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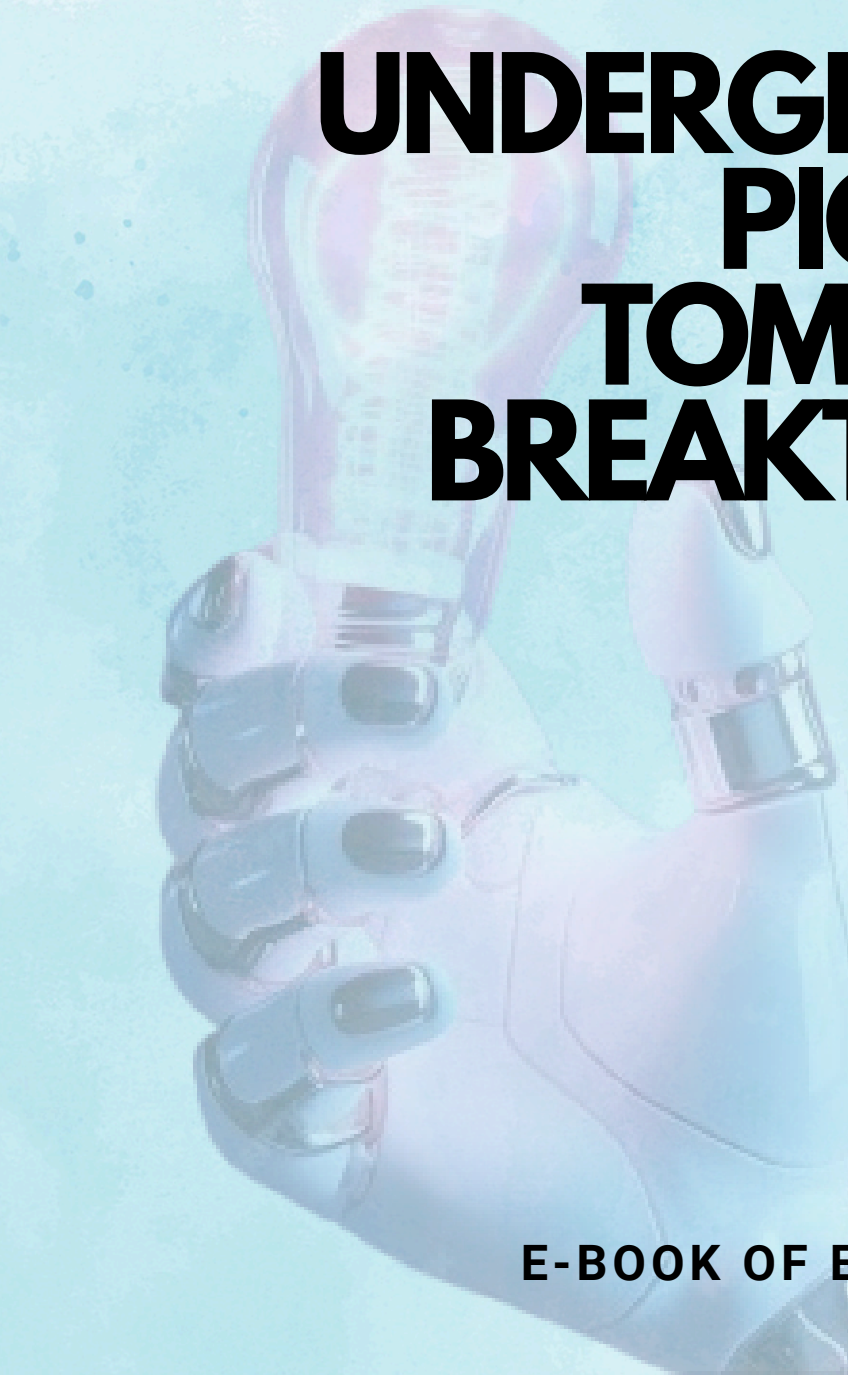

