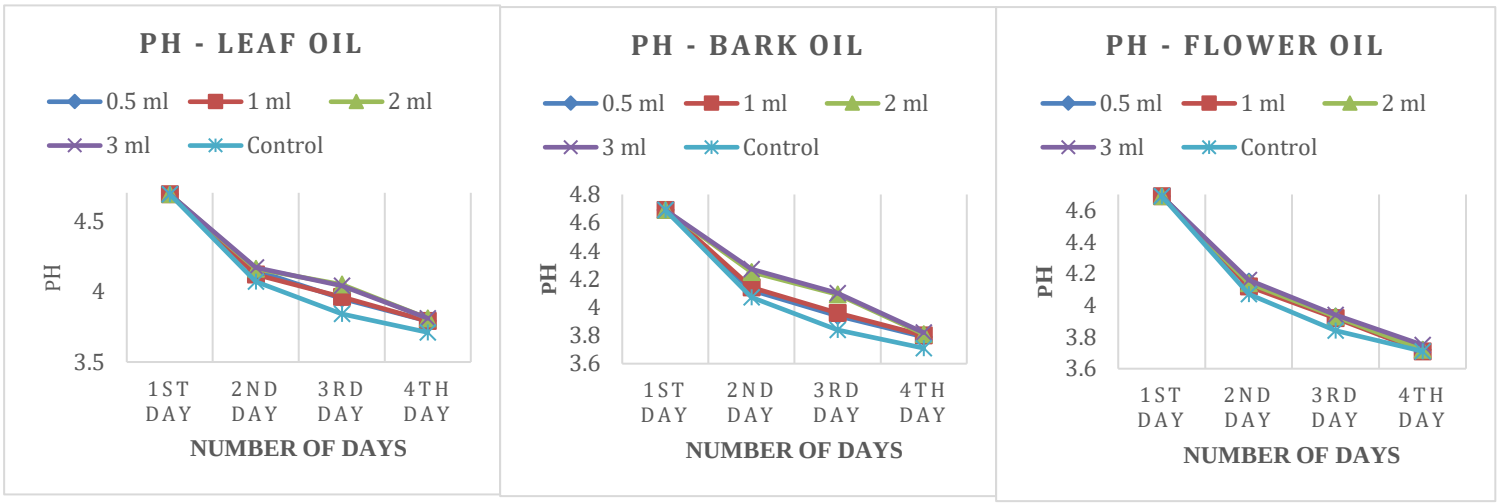

Fig. 2. Changes in the pH of *Caryota* inflorescence sap when treated with varying amounts of *Acronychia pedunculata* leaf oil, bark oil, and flower oil, including 0.5 ml, 1 ml, 2 ml, and 3 ml.

Effect of *Acronychia pedunculata* Leaf, Bark, and Flower Oils on the Turbidity of *Caryota urens* Inflorescence Sap

The mean turbidity of *Caryota urens* inflorescence sap treated with *Acronychia pedunculata* leaf oil at concentrations of 2 ml and 3 ml was significantly higher than that of the 0.5 ml, 1 ml, and control treatments after 3 days. By the 4th day, all treatments reached the maximum turbidity value measurable by the equipment (801 ATU). Similarly, the mean turbidity of *Caryota urens* inflorescence sap with *Acronychia pedunculata* bark oil at 2 ml and 3 ml concentrations was significantly higher than at 0.5 ml, 1 ml, and the control after 4 days. On the 3rd and 4th days, turbidity values for the 0.5 ml and 1 ml bark oil treatments, as well as the control, reached the maximum value of 801 ATU (Figure 3). For *Acronychia pedunculata* flower oil, the mean turbidity of *Caryota urens* inflorescence sap with 2 ml and 3 ml concentrations was significantly higher than with 0.5 ml, 1 ml, and the control after 3 days (Fig. 3). By the 4th day, all concentrations of flower oil also reached the maximum turbidity value of 801 ATU.

Accordingly, most treatments with *Acronychia pedunculata* oils reached the maximum turbidity value of 801 ATU by the 4th day, with the exception of the 2 ml and 3 ml concentrations of bark oil. On the 1st and 2nd days, all concentrations of *Acronychia pedunculata* oils exhibited higher turbidity compared to the control.

