

Antibacterial and Antioxidant Activities of *Typhonium Flagelliforme* (Lodd.) Blume Tuber

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Abstract: Problem Statement: Multiple drug resistance in human pathogenic micro organisms has developed due to indiscriminate use of modern antimicrobial drugs generally used in the management of infectious diseases. This increases the importance of exploiting the natural sources instead modern drugs. **Approach:** The antibacterial and antioxidant activity of different extracts from of *Typhonium flagelliforme* (L.) Blume tuber (family: Araceae) commonly called 'Rodent Tuber' was assessed towards selected bacteria as well as in different antioxidant models. The antibacterial screening was carried out by disc diffusion method. Two complementary test systems, namely DPPH free radical scavenging and total phenolic compounds, were used for the antioxidant analysis. **Results:** Except hexane extract none of the other extracts shown anti bacterial activity against the selected strains. The hexane extract from *Typhonium flagelliforme* tuber had interesting activity against both the gram negative bacteria, *Pseudomonas aeruginosa* (11±1.0 mm diameter) and *Salmonella choleraesuis* (12±1.1 mm diameter). The positive control, Streptomycin had shown zone of inhibition of 20±1.5 mm, 20±1.3 mm, 23±1.5 mm and 23±1.0 mm in Methicillin Resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella choleraesuis* and *Bacillus subtilis* respectively. All the extracts were subjected to screening for their possible antioxidant activity. The DPPH assay showed that the inhibitory activity of ethyl acetate (77.6±0.9%) and dichloromethane (70.5±1.7%) extracts were having comparatively admirable inhibition capacity when compared to the positive control BHT (95.3±1.3%). Total phenolic content of all extracts was also evaluated, and dichloromethane extracts (5.21±0.82 GAE mg/g extract) was superior to all other extracts, followed by hexane and ethyl acetate. **Conclusion:** Considering all the results collectively *T. flagelliforme* appears to be a promising plant demonstrating antibacterial and antioxidant activity that requires further investigation.

Key words: *Typhonium flagelliforme*, rodent tuber, antibacterial activity, antioxidant activity, fatty acids

INTRODUCTION

Plants are more important in human's life and fulfil his every day's needs. They are used as cosmetic, food, flavours, ornamental and medicine. The higher plants are used to treat a number of infectious diseases around the world. They provide an innumerable of natural products, which are used in extensive applications in combating the diseases.

Medicine is used for treating diverse ailments. Malaysia possesses an immense number of medicinal plants^[1]. Even if medicinal plants are used and distributed throughout the world, they are more

plentifully present in tropical countries like Malaysia. These medicinal plants are one of the best resources for the invention and development of novel bioactive substances^[2]. Several studies indicated that medicinal plants contain substances like peptide, unsaturated long chain fatty acids, aldehydes, alkaloids, essential oils, phenols and water or ethanol soluble compounds. These compounds are potentially significant in therapeutic applications against human and animal pathogens, including bacteria, fungi and viruses^[3,4].

Oxidation is essential in many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen

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derived free radicals is involved in the onset of many diseases such as atherosclerosis, rheumatoid arthritis, and cancer as well as in degenerative processes associated with aging^[5]. Almost all organisms are well protected against free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherols and glutathione^[6]. When the mechanism of antioxidant protection becomes unbalanced by factors such as aging, deterioration of physiological functions may occur resulting in diseases and accelerated aging. However, the antioxidants present in human diet are of great interest as possible protective agents to help the human bodies reduce oxidative damage.

Researchers have reported the antimicrobial activity of several herbal plants^[7,8]. In recent years, multiple drug resistance in human pathogenic micro organisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents^[9]. The cost of production of synthetic drugs is also high and they produce adverse effect compared to plants derived drugs. Hence much attention has been paid recently, to the biologically active compounds derived from plants used in herbal medicine^[10].

