Journal Of Research Technology & Engineering
www.jrte.org

Production of plant-derived banana ripening spray using Kappetiya leaves (*Croton laccifer*)

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Received:05 Sep 2023; Revised: 11 Sep 2023; Accepted: 27 Sep 2023; Available online: 10 Oct 2023

Abstract: Fruit ripening is a natural process that includes physiochemical changes such as tissue softening, aroma and flavor production, pigment changes, etc. Induced ripening is essential in commercial -scale banana cultivation and distribution to assure good texture, flavor, and uniform peel color. However, the use of potentially toxic ripening agents such as ethylene gas, acetylene gas (calcium carbide-based), and ethephon can affect fruit quality and health-related issues (induction of cancer, cardiovascular dysfunction, diabetic mellitus, etc.). In Sri Lanka, smoke induced by Kappetiya (*Croton laccifer*) leaves is traditionally used in banana ripening. This study focuses on the development of a non-toxic, Kappetiya-derived banana ripening spray (KBRS) using Kappetiya leaves. Kappetiya crude was extracted from Soxhlet extraction using two different solvents (pure ethyl acetate and distilled water). According to FTIR analysis, C-H/N-H (2918.10 cm^{-1}, 2850.65 cm^{-1}), C=O (1711.75 cm^{-1}), NH2 (1375.18 cm^{-1}), C-O (1239.08 cm^{-1}), C-N (1044.88 cm-1), and C-X (608.65 cm^{-1}, 523.33 cm^{-1}) stretching peaks were obtained from the crude extract using ethyl acetate. KBRS has been tested on "Ambon banana" (*Musa acuminata*) as a common commercially available variety that takes more time to ripen in comparison to other banana varieties. KBRS has been prepared in a concentration series of 4000 ppm, 2000 ppm, 1500 ppm, 1000 ppm, 500 ppm, and 100 ppm and applied to green Ambon bananas and tested for physical parameters (peel color, black marks, color of the stalk, texture). The green Ambon banana applied with 100 ppm, 500 ppm, and 1500 ppm KBRS took more time (4 to 10 days) to ripen, while the banana applied with 1000 ppm KBRS ripened within 2 days without any black marks on the peel or stalk of the banana. However, the green Ambon banana applied with 4000 ppm and 2000 ppm of KBRS had black marks on the peel and stalk, and the fruits had bad texture. Accordingly, it concludes that 1000 ppm Kappetiya-derived banana ripening spray is the best selection to ripen the Ambon banana variety in Sri Lanka.

Index Term: Key Ambon banana (*Musa acuminata*), banana ripening, calcium carbide, Kappetiya (*Croton laccifer*), plant- derived

1 INTRODUCTION

Ripening is a biochemical process that involves a series of physiological changes in color, aroma, flavor, and texture that make the fruits both attractive and tasty. Bananas are one of the most consumed and popular fruits in the world. Once harvested, it is highly perishable, with a short shelf life leading to high post-harvest losses of 20% to 50% due to poor handling and

quality deterioration. To reduce these high post-harvest losses, bananas are harvested when green but mature and artificially ripened when needed with the use of ripening agents.[1]

In developing countries, the use of potentially toxic ripening agents is common because they provide fruits with the desired color and taste within a short period of time. Ripening agents are substances that accelerate the ripening process, and they come in different forms such as ethylene gas, ethephon, ethylene glycol, and calcium carbide[1]. Among other artificial ripening agents, calcium carbide (CaC_2) is the most widely used by vendors. It has been widely studied for its effect on fruit quality and health-related issues such as the induction of cancer, cardiovascular dysfunction, diabetes mellitus, etc. Researchers showed that CaC_2 contains impurities such as arsenic and other toxic and carcinogenic chemicals.[2] But even today, especially in developing countries, many vendors may consider the toxic effects negligible. Because of the potential health hazards related to the ripening agents, artificial banana ripening is highly debatable throughout the world. Because of that, most of the SAARC (South Asian Association for Regional Cooperation) countries have banned the use of calcium carbide as an artificial fruit ripening agent[3].

Therefore, there is an essential need to produce plant-derived, non-toxic, environmentally friendly, low- cost banana ripening syrup. The renewable nature of plant extracts and eco-friendly sprays makes them advantageous over other hazardous artificial agents.

