

ANTIOXIDATIVE CONSTITUENTS FROM TWIG AND LEAVES OF *JASMINUM SAMBAC*

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Abstract

This study aimed to investigate new antioxidative constituents from twig and leaves of *Jasminium sambac* (Oleaceae family) as potent antioxidant sources. The antioxidant behaviour of each extract from twig and leaves of *J. sambac* was assessed qualitatively by spraying the thin layer chromatography (TLC) of extracts with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and quantitatively by DPPH radical scavenging method. The collection of antioxidative constituents was carried out using centrifugal chromatography. The chemical structures of all constituents were identified from Gas Chromatography Mass Spectrometer (GCMS). The qualitative Thin Layer Chromatography (TLC) screening of antioxidants showed that all types of extracts from *J. sambac* leaves demonstrated moderate antioxidant behaviour as well as the extracts from twig parts. The quantitative DPPH radical scavenging activity of leaves and twig extracts revealed dichloromethane (DCM) extract scavenged DPPH free radicals more effective at IC₅₀ of 6.24 ppm and 5.22 ppm respectively compared to petroleum ether extract and methanol extract where both extracts exhibited IC₅₀ more than 100 ppm. The antioxidative constituents isolated from DCM extract of twig of *J. sambac* were recognized as isoamyl nitrite and benzophenone whereby benzophenone was also found in DCM extract of *J.sambac* leaves. Thus, *J. sambac* can be considered as a promising source of bioactive compounds with potent antioxidant properties which can be utilized as baseline information to overcome health disorders.

Keywords: Antioxidant Activity, Essential Oil, *J.sambac*, Oleaceae, Radical Scavenging

Introduction

Jasminum sambac is a *Jasminum* genus belongs to the Oleaceae family. It is also commonly known as Arabian jasmine and has been widely used around the world in medicine and aromatherapy since ancient times (Sengar et al., 2015). It has more than two hundreds other species and mainly found in South Asia (Shekhar and Prasad, 2015). Modern research claimed that Jasmine exhibited antioxidant properties. Antioxidant is a chemical which inhibits oxidation caused by free radical that can damage body cells. Free radicals are highly reactive substances which induced cancer and gene mutation (Sikiru et al., 2013). Some active compounds in Jasmine can be identified from phytochemicals screening. Phytochemical screening is an analysis involving extraction and identification of bioactive substances which naturally found in plants (Ndam et al., 2014).

Secondary metabolites are organic compounds which are not essential in growth and metabolisms of plants but may be beneficial to human health. Examples of secondary metabolites found in *J. sambac* are alkaloids, flavonoids, terpenoids, carbohydrates, proteins, phenols, tannins, saponins and phytosterols (Shekhar and Prasad, 2015). Traditionally this plant used in blood shot eyes, regulates menstrual flow, to lower blood glucose level, to treat diarrhea, fever, cough, abdominal distension and indolent ulcer. Previous research reported by Sabharwal et al., (2013) indicated that *J. sambac* was antioxidative, antimicrobial, antitumor, antidiabetic and antiacne properties. All parts of this plant such as flower, stem, root, twig and leaves of this plant has their own benefit. The flower and leaves have antipyretic and decongestant properties. Its flower also used for treatment of diarrhea, abdominal pain, conjunctivitis and dermatitis while their leave and root are used for treatment of diarrhea, fever, pain and as an anesthetic. Most of *Jasminum* plants commonly present in pink, white or yellow color are strongly scented and others are unscented (Jain et al., 2011).

Materials and Methods

Plant Extraction for Crude Extracts

The leaves and twig parts of *J. sambac* were collected at Bandar Jengka Pahang and were subjected to open drying process for one week. About 200 g of dried powdered leaves and twig were weighed separately and soaked consecutively for three weeks using petroleum ether, dichloromethane and methanol in three times. After soaking process was completed, the macerations were filtered and subjected to dryness using rotary evaporator to yield crude extract. The crude extracts were safely kept prior to use for Thin Layer Chromatography (TLC) analysis and antioxidant assay.

