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# Investigations of Antioxidant and Antibacterial Activities of *Typhonium flagelliforme* (Lodd.) Blume Leaves

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**Abstract:** The antioxidant and antibacterial activity of different extracts from of *Typhonium flagelliforme* (L.) Blume leaves (family: Araceae) commonly called 'Rodent Tuber' was assessed towards different antioxidant models as well as in selected bacteria. None of the extracts showed significant activity against the selected strains. The only exception is hexane extract (2.0±0.15 mm diameter) against *Pseudomonas aeruginosa.* The positive control, Streptomycin had shown zone of inhibition of 20±1.5, 20±1.3, 23±1.5 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. Two complementary test systems, namely DPPH free radical scavenging and total phenolic compounds, were used for the analysis. The results showed that the inhibitory activity of Dichloromethane (60.7±3.2%) and Methanol (60.1±2.3%) extracts were comparatively commendable inhibition capacity when compared to the positive control BHT (95.3±1.3%). The total phenolic content of Methanol extracts (5.69±0.15 GAE mg $g^{-1}$ extract) was superior to all other extracts, followed by dichloromethane and ethyl acetate. Considering all the results collectively T. *flagelliforme* appears to be a promising plant demonstrating antioxidant activity that requires further investigation.

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

## 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 (Lin, 2005). 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 (Tomoko *et al*., 2002). 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 (Parvez *et al*., 2005; Khan *et al*., 2003).

Oxidation is essential in many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen 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 (Halliwell and Gutteridge, 1984). 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 (Mau *et al*., 2002). When the mechanism of antioxidant protection becomes unbalanced by factors

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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 (Shaia *et al*., 2008; Firas and Al-Bayati, 2008). In recent years, multiple drug resistance in human pathogenic microorganisms 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 (Karaman *et al*., 2003). 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 (Essawi, 2000).

*Typhonium flagelliforme* (Lodd.) Blume (Araceae) is a herbal plant which grows up to 30 cm 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 (Jerome *et al*., 2001). This plant grows wild in wasteland and is native to the South East Asian countries and the southern part of India and Sri Lanka (Chee *et al*., 2001b). 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 (Choon *et al*., 2008).

Hexane extract of *T. flagelliforme*, displayed poor cytotoxic activity against *in vitro* P 388 murine leukemia cells (Chee *et al*., 2001a). 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* (Chee *et al*., 2001b). *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 (Zhong *et al*., 2001).

Several chemical constituents had been identified from *T. flagelliforme*. The hexane extract was reported to contain saturated hydrocarbons and aliphatic acids (Chee *et al*., 2001b), while the ethyl acetate extract was found to contain aromatic fatty acids (Chen *et al*., 1997). 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 (Huang *et al*., 2004). Pharmacological studies conducted on rats also indicated that the juice extract was able to prevent hepatocarcinogenesis (Choon *et al*., 2008).

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

## MATERIALS AND METHODS

**Collection of plant materials:** *T. flagelliforme* (Lodd.) Blume leaves 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-L100156 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 tuber before drying. The leaves were air dried and then oven dried at reduced temperature. The fully dried plant were powdered and weighed before cold maceration. The powdered leaves (284 g) were 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, Universiti 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 (Sahoo *et al*., 2006;

Prusti *et al*., 1999). The obtained microorganism strains were inoculated in a Petri dish containing nutrient broth at 370°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 600 nm 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 (0.05 mL from 100 mg $mL^{-1}$ extract) to achieve desired concentration and placed in inoculated agar. Streptomycin (10 μg $disc^{-1}$) 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 (Djeridane *et al*., 2006). Stock solutions of *T. flagelliforme* leaves extracts were prepared in a concentration of 20 mg $mL^{-1}$, 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 ($NaHCO_3$ 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 $g^{-1}$ 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.* (2008). Extract stock solutions were prepared in 100 mg $mL^{-1}$ 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. A 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 Hydroxytoluene (BHT) was also tested against DPPH and used as a reference.

