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Preliminary study of the selected plants from Besut coastal forest for controlling pre and post-harvest fungal pathogen

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Plant pathogenic fungi are major problems in the agricultural sector in Malaysia. During storage, fruits and vegetables are often subject to varying levels of microbial decay, mainly due to pathogenic fungi, which usually infect the host through wounds sustained during harvest, handling and processing. Besut coastal area has a beach forest which content plants that have growing on BRIS (Beach Ridges Interspersed with Swales) soil with the characteristic of sand, low fertility status, excessive leaching and high surface. The present study aimed to evaluate the antifungal properties of the selected plant that has grown on BRIS soil at Besut beach forest in controlling postharvest fungal pathogen. Seven plants were selected: *Melaleuca cajuputi* (Gelam plant), *Baeckea frutescens* (Cucur atap plant), *Anacardium occidentale* (Gajus plant), *Carallia suffruticosa* (Sisik puyuh plant), *Chromolaena odorata* (Kapal terbang plant), *Garcinia nervosa* (Manggis hutan plant), and *Catunaregam tomentosa* (Badang plant). Various concentrations of leaf extracts were evaluated using the well diffusion method and disk diffusion method against *Curvularia* sp., *Colletotrichum* sp., *Fusarium* sp., *Phytium* sp., and *Rhizopus* sp. of pathogenic fungi. Results indicated that the mycelial growth of most of the selected fungi was reduced in all culture media contain the selected plant's extract. Furthermore, through two antifungal test method, plant extract from *Baeckea frutescens* shows the highest antifungal activity among other plant extracts with a minimal inhibitory concentration (MIC) of 100 mg**.** Inhibition zone for *Colletotrichum* sp, by well diffusion method *B. frutescencs* (29.8%)> *M. cajuputi* (12.3%)> *C. suffruticosa* (11.5%)> *C. tomentosa* (9.0%)> *A. occidentale* (9.2%) > *G. nervosa* (9.0%) > *C. odorata* (8.0%) conclusion the selected plants from Besut Coastal has antifungal properties in controlling postharvest pathogen.

Keywords: pathogen, antifungal, postharvest, coastal forest

INTRODUCTION

Despite the use of sophisticated agricultural technologies, storage facilities, and processes, global agricultural production losses owing to damaging plant fungi are estimated to range from 20% to 40% each year (Alotibi et al. 2020). The impact on plant wellbeing is a significant and long-term danger to food production and the global economy (Agrillo et al. 2019). Microbe infection can occur during the growing season, during harvest, during handling, storage, transport, and marketing, or even after the consumer has purchased the product. Microbial infections are the leading cause of 20% production loss and 10% postharvest loss in key food crops (Bleackley et al. 2019). Fruit and vegetable postharvest

infections frequently cause them to rot, which has a detrimental impact on their storage, shelf life, and, ultimately, their economic value. To avoid excessive output losses and a somewhat consistent crop protection method, producers grew highly relied on agrochemicals such as insecticides. This contributes to the financial stability of their operations. However, chronic use of chemical fungicides, pesticides, herbicides, and fertilisers is posing serious health and environmental concerns (Agrillo et al. 2019), and when genetically engineered, the plant develops resistance to broad-spectrum herbicides (Alotibi et al. 2020).

There are several fungal pathogens that associate with loss during pre and postharvest. *Colletotrichum* spp., for example, is an imperfect fungus belonging to the *Melanconiales* family, which contains some of the most successful plant pathogenic fungal species worldwide, causing high economic losses to a wide range of woody and herbaceous crops, especially fruit, vegetables, and ornamentals, both in tropical and temperate regions. These diseases are commonly referred to as anthracnose. (Landum et al. 2016; Morsy and Elshahawy, 2016). Major pineapple production in Brazil faced serious problem due to fusariosis, a disease caused by the fungus, *Fusarium guttiforme* Nirenberg & O'Donnell (*F. guttiforme*) (Sales et al. 2016). *Fusarium* is recognized to affect crops, resulting in jeopardized food security and less agricultural crop yields (Mongalo et al. 2018).

The ways to reduce pre and postharvest losses is by using the antimicrobial agent. There are numerous antimicrobial agents on the market, including metal ions (silver, copper, gold, and platinum), metal oxides (TiO_2, ZnO, and MgO), essential oils (thyme, oregano, pimento, clove, citron, lemon verbena, lemon balm, and cypress leaf), plant extracts (grape seed, green tea, pomegranate peel/rind, acerola, pine bark, bearberry) (Vilela et al. 2018). Nowadays more people preferred about natural-based antimicrobial agent.