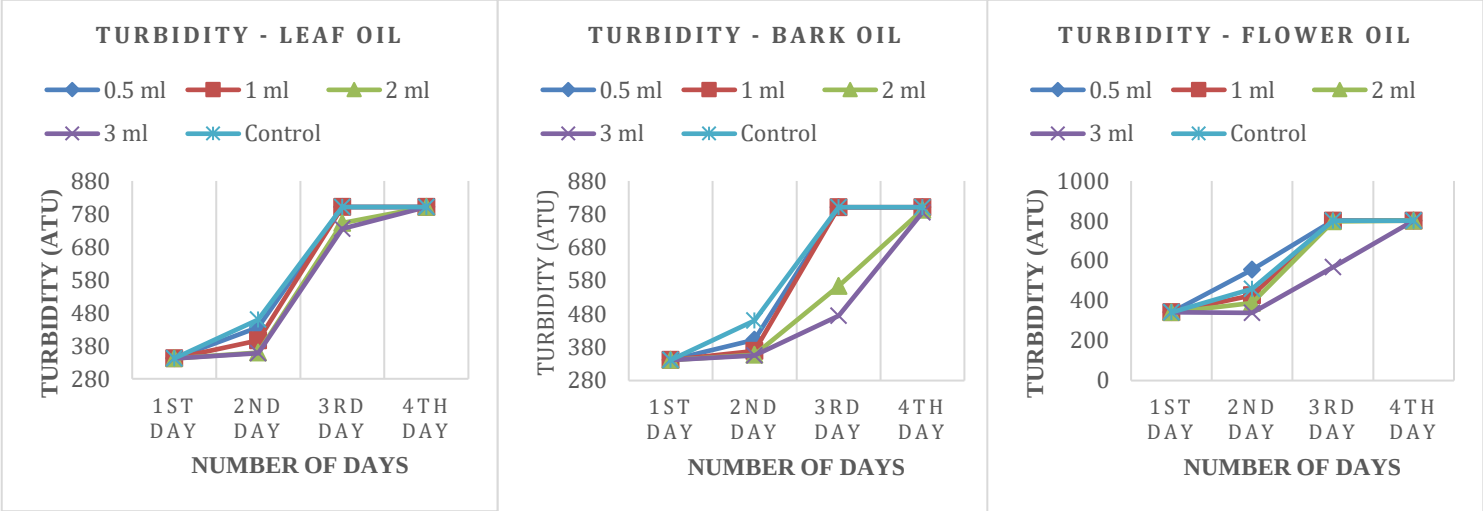

Fig. 3. Changes in the turbidity of *Caryota* inflorescence sap when treated with varying amounts of *Acronychia pedunculata* leaf oil, bark oil, and flower oil, including 0.5 ml, 1 ml, 2 ml, and 3 ml.

Effect of *Acronychia pedunculata* Leaf, Bark, and Flower Oils on the Alcohol Content of *Caryota urens* Inflorescence Sap

As shown in Fig. 4, the mean alcohol content of *Caryota urens* inflorescence sap treated with *Acronychia pedunculata* leaf oil at concentrations of 0.5 ml, 2 ml, and 3 ml was significantly lower than that of the 1 ml and control treatments after 4 days. Among these concentrations, the 3 ml treatment of leaf oil showed the most favorable results in reducing alcohol content. For *Acronychia pedunculata* bark oil, the mean alcohol content of *Caryota urens* inflorescence sap at concentrations of 0.5 ml, 1 ml, 2 ml, and 3 ml was significantly lower than the control after 4 days. On the 4th day, the 3 ml concentration of bark oil resulted in the lowest alcohol content compared to other treatments. Additionally, the 3 ml bark oil also demonstrated the highest pH value among all oil treatments. Similarly, the mean alcohol content of *Caryota urens* inflorescence sap with *Acronychia pedunculata* flower oil at concentrations of 0.5 ml, 1 ml, 2 ml, and 3 ml was significantly lower than that of the control after 4 days. On the 4th day, the 3 ml concentration of flower oil showed the lowest alcohol content among the flower oil treatments.

Consequently, all three *Acronychia pedunculata* oils (leaf, bark, and flower) demonstrated a reduction in alcohol content compared to the control. Among the different concentrations tested, the 3 ml concentration consistently yielded the most effective results in reducing alcohol content for each type of oil. Specifically, the 3 ml concentration of *Acronychia pedunculata* bark oil was the most effective in reducing alcohol content compared to other oil types and concentrations.

