วารสารมหาวิทยาลัยศรีนครินทรวิโรฒ (สาขาวิทยาศาสตร์และเทคโนโลยี) ปีที่ 12 ฉบับที่ 24 กรกฎาคม-ธันวาคม 2563

การตรวจหาสารพฤกษเคมีและฤทธิ์ต้านจุลินทรีย์ของสารสกัดเท้ายายม่อม
(Tacca leontopetaloides (L.) Kuntze.)
PHYSICOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF THAO YAI MOM
(TACCA LEONTOPETALOIDES (L.) KUNTZE.) EXTRACTS

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# บทคัดย่อ

การตรวจสอบสารพฤกษเคมีเบื้องต้นของหัวเท้ายายม่อม พบว่าสารสกัดจากฟลาวร์ประกอบด้วย แอลคาลอยด์ ซาโพนิน ฟลาโวนอยด์ และแทนนิน โดยสารพฤกษเคมีทั้งหมดข้างต้นยกเว้นแอลคาลอยด์พบในสาร สกัดจากเปลือกเช่นกัน การทดสอบเฟอร์ริกคลอไรด์ไม่สามารถตรวจพบสารประกอบฟืนอลิกจากสารสกัดทั้งสอง ส่วน การวิเคราะห์สารประกอบฟืนอกลิกและแทนนินทั้งหมดด้วยวิธี Folin-Ciocalteu colorimetric พบว่าสารสกัด จาก ฟลาวร์และเปลือกมีปริมาณฟืนอลิกทั้งหมด 2.70 และ 8.11 มิลลิกรัมสมมูลกรดแกลลิก / กรัมน้ำหนักแห้ง ์ตามลำดับ และมีปริมาณแทนนินทั้งหมดเท่ากับ 2.62 และ 8.68 มิลลิกรัมสมมูลกรดแทนนิก / กรัมน้ำหนักแห้ง ตามลำดับ ขณะที่ปริมาณฟลาโวนอยด์ในสารสกัดจากเปลือกวิเคราะห์ได้ 20.79 มิลลิกรัมสมมูลเคอร์ซิทิน / ้น้ำหนักแห้ง ซึ่งสูงกว่าปริมาณฟลาโวนอยด์ในสารสกัดจากฟลาวร์ (0.79 มิลลิกรัมสมมูลเคอร์ซิทิน / น้ำหนักแห้ง) ถึง 26 เท่า สารสกัดจากเปลือกแสดงฤทธิ์ยับยั้งการเจริญของจุลินทรีย์อย่างมีนัยสำคัญเมื่อทดสอบด้วยวิธี Broth dilution โดยสามารถยับยั้งการเจริญของ Candida lipolytica ได้ร้อยละ 99.70, Bacillus subtilis ร้อยละ 76.58, Enterococcus faecalis TISTR 379 ร้อยละ 72.79, Staphylococcus aureus ร้อยละ 69.23 และ Salmonella sp. ร้อยละ 56.92 แบคทีเรียแกรมลบตัวอื่น ได้แก่ Pseudomonas fluorescens TISTR 904 และ Escherichia coli ้ มีความต้านทานต่อสารสกัดจากเปลือกมากกว่า Salmonella sp. สารสกัดจากฟลาวร์มีฤทธิ์ยับยั้งจุลินทรีย์ต่ำกว่า สารสกัดจากเปลือก โดยแสดงฤทธิ์ยับยั้งการเจริญของจุลินทรีย์อย่างมีนัยสำคัญต่อ C. lipolytica คิดเป็นร้อยละ 45.86 และ E. faecalis TISTR 379 คิดเป็นร้อยละ 13.55 การทดสอบ MIC พบว่า S. aureus ไวต่อสารสกัดจาก เปลือกมากที่สุด เนื่องจากมีค่า MIC ต่ำที่สุด (12.5 มิลลิกรัม / มิลลิลิตร) ตามด้วย *E. faecali*s TISTR 379 และ C. lipolytica (25 มิลลิกรัม / มิลลิลิตร) และ B. subtilis (50 มิลลิกรัม / มิลลิลิตร) ส่วนแบคที่เรียแกรมลบ Salmonella sp. มีความไวต่อสารสกัดน้อยที่สุด โดยมีค่า MIC สูงสุด (100 มิลลิกรัม / มิลลิลิตร) ผลการศึกษา แสดงให้เห็นว่าเท้ายายม่อมมีฤทธิ์ต้านจุลินทรีย์และอาจใช้เป็นสารสกัดจากธรรมชาติที่สามารถยับยั้บจุลินทรีย์ใด้

