ANTI-SOFT ROT AND ANTIOXIDANT BEHAVIOUR OF NINE MEDICINAL PLANTS FROM PAYA BUNGOR, PAHANG

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Abstract

Medicinal plants have been gaining attention for their ability to control plant diseases. In searching of an alternative anti-soft rot agent from natural sources to substitute hazardous synthetic bactericides, we aimed to investigate the potential of plant extracts from nine medicinal plants from Paya Bungor, Pahang, as a potent anti-soft rot and antioxidant agent. Extracts were tested in-vitro against one phytopathogenic bacteria namely *Erwinia chrysanthemi*, a causal agent of soft rot disease. The antibacterial assay and antioxidant work were held using agar diffusion method and qualitative dot blot assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent was used to screen antioxidant properties. Among the nine plant species, the methanol extract from *Coscinium fenestratum* (akar sekunyit) exhibited a marked inhibitory effect in-vitro on *E. chrysanthemi* pathogen with the inhibition zone of 19.67 mm. The antioxidative behavior could be seen in all types of the plant extracts. Therefore, the use of these plants against *E. chrysanthemi* to inhibit bacteria growth could be exploited in the future to replace chemical pesticides apart from antioxidative source. The data could be used as a basic scientific information to ensure the use of medicinal plant in traditional practice as well as the development of natural pesticides and medicines in future.

Keyword: Antioxidant, anti-soft rot, medicinal plant

Introduction

Nowadays, medicinal plants are widely used as alternative tools other than synthetic bactericides to control plant disease. The use of medicinal plant as a natural origin does not adversely affect the environment compared to the existing chemical bactericides which are often associated with environmental pollution and loss of beneficial soil biodiversity. Therefore, the use of medicinal plant in controlling plant disease has been gaining popularity despite its application in substituting chemical or synthetic pesticides being considered challenging.

As it is known, most medicinal plants contain important natural active ingredients such as alkaloid, phenolic and terpenoid that act as antimicrobial sources and antioxidants. The presence of alkaloid and phenolic would contribute to antioxidant properties while terpenoid would contribute to antimicrobial properties (Ndivo et al., 2018). Soft rot disease caused by plant phytopathogens has caused huge losses in the agriculture sector. It was reported that the use of medicinal plants to possess antimicrobial action against plant pathogen of *Sclerotium rolfsii* (Sacc.) showed less severity of collar rot disease on tomatoes (Mahato et al., 2018).

A study also investigated the efficacy of medicinal plants such as *Zingiber officinale* Rosc., *Piper nigrum* Linn., *Azadirachta indica* A. Juss., *Nicotiana tabacum* Linn and *Carica papaya* Lam. as well as a synthetic fungicide (mancozeb) using various concentrations for the management of yam tuber rot fungal pathogen caused by *Penicillium expansum* isolated from rotted yam tubers in storage (Gwa et al., 2018). It was found that among the extracts tested, *P. nigrum* was the most effective one with the highest growth inhibition of 76.29% at 90 g/L concentrations followed by *Z. officinale* with the highest growth inhibition of 72.06% at 90 g/L too.

Yang and Zhang (2019) recently reported the use of polyphenolic compound from green tea leaves. The phenolic compounds which were flavonoids were known as catechins: epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) extracted from tea exhibited antimicrobial properties against a wide spectrum of plant pathogens including *Erwinia* species.

Soft rot disease caused by phytopathogen such as *Pectobacterium carotovorum and E. amylovora* has been observed in various crops which led to yield shortages and economic losses. Investigation revealed the alkaloid extract from medicinal plants reduced the severity of the disease on potato, pear and onion caused by these phytopathogens (Shaheen and Issa, 2020).

Since many studies have been done to inhibit the growth of plant pathogens using various medicinal plants, we present our study on soft rot plant disease caused by *E. chrysanthemi* using nine medicinal plants from Paya Bungor, Pahang, Malaysia, to examine their potential as a natural anti soft rot agent.

Materials and Methods

Preparation of plant extracts

All of the selected medicinal plants namely *Mapania cuspidate*, *Cinnamomum camphora, C. fenestratum*, *Cratoxylon ligustrinum*, *Homalomema sagittifolia*, *Tacca integrifolia, Tetracera indica, Ziziphus oenoplia* and *Fagrea elliptica* were obtained at Paya Bungor Forest, Kuantan Pahang, and were authenticated by botanist Dr Shamsul Kamis from Universiti Kebangsaan Malaysia. The leaves were washed, dried and ground into fine powder. The powdered samples were then soaked consecutively with hexane, dichloromethane and methanol until they became colorless. The maceration was filtered and evaporated using rotary evaporator until viscous colored liquid was formed. The hexane, dichloromethane and methanol crude extract were properly labelled prior to use.