Fourier Transform Infra-Red (FTIR) Analysis of Crude Extract

The FTIR model of Perkin Elmer Spectrum 100 FT-IR. ATR- FTIR was used to identify the functional groups in methanol, dichloromethane and petroleum ether extract of *J. sambac* leaves and twig. The expected functional groups were alkane, alkene, alcohol, carboxylic acid, ester, benzene aromatic ring, amine and carbonyl group.

Qualitative DPPH Radical Scavenging Assay

All types of extracts from both leaves and twig of *J. sambac* were subjected to TLC chromatographic analysis by developing the TLC sheets in suitable binary solvent until the constituents were well separated on it. As soon as the drying process of TLC was over, the top surface of TLC was sprayed directly with 0.6% DPPH in methanol and was left for several minutes. The yellow emerging color against purple background indicated the antioxidant behavior of the extracts.

Quantitative DPPH Radical Scavenging Assay

The tested sample of methanol extract was prepared at different concentrations ranging from 100, 50, 25, 12.5, 6.25 and 3.125 ug/ml. About 1 ml of different concentration of extract was mixed with 3 ml of 0.004% methanolic solution of DPPH in separate test tube. The solution mixture was allowed to stand in the dark room for 30 minutes. The absorbance of the mixture was measured at 517 nm using UV-visible spectrophotometer of Shimadzu UV-1800 spectrophotometer. About 1 ml methanol in 3 ml of DPPH solution was used as control (Ramesh et al., 2015). The same process

was repeated for petroleum ether extract and dichloromethane as well as ascorbic acid which serve as a standard. The scavenging behavior was observed when DPPH solution changed its color from violet to light yellow. The concentration of the extract that scavenged 50% of DPPH radical was determined from the plotted graph of percent inhibition against concentration (Al-Owaisi et al., 2014). The calculated percent inhibition was performed using the equation below:

$$\% \text{ Inhibition} = (A_0 - A_s / A_0) \times 100\%$$

A_0 : Absorbance of the control

A_s : Absorbance of the tested sample

Isolation of Antioxidative Compounds Using Circular Chromatography

Chromatography involved separation of compound from a mixture in which the mixture was dissolved in selected mobile phase and travelled through the stationary phase. The compounds separated based on differential partitioning at different speeds. Dichloromethane extract was used in the isolation of compounds. Firstly, the rotor was wetted with n-hexane until completed. Then dichloromethane extract was pumped in with n-hexane as eluting solvent. Then, 100 ml of n-hexane was used to separate non-polar compound and collected by using vials. The method was repeated by using different solvent.

Structure Identification Using Gas Chromatography Mass Spectrometer (GC-MS)

Isolated antioxidative compounds and essential oils from leaves and twig were dissolved in a suitable solvent and were analysed using GCMS model of Agilent Technologies GCMS 5977A MSD with carrier gas of helium with flow rate of 1.0 mL/min. Inlet temperature was 250°C and MSD detector temperature was 150°C. The GC oven temperature program has initial temperature of 100°C and increased to 270°C at 4°C/min and finally was held for 7.5 min. 1 µL was injected using a split technique. The chemical constituents in *J. sambac* were identified by comparison of their mass spectral fragmentation patterns in MS library (Al-Owaisi et al., 2014; Wei et al., 2015).

Results and discussion

Fourier Transform Infra-Red (FTIR) Analysis

The FTIR analysis was used as a primary method to characterize the compounds in leaves and twig of *J. sambac* by means of the presence of functional groups in each extracts. The functional groups are usually groups of atoms in the chemical structure that responsible for all chemical reactions. The compounds from *J. sambac* can be classified differently according to the presence of different functional groups. The types of functional groups in leaves and twig of *J. sambac* for each extract is depicted in **Table 1**.

Table 1 depicted common functional groups available in leaves and twig of *J. sambac* in all types of extracts which are OH, C=O, C=C aromatic CH₃, CH₂ and C-O except C=O did not appear in methanol extract of twig part. These functional groups are commonly found in most of organic compounds of secondary metabolites. If we focusing on the absorption frequency of C=O stretching vibration, the value is quite low with that of normal value which is around 1715 cm⁻¹. Generally, it was most probably due to phenomenon of conjugation effect that makes the C=O more single bond character and hence weaken the bond to lower the absorption frequency. The presence of functional group of OH hydroxyl and the carbonyl group C=O might contribute to bioactivity as well as antioxidant activity.