Typhonium flagelliforme (Lodd.) Blume (Araceae) is a herbal plant which grows up to 30cm in height. It has an oblong whitish tuber, triangular leaves and a spathe. The occasionally beautiful and often bizarre combination of spathe and spadix called the inflorescence, sometimes referred to as a flower is a distinguishing feature of all aroids, which has been used for trapping their pollinators because of their particular morphology and organization of their inflorescences^[11]. This plant grows wild in wasteland and is native to the South East Asian countries and the southern part of India and Sri Lanka^[12]. As a general practice, the juice of the fresh whole *Typhonium flagelliforme* plant is prepared in honey to be consumed as a drink. There are also other practices where the leaves are wrapped in Longan flesh and taken raw^[13].

Hexane extract of *T. flagelliforme*, displayed poor cytotoxic activity against *in vitro* P 388 murine leukemia cells^[12]. A low cytotoxic activity has been exhibited by the polar fraction of this plant, with crude water extract being able to reduce lymphoidal cells growth *in vitro*^[14]. *T. flagelliforme*, were also used to provide relief in cough and asthma, which was experimentally verified that water, alcohol and ester extract could significantly decrease cough times,

prolong asthma incubation period, decrease twisting times, inhibit ear swelling and decrease autonomic action times^[15].

Several chemical constituents had been identified from *T. flagelliforme*. The hexane extract was reported to contain saturated hydrocarbons and aliphatic acids^[16], while the ethyl acetate extract was found to contain aromatic fatty acids^[16]. No biological activities were indicated for these compounds. In addition, phenylpropanoid glycosides, sterols, and a cerebroside which has anti hepatotoxic activity were reported from the root of this plant^[17]. Pharmacological studies conducted on rats also indicated that the juice extract was able to prevent hepatocarcinogenesis^[13].

The aim of this study was to evaluate the *in vitro* antibacterial activity of the *T. flagelliforme* tuber against Gram-positive and Gram-negative bacteria and its antioxidant activity.

MATERIALS AND METHODS

Collection of plant materials: *T. flagelliforme* (Lodd.) Blume tubers were collected in July 2007 from the state of Selangor, Malaysia. Authentication was done at the Department of Botany, Faculty of Science, University Putra Malaysia where voucher specimen TF-T100156 was deposited.

Extraction procedure: Fresh Plant (10 kg) were harvested and washed thoroughly with running tap water and then distilled water, followed by separation in to aerial parts as well as tubers before drying. The tubers were air dried and then oven dried at reduced temperature. The fully dried plant were powdered and weighed before cold maceration. The powdered tuber (398 gm) was extracted with different solvents in the order of increasing polarity. The solvents used were hexane, dichloromethane, ethyl acetate and methanol. The extraction was done for 7 days with occasional shaking and the process was repeated for three times. The combined extracts were filtered through Whatman® No. 41 filter paper (pore size 20-25 µm) and dried under vacuum using a rotary evaporator and then weighed to calculate the yield of the extracts and stored at 4°C until required.

Antibacterial activity:

Bacterial strains: The antibacterial activity of plant extracts was evaluated using two Gram-positive bacteria, Methicillin Resistant *Staphylococcus Aureus* (MRSA) and *Bacillus subtilis* B29, and other two Gram-negative bacteria, *Pseudomonas aeruginosa* 60690 and *Salmonella choleraesuis*. All the bacterial

strains were obtained from Laboratory of Molecular Biomedicine, Institute of Bioscience, University Putra Malaysia, Serdang, Malaysia.

Antibacterial Assay: The screening of the extracts on antibacterial effect was carried out by determining the zone of inhibition using paper disc (6 mm in diameter, Whatman No. 1) diffusion method^[18,19]. The obtained micro organism strains were inoculated in a Petri dish containing nutrient broth at 37°C for 24 h and were referred as seeded broth. The density of the bacterial suspension was standardized by standard method and the concentrations of the cultures were adjusted turbidometrically at wavelength of 600nm to 500,000-1000,000 colony forming unit per mL (CFU mL⁻¹). The extracts were dissolved in dimethyl sulphoxide which was previously tested for antibacterial activity against all test bacteria and found to have no antibacterial activity. The extracts were diluted to concentration of 100 mg mL⁻¹ and finally sterilized by filtration using 0.45 µm millipore filters. The sterile discs were impregnated with extract solution (0.05mL from 100mg mL⁻¹ extract) to achieve desired concentration and placed in inoculated agar. Streptomycin (10 µg/disc) was used as standard. The controls were prepared using the same solvents without extracts. The inoculated plates contain the test and standard discs were incubated at 37°C for 24 h.