Our study focused on the production of plant-derived banana ripening spray using Kappetiya (*Croton laccifer), which* falls into the category of non-food crops. Kappetiya (*Croton laccifer*) is a common plant species found in the deserted lands of Sri Lanka. In Sri Lanka, smoke generated from burning Kappetiya green leaves is traditionally used in banana ripening. That's why Kappetiya leaves were chosen to produce this banana-ripening spray. From there, we can determine whether the traditional method is actually true or false. And also, "Ambon banana (*Musa acuminata*)" was chosen to apply the prepared spray because it is a commercially very important banana variety and it takes a long time to ripen (7–10 days).

Soxhlet extraction was used to extract Kappetiya leaves due to the high yield; there was no need to filtrate the extracts[4], requires a smaller quantity of solvent compared to maceration[5] requires a smaller quantity of plant material compared to hydrodistillation. Soxhlet extraction is a conventional extraction method that is also called conventional Soxhlet extraction (CSE). This is a standard technique that has been used for a long time.[6]. Soxhlet extraction is commonly used in small research settings or at the small manufacturing enterprise (SME) level[5].

Fourier transform infrared spectroscopy FTIR spectroscopy was used to analyze *Croton laccifer* crude due to some advantages such as being relatively simple, reagent-free, low-cost, label-free, and nondestructive, which requires a low amount of sample to produce highly sensitive and reproducible results[7]. FTIR was originally a spectroscopic technique used to identify the functional groups of the chemical constituents[8]. This is a widely used technique for the analysis of samples in the research field.

The objectives and aims of the research were to extract the crude of the *Croton laccifer* leaves with high purity using the Soxhlet extraction method using two solvents such as ethyl acetate and distilled water, to identify the functional groups present in the *Croton laccifer* leaves through FTIR spectroscopy, to make a non-toxic, eco-friendly, and cost-effective spray, and to test the sprays in the laboratory to determine their effectiveness and choose the best spray to ripen banana.

2 MATERIALS AND METHODS

2.1 Plant Material

Kappetiya (*Croton laccifer*) leaves were collected in the Avissawella, Gampola and Galgamuwa area. Collected leaf samples were separated from the stem and hung to expose the plant to air at ambient temperature about 2 weeks to preserve heat-labile compounds. Then ground into powder form using a grinder and stored in a cellophane bag until used.

2.2 Extraction of the Plant material

Thimble chamber (250 ml) and round bottom flask (1L) were washed and kept in the oven until dry. After drying the round bottom flask, the weight of the flask was measured using an electrical balance. Approximately 15-25 g of dried and ground *Croton laccifer* leaves sample was weighed using an analytical balance and placed in a thimble made from cotton wool and then it was placed in the thimble chamber of the Soxhlet apparatus. Then set the apparatus after filling with 300ml of extraction solvent (ethyl acetate or distilled water) and switched on the heating mantle after opening the tap line. The optimum temperature was kept according to the boiling point of the solvent used. Extraction process proceeded for about 10 hours. After extraction, a rotary vacuum evaporator (at 60 ◦C for ethyl acetate trial and 80 ◦C for distilled water) was used in order to remove solvent. After that, the weight of the flask (1L round bottom flask which includes the extracted crude) was taken and weight of the extracted crude was calculated by reducing the empty flask's weight. The extracted crude was collected into a MC bottle by using a little amount of solvent (ethyl acetate or distilled water) and this solvent was evaporated using a water bath. Finally, the MC bottle with extracted crude was labelled and covered with an aluminum foil and stored in the refrigerator at 4°C.

2.3 Spray Preparation and application

1 g of the plant extract was measured using an electrical balance and dissolved in a 250 ml of 95% of ethanol solution (concentration of the plant extract in the solution is 4000 ppm) using a stirrer. A concentration series (4000 ppm, 2000 ppm, 1500 ppm, 1000 ppm, 500 ppm, 100 ppm) was prepared by dissolving 50 ml of stock solution with 50 ml of distilled water for 2000 ppm, 37.5 ml of stock solution with 62.5 ml of distilled water for 1500 ppm, 25 ml of stock solution with 75 ml of distilled water for 1000 ppm, 12.5 ml of stock solution with 87.5 ml of distilled water for 500 ppm and 2.5 ml of stock solution with 97.5 ml of distilled water for 100 ppm using a stirrer and 100 ml of stock solution was taken directly for 4000 ppm spray. Prepared sprays were applied to the Ambon Banana samples placed in trays (three replications for each spray) using spray bottles and a bunch with 2 fruits was kept as a control sample (non - treated sample). Observations were taken every 24 hours until a sample.