E-BOOK OF EXTENDED ABSTRACTS

POTENTIAL OF *SYZYGIUM AROMATICUM* (CLOVE) AS A NATURAL ANTIBACTERIAL AND ANTIOXIDANT REMEDY

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ABSTRACT

Syzygium aromaticum (Clove) is a species of the Myrtaceae family, also known as Cengkih in Malaysia. Chemical compounds in clove have been discovered to have a lot of potential metabolites to cure many diseases and illnesses such as colds, eye sties, bronchitis, sinus conditions, cough and asthma. The research focused on the extraction, phytochemical analysis and bioactivity properties of *S.aromaticum*. The extraction process used different polarity solvents such as petroleum ether, chloroform and methanol. Phytochemical screening was performed using several chemical tests, and the presence of alkaloids, flavonoids, tannins, saponins, steroids and phenols in crude extracts of clove was successfully detected. The antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* was determined in this study. The methanol crude extracts displayed the highest antibacterial activity against *B. cereus* with an inhibition zone of 12 mm. The antioxidant activity of *S. aromaticum* extract was carried out using the DPPH method. The petroleum ether extract showed strong DPPH radical scavenging activity with an IC_{50} value of 59.59 µg/mL. This study shows that the extracts of *S.aromaticum* contain medicinally bioactive compounds and have the potential as antibacterial and antioxidant agents that may benefit human health.

Keywords: Syzygium aromaticum, Myrtaceae, Phytochemical, Antibacterial, Antioxidant

INTRODUCTION

Natural product is substances that originate from animals, plants and microorganisms. New therapeutic compounds have come from nature due to the tremendous chemical diversity found in various species of plants. Since the beginning of the 20th century, the extraction or powder of medicinal plants has been used as the main active ingredient in medicinal products because they are considered a powerful source of drugs that have no side effects when applied to patients (Brown et al., 2010). *Syzygium aromaticum* is a tropical evergreen plant from the Myrtaceae family that has been commonly used as a spice for human nutrition and a flavouring agent since ancient times. It is also used for treating several illnesses in folk medicine such as colds, eye sties, bronchitis, sinus conditions, cough and asthma (Cortés-Rojas et al., 2014). Clove buds are believed to be an effective antioxidant and antibacterial herbal because they contain the phenolic compounds, eugenol. This compound was reported to be the major constituent present in the clove oil, which is responsible for its biological activity (El-maati et al., 2016). In this study, the phytochemical screening was done on *S.aromaticum* extracts to detect the presence of secondary metabolites and demonstrate their biological activities (antioxidant and antibacterial).

OBJECTIVE

This study aims to extract phytochemicals from the *S.aromaticum* buds using different polarities of solvents such as petroleum ether, chloroform and methanol. Phytochemical screening has been done to identify the presence of phytochemicals from the extracts of *S.aromaticum*. Besides, the antioxidant and antibacterial

activities of *S.aromaticum* extracts were also demonstrated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) and disc diffusion methods, respectively.

METHODOLOGY

Plant Extraction

The buds of *S.aromaticum* were dried and ground into fine powder. It has been weighed and extracted sequentially with petroleum ether, chloroform and methanol. The extracts were filtered through a filter paper and concentrated using a rotary evaporator to obtain the crude extract.

Phytochemical Screening

Chemical tests for the screening and identification of bioactive chemical constituents such as alkaloids, flavonoids, tannins, saponins, steroids and phenols on *S.aromaticum* extracts were carried out by using a standard procedure (Khan et al., 2022).

Antioxidant Assay

DPPH radical scavenging assay was utilised to determine the antioxidant activity of *S.aromaticum* with some modifications (Ghadermazi et al., 2017). Each sample (1.0 mg) was dissolved in methanol (1 mL) to obtain a stock solution with a concentration of 1000 µg/mL. A series of diluted solutions was prepared from the stock solution with methanol starting from 1000, 500, 250, 125, 62.5, 31.3,15.63 and 7.81 µg/mL. The sample solutions with various concentrations (0.2 mL) were mixed with 3.8 mL of methanolic DPPH solution (50 µM). The mixture was incubated for 30 minutes at room temperature in the dark. After 30 minutes, the absorbance of the reaction mixture was recorded at 517 nm.

Antibacterial Assay

The antibacterial activity of the crude extracts of *S.aromaticum* was determined using the disc diffusion method with slight modification (Saikumari et al.,2016). The activity was tested against two Gram-positive bacteria, *B. cereus* and *S.aureus* as well as two Gram-negative bacteria, *E. coli* and *S. typhi.*

RESULTS AND DISCUSSION

Extraction of the sample

The crude extracts of *S.aromaticum* from the extraction process were weighed using an analytical balance. The percentage and yield of each extract were calculated. Table 1 below shows the results of the sample extraction of different solvents. Methanol demonstrated the highest percentage yield at 10.83%, indicating effective extraction of a diverse range of polar compounds. Chloroform exhibited moderate efficiency with a percentage yield of 7.23%, while petroleum ether showed a relatively lower yield of 5.57%, suggesting lower solubility of non-polar compounds in this solvent.