Antioxidant Assay:

Amount of total phenolic compounds: The amount of Total Phenolics (TPC) in the extracts was determined using the Folin-Ciocalteu reagent method^[20]. Stock solutions of *T. flagelliforme* extracts were prepared in a concentration of 20 mg/ml, a 50 µL from this solution was transferred to a test tube (n = 3). To this tube, 0.4 mL of Folin-Ciocalteu reagent (1:10) was added and the tube was shaken thoroughly. After 1 min, 0.8 mL of sodium bicarbonate solution (NaHCO₃ 7.5%) was added and the mixture was allowed to stand for 30 min with intermittent shaking. Absorbance was measured at 765 nm using a Shimadzu UV-Vis spectrophotometer. The total phenolic content was expressed as Gallic Acid Equivalents (GAE) in mg per g extract from the calibration curve of gallic acid standard solution. For the gallic acid, the curve was established by plotting concentration (mg mL⁻¹) versus absorbance (nm) ($y = 5.145x + 0.014$; $R^2 = 0.9975$). Here $y =$ Absorbance and $x =$ Concentration.

DPPH radical scavenging assay: Radical scavenging activity of plant extracts against stable DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate, Sigma-Aldrich Chemie, Steinheim, Germany) was determined spectrophotometrically. When DPPH reacts with an

antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep-violet to light-yellow) were measured at 517 nm wavelength.

Radical scavenging activity of extracts was measured by slightly modified method of Changwei *et al.*^[21] as described below. Extract stock solutions were prepared in 100mg/ml in ethanol. Methanol extract was not fully soluble in ethanol (even after treating solutions for 5 min in an ultrasonic bath), therefore it was dissolved in dimethylsulphoxide. The working solution was prepared using methanol in a concentration of 500 µg mL⁻¹. The solution of DPPH in methanol 2.5 mg mL⁻¹ was prepared daily, before UV measurements. 5 µL of this solution were mixed with 100 µl extract solution 96 well plate. The samples were kept in the dark for 30 min at ambient temperature and then the decrease in absorption was measured by using microtitre plate reader (Labsystems iEMS Reader MF). Absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured daily. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula:

$$\% \text{ Inhibition} = [(A_B - A_A) / A_B] \times 100$$

where:

A_B = Absorption of blank sample (t = 0 min)

A_A = Absorption of tested extract solution (t = 30 min).

Commercial standard antioxidant Butylated Hydroxyl Toluene (BHT) was also tested against DPPH and used as a reference.

RESULTS

In this study, some of biological activities of *T. flagelliforme* tuber have been investigated, whereby; hexane, dichloromethane, ethyl acetate and methanol extracts of *T. flagelliforme* tuber were assayed for their antibacterial and anti-oxidant properties using disc diffusion method, DPPH assay and total phenolic compounds, respectively.

The antibacterial activity results are shown in Table 1. Our findings showed that except hexane extract none of the other extracts shown any activity against the selected strains. The hexane extract from *T. flagelliforme* tuber had interesting activity against gram negative bacteria. In which the activity was against *Pseudomonas aeruginosa* 11±1.0 mm diameter and *Salmonella choleraesuis* 12±1.1 mm diameter. The positive control, Streptomycin had shown zone of inhibition of 20±1.5 mm, 20±1.3mm, 23±1.5mm and 23±1.0 mm in Methicillin Resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella choleraesuis* and *Bacillus subtilis* respectively.

Table 1: Paper disk diffusion of *T. flagelliforme* tuber growth^a

	Bacterial Strains			
	Diameter of Inhibition (millimetre)			
	MRSA	PA	SC	BS
<i>T. flagelliforme</i> tuber				
Hexane	-	11±1.0	12±1.1	-
Dichloromethane	-	-	-	-
Ethyl Acetate	-	-	-	-
Methanol	-	-	-	-
Control (Streptomycin 10 µg/disc)	20±1.5	20±1.3	23±1.5	23±1.0

^aThe screening of the extracts antibacterial effect was carried out by determining the zone of inhibition using paper disc (6 mm in diameter, Whatman No. 1) diffusion method (n = 2). MRSA: Methicillin Resistant *Staphylococcus aureus*, PA: *Pseudomonas aeruginosa*, SC: *Salmonella choleraesuis* and BS: *Bacillus subtilis*.

