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FULL PAPER

Antibacterial and antioxidant activities of *Homalomena josefii* P.C. Boyce and S.Y. Wong rhizome extract

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Abstract

The rhizome of *Homalomena josefii* P.C. Boyce and S.Y. Wong is commonly used in Borneo folk medicine. It is potent in treating stomach aches, headaches, and snake bites. The lack of information about their biological activities leads to an investigation to determine its antibacterial and antioxidant activities of *H. josefii* rhizome extract. This study aimed to ascertain antibacterial activity in opposition to foodborne pathogens namely *Bacillus megaterium* ATCC14581, *Bacillus pumilus* ATCC14884, *Proteus mirabilis* ATCC21100 and *Klebsiella pneumoniae* ATCC13773 and its antioxidant properties of *H. josefii* rhizome extracts. Extraction of dried powdered *H. josefii* rhizome was carried out through the maceration method using methanol as a solvent to produce crude extracts. The crude extracts were then tested for antibacterial and antioxidant activities. The antibacterial activity was conducted in terms of disc diffusion assay (DDA), minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and time-kill assay. All were performed following the Clinical and Laboratory Standard Institute (CLSI) procedure to ensure liable results. Total phenolic compounds (TPC) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) scavenging assay were used to stimulate the antioxidant activity. The results show that DDA inhibition zone of the methanolic extracts ranged from 9.00±0.58 to 10.00±0.00 mm. The extract was able to inhibit the growth of all tested bacteria with MICs value ranging from 0.31 to 5.00 mg/mL. Meanwhile, the extract is able to kill all tested bacteria with MBC values that range from 0.63 to 5.00 mg/mL. Time-kill assay curve analysis results showed that the extract was able to completely kill the bacterial growth at 4 × MIC for 4 hours. The total phenolic compound (TPC) of methanolic extract was 435.138 mg GAE/g and IC_{50} of the extract was 11.809 µg/mL. In conclusion, the methanolic extract of *H. josefii* rhizome extract exhibited antibacterial and antioxidant activities, thus it can further be developed as a natural preservative.

1. Introduction

Natural products extracted from plants as medication have been around for a long time and have been known to cure those suffering from ailments such as chronic stomach illness, fever, inflammatory problems and more. Present-day medications were made possible through phytochemistry and medicinal chemistry first practised in the rural areas of our ancestors. Understanding the biological factors of effecting cells becomes a major scientific significance to prolong human health. Research has shown that around 80% of the Asian or African populations still relies on traditional medicine medication as compared to western medication (Oyebode *et al.*, 2016). Furthermore, bacterial proliferation consistently mutates and grows resistant to multiple drugs, these are known as antimicrobial resistance (AMR) (World Health Organization, 2018).

In addition, the overwhelming use of antibiotics without professional guidance raises the concerns of antibiotic-resistant bacteria. These concur with viral

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infections that do not correlate with the use of antibiotics. This occurs when the public is misinformed about the differences between bacterial and viral infections. Misconceptions as such leads to having one's body developing resistance towards a drug at a certain period of time. Thus, long-term use of synthetic drugs is not useful against common bacterial diseases such as foodborne bacteria that can cause food poisoning. There are various approaches available to tackle the growth of food pathogens through preservatives (Hasheminya and Dehghannya, 2019). However, several side effects due to synthetic preservatives are a growing concern among consumers. Therefore, natural compounds are increasingly in favour (Houicher *et al*., 2018).

On top of that, antioxidants play an important role as food additives to avoid prevention of diseases, ageing and degradation as they are associated with active oxygen and lipid peroxidation (Ahmed *et al.*, 2019). Lipid oxidation and microbial proliferation, which are deteriorative chemical reactions present in food, will make it inedible and toxic (Bazder *et al.*, 2018). Nevertheless, essential oils prepared through hydrodistillation, steam distillation or mechanical processes are antibacterial, antifungal, antiparasitic, insecticidal, and antiviral and they possess the ability to reduce the risks of diseases such as diabetes, cardiovascular diseases and cancer (Rezaie *et al.*, 2015; Hasheminya and Dehghannya, 2020). All of which are products of oxidative stresses in the human body from the generation of free radicals. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are responsible for the damaging effects on deoxyribonucleic acid (DNA) as it leads to the oxidation of lipid and proteins (Li *et al.*, 2015). Imbalances between oxidation and antioxidation lead to degenerative diseases such as cancer.