Table 1: Result of sample extraction

Extract	Weight of ground sample (g)	Weight of crude extract (g)	Percentage yield (%)
Petroleum Ether	400.00	22.27	5.57
Chloroform	362.75	26.24	7.23
Methanol	341.59	36.99	10.83

Phytochemical screening of S.aromaticum

S.aromaticum has been reported to contain many active compounds. In this study, phytochemical screening was carried out to detect the presence of secondary metabolites in petroleum ether, chloroform and methanol. Moreover, phytochemical screening confirmed the presence of various bioactive compounds in clove, including alkaloids, flavonoids, saponins, tannins and phenols. These compounds contribute to the potential health benefits associated with clove. Table 2 shows the result of the phytochemical analysis of those extracts.

Table 2: Phytochemical screening of *S.aromaticum*

Test	Crude extract			Observation
	Pet. ether	Chloroform	Methanol	
Alkaloid	-	+	+	Reddish brown
Flavonoid	+	+	+	Light yellow
Phenol	+	+	+	Dark green
Steroid	+	+	+	Red color
Saponin	-	-	+	Frothing
Tannin	-	-	+	Dark Green

Key: presence (+), absence (-)

Antioxidant Activity

The DPPH radical scavenging assay was employed to evaluate the antioxidant properties of *S.aromaticum.* The data on the DPPH radical scavenging activity of petroleum ether, chloroform, and methanol extracts from *S.aromaticum* leaves, along with the standard antioxidant, ascorbic acid, is presented in Table 3. Petroleum ether extract demonstrated strong radical scavenging activity with an IC_{50} value of 59.59 µg/mL. The chloroform extract exhibited significant DPPH radical scavenging activity, with an inhibition rate of 91.47% at 1000 µg/mL with a corresponding IC_{50} value of 66.75 µg/mL. Methanol extract was presented the lowest IC_{50} value in this experiment with 71.70 µg/mL.

Table 3: DPPH radical scavenging of the extract of S.aromaticum

Extract	% Inhibition at 1000µg/mL	IC_{50} (µg/mL)
Petroleum ether	83.88 ± 0.36	59.59 ± 0.58
Chloroform	91.47 ± 0.22	66.75 ± 0.79
Methanol	90.59 ± 0.57	71.70 ± 0.88
Ascorbic acid	97.18 ± 0.02	57.23 ± 0.14

Data present mean ± standard deviation of three replicate experiments.

Antibacterial Activity

The diameter of the inhibition zone for each extract was measured. Table 4 showed the bacterial activity of *S.aromaticum* extract against selected bacteria, with a diameter inhibition in the range of 7.0 to 12.0 mm. The methanol crude extract displayed the highest antibacterial activity against *B. cereus* with an inhibition zone of 12.0 mm compared to other extracts. In contrast, there is no inhibition zone (6.0 mm) observed for the methanol extract against *S.typhi*.

Table 4: Antibacterial activity of S. aromaticum extracts

Extract	Diameter of Inhibition Zone (mm)			
	B. cereus	***S. aureus***	***E. coli***	***S. typhi***
Petroleum ether	9.0	8.0	8.0	7.0
Chloroform	7.0	10.0	8.0	8.0
Methanol	12.0	11.0	11.0	6.0
Streptomycin[a]	20.0	18.0	16.0	15.0

Inhibition zone diameter (mm) including diameter of disc 6 mm; [a]Positive control

CONCLUSION

The phytochemical screening has revealed the presence of various secondary metabolites in *S.aromaticum* extract, including alkaloids, flavonoids, saponins, phenols, tannins, and steroids. An antibacterial study was conducted using the disc diffusion method against *B. cereus*, *S. aureus*, *E. coli* and *S. typhi.* In addition, the result showed that the methanol extract displayed the highest antibacterial activity against *B. cereus* with an inhibition zone of 12 mm. This result was supported by the study of Ajiboye and team (2016). Meanwhile, the antioxidant study revealed that the petroleum ether extract demonstrated strong radical scavenging activity with an IC_{50} value of 59.59 µg/mL, followed by chloroform extract (IC_{50} 66.75 µg/mL)

and methanol extract (IC_{50} 71.70 µg/mL), respectively. The results showed that the extract of *S.aromaticum* has potential as an antibacterial and antioxidant agent for pharmaceutical purposes (Shekhar et al, 2018).

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