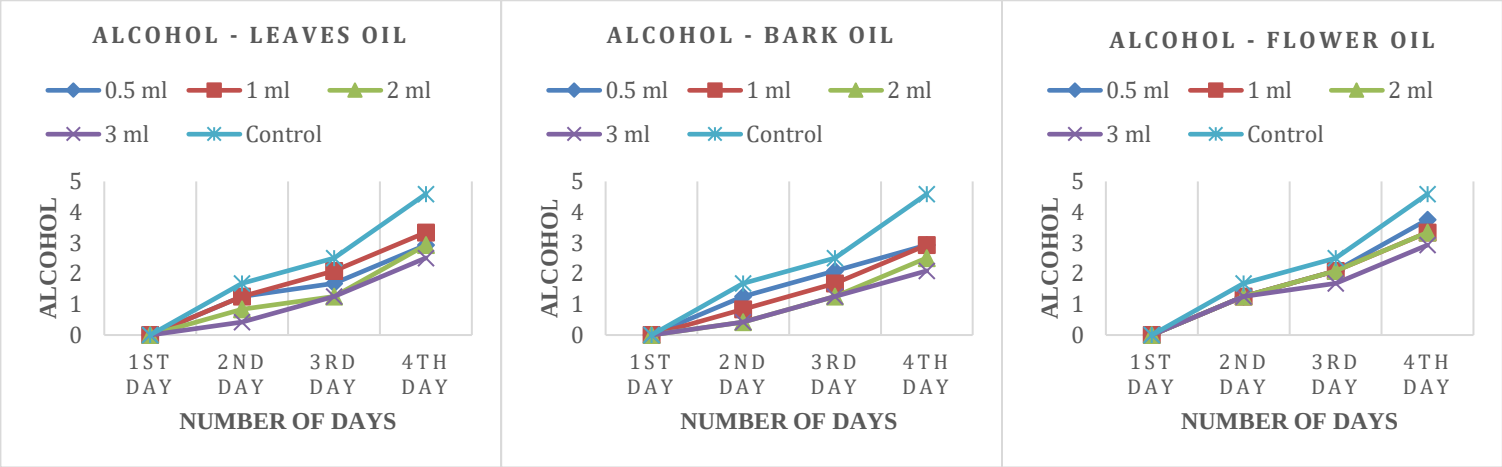

Fig. 4. Changes in the alcohol content of *Caryota* inflorescence sap when treated with varying amounts of *Acronychia pedunculata* leaf oil, bark oil, and flower oil, including 0.5 ml, 1 ml, 2 ml, and 3 ml.

Effect of *Acronychia pedunculata* Leaf, Bark, and Flower Oils on the Brix of *Caryota urens* Inflorescence Sap

The mean Brix values of *Caryota urens* inflorescence sap treated with *Acronychia pedunculata* leaf oil at concentrations of 0.5 ml, 1 ml, 2 ml, and 3 ml were significantly higher than those of the control after 4 days. Among these concentrations, the 2 ml and 3 ml treatments consistently yielded higher Brix values compared to the 0.5 ml and 1 ml treatments throughout the observation period. Similarly, the mean Brix values of *Caryota urens* inflorescence sap with *Acronychia pedunculata* bark oil at concentrations of 0.5 ml, 1 ml, 2 ml, and 3 ml were significantly higher than the control after 4 days. The 2 ml and 3 ml concentrations showed the highest Brix values, outperforming the 0.5 ml and 1 ml treatments on all measurement days. For *Acronychia pedunculata* flower oil, the mean Brix values of *Caryota urens* inflorescence sap at concentrations of 0.5 ml, 1 ml, 2 ml, and 3 ml were significantly higher than those of the control after 4 days. Among these, the 1 ml concentration exhibited the highest Brix values consistently compared to the 0.5 ml, 2 ml, and 3 ml concentrations (Fig. 5).

Overall, all concentrations of *Acronychia pedunculata* oils demonstrated positive effects on the Brix values of *Caryota urens* inflorescence sap. However, Brix values showed a notable decline starting from the 3rd day. Among the oils, *Acronychia pedunculata* bark oil at a 3 ml concentration proved to be the most effective in maintaining higher Brix values compared to other treatments.