The *Homalomena* (Family Araceae) is made up of 150 species that can be found around the globe. Usually found abundantly in tropical and subtropical regions such as America, Africa and Southwestern and Eastern China, more likely in the Yunnan, Guangdong and Guangxi provinces (Zhao *et al.*, 2016). In China *Homalomena occulta,* commonly known as Qian-nian-jian is widely consumed to treat rheumatoid arthritis, strengthen tendons and bones, and is reported to protect kidneys and liver (Yang *et al.,* 2019). This is proof that many indigenous groups utilize this extensive range of species as sustenance and medicinal source. In Assam, Northeast India, many tribes around the region reportedly consume the leaves and rhizomes of the *Homalomena aromatica* Schott in the form of paste as they are known for medicinal properties such as anti-depressants, anti-inflammatory, antiseptic, sedative and analgesic (Policegourda *et al.*, 2012). These species were found to have active ingredients such as linalool, terpineol α-cadinol, β-pinene, oplopanone, linalyl acetate and limonene among others (Zhou *et al.*, 1991; Singh *et al*., 2000; Kehie *et al.*, 2016).

In Malaysia, there are limited studies on the *Homalomena* species found in the country. The most common species utilized by people living in rural areas is the *H. sagittifolia* Jungh. A recent study conducted by Wong *et al.* (2011), discovered a new species such as the *H. josefii*. The plant is based in damp parts of the Malaysian forests. It is about 30-60 cm tall with dark green waxy, arrow-shaped leaves. The rhizomes, in particular, are pleasantly aromatic and are used as native medicine to the indigenous groups, especially in Sarawak and Sabah. Further studies should be conducted to identify the essential oils in *H. josefii*. Therefore, this research was conducted to identify the antimicrobial and antioxidant activity present in the rhizome of *H. josefii*.

2. Materials and methods

2.1 Materials

Rhizomes of *H. josefii* P.C. Boyce and S.Y. Wong were obtained home-grown and from Sarawak. The plant was first identified by Bogner, J. from Botanische StaatssammLung München (M), Germany, with voucher number M0089835 and deposited at the herbarium of the Laboratory of Natural Products, Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM) until further use.

2.2 Solvent extraction

Maceration methods were adapted from Rukayadi *et al.* (2008). Methanolic extraction (Chemiz, 99%) was used to acquire the essential oils from *H. josefii.* Samples were firstly air-dried and grounded prior to maceration. A 100 g of the sample was dissolved into 400 mL of solvent for 48 hrs at room temperature in the shaker. The extracts were then filtered and concentrated with a rotary vacuum evaporator (BUCHI Rotavapor R-200, Switzerland).

2.3 Preparation of extract

A 1% (w/v) concentration of the extract was prepared with 10% dimethyl sulfoxide (DMSO) (Sigma-Aldrich) as solvent. DMSO was known not to have any antibacterial properties at 100%.

2.4 Antibacterial assay

2.4.1 Disc diffusion assay (DDA)

This method used was adapted from Shanmugam *et al.* (2016) with marginal modifications and conducted according to regulations by the Clinical and Laboratory

Standard Institute (CLSI) (2012). The antibacterial activity crude extract was evaluated against *Bacillus megaterium* ATCC14581, *Bacillus pumilus* ATCC14884, *Proteus mirabilis* ATCC21100 and *Klebsiella pneumoniae* ATCC13773. Microorganisms were obtained from American Type Culture Collection (ATCC) (Rockville, Maryland, USA). The sub-cultured microorganisms were maintained on Muller-Hinton agar (MHA) to approximately 10^6 CFU/mL. Chlorhexidine (CHX) was used as a positive control at 1% (w/v). Chlorhexidine has been used at 0.12 - 0.20% in several antiseptic medications to prevent the accumulation of biofilm (Shirato *et al.*, 2017). While dimethylsulfoxide (DMSO) was used as a negative control at a concentration of 10% (v/v). Fresh bacterial strains were firstly cultured onto the MH agar prior to the inoculation of the sterile paper disc. Each paper disc (Whatman Antibiotic Assay Discs, 6 mm in diameter) was infused with 10 µL of 1% concentration *H. josefii* extract, 1% CHX and 10% DMSO respectively. The plates were then incubated at 37°C for 24 hrs, where the results obtained based on the diameter of the zone of inhibition and was recorded in millimetres (mm). This is clearly observed when the inhibition zone is larger than 6 mm, concluding that antibacterial activity took place. The assay was performed in triplicates.