2.4 Plant Extracts analysis

Plant extracts obtained using ethyl acetate and distilled water solvents were analyzed using FTIR spectroscopy.

3 RESULTS AND DISCUSSION SECTION

In order to estimate the effect of Kappetiya (*Croton laccifer*) leaves on banana ripening, Soxhlet extraction was performed. The dark green Soxhlet extracts were obtained using ethyl acetate, and the dark brown Soxhlet extracts were obtained using distilled water. Both extracts were semi-solid under ambient conditions and had two different specific odors, different from those of the natural plant leaves' odor.

The yield obtained from the Soxhlet extraction is calculated as y = (input/output) 100%. Table 1 shows the yield obtained from Soxhlet extraction using ethyl acetate and distilled water as the solvents, according to the weight of the plant material used. C1T1, C2T1, and C3T1 yields were obtained as 12.53 %, 11.88 %, and 8.60 %, respectively, for three replications using ethyl acetate (11% of the average yield), and C1T2 yields were obtained as 26.79 % from the very first replication using distilled water. According to the results, the yield obtained using ethyl acetate is much lower than the yield obtained using distilled water.

Solvent used for extraction	Replication	Sample name	Weight of the plant material(g)	Weight of theextracts (g)	Yield (%)
Ethyl acetate	1	C1T1	15.0059	1.88	12.53
	2	C2T1	25.0088	2.97	11.88
	3	C3T1	25.0041	2.15	8.60
Distilled water	1	C1T2	25.0070	6.70	26.79

Table 1. Table of the yield obtained using Soxhlet extraction

Prepared Kappetiya-derived banana ripening sprays (KBRS) were applied to the Ambon banana samples and tested for physical parameters (peel color, black marks, color of the stalk, texture). To determine the color of the banana samples, a banana ripening color chart (Figure 1) was used. According to the chart, S4- stage ripened bananas are ready for retail display, and S5 - stage ripened bananas are most popular for consumer purchase. But in Ambon bananas, S4 -stage ripened bananas are also most popular with consumers. The Ambon banana samples treated with KBRS prepared from the crude obtained using ethyl acetate were given the following observations: the green Ambon banana applied at 100 ppm, 500 ppm, 1500 ppm, and 2000 ppm KBRS took more time to ripen (4 to 10 days took to ripen to S4 stage), while the KBRS with 1000 ppm concentration applied banana ripened within 2 days (ripened to S4 stage after 48 hours). The green Ambon banana applied with 4000 ppm and 2000 ppm of KBRS had black marks on the peel, the stalk got a black color, and had a bad texture, while the KBRS with 1000 ppm concentration applied banana ripened without any black marks on the peel or stalk of the banana and was good in texture. The Ambon banana samples treated with KBRS prepared from the crude obtained using distilled water were given observations such as the green Ambon banana applied at 100 ppm, 500 ppm, 1000 ppm, and 2000 ppm. KBRS took more time to ripen (8 to 10 days took to ripen to S4 stage), and the KBRS with 1500 ppm and 4000 ppm concentration applied banana took 7 days to ripen (ripened to S4 stage after 144 hours), there were no black marks on the peel or stalk, and all the samples were good in texture. The control sample (non-treated, i.e., the Ambon banana sample, which has not been treated with any of the spray) had begun to ripen on the 7^{th} day (ripen to S3 stage), and it had ripened to S4 stage on the 8^{th} day.

However, according to the observations, sprays prepared from the crude obtained using ethyl acetate give better results than sprays prepared from the crude obtained using distilled water. The best spray to ripen bananas is KBRS with 1000 ppm because it ripened bananas within 2 days

without any black marks on the peel and with good texture. It might be due to the optimum concentration of Kappetiya (*Croton laccifer*) leaf extract. Because the sprays prepared from the crude obtained using distilled water took 7 days to ripen bananas and the non-treated samples also ripened within 7 days, they may not have much effect on the ripening of bananas.