Table 2: Total phenolic content in *T. flagelliforme* tuber extracts

<i>T. flagelliforme</i> Tuber	Total phenolic content as gallic acid equivalents (GAE mg g ⁻¹ extract)
Extracts	
Hexane	3.27±0.85 ^b
Dichloromethane	5.21±0.82 ^b
Ethyl Acetate	2.49±0.33 ^a
Methanol	2.20±0.25 ^c

Each value in the table is represented as mean ± SE (n = 3); different letters in the same column indicate significant difference (P < 0.05)

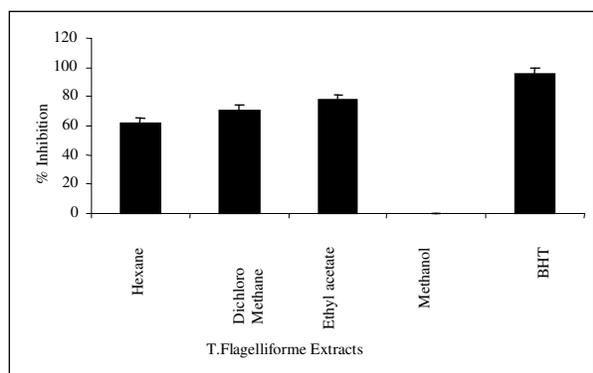


Fig. 1: Free radical scavenging capacities of the *T. flagelliforme* tuber extracts measured in DPPH assay

All the extracts were subjected to screening for their possible antioxidant activity. Two complementary test systems, namely DPPH free radical scavenging and total phenolic compounds were used for the analysis. DPPH, a stable free radical with a characteristic absorption at 517 nm, was used to study the radical scavenging effects of extracts. As antioxidants donate protons to these radicals, the absorption decreases. The decrease in absorption is taken as a measure of the extent of radical scavenging. Free radical scavenging capacities of the extracts, measured by DPPH assay, are

shown in Fig. 1. The results showed that the inhibitory activity of ethyl acetate 77.6±0.9% and dichloromethane 70.5±1.7% extracts were comparatively admirable inhibition capacity when compared to the positive control BHT 95.3±1.3%. The total phenolic content of dichloromethane extracts 5.21±0.82 GAE mg g⁻¹ extract was superior to all other extracts, followed by hexane and ethyl acetate (Table 2).

DISCUSSION

Our results showed considerable antibacterial activity of the hexane extract, especially against gram negative strains. This is probably due to saturated fatty acids present in the extract, which was well documented^[16]. Saturated fatty acids have been studied extensively on their antibacterial activity^[22,23]. Due to the continuous emergence of antibiotic-resistant strains there is continual demand for new antibiotics. In many developing countries about 80% of available drugs come from medicinal plants and in industrialized countries plants make up the raw material for processes, which synthesize pure chemical derivatives^[24].

Both the complementary test reveals that dichloromethane and ethyl acetate extracts are showing good anti oxidant activity. The growing interest in the substitution of synthetic food antioxidants with natural ones has fostered research on plant sources and screening of raw materials to identify new antioxidants. In this view some biological properties such as anticarcinogenicity, antimutagenicity, antiallergenicity and antiaging activity have been reported for natural and synthetic antioxidants^[25]. Polyphenols are the major plant compounds with antioxidant activity, although they are not the only ones. The antioxidant activity of phenolic compounds is reported to be mainly due to their redox properties^[26,27], which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

CONCLUSION

The experiments described above demonstrated that *T. flagelliforme* tuber extracts possess compounds with significant antibacterial and antioxidant effect which could be purified from the respective extracts. The further isolation of such bioactive components could perhaps clarify the pharmacological properties of *T. flagelliforme* tuber and be further exploited for pharmaceutical use.

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