2.4.2 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC was carried out through a microdilution method in 96-wells round bottom microtiter plate as modified from Ramasamy *et al.* (2013). The first column was filled with 200 µL of MHB as a negative growth control. Next, the second column was filled with 200 µl bacterial suspension as positive growth control. Microdilution was performed at concentrations that ranged from 5 mg/mL to 0.01 mg/mL. The plate was then incubated for 24 hours at 37°C. MIC value was recorded and regulated by the lowest extract concentration that had produced no observable growth of turbidity after the period of incubation in the wells.

MBC was determined by sub-culturing the suspensions from each well from MIC onto an MH agar plate with a volume of 10 µL. The plates were then incubated for 24 hrs at 37°C. MBC was determined as the lowest concentration at which no growth had occurred on the MH agar plate.

2.4.3 Determination of time-kill curve

Time-kill assay was performed using the crude extract against all selected bacteria. Firstly, the inoculum suspension of approximately 10^6 CFU/mL was prepared. Mueller-Hinton Broth (MHB) medium containing inoculum were then diluted to obtain final concentrations of 0.0 × MIC, 0.5 × MIC, 1.0 × MIC, 2.0 × MIC and 4.0 × MIC. 1% phosphate-buffered saline (PBS) was used as a medium to serially dilute the aliquot in 1:100 ratios and plated with 10 µL onto MH agar. These concentrations were conducted over a period of 4 hrs to identify the efficiency of the extract to eliminate the growth of bacteria. The number of colonies that were formed on the plates after 24 hrs of incubation was recorded and the graph of Log_{10} CFU/mL versus time was plotted.

2.5 Antioxidant assay

2.5.1 Determination of total phenolic content (TPC)

TPC was measured by the Folin-Ciocalteu method with some modifications (Puangbanlang *et al.*, 2019). Reference standard applied during the procedure was gallic acid as seen in other researches. The TPC of the extract acquired from the conventional assays were expressed as a gallic acid equivalent (GAE, mg GAE/g samples). 100 µL of Folin-Ciocalteu reagent was added with 20 µL of extract. After 5 mins, 80 µL of 7.5 sodium carbonate, Na_2CO_3 was added. The mixture was allowed to stand for 30 mins before the reading was taken at 750 nm in a microplate reader. A standard curve was plotted using gallic acid. The TPC results were calculated using the following equation (1):

$$\text{TPC} = \frac{concentration\left(mg\frac{GAE}{mL}\right) \times volume\ used\ (mL)}{mass\ weight\ (g)} \quad (1)$$

The correlation between the antioxidant test and total phenolic content was analysed through the simple linear regression and the correlation coefficient (R^2) was calculated.

2.5.2 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay

The percentage of antioxidant activity of the extract was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay according to the method reported by Sridhar and Charles (2019), with slight modifications. Three 200 µL replicates of MeOH extracts were made. The extracts were resuspended in respective solvents before 200 µL DPPH was added with the absorbance of 517 nm in a 96-well flat-bottom plate. As a reference, control was made by DPPH solution was stirred in the absence of the extracts under similar conditions. The mixtures were incubated for 30 mins at room temperature. The results obtained from the absorbance were measured using a microplate reader (TECAN, Infinite F200 Pro). The DPPH radical-scavenging activity (%) was calculated using the following equation (2):

$$\%\text{DPPH Scavenging} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100 \quad (2)$$

Where, $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of extract and DPPH.

Next, the concentrations of adsorbed 2,2-diphenyl-1-picrylhydrazyl free radical from the extracts were calculated by distinctions in concentration of 2,2-diphenyl-1-picrylhydrazyl in the presence and absence of the extracts. The following equation of DPPH adsorption was adapted from Zamani *et al.* (2018) .

$$DPPH\ adsorption\ (\%) = \frac{C\,ref - C\,s}{C\,ref} \times 100 \quad (3)$$

Where, C_{ref} is the concentration of mixture without DPPH solution and C_s is the concentration of mixture including with extract and 2,2-diphenyl-1-picrylhydrazyl solution.