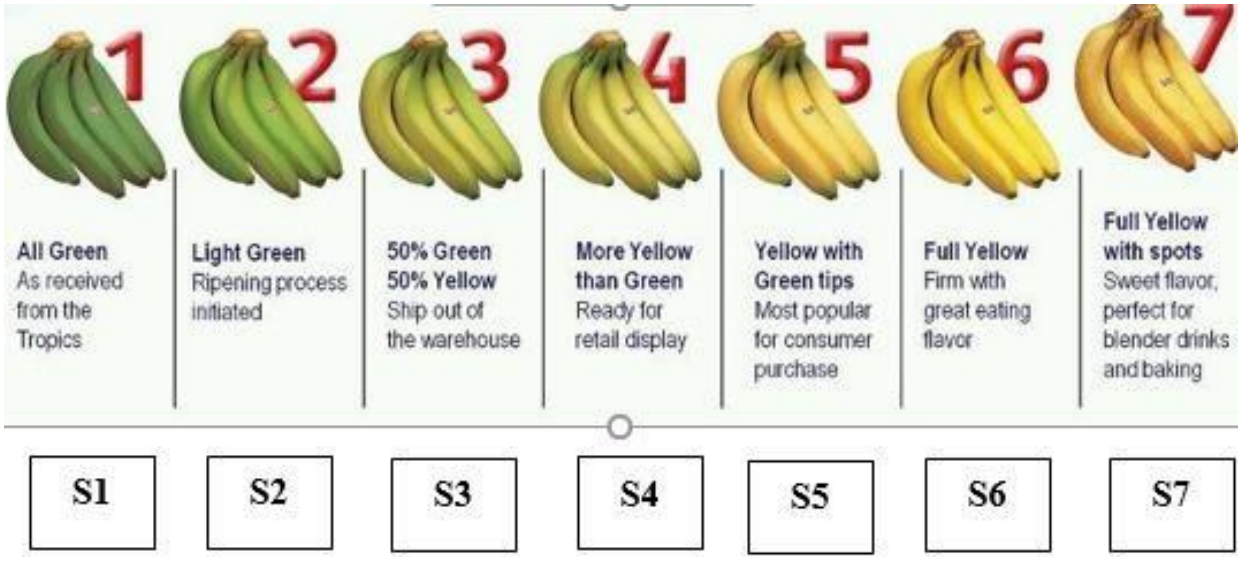

Fig. 1. Banana ripening color chart

Figure 2 and 3 shows the FTIR analysis of the extracted crudes. It also shows the different functional groups from the ethyl acetate (semi-polar solvent) and distilled water (polar solvent). Alkane (C-H stretching) or amine salt (N-H stretching) was resulted at the wavenumber 2918.10 cm^{-1} and 2850.65 cm^{-1} at the wave number range 3000-2800 cm^{-1} and 3000-2840 cm^{-1}, Carboxylic acid or aliphatic ketone (C=O stretching) was resulted at the wave number 1711.75 cm^{-1} at the wave number range 1725-1705 cm^{-1} and 1720-1706 cm^{-1}, (NH_2 stretching) was resulted at the wave number 1375.18 cm^{-1}, alkyl aryl ether (C-O stretching) was resulted at the wave number 1239.08 cm^{-1} at the wave number range 1275-1200 cm^{-1}, Amine stretching (C- N) was obtained at the wave number 1044.88 cm^{-1} at the wave number range 1250-1020 cm^{-1}, alkyl halides (C-X stretching) was obtained at the wavenumber 608.65 cm^{-1} and 523.33 cm^{-1} at the wavenumber range 690-515 cm^{-1} and 600-500 cm^{-1} from the crude obtained using ethyl acetate. Alcohol (O-H stretching) was resulted at the wave number 3268.96 cm^{-1} at the wave number range 3550-3200 cm^{-1}, Conjugated alkene or cyclic alkene (C=C stretching) was resulted at the wave number 1602.34 cm^{-1} at the wave number range 1650-1600 cm^{-1}, primary alcohol (C-O stretching) or (C-N bending) was obtained at the wavenumber 1397.11 cm^{-1} and 1319.89 cm^{-1}, alkyl halides (C-X stretching) was obtained at the wave number 581.86 cm-

1 and 529.18 cm^{-1} at the wave number range 690-515 cm^{-1} and 600-500 cm^{-1} from the crude obtained using distilled water. According to the Subhankar Das et al., FTIR analysis of *Croton caudatus*, it shows N-H stretching, C-H stretching, C=O groups, NH_2 stretching, C-O bending, C=C stretching, C-N bending, C-X stretching of alkyl halides. So that we can conclude that these FTIR results are correct because both *Croton laccifer* and *Croton caudatus* are *Croton* species and these two show somewhat the same results.