Antibacterial Activity

The antibacterial activity of all extracts of the selected plants against *E. chrysanthemi* was slightly modified from Maha Yudden et al. (2019). A concentration of 200 mg/ml of each crude extract was used in the preparation of sterile impregnated disc. The discs were used in disc diffusion method to evaluate the effectiveness of extracts by measuring the zone of inhibition around the disc after 24 hours of incubation at room temperature. The greater the diameter of the inhibition zone measured, the more effective the extracts were.

Screening of phytochemicals

The screening was conducted by means to detect the existence of alkaloid, terpenoid, saponin and phenolic in the leaves part of each species (Maha Yudden et al., 2019). Reagents such as Dragendorff's reagent, ferric chloride solution, vanillin/H_2SO_4 and water solution were used to screen alkaloid, phenolic, terpenoid and saponin, respectively.

Screening of antioxidant using qualitative dot blot assay

The screening of antioxidant was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent which was sprayed on the thin layer chromatography (tlc) of extracts. About 50 ul of each extract with a concentration of 200 mg/ml was dropped on tlc. The yellow color against purple background authenticated the presence of antioxidants in the extract (Harun et al., 2020).

Result and Discussion

Antibacterial Activity

The results of antibacterial activities were obtained from the disc diffusion assay and it described the effectiveness of extracts by referring to the size of the clear zone around the disc. The larger the inhibition zone measured, the more effective the extract was towards bacteria. In this experiment, two types of extracts which were dichloromethane (DCM) and methanol (MeOH) extract with a concentration of 100 mg/ml for each species were evaluated on their effectiveness on *E. chrysanthemi*. According to **Table 1**, generally, all tested species in the form of DCM extract and MeOH extract have potentials to inhibit the growth of *E. chrysanthemi*. According to the result from DCM extract, *C. camphora, C. fenestratum* and *C. ligustrium* exhibited the highest antibacterial activity with the inhibition of 15 mm. Methanol extract of *C. fenestratum* seemed to be the more active extract with the inhibition zone of 19.67 mm compared to other species. The methanol extracts of *C. ligustrium* and *T. indica* were not active towards *E. chrysanthemi* since the 6 mm size of inhibition zone indicated that there was no clear zone observed around the disc. The potent antibacterial activity demonstrated by all nine species might be due the presence of remarkable secondary metabolites such as terpenoid and phenolics. As far as it is concerned, hydroxyl group is usually found in most terpenoid and phenolic structures which may cause a promising antibacterial activity. It was reported that hydroxyl group which could be found in terpenoid or phenolic compounds had a disruptive effect on the cell membrane of *E. chrysanthemi* which thus led to its serious damage (Ceruso et al., 2020). The suppression of bacterial growth of *E. chrysanthemi* might also be attributed by the synergistic action of the combination of terpenoid and phenolic compounds (Daud et al., 2021).

Table 1 Anti-bacterial activity of DCM and MeOH extract against *Erwinia chrysanthemi*

Species (200mg/ml)	**Local name**	**Inhibition zone (mm) ± S.D DCM extract**	**Inhibition zone (mm) ± S.D MeOH extract**
Mapania cuspidate	Pandan serapat	10.83± 0.34	6±0
Cinnamomum camphora	Medang	15±3.26	14.67±0.30
Coscinium fenestratum	Akar sekunyit	15±0	19.67±0.31
Cratoxylon ligustrinum	Geronggang selunsur	15±0	6±0
Homalomema sagittifolia	Keladi kemoyang	9.33±7.24	11±1.41
Tacca integrifolia	Keladi murai	9.83±6.38	15.17±0.51

Tetracera indica	Mempelas	12.83±0.9	6±0
Ziziphus oenoplia	Kekait	9.67±1.74	15.33±0.57
Fagrea elliptica	Tembusu hutan	11.33±2.64	13.67±0.95
Standard ampicillin		19.66±0.7	19.66±0.7

DCM : dichloromethane; MeOH : methanol; S.D : standard deviation
6 mm : not active (no inhibition zone); 7-10 mm : weakly inhibited; 11-14 mm : moderately inhibited ; >15 mm : highly inhibited

Phytochemicals and antioxidant properties

Table 2 demonstrates the presence of phytochemicals as well as its antioxidant screening result. The results revealed that all species contained terpenoid and phenolic compounds with a moderate to high antioxidant content. *C. fenestratum, C.ligustrinum, H. sagittifolia*, *T. integrifolia* and *Z. oenoplia* were among the species that were screened to exhibit higher antioxidants from their methanol extracts. **Table 2** and **Figure 1** depict the presence of antioxidant in all species in which most of the species were qualitatively screened to exhibit higher antioxidant content which was indicated by the high yellow color intensity. The low intensity of the yellow color might be attributed by the lesser antioxidant content. The existence of antioxidant in all types of extract might be due to the phenolic and terpenoid content in both DCM and MeOH extracts. Generally, both have a specific group such as hydroxyl group to manifest their antioxidant properties. The more the hydroxyl groups present, the more powerful the antioxidants were. As far as it is concern, phenolic and terpenoid are among antioxidant phytochemicals that protect our body cell from being damaged by free radicals, thus possibly reducing the risk of cancer (Cummins and Tangney, 2013). Theoretically, these antioxidants donate their hydrogen or electron through hydrogen atom transfer (HAT) or single electron transfer (SET) to neutralize the free radical from perturbing body cell through oxidative stress (Dontha, 2016).