Table 1 FTIR absorption frequency of functional groups for leaves and twig of *J. sambac*

	Petroleum extract		Dichloromethane extract		Methanol extract	
Leaves	Absorption frequency (cm ⁻¹)	Functional group	Absorption frequency (cm ⁻¹)	Functional group	Absorption frequency (cm ⁻¹)	Functional group
	2916.60	CH ₂	3267.31	OH	3267.31	OH
	2849.16	CH ₃	2918.25	CH ₃	2928.06	CH ₃
	1704.80	C=O	2849.84	CH ₂	1606.84	C=O
	1462.81	CH ₃ bending	1706.23	C=O		
	1377.16	CH ₂ bending	1621.42 and 1461.95 and 1164.93	C=C aromatic C-O		
	3430.88	O-H	2849.07	C-H aldehyd	3273.83	OH
Twig	2916.74	C-H aldehyd	1706.90	C=O	2933.81	C-H
	1704.76	C=O	1634.45 and 1462.69	C=C aromatic	1628.44	C=C
	1052.50	C-O	1035.35	C-O	1020.13	C-O

Quantitative DPPH Radical Scavenging Assay of Leaves and Twig of *J. Sambac*

Antioxidant is a substance that can hinder oxidation of other molecule from producing free radicals. When chain reaction occurs in the body cell, it can cause damage to the cell. Antioxidant will halt the chain reactions by removing free radical intermediate. Usually 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant assay is used to identify antioxidant activity. It is the capability of stable free radical DPPH to react with hydrogen donors and can be determined under UV-Vis spectroscopy (Shekhar and Prasad, 2015).

The DPPH scavenging process is a process to test an ability of compound as a radical scavenger or hydrogen donors as well as evaluation of antioxidant activity. The DPPH scavenging assay method is based on the reduction of DPPH, a stable and nitrogen centered violet colored free radical that upon reduction is converted to yellow by electron or hydrogen donating ability of the antioxidant compound found in the extract. The degree of discoloration indicates the scavenging potential of antioxidant compounds of extract in terms of hydrogen ability. The more the discoloration the more is the reducing ability. **Figure 1** and **Figure 2** show the relation of the percentage of DPPH scavenging of different extracts with different serially diluted concentrations of leaves and twig extracts of *J. sambac*.