## RESULTS

In this study, some of biological activities of *T. flagelliforme* leaves have been investigated, whereby; hexane, dichloromethane, ethyl acetate and methanol extracts of *T. flagelliforme* leaves were assayed for their antibacterial and antioxidant 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 none of the extracts showed significant activity against the selected strains. The only exception is hexane extract (2.0±0.15 mm diameter) against *Pseudomonas aeruginosa.* The positive control, Streptomycin had shown zone of inhibition of 20±1.5, 20±1.3, 23±1.5 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. 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

Table 1: Paper disk diffusion of *T. flagelliforme* leaves growth[a]

| | Diameter of inhibition (millimetre) | | | |
|---|---|---|---|---|
| | Bacterial strains | | | |
| *T. flagelliforme* leaves | MRSA | PA | SC | BS |
| Hexane | - | 2.0±0.15 | - | - |
| Dichloromethane | - | - | - | - |
| Ethyl acetate | - | - | - | - |
| Methanol | - | - | - | - |
| Control (Streptomycin 10 μg disc$^{-1}$) | 20±1.5 | 20±1.3 | 23±1.5 | 23±1.0 |

[a]The 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* leaves extracts

| *T. flagelliforme* leaves extracts | Total phenolic content as gallic acid equivalents (GAE mg g$^{-1}$ extract) |
|---|---|
| Hexane | 2.20±0.32[b] |
| Dichloromethane | 5.31±0.82[b] |
| Ethyl acetate | 4.24±0.26[a] |
| Methanol | 5.69±0.15[c] |

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

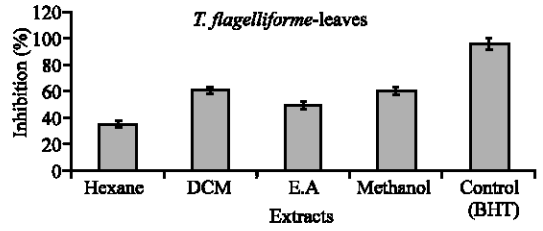


Fig. 1: Free radical scavenging capacities of the *T. flagiliforme* leaves extracts measured in DPPH assay

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 Dichloromethane (60.7±3.2%) and Methanol (60.1±2.3%) extracts were comparatively commendable inhibition capacity when compared to the positive control BHT (95.3±1.3%). The total phenolic content of Methanol extracts (5.69±0.15 GAE mg g$^{-1}$ extract) was superior to all other extracts, followed by dichloromethane and ethyl acetate (Table 2).

## DISCUSSION

Our results showed no antibacterial activity of *T. Flagelliforme* leaves against the selected strains. The only exception is hexane extract (2.0±0.15 mm diameter) against *Pseudomonas aeruginosa* and which is not considerable too.

Both the complementary test reveals that dichloromethane and methanol extracts are showing good antioxidant activity. At the same time it is interesting to note that the same extracts give the higher total phenolic content as well. 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 (Moure *et al*., 2001). 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 (Galato *et al*., 2001; Zheng and Wang, 2001), 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* leaves extracts possess compounds with significant antioxidant effect which could be purified from the respective extracts. In addition to being a good antioxidant, further studies are needed to be done before reaching to any concrete conclusion. Efforts are being in progress to evaluate this extracts in number of other assays and to identify the active principles, responsible for their bioactivity by different spectroscopic method.

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## REFERENCES

Changwei, A., L. Anping, A.E. Abdelnaser, D.X. Tran and T. Shinkichi, 2008. Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa* L. fil. Extract. Food Control., 19 (10): 940-948. DOI: 10.1016/ j.foodcont.2007.09.007.

Chee, Y.C., L.C. Kit., W.S. Teng, H. Yukio and T. Koichi, 2001a. The cytotoxicity and chemical constituents of the hexane fraction of *Typhonium flagelliforme* (Araceae). J. Ethnopharmacol., 77: 129-131. PMID: 11483390.

Chee, Y.C., L.C. Kit., T. Koichi and I. Hideji, 2001b. Cytotoxic activity of *Typhonium flagelliforme* (Araceace). Phytother. Res., 15: 260-262. PMID: 11351365.

Chen, S.X., C.J. Gohand and O.L. Kon, 1997. Fatty acids from Typhonium flagelliforme. Planta Med., 63: 580-583. PMID: 9434618.

Choon, S.L., H.M.H. Rosemal, N.K. Nair, M.I.A. Majid, S.M. Mansor and V. Navaratnam, 2008. *Typhonium flagelliforme* inhibits cancer cell growth *in vitro* and induces apoptosis: An evaluation by the bioactivity guided approach. J. Ethnopharmacol., 118: 14-20. PMID: 18436400.

Djeridane, A., M. Yousfi, B. Nadjemi, D. Boutassouna, P. Stocher and N. Vidal, 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem., 97: 654-660. DOI: 10.1016/j.foodchem.2005.04.028.

Essawi, T., 2000. Screening of some Palestinian medicinal plants for antimicrobial activity. J. Ethnopharmocol., 70: 343-349. DOI:10.1016/S0378-8741(99)00187-7.

Firas, A. and Al-Bayati, 2008. Synergistic antibacterial activity between Thymus vulgaris and Pimpinella anisum essential oils and methanol extracts. J. Ehnopharmacol.,116: 403-406. DOI: 10.1016/j.jep.2007.12.003.