This study emphasizes the use of plant extract as one of the antimicrobial agents as it is more safe and eco-friendly. Currently, research into natural products for pest management, particularly aromatic and medicinal plants (Sales et al. 2016), is gaining a lot of traction because plant extracts pose no health risk or pollution and are less expensive than chemicals with minimal or no adverse side effects on hosts (Sales et al. 2016). (Alotibi et al. 2020). Plant extract has been shown to be an effective antibacterial agent in numerous researches. Plant extract, for example, has been shown to help reduce rice blast disease caused by the pathogen *Magnaporthe grisea* in a study by Dar et al. (2018).

The East Coast region of Peninsular Malaysia comprises Kelantan state, Terengganu state, Pahang state and a small part of Johor state. This region is remarkable in that it has a coastal forest that is not found anywhere else in Malaysia, and it is made up of dune ridge soil with a sand content of more than 90% that is dispersed across Kelantan (17,806 ha), Terengganu (67,582 ha), Pahang (36,017 ha), and a small part of Johor (Jamilah et al. 2014). Due to its sandy texture, Coastal Forest is infertile and is usually regarded as inappropriate for agricultural use and is left under a forest of grassland vegetation. Coastal Forest has been populated and used for agriculture on the East Coast region of Peninsular Malaysia, unlike in other places, due to a lack of arable native land and rising demand for food (Hafiz et al. 2017). A total of 41 genera of woody plants from 22 families have been reported, comprised 44 identified species with 451 individual stems. The five most dominant families were Dipterocarpaceae, Myristicaceae, Ebenaceae, Annonaceae, and Sapindaceae (Jamilah et al. 2014).

Besut Beach Coastal Forest in Terengganu East Coast of Peninsular Malaysia is unique for its coastal forest is used for agricultural activities and the variety of plants that can grow on dune ridge. Some of the native vegetation in Beach Forest has been taken down, even though these plants are beneficial to local agricultural activity. The goal of this study was whether some plants in the Besut Beach Coastal Forest could prevent pre- and postharvest pathogens for agricultural purposes. As a result, seven plants were chosen based on their plentiful abundance in the Besut Beach Coastal Forest.

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Table 1: Ethno botanical uses of selected plants

Plant names	Family	Common uses	References
Melaleuca cajuputi	Myrtaceae	treat cholera, muscular pain, diarrhoea, scabies, and intestinal worms	(Al-Abd et al. 2016)
Anacardium occidentale	Anacardiaceae	Folk remedy for treating diabetes mellitus, diarrhoea, skin diseases, fevers, aches and pains and various inflammatory conditions such as arthritis	(Jaiswal et al. 2017)
Carallia suffruticosa	Rhizophoraceae	Leaves are applied as a poultice to boils and to reduce fever. A decoction of the leaves is taken to expel worms from the intestines	(Rusli et al. 2019)
Chromolaena odorata	Asteraceae	treatment of coughs and colds, skin infections, dysentery, wounds, toothache, malaria, stomach problems, diarrhoea, stomach ulcers, and also bacterial and fungal infections	(Omokhua et al. 2017)
Garcinia nervosa	Clusiaceanative	Treatment of abdominal pain, infected wound, dysentery, diarrhoea, suppuration, leucorrhoea, chronic ulcer and gonorrhoea	(Seruji et al. 2013)
Catunaregam tomentosa	Rubiaceae	Leaves used to cool down, cure yellow urine, urinating, boiling the stomach	(Weerapreeyakul et al. 2016; Nassar and Adss, 2016)
Baeckea frutescens	Myrtaceae	Remedies for influenza, malaria, fever, headache, abdominal pain dysentery, jaundice, measles, irregular menstrual cycles, massaging postpartum women, rheumatism and snake bites	(Adib et al. 2014; Ito et al. 2016)

MATERIALS AND METHODS

Plant Leaves Sample Collections

Fresh matured leaves of seven plants including *Melaleuca cajuputi* (Gelam plant), *Baeckea frutescens* (Cucur atap plant), *Anacardium occidentale* (Gajus plant), *Carallia suffruticosa* (Sisik puyuh plant), *Chromolaena odorata* (Kapal terbang plant), *Garcinia nervosa* (Manggis hutan plant),) and *Catunaregam tomentosa* (Badang plant) were randomly selected and collected from the wild surround Besut beach coastal area. Ethnobotanical information of these plants listed in Table 1. About 1 kg of fresh leaves per plant were harvested, rinsed with tap water, and dried in an oven dryer for 24 hours at 40^{o}C. Using an electric blender, the dried plant components were mashed into a fine powder and stored in an airtight container.