3. Results and discussion

3.1 Antibacterial assay

3.1.1 Disc diffusion assay (DDA)

Antibacterial activity of *H. josefii* extract was investigated against foodborne pathogens using disc diffusion method. The diameter of the zone of inhibition in millimetre for different bacteria against the extract was determined as depicted in Table 1. This was carried out by sub-culturing selected bacteria onto MHA and placing 10 µL of solvents and extract onto a 6 mm paper disc and were inverted during incubation for 24 hours at 37°C (Saravanan *et al.*, 2011). As displayed in Table 1, the results for DDA showed that the methanol extracts exhibit large inhibition zone where *B. megaterium* and *B. pumilus* were both 9.68±0.58 mm respectively, *K. pneumoniae* was 10.00±0.00 mm, while *P. mirabilis* was 9.00±1.00 mm. From the study conducted, it was perceived that the essential oils of *H. josefii* exhibited a more inhibitory effect against the Gram-positive pathogen when compared with the Gram-negative pathogens. This corresponds to the results from previous research conducted on *H. aromatica,* whereby Gram-positive bacteria are more susceptible, due to its cell wall lipopolysaccharide (Laishram *et al.*, 2006). An *in vitro* comparison study of extract against chlorhexidine is shown in Table 1.

Table 1. Mean values of zone of inhibition of DDA

Bacterial Strains	Methanol Extract (mm)	CHX (mm)
B. megaterium ATCC14581	9.68±0.58	18.33±1.15
B. pumilus ATCC14884	9.68±0.58	18.00±1.00
K. pneumoniae ATCC13773	10.00±0.00	16.00±1.73
P. mirabilis ATCC21100	9.00±1.00	20.00±3.00

Values are the mean±S.D. of replications (n = 3), CHX = chlorhexidine

3.1.2 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The serial dilution for MIC technique was employed onto bacteria and incubated overnight at 37°C. The overnight cultures were diluted in Mueller-Hinton Broth (MHB) to ensure the CFU of the bacteria were 10^{-6}. The MIC results for *B. megaterium*, *B. pumilus*, *K. pneumoniae* and *P. mirabilis* based Table 2 with concentrations of 0.42, 0.52, 0.52, 4.17 mg/mL, respectively. On the other hand, MBC results from the following bacteria were 0.83, 1.04, 1.67, 5.00 mg/mL, respectively. This would indicate that the crude extract is more effective on the Gram-positive bacteria, as opposed to the Gram-negative bacteria as the concentration needed in inhibiting growth and bactericidal actions are lower.

Table 2. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *H. josefii* extracts on food borne bacteria. (MIC and MBC values expressed as mg/mL)

Bacterial Strains		Methanol Extract (mg/mL)	CHX (mg/mL)
B. megaterium	MIC	0.42	0.01
ATCC14581	MBC	0.83	0.03
B. pumilus	MIC	0.52	0.02
ATCC14884	MBC	1.04	0.03
K. pneumoniae	MIC	0.52	0.03
ATCC13773	MBC	1.67	0.03
P. mirabilis	MIC	4.17	0.01
ATCC21100	MBC	5.00	0.02

Values are the mean±S.D. of replications (n = 3), CHX = chlorhexidine

3.1.3 Time-kill curve

Figure 1 illustrates the susceptibility of both the Gram-positive as well as the Gram-negative bacteria affected by the *H. joseffi* extract at concentrations prepared based on the lowest concentrations resulting from MIC tests. Concentrations applied on *B. megaterium*, *B. pumilus*, *K. pneumoniae* and *P. mirabilis* are 0.42, 0.52, 0.52 and 4.17 mg/mL, respectively. As seen in Figure 1, bacterial growth has exponentially dropped to null after 4 hours at 4× MIC concentrations. By observing the resulting growth after 24 hours of incubation at 37°C, it was noted that there were no signs of growth on the MH agar for petri dishes labelled 4x MIC 4 hours. Hence, the method conducted in accordance with Bax *et al.* (2017), has been theoretically proven in this research.

The present study revealed high antibacterial activity of *H. joseffi* methanolic extract against the selected Gram -positive and Gram-negative bacteria with an effect beginning within the first 24 hrs in low concentrations.