Keawpradub et al. (2005) had reported that the presence of alkaloid berberine was responsible for the moderate antioxidant activity of the methanol extract of *Cosinium blumeanum*. The structure of alkaloid berberine from *C. fenestratum* had been determined by Tushar et al. (2008) and it is shown in **Figure 2**. According to Opeyemi et al. (2019), the main cause for the antioxidant activity of methanol extract of *Entada spiralis* was attributable to the presence of phenolic compounds such as 3,4′,5,7-tetrahydroxyflavone known as kaempferol, catechin and epicatechin as well as new antioxidants known as pachypodol and gallic acid. It is therefore assumed that antioxidant properties of the selected species tabulated in **Table 2** might be due to the existence of alkaloid and phenolic compounds from methanol extract.

Table 2 Phytochemicals and antioxidant profile

	DCM extract				**MeOH extract**			
Species	**Alk**	**Ter**	**Phe**	**AO**	**Alk**	**Ter**	**Phe**	**AO**
Mapania	x	√	√	+	x	√	√	+

cuspidata								
Cinnamomum camphora	√	√	√	++	x	√	√	++
Coscinium fenestratum	√	√	√	+++	√	√	√	+++
Cratoxylon ligustrinum	x	√	√	+++	x	√	√	+++
Homalomema sagittifolia	x	√	√	+	x	√	√	+++
Tacca integrifolia	x	√	√	+	x	√	√	+++
Tetracera indica	x	√	√	+	x	√	√	++
Ziziphus oenoplia	x	√	√	+	x	√	√	+++
Fagrea elliptica	x	√	√	+	x	√	√	++

Alk: alkaloid; Ter: terpenoid; Phe: phenolic; AO: antioxidant; DCM: dichloromethane; MeOH: methanol.
x : not exist; √ : exist
(+): low yellow color intensity; (++): medium yellow color intensity; (+++):high yellow color intensity.

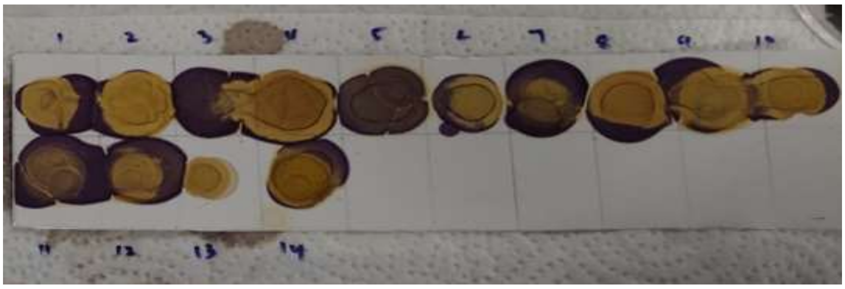

Figure 1 Screening of antioxidant properties of methanol extract of plants species. Yellow color indicates the antioxidant properties.
4: *H. sagittifolia* ; 5: *M. cuspidate* ; 6: *F. elliptica;* 8: *T. integrifolia;* 9: *C. ligustrinum*; 10: *Z. oenoplia* ;12: *C. camphora* ; 13: *T.* indica; 14: *C. fenestratum*

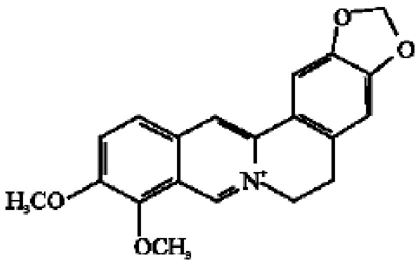

Figure 2 Chemical structure of alkaloid berberine (Tushar et al., 2008)

Conclusion

The investigation concludes that *C. fenestratum* is the most active antibacterial agent against *E. chrysanthemi* with the inhibition zone of 19.67 mm and all species were proven to have antioxidant properties. For future work, the antibacterial activity and antioxidant activity need to be explored in detail using various concentration of extracts as well as other methods to establish this research work. It is also imperative to further this work for the identification of anti-soft rot and antioxidant constituents.

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Conflict of interests

The author declares that this paper has no conflict of interest.

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