Galato, D., K. Ckless, M.F. Susin, C. Giacomelli, R.M. Ribeiro do Valle and A. Spinelli, 2001. Antioxidant capacity of phenolic and related compounds: Correlation among electrochemical, visible spectroscopy methods and structure-antioxidant activity. Redox Report, 6: 243-250. DOI: 10.1179/135100001101536391.

Halliwell, B. and J.M.C. Gutteridge, 1984. Lipid Peroxidation, Oxygen Radicals, Cell Damage and Antioxýdant Therapy. Lancet, 323 (8391): 1396-1397. PMID: 6145845.

Huang, P., G. Karagianis and P.G. Waterman, 2004. Chemical constituents from *Typhonium flagelliforme*. Zhongyaocai, 27: 173-175. PMID: 15272778.

Jerome, A., Q. Angelique and G. Marc, 2001. Pollination ecology of *Arum italicum* (Araceae). Botanical J. Linnean Soc., 141: 205-214. DOI: 10.1046/j.1095-8339.2003.00139.x.

Karaman, F. Sahin, M. Güllüce, H. Ögütçü, M. Sengül and A. Adýgüzel, 2003. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. J. Ethnopharmacol., 85: 213-235. DOI:10.1016/S0378-8741(03)00006-0.

Khan, M., M. Kibara and B. Oinoloso, 2003. Antimicrobial activity of the alkaloidal constituents of the root bark of Eupomatia lourina. Pharmaceut. Biol., 41: 277-280. DOI: 10.1076/phbi.41.4.277.15671.

Lin, K.W., 2005. Ethnobotanical study of medicinal plants used by the Jah Hut peoples in Malaysia. Indian J. Med. Sci., 59: 156-161. http://www.indianjmedsci.org/temp/IndianJMedSci594156-8479652_233316.pdf.

Mau, J.L., H.C. Lin and S.F. Song, 2002. Antioxidant properties of several specialty mushrooms. Food Res. Int.,35: 519-526. DOI: 10.1016/S0963-9969(01)00150-8.

Moure, A., J.M. Cruz, D. Franco, J.M. Dominguez, J. Sineiro, H. Dom´ýnguez, M.J. ´U˜nez and J.C. Paraj´o, 2001. Natural antioxidants from residual sources. Food Chem., 2: 145-171. DOI:10.1016/S0308-8146(00)00223-5.

Parvez, M., H.K. Mahboob, I. Zahurul and M.H. Shek, 2005. Antimicrobial activities of the petroleum ether, methanol and acetone extracts of kaempferia galangal. rhizome. J. Life Earth Sci., 1: 25-29. http://www.ru.ac.bd/flife/06.%20Antimicrobiol.pdf.

Prusti, A., S.R. Mishra, S. Sahoo and S.K Mishra, 2008. Antibacterial Activity of Some Indian Medicinal Plants. Ethnobotanical Leaflets, 12: 227-230. http://www.siu.edu/~ebl/leaflets/abp.htm.

Sahoo, S., D.M. Kar, S. Mohapatra, S.P. Rout and S.K. Dash, 2006. Antibacterial activity of Hybanthus enneaspermus against selected UTI pathogens. Ind. J. Pharmacol. Sci., 68 (5): 653-655. http://www.ijpsonline.com/temp/IndianJPharmSci685653-2204888_060728.pdf.

Shai, L.J., L.J. McGawa, M.A. Aderogbaa, L.K. Mdeea and J.N. Eloff, 2008. Four pentacyclic triterpenoids with antifungal and antibacterial activity from Curtisia dentata (Burm.f) C.A. Sm. Leaves. J. Ethnopharmacol., 119: 238-244. DOI: 10.1016/j.jep.2008.06.036.

Tomoko, N., A. Takashi, T. Hiromu, I. Yuka, M. Hiroko, I. Munekazu, T. Totshiyuki, I. Tetsuro, A. Fugio, I. Iriya, N. Tsutomu and W. Kazuttito, 2002. Antibacterial activity of extracts prepared from tropical and sub tropical plants on methicillin resistant *Staphylococcus aureus*. J. Health Sci., 48: 273-289. http://jhs.pharm.or.jp/data/48(3)/48_273.pdf.

Zheng, W. and S.Y. Wang, 2001. Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem., 49: 5165-5170. PMID: 11714298.

Zhong, Z., G. Zhou, X. Chen and P. Huang, 2001. Pharmacological study on the extracts from *Typhonium flagelliforme*. J. Chinese Med. Materials, 24: 735-738. PMID: 11822289.