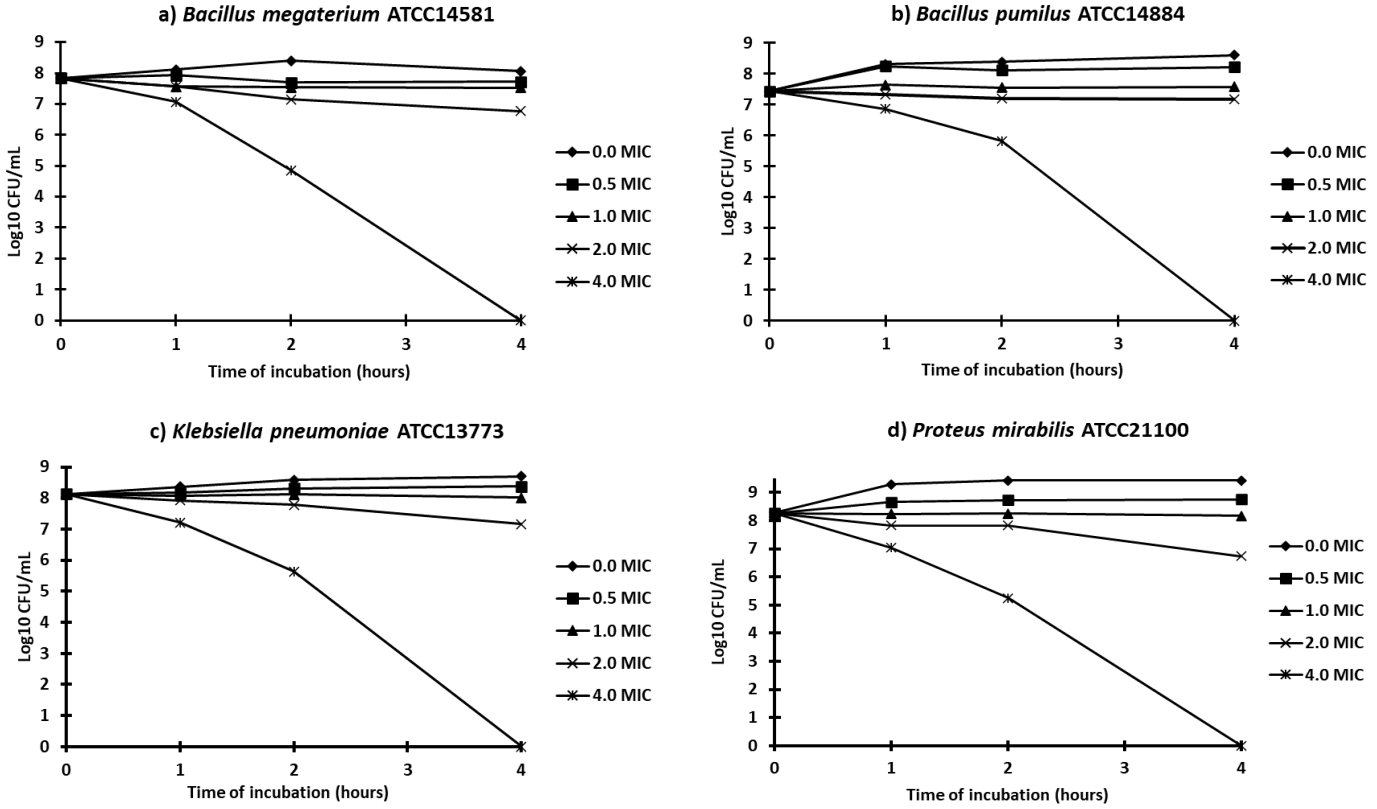

Figure 1. Time kill curve assay CFU/mL plot of a) *B. megaterium* ATCC14581, b) *B. pumilus* ATCC14884, c*) K. pneumoniae* ATCC13773 and d) *P. mirabilis* ATCC21100 for 0 × MIC, 0.5 × MIC, 1.0 × MIC, 2.0 MIC and 4.0 MIC of methanolic *H. joseffi* extract at 0 to 4 hours incubation time.