Extraction method

In this study, the maceration method was used for extraction (Moharrami and Hashempour, 2021). Methanol was added to leaf powder at a concentration of 99 percent and macerated for 24 hours, with three replications for a total of 72 hours in the dark at room temperature. Filtration was used to collect the extract solution. The extracts were then dried in a rotary vacuum evaporator to remove the solvent.

Antifungal screening method

Two approaches were utilised to screen antifungal activity: the agar-disk diffusion method and the agar well diffusion method.

Agar-Disk Diffusion Method

Microbiology experiments are conducted using the official method of agar disc diffusion testing (Balouiri et al. 2016). In this investigation, a 120-hour old fungal plug from the culture agar plate was placed in the centre of the test agar plate using a 1 mm diameter (cut using a cork borer) fungal plug from the culture agar plate. Then, 3 paper discs (about 6 mm in diameter) containing the test compound (plant extract) at a concentration of 100 mg/mL were placed on the agar surface (25 mm from the centre), with 5 replicates.

Agar Well Diffusion Method

The antimicrobial activity of plants or microbial extracts is frequently assessed using the agar well diffusion method. 1 mm (plug using cork borer) of 120-hour old fungi from the culture agar plate was placed in the centre of the test agar plate, similar to the procedure employed in the disk-diffusion method. Then, using a sterile cork borer, 3 holes with a diameter of 6 mm were punched aseptically, and a volume (20–30 uL) of the antimicrobial agent or extract solution at a concentration of 100 mg/mL was put into the well at a distance of 25 mm from the centre. Then, depending on the test microorganism, agar plates were incubated under the appropriate conditions. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. The tests were run in five replicates. The inhibition zone was calculated using the following formula: (C – T)/ C x 100, where C represents the radial growth of the test pathogen in the control plate (measured in mm) and T represents the radial growth of the test pathogen in the test plates (mm) (Tamreihao et al. 2018).

Statistical Analysis

This study used a complete randomised design with 5 replicates per extract per fungi. The data presented are the mean and standard error (mean S. E.). The statistical significance of the differences between samples was determined using the Duncan test at a significance level of P = 0.05 in SPSS version 24.0. (IBM Corp, Armonk, USA). When P 0.05, a difference was considered statistically significant and was denoted by a different letter.

RESULTS

The result presented in Figure 1 showed that the mycelial growth inhibition of the 5 fungi by 7 plant extracts in two antifungal screening method. A significant difference in the *Baeckea frutescens* leaf extract against fungi was noticed compared with other plant extracts in mycelial growth inhibition of *Colletotrichum* sp., *Curvularia* sp., *Fusarium* sp., *Phytium* sp in Disk diffusion method and *Rhizopus* sp. Among 5 fungi, *B. frutescens* leaf extract to show the highest mycelial growth inhibition of *Colletotrichum sp.* with 25% by disk diffusion method and 29.8% by well diffusion method. While *Melaleuca cajuputi* shows the highest mycelial growth inhibition of *Phytium* sp. Compared with *B. frutescens* by 11.4% (disk diffusion method) and 20.6% (well diffusion method). Interestingly all plant extracts detected are known to have some antifungal properties with *Baeckea frutescens* show the highest antifungal activities.

From Table 2 and 3, can be seen that the radial growth of *Colletotrichum* sp. in the test plate

consists of *B. frutescens* extract show the slowest compared to control and other extracts. Even on Day 5 and 6 (Figure 2 and 3), all the test plate pathogen and control reach the test disk and well except the test plate with *B. frutescens*.