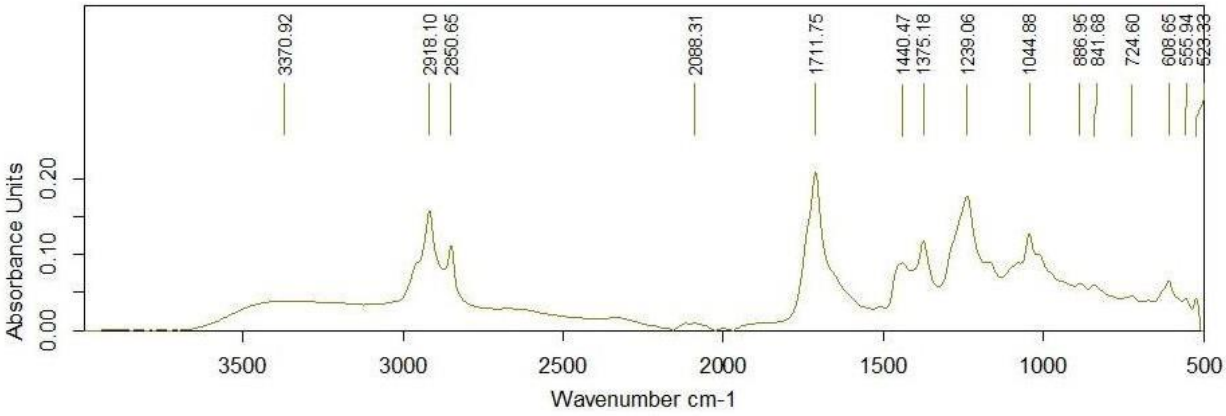

Fig. 2. FTIR spectra of the extracted crude obtained using ethyl acetate

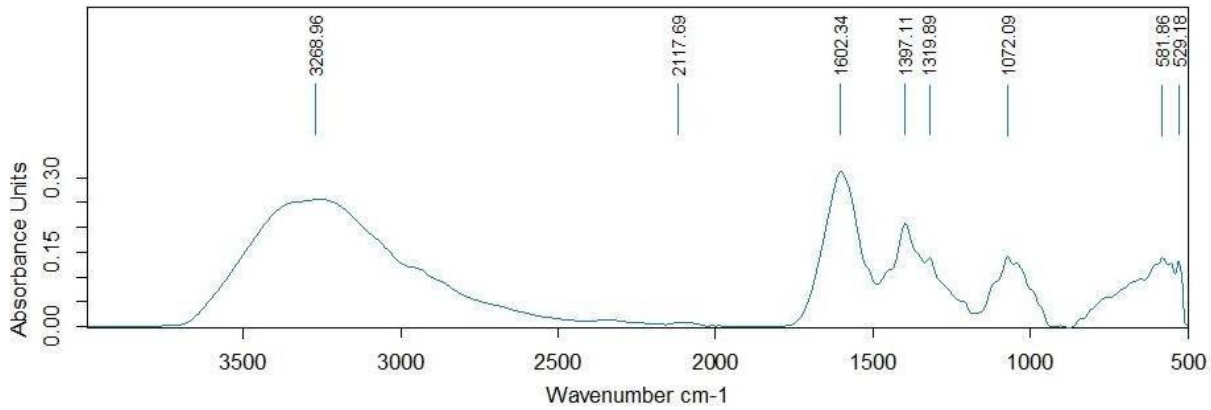

Fig. 3. FTIR spectra of the extracted crude obtained using distilled water

8 CONCLUSION

Kappetiya (*Croton laccifer)-derived* spray with a 1000 ppm concentration prepared from the crude obtained using ethyl acetate is the best spray to ripen bananas. Kappetiya (*Croton laccifer)-derived* sprays prepared from the crude obtained using distilled water have little effect on banana ripening. The traditional method of banana ripening using Kappetiya (*Croton laccifer*) leaves is a scientifically true method because Kappetiya (*Croton laccifer*) has an effect on banana ripening. According to FTIR

analysis, C-H/N-H, C=O, NH_2, C-O, C- N, and C-X stretching peaks were obtained from the crude extract using ethyl acetate.

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