The antibacterial properties may be due to synergistic or additive effects derived from the phytochemical compounds from the rhizomes itself (Delgado *et al.*, 2004). Studies conducted on different *Homalomena* species have shown that the rhizomes and leaves have an accumulative number of terpenes, flavonoids, sesquiterpenes and more which were responsible for its antibacterial activity (Elbandy *et al.*, 2004). For example, isolated natural compounds from *H. occulta* include sesquiterpenoids, triterpenoids, alkaloids and phenolic acids (Yang *et al.*, 2019). These compounds were found to have bioactive properties and exhibit such effects strongly when isolated from essential oils.

Inhibitory effects are associated with cell wall disruption by damaging it deep within the cell membrane. Owing to a fact that Gram-positive bacteria cell all lipopolysaccharide is more susceptible (Laishram *et al.*, 2006). In fact, as per Conner (1993), essential oils studied previously such as clove, cinnamon, oregano have shown strong antibacterial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus.* When extracted from medicinal aromatic plants, they have been reported to be effective against both Gram-positive and Gram-negative bacteria (Tariq *et al.*, 2019).

In addition to that, the major compounds found in most *Homalomena* rhizome species are linalool (61.9%) and terpinen-4-ol (2.5%) (Wong *et al.*, 2004). In short, these major compounds would be responsible these antibacterial properties as seen in Figure 1. The rhizome extract of *H. josefii* in comparison to *H. sagittifolia* J. could potentially possess the same number of chemical components that allows it to fight off food borne pathogens. In a study conducted previously, linalool has displayed algicidal properties on *Microcystis aeruginosa* and *Chlamyomonas reinhardtii* by exhibiting prominent inhibitory and lethal effects (Chen *et al.*, 2018). As for the presence of terpen-4-ol, studies have shown that it does not only have antibacterial properties, but also antifungal properties. Antifungal properties were reported by An *et al*. (2019), stating that tea tree essential oil extract has strong antifungal efficacy against *Aspergillus niger in vitro*. It also acts as a natural preservative; hence the application on food is impeccable to ensure food safety against foodborne pathogens. Based on that research, *H. joseffi* extract would exhibit equal effects as the tea tree oil extract deduced from terpen-4-ol.

Additionally, results indicate the mechanisms responsible for its antibacterial properties could potentially be due to the constituents of the essential oil. It has been reported to weaken the cellular structure, ceasing the functions of its membrane integrity (Turina *et al.*, 2006). This then leads to the wrecking of energy production, membrane transport and various important processes such as the natural process of nutrient and

macromolecule synthesis and of proliferation regulators and respiratory processes (Oussalah *et al.*, 2006). Besides that, essential oils have also been reported to influence the external envelope of the cell and cytoplasm due to its lipophilic nature, allowing it to penetrate through cell membranes with ease. Radünz *et al.* (2019) confirmed that the relationship between the oil and the lipids of the cell membrane of the bacterium allows for the alteration of its permeability. Reports have shown that this had caused leakage of cellular components and loss of essential ions such as potassium ions (K^+) (Saad *et al.*, 2013).

Moreover, the hydrophilic nature of the cell membrane Gram-negative bacteria obstructs the contact of the hydrophobic constituents of essential oils with the bacterial cell; therefore some research has modulated its attacks through its efflux mechanism (Saei-Dehkordi *et al.*, 2010; Devi *et al.*, 2010). Essential oils are able to slip through cell walls and cytoplasm membrane, disrupting the positioning of fatty acids and phospholipid bilayers, resulting in the degradation of bonds between the lipid and proton layer (Longbottom *et al.*, 2004). Hence, these damages are linked to the damaging effects of proton pumps and decrease in ATP production which drives it through a domino effect, damaging other cell organelles, resulting in bactericidal or bacteriostatic effects (Turina *et al.*, 2006).

Despite the labour-intensive method through time-kill assays than MBC, it is acknowledged and essential to provide a better insight of characterisation of cell eradication potential from antibacterial extracts (Arhin *et al.*, 2009). These results will determine whether the extract gives bacteriostatic or bactericidal properties. This study will also be an advantage in producing naturally occurring antimicrobial compounds to combat emerging multiple multidrug-resistant pathogens. Longer incubation periods in vitro significantly reduced the growth of all tested concentrations and began mostly after the maximum effect within 4 hrs. The present study demonstrated that extracts at concentrations less than 1% had a significant effect against *B. megaterium*, *B. pumilus*, *K. pneumoniae* and *P. mirabilis.* As such, the hypothesis formulated was accepted and the methanolic extract can be considered as an alternative antibacterial treatment agent in food.