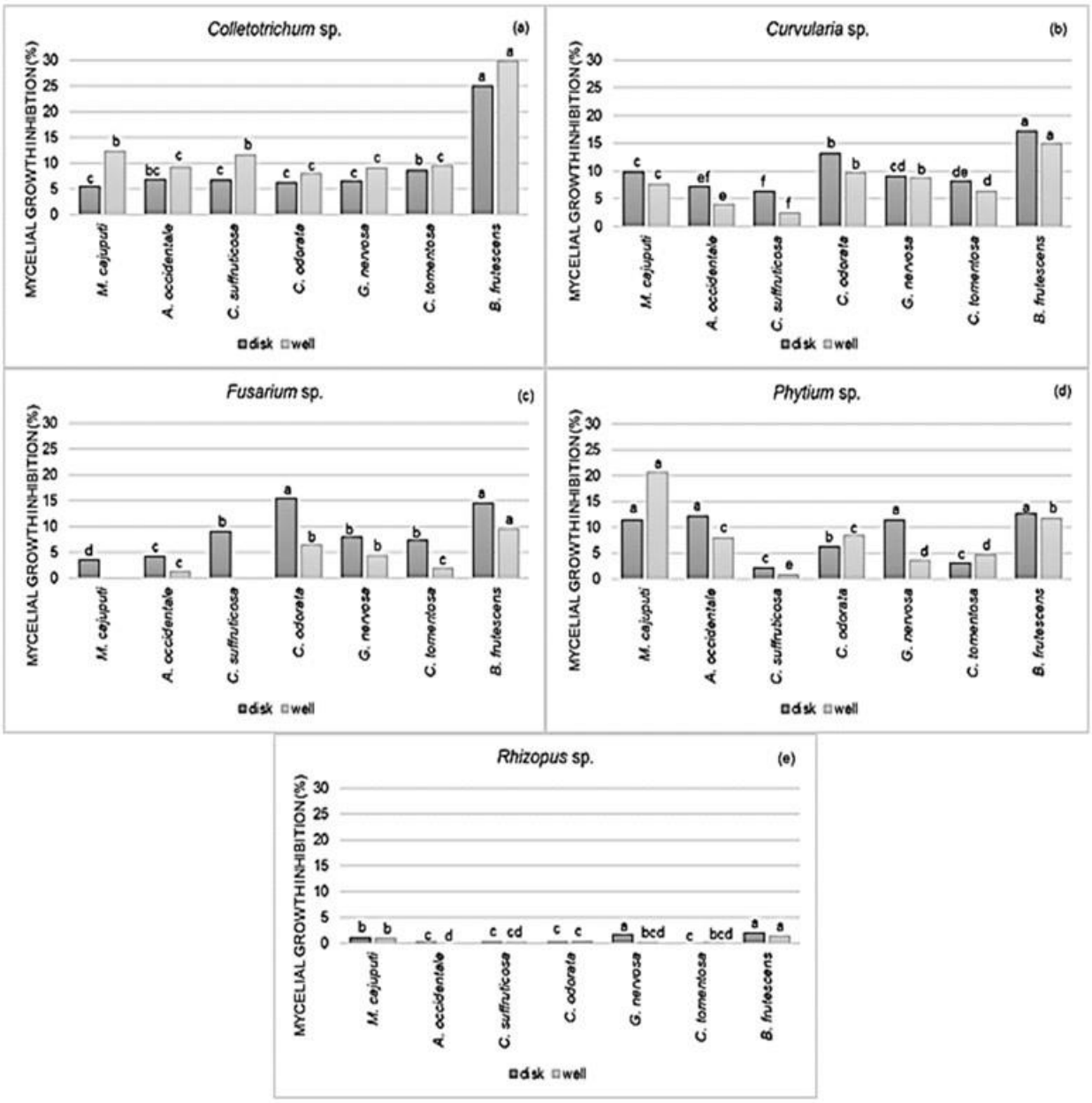

Figure 1: the mycelial growth inhibition of the 5 fungi (a) *Colletotrichum* sp. (b) *Curvularia* sp. (c) *Fusarium* sp. (d) *Phytium* sp. and (e) *Rhizopus* sp. by 7 plant extracts in two antifungal screening method (disk and well diffusion) by the same letter are not significantly different based on LSD at p = 0.05 according to Duncan's multiple range test.