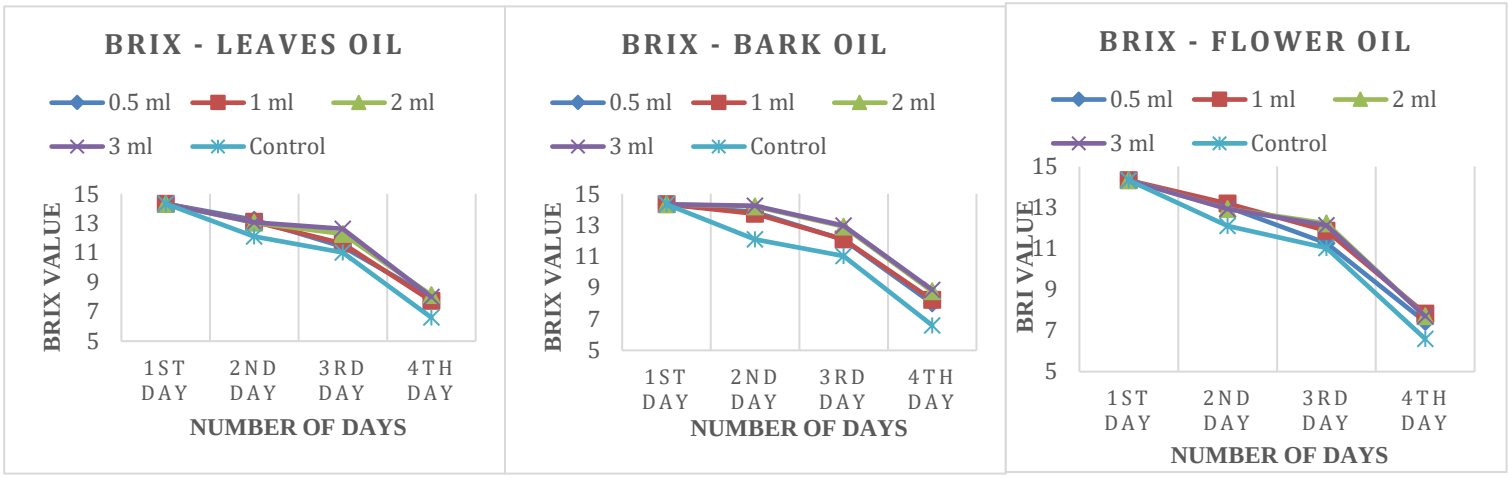

Fig. 5. Changes in the Brix value of *Caryota* inflorescence sap when treated with varying amounts of *Acronychia pedunculata* leaf oil, bark oil, and flower oil, including 0.5 ml, 1 ml, 2 ml, and 3 ml.

Microbial Test Results

The mean number of microbial colonies observed in *Caryota urens* inflorescence sap treated with the control was significantly higher compared to those treated with *Acronychia pedunculata* leaf oil, and bark oil. Petri dishes containing bark oil exhibited a markedly lower number of microbial colonies, even after three days of incubation (Fig. 6). This suggests that the polyphenols and terpenes present in the bark oil contribute significantly to its antimicrobial activity. [8-13]

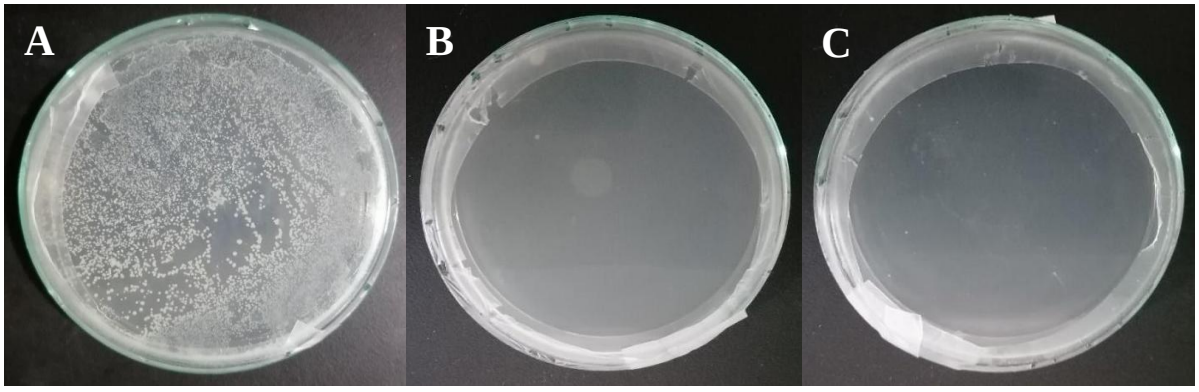

Fig. 6. Microbial Test Results for A) Control B) *Caryota urens* inflorescence sap treated with *Acronychia pedunculata* bark oil C) *Caryota urens* inflorescence sap treated with *Acronychia pedunculata* leaf oil

4 CONCLUSION

This study evaluated the effectiveness of oils extracted from the leaves, bark, and flowers of *Acronychia pedunculata* in inhibiting the fermentation of *Caryota urens* inflorescence sap. Among the oils tested, *Acronychia pedunculata* bark oil proved to be the most effective in inhibiting fermentation. While *Acronychia pedunculata* leaf oil also exhibited some potential for fermentation inhibition, it was less effective compared to the bark oil. Conversely, *Acronychia pedunculata* flower oil demonstrated the least effectiveness in all parameters tested and is not recommended for this application, partly due to its seasonal availability and lower efficacy. FTIR analysis suggested that the oils contained polyphenols (such as flavonoids and tannins) and terpenes. Polyphenols were indicated by aromatic peaks, suggesting their role in antimicrobial activity, while terpenes were indicated by alkenes and C=C bending peaks, known for their broad-spectrum antimicrobial effects. This study recommends *Acronychia pedunculata* bark oil as an effective fermentation inhibitor for *Caryota urens* inflorescence sap, providing valuable insights for enhancing the production of jaggery and treacle in Sri Lanka.

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