3.2 Antioxidant assay

3.2.1 Total phenolic content (TPC)

The antioxidant potency of the extract depends on the composition of the extract and the tests done to ensure the results are not highly influenced by extrinsic and intrinsic factors. Thus, a reliable protocol requires methods that work alongside each other to confirm its validity. TPC method was utilized to regulate and compare the total phenolic content of both solvent extracts. It is based on the transfer of electrons in alkaline media from the phenolic compounds to the Folin -Ciocalteu reagent (Singleton and Rossi, 1965). Table 3, clearly depicts the total phenolic content results from the methanolic extract. These results indicate a very high concentration at 432.138±68.01 mg GAE/g. This value is evidently lower as compared to those reported by Zeng *et al.* (2011) on a different species (*Homalomenae*) at 141±1.21 mg GAE/g. This could be due to degradation during drying, grinding and storing procedure of the samples.

Table 3. Total phenolic content (TPC)

Variables	Results
R^2	0.9958
Methanol extract (mg GAE/g)	435.138±68.01

3.2.1 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay

A 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay assesses free radical scavenging potential and is considered to be a standard-easy colorimetric method used to determine the antioxidant properties from *H. joseffi*. (Mishra *et al.*, 2012). As displayed in Figure 2, the DPPH radical activity of methanol was at its maximum at 96.32% at a concentration of 1 mg/mL. Besides that, methanol extract displayed positive antioxidant activity in the DPPH scavenging assay with IC_{50} 11.809 µg/mL (Table 4). As the DPPH radical eliminating ability increases at first, and reaches a maximum, this could indicate that the ability of electron or hydrogen donor has a saturation point (Zeng *et al.*, 2019).

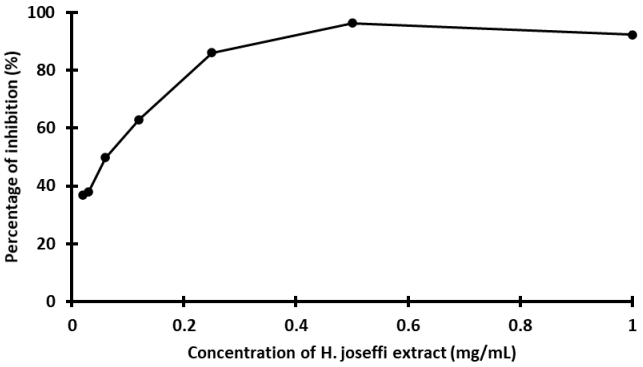

Figure 2. Antioxidant capacity of *H. joseffi* extract

Table 4. DPPH scavenging assay

Variables	Methanol	Gallic acid standard
R^2	0.9894	0.9884
IC_{50}	11.809 µg/mL	0.43 µg/mL
Inhibition (%)	96.32	85.35

A simple linear regression analysis (Figure 2) was utilized to analyse the correlation between antioxidant abilities and the total phenolic content. According to earlier studies, the correlation coefficient R^2 between DPPH values and TPC were usually high in methanolic extracts (Guleria *et al.*, 2012). The presence of several phenol groups is very reactive in neutralizing free radicals through the donation of an electron or hydrogen atom and chelating metal ions in aqueous solutions (Li *et al.*, 2013). According to a study by Zeng *et al.* (2011), high contents of phenolic compounds within the rhizomes together with strong antioxidant activity is attributed to strong antioxidant potency in the extract. Therefore, the source of phenolic and flavonoid contents can easily be found in the rhizomes of *H. josefii* through further research. It was also clear that the high yield of phenolic compounds with high antioxidant activity can be achieved by using the appropriate solvent.

4. Conclusion

As demonstrated by the growth curve analysis, disc diffusion assay, minimum inhibitory concentration and minimum bactericidal concentration testing, the methanolic extract of *Homalomena joseffi* depicts an exceptionally strong action and is highly active against *B. megaterium, B. pumilus, K. pneumoniae* and *P. mirabilis* isolates. Besides that, antioxidant tests display the effectiveness of the extract at low concentrations. Further studies should include its thorough chemical compound characterisation and application as a possible natural food preservative. Cytotoxic tests should be considered in future research to ensure the safety of consumption when applied to food.

Conflict of interest

The authors declare no conflict of interest.

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