Table 2: Comparison of the radial growth of *Colletotrichum* sp. by disk diffusion method (cm) for 6 days

Plant Extract	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
M. cajuputi	1.53±0.17^{bc}	8.07±0.15d^{e}	14.33±0.13^{d}	20.20±0.31^{b}	23.00±0.00^{b}	23.00±0.00^{b}
A. occidentale	1.53±0.12^{bc}	7.47±0.17b^{c}	13.73±0.12^{c}	19.93±0.18^{b}	23.00±0.00^{b}	23.00±0.00^{b}
C. suffruticosa	1.67±0.16^{c}	7.53±0.23^{bcd}	13.60±0.29^{c}	20.13±0.22^{b}	23.00±0.00^{b}	23.00±0.00^{b}
C. odorata	1.87±0.13^{c}	7.93±0.15^{cde}	13.93±0.15^{cd}	19.60±0.29^{b}	23.00±0.00^{b}	23.00±0.00^{b}
G. nervosa	1.67±0.19^{c}	7.80±0.20^{cd}	13.60±0.27^{c}	20.00±0.34^{b}	23.00±0.00^{b}	23.00±0.00^{b}
C. tomentosa	1.13±0.09^{ab}	7.20±0.11^{b}	13.00±0.20^{b}	19.80±0.20^{b}	23.00±0.00^{b}	23.00±0.00^{b}
B. frutescens	1.00±0.00^{a}	5.07±0.18^{a}	10.07±0.12^{a}	15.00±0.20^{a}	18.93±0.35^{a}	21.40±0.25^{a}
control	2.27±0.12^{d}	8.47±0.19^{ef}	16.47±0.19^{e}	22.07±0.28^{c}	23.00±0.00^{b}	23.00±0.00^{b}

Data represented as mean ± standard error. Means in the same column followed by the same letter are not significantly different based on LSD at p = 0.05 according to Duncan's multiple range test.

Table 3: Comparison of the radial growth of *Colletotrichum* sp. By well diffusion method (cm) for 6 days

Plant Extract	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
M. cajuputi	1.80±0.15^{b}	7.20±0.11^{bc}	12.47±0.22^{b}	19.47±0.17^{b}	22.93±0.07^{b}	23.00±0.00^{b}
A. occidentale	1.93±0.15^{b}	7.73±0.25^{cd}	12.87±0.26^{bc}	21.40±0.21^{d}	23.00±0.00^{b}	23.00±0.00^{b}
C. suffruticosa	1.73±0.12^{b}	7.07±0.23^{b}	12.60±0.41^{b}	20.47±0.37^{c}	22.80±0.11^{b}	23.00±0.00^{b}
C. odorata	2.80±0.24^{c}	8.40±0.35^{e}	13.60±0.57^{cd}	20.53±0.26^{c}	22.80±0.15^{b}	23.00±0.00^{b}
G. nervosa	1.87±0.17^{b}	8.20±0.18^{de}	14.00±0.14^{d}	20.40±0.16^{c}	22.67±0.13^{b}	23.00±0.00^{b}
C. tomentosa	1.67±0.16^{b}	7.67±0.13^{bcd}	14.00±0.24^{d}	20.53±0.24^{c}	22.80±0.15^{b}	23.00±0.00^{b}
B. frutescens	1.13±0.10^{a}	5.27±0.12^{a}	9.93±0.21^{a}	15.07±0.15^{a}	18.07±0.27^{a}	20.07±0.21^{a}
control	3.80±0.26^{d}	9.60±0.19^{f}	16.73±0.15^{e}	22.93±0.07^{e}	23.00±0.00^{b}	23.00±0.00^{b}

Data represented as mean ± standard error. Means in the same column followed by the same letter are not significantly different based on LSD at p = 0.05 according to Duncan's multiple range test.