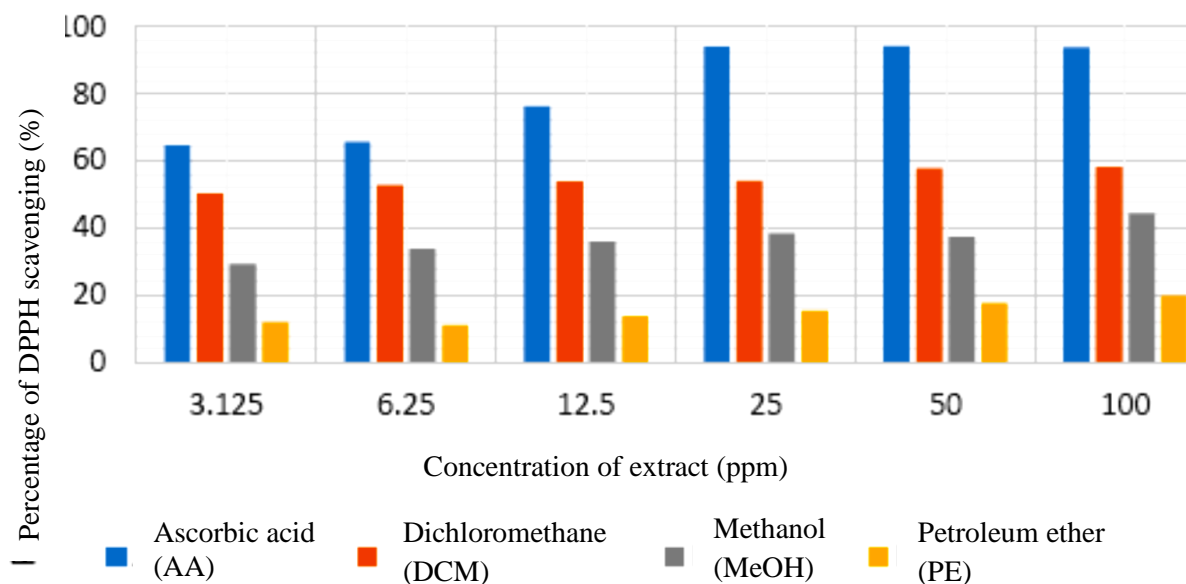


Figure 1 Percentage of DPPH scavenging at different concentration of *J. sambac* leaves

For leaves extract, according to the **Figure 1**, generally, as the concentration changed, the percentage of DPPH scavenging was also change in a concentration dependent manner whereby, the more the concentration of extracts, the higher the percentage scavenging which indicates the increment of antioxidants. Ascorbic acid has the highest DPPH scavenging for all concentrations compared with extracts and among extracts, dichloromethane extract showed higher percentage scavenging in the same manner as ascorbic acid.

Dichloromethane extract is considered as moderately antioxidative because at 3.125 ppm, the DPPH has already scavenged DPPH by 50.2%. Methanol has the second least antioxidant content due to the DPPH scavenging is less than 50% at 100 ppm. This means methanol extract of *J. sambac* leaves is a weak antioxidant because it requires high concentration of extract to scavenge the DPPH by 50%. Petroleum ether has the least scavenging which is less than 20% even at concentration of 100 ppm. Hence, petroleum ether extract and methanol extract of *J. sambac* leaves were not effective enough to be a source of antioxidant.

The scavenging trends of all extracts from twig parts are about in the same manner as it happened in leaves extract. The dichloromethane extract won the competition of scavenging activity compared to other extracts with highest percentage scavenging at about 43% at 50 ppm. This phenomena could possibly because of antioxidants were more abundant in dichloromethane extract.

The quantitative measurement of concentration in inhibiting the DPPH free radicals by half is recognized as inhibitory concentration (IC_{50}). The value of IC_{50} of leaves and twig extracts are shown in **Table 2**. In DPPH scavenging assay, the twig and leaves extracts of *J. sambac* were investigated through free radical scavenging activity via their reaction with the stable DPPH radicals. The low IC_{50} value indicated strong ability of the extract or antioxidant to act as DPPH scavenger and high IC_{50} value indicated low scavenging activity of scavengers as more amounts of the scavengers were required to achieve 50% scavenging reaction. This means, the scavengers are less effective in scavenging the DPPH radicals. According to **Table 2**, the IC_{50} value of dichloromethane of both leaves and twig is lower compared to other extracts and therefore this

extract can work effectively as an antioxidant compared to other extract. When comparing between both parts, the IC₅₀ of dichloromethane extract from twig (5.22 ppm) is slightly lower compared to leaves extract (6.24 ppm). It could be due to the presence of alkaloid in twig part of *J. sambac*. Other extract can be regarded as not effective antioxidant as IC₅₀ values exceed 100% scavenging.