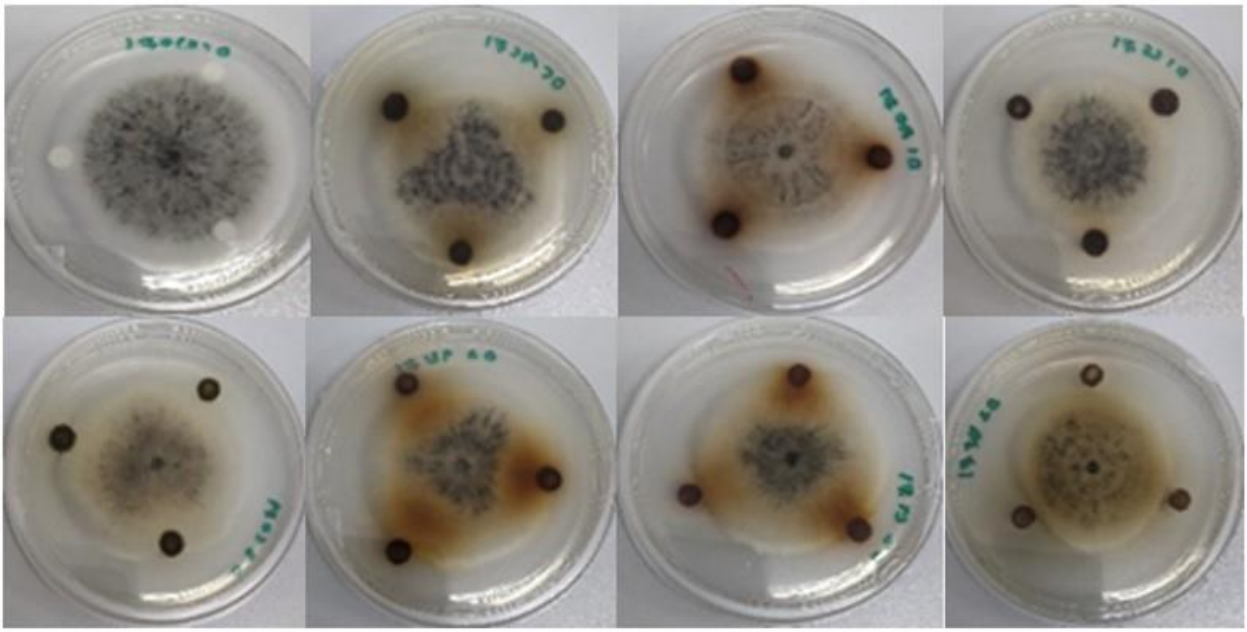

Figure 2: Radial growth at day 6 of *Colletotrichum* sp. by disk diffusion method (a)Control, (b)*M. cajuputi*, (c)*A. occidentale*, (d)*C. suffruticosa*, (e)*C. odorata*, (f)*G. nervosa*, (g)*C. tomentosa* and (f)*B. frutescens.*

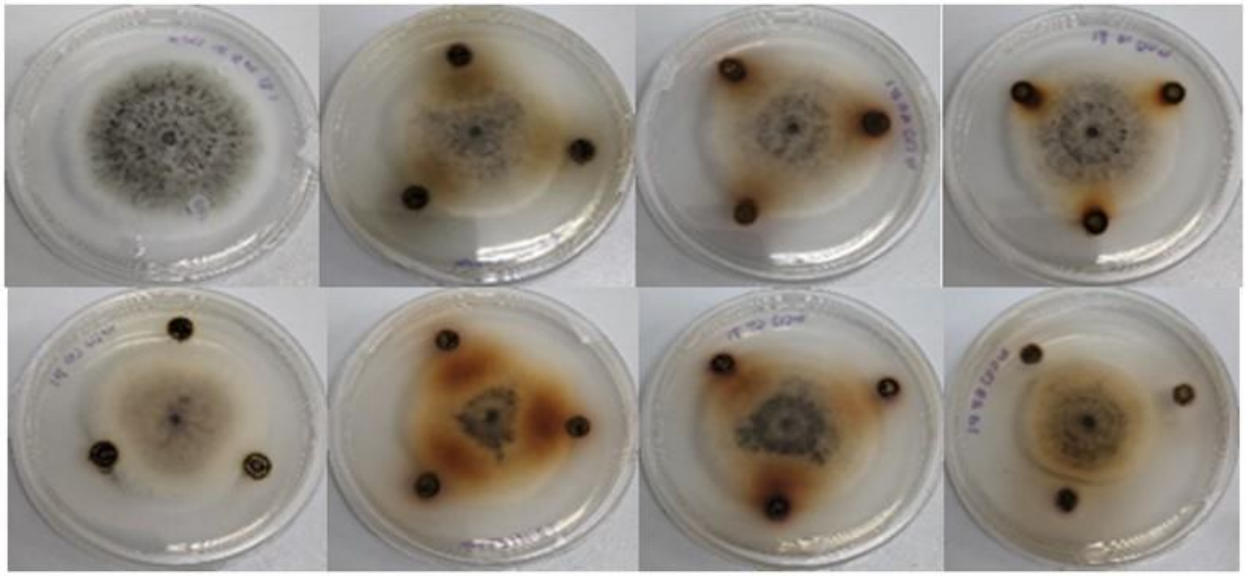

Figure 3: Radial growth at day 6 of *Colletotrichum* sp. by well diffusion method (a)Control, (b)*M. cajuputi*, (c)*A. occidentale*, (d)*C. suffruticosa*, (e)*C. odorata*, (f)*G. nervosa*, (g)*C. tomentosa* and (f)*B. frutescens.*