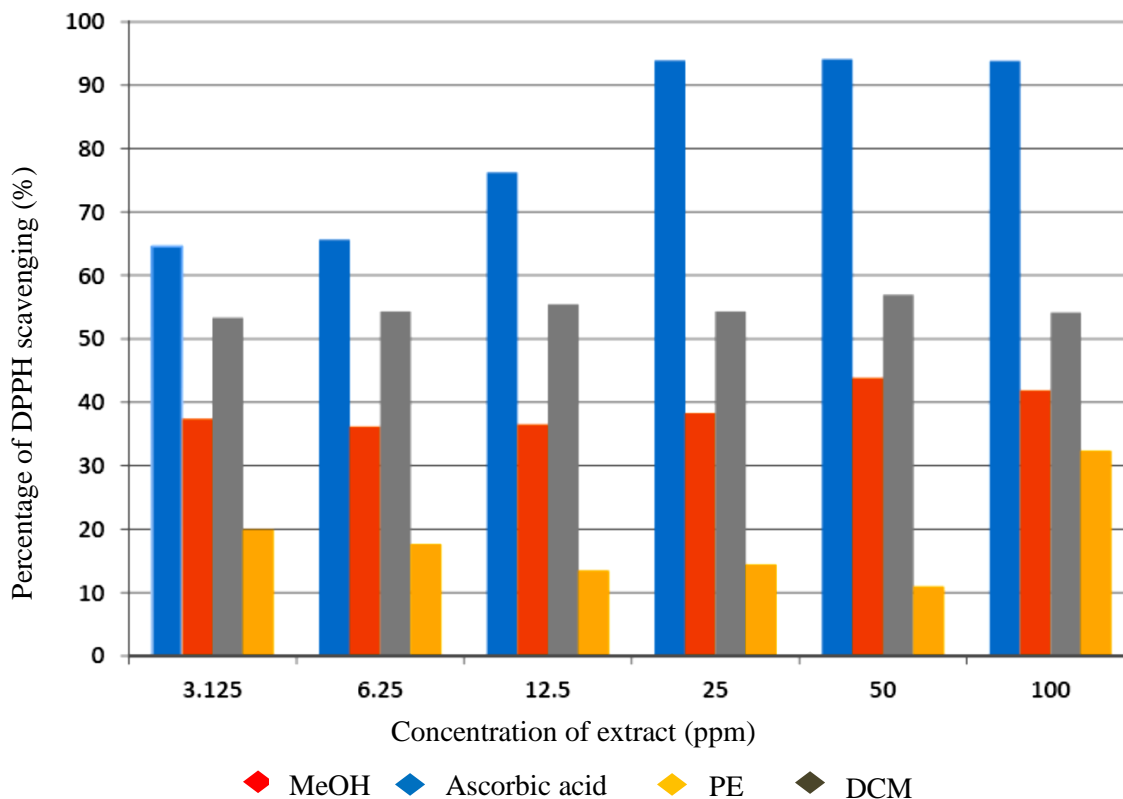


Figure 2 Percentage of DPPH scavenging at different concentration of *J. sambac* twig

Table 2 IC₅₀ of crude extracts of leaves, twig *J. sambac* and standard ascorbic acid

Type of sample	Inhibitory concentration, IC ₅₀ , ppm	
	Leaves	Twig
Ascorbic acid	2.90	2.90
Dichloromethane	6.24	5.22
Methanol	>100	>100
Petroleum ether	>100	>100

Further explanation on how antioxidative compounds react to scavenge radicals can be understood by the mechanism of antioxidant and DPPH. Theoretically, the mechanism of the radical scavenging starts when a DPPH which is known as 2,2-diphenyl-1-picrylhydrazyl of dark purple crystalline powder. 2,2-diphenyl-1-picrylhydrazyl accepts one hydrogen from antioxidant compound/extracts and become 2,2-diphenyl-1-picrylhydrazine which is yellow in color. During radical scavenging process, the nitrogen atom from DPPH will accept an electron from hydrogen

of the –OH group. The loss of electron will form radical oxygen which allows the delocalization of electron to form double bond. Therefore this mechanism also can be used to explain how antioxidant from leaves and twigs of *J. sambac* works on free radicals.

Antioxidatives Constituents from Leaves and Twig of *J.Sambac*

The antioxidative constituent from leaves part was successfully isolated from dichloromethane crude extracts using circular chromatography. The antioxidative behaviour of this extract was screened from Thin layer Chromatography (TLC) qualitative analysis as illustrated in **Figure 3**. The appearance of yellow colour against purple background indicated the antioxidative behaviour of constituents in the extract. According to (TLC) diagram, the yellow colour can be seen at different places on TLC which indicated different antioxidative constituents. However only one pure antioxidative constituent was successfully isolated from leaves part and the Wiley Database from GCMS was recognized the isolated compound as benzophenone after subjected to GCMS analysis. Benzophenone is non-polar aromatic ketone which soluble in polar solvent such as ethyl acetate since it has polar carbonyl group which can form dipole-dipole forces with ethyl acetate. According to Dimitrina et al., (2013), benzophenone from *Hypericum elegans* is antioxidative and has potential to be implemented in free radical pathologies and neurogenerative disorders. **Figure 4(c)** illustrated the benzophenone structure from *J. sambac* leaves.