DISCUSSION

There is a growing interest in the use of biological control agents in agriculture. As one of the agents, many plant extracts have been studied. Current control measures are based on the use of chemical treatments as a preventive measure, the selection of resistant cultivars, and early harvesting (Landum et al. 2016). In this study, two methods of the antifungal test were performed to select the best plant extract to inhibit the pathogenic fungal growth with 7 plant extracts and 5 pathogenic fungi. Agar disk diffusion is a well-known official method for routine antimicrobial susceptibility testing in many clinical microbiology laboratories, but it is ineffective for determining the minimum inhibitory concentration (MIC) because it is impossible to quantify the amount of antimicrobial agent diffused into the agar medium. As a result, the agar well diffusion method was used to support the results of the agar disc diffusion method. This approach has long been used to assess the antibacterial activity of plant or microbial extracts (Balouiri et al. 2016).

Plant extracts possess biological activities that are beneficial to agricultural production. Many plant diseases caused by fungi have been inhibited by plant extracts. Dar et al. 2018 discovered that leaf extracts from most of the plants examined have antifungal activities and might be utilised as a seed and foliar treatment. The results show that different plant extracts varied in their effectiveness in inhibiting fungi growth. This because the diversity in the phytochemical components of plant extract, for example, the secondary metabolites of plants, even those obtained from the same species, may result in different responses, especially with esteem the potential for microorganism inhibition. Particularly plant reported to have antimicrobial properties contain a range of secondary metabolites such as alkaloids, quinones, flavonoids, glycosides, saponins, tannins and terpenoids. The concentration of these bioactive compounds in each plant species depends on the environmental conditions and pathosystem (Sales et al. 2016). In agreement with our results, previous study on the plant extracts of *Rumex vesicarius* L. and *Ziziphus spina-christi* (L) Desf. exhibited antifungal activity against *Fusarium*, *Helminthosporium*, *Alternaria*, and *Rhizoctonia* species (Alotibi et al. 2020). Besides, a study on the South African medical plants extract has concluded that the plant species with good antifungal properties and antioxidant activity are potent and may be used to inhibit the growth of such fungal strains by blocking decay of foodstuffs (Mongalo et al. 2018).

Colletotrichum sp. has been ranked in the top ten fungal pathogens due to their scientific and economic importance they can cause losses not only in the field (pre-harvest) but also during storage (post-harvest) (Landum et al. 2016). Sesquiterpenes, flavonoids, flavones, phloroglucinols, chromones, and chromanones are abundant in *B. frutescens*. This species'

phytochemical investigation has resulted in the isolation of new phloroglucinols, including baeckenone B, which has antibacterial activity against *Bacillus subtilis* (Ito et al. 2016). As a result, for antifungal properties, *B. frutescens* was suggested to have a special phytochemical that inhibited the mycelial growth of the pathogen fungal. According to Razmavar et al. (2014), *B. frutescens* extract contains phytochemical components with the most important applications against human pathogens, including those that cause endocrine disruption. Furthermore, total flavonoids have been identified as the most important bioactive components, with anti-inflammatory and enhanced antioxidant activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) enzymes (Jia et al. 2014).

CONCLUSION

The plant extract's antifungal activities have provided promising evidence of its potential as a promising safe and environmentally friendly candidate for future applications as a bio control agent in minimising postharvest losses caused by fungal plant pathogens, and thus serve as a natural alternative to conventional fungicides in agriculture. However, more research is needed to identify and describe the key phytochemical molecule that inhibits fungal development, as well as its involvement in disease prevention.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

NNNMN designed and performed the experiments and also wrote the manuscript. KMS main supervised, designed experiments and reviewed the manuscript. MHS plant pathology supervised. NEMN and NAMR postgraduate researcher group experiment material support. All authors read and approved the final version.

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