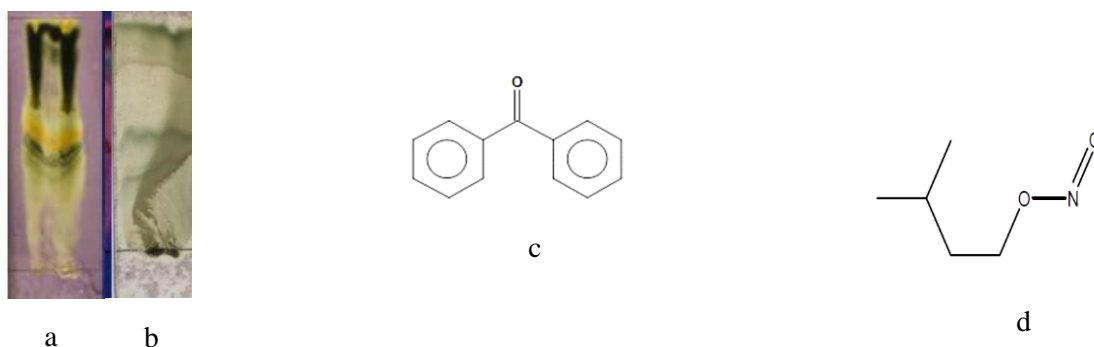


Figure 3 (a): Antioxidative TLC diagram of DCM crude extract *J. sambac* leaves; (b): Antioxidative TLC diagram of DCM crude extract *J. sambac* twig; (c): Benzophenone; (d): Isoamyl nitrite

Isolation and determination of antioxidative constituents from twig part revealed two constituents namely as isoamyl nitrite and benzophenone which is same as it found in leaves part. Both constituents were also comes from dichloromethane extract of twig part. Figure 4(d) depicted the structure of isoamyl nitrite from GC-MS analysis. Since the structure is nitrogen based, isoamyl nitrite is also known as derivative alkaloids. Alkaloid is a group of naturally occurring chemical compound that mostly contain basic nitrogen atom. Besides having nitrogen, an alkaloid structure can have oxygen, sulfur and other elements such as chlorine, bromine and phosphorous. Previously, methanolic extract having phytoprinciples such as tannis, alkaloids, flavonoid, phenolic compound, reducing sugar and protein were responsible for the antioxidant activity in *J. sambac* species (Sikiru et al., 2013). Therefore it is suggested that isoamyl nitrite from twig of *J. sambac* is in agreement with this finding since isoamyl nitrite is also an alkaloid which is known

to be biologically active compound that responsible for antioxidant activity. Alkaloids extract from the *J. sambac* showed strong antioxidant activity, and with its strong radical scavenger power, they can be used as natural and good sources of natural antioxidants for medicinal and commercial purposes (Gowdhami et al., 2015).

Previous studies also revealed antioxidant activity in methanolic extract of *J. sambac* leaves and essential oil (Mittal et al., 2011). The extract was tested by DPPH free radical (2,2-dipheynl-1-picrylhydrazyl) and β -carotene-linolic acid assay. A previous study from Shekhar and Prasad, 2015 revealed methanolic extract of *J. sambac* showed better antioxidant activity than ethanolic extract. However, our present study revealed dichloromethane extract has better antioxidant activity compared to methanolic extract in same species. Since our finding was deviated from the previous study, it would suggest that benzophenone and isoamyl nitrite is new antioxidative constituents from *J. sambac* species.

Conclusion

As the conclusion, two new antioxidative constituents were successfully isolated and determined from leaves and twig parts of *J. sambac* which is known as benzophenone and isoamyl nitrite. The lower IC₅₀ value of dichloromethane extracts from leaves and twig is hoped to provide new information about how to combat current human disease caused by toxic free radicals. However other spectroscopic analysis should be further conducted such as 1D and 2D Nuclear Magnetic Resonance (NMR) as well as Liquid Chromatography Mass Spectrum (LCMS) for structure confirmation.

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Conflict of interests

Author declares no conflict of interest.

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