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**คำสำคัญ:** เท้ายายม่อม ฟืนอลิก แทนนิน ฟลาโวนอยด์ ฤทธิ์ยับยั้งจุลินทรีย์

## **Abstract**

Phytochemical screening of Thao Yai Mom (Tacca leontopetaloides (L.) Kuntze) indicated that its flour extracts contained alkaloids, saponins, flavonoids and tannins. These compounds except alkaloids were also found in the peel extracts. The ferric chloride test could not detect phenolic in both extracts. Total phenolic contents of the flour and peel extracts determined by Folin-Ciocalteu colorimetric method were 2.70 and 8.11 mg GAE/g dry sample, respectively. Tannins were evaluated at 2.62 and 8.68 mg TAE/g dry sample of flour and peel, respectively. Whereas total flavonoid content of the peel extracts was 20.79 mg QE/g dry sample, which was about 26 times higher than that of the flour extracts (0.79 mg QE/g dry sample). The significant microbial growth inhibition, tested with broth dilution method, by the peel extracts were observed for Candida lipolytica at 99.70%, Bacillus subtilis at 76.58%, Enterococcus faecalis TISTR 379 at 72.79%, Staphylococcus aureus at 69.23% and Salmonella sp. at 56.92%. Other gramnegative bacteria, Pseudomonas fluorescens TISTR 904 and Escherichia coli showed much more resistant to the peel extracts than Salmonella sp. The flour extracts possessed less potential antimicrobial activity than the peel extracts and gave significant inhibition only on C. lipolytica at 45.86% and E. faecalis TISTR 379 at 13.55%. The MIC test of peel extracts indicated that S. aureus was the most sensitive microorganism giving the lowest MIC value (12.5 mg/ml) followed by E. faecalis TISTR 379 and C. lipolytica (25 mg/ml) and B. subtilis (50 mg/ml), respectively. Gram-negative Salmonella sp. was observed less sensitive to the extracts with the highest MIC value (100 mg/ml). This study gave evidence that Thao Yai Mom might be applicable as natural source of antimicrobial agent.

Keywords: Thao Yai Mom, Phenolic, Tannin, Flavonoid, Antimicrobial activity

#### Introduction

Tacca leontopetaloides (L.) Kuntze (commonly called 'Thao Yai Mom' in Thai) is also named as Tacca pinnatifida Forst. [1]; and Tacca involucrata (Schumach and Thonn, 1827) [2]. Under the revised APG II system, Taccaceae is included in the expanded Dioscoreaceae (yam family) [3]. In Thailand, Thao Yai Mom plants naturally grow around eastern coasts such as Chonburi and Rayong Province. Thao Yai Mom tubers contain starch that can be processed and consumed as food or medical treatment. The traditional processing method of Thao Yai Mom starch involves peeling and pulping the tubers. This is followed by several soaking and washing cycles of the pulps to remove poisonous anti-nutritional substances which are usually found in peels and raw tubers. The extracts of unpeeled Tacca tuber were previously reported having antioxidant activity along with phytochemical constituents of alkaloids, vitamin C, vitamin E, flavonoids, phenols, glycosides, saponins and volatile oils [4]. Based on chemical analysis [5], it was indicated that Tacca peel had high content of antinutrients such as phytate, oxalate, saponin, haemagglutinin and especially cyanogenic glycoside. Some other Dioscoreaceae tubers were reported having antimicrobial and antioxidant activity [6-8]. The antioxidant activity was possibly identified through

the presence of bioactive phytochemicals such as phenolic compounds which directly influence the antimicrobial efficiency of the extracts [7]. *Tacca* rhizomes possess higher values of total polyphenols than those of some other yam varieties [9]. Many aspects of *Tacca*, such as chemical compositions, phytochemicals, antioxidant properties, have been studied in various areas. However, antimicrobial activities of *Tacca* have been rarely examined. According to Thai local indigenous knowledge, raw Thao Yai Mom starch is mixed with warm water to make a paste for treating wound or abscess. In this study, Thao Yai Mom tuber and its bioactive compounds are of interest in antimicrobial aspects.

# **Objectives**

This study aims to investigate the phytochemical compounds and antimicrobial activity of Thao Yai Mom extracts for use as antimicrobial agents.

### **Methods**

# Sample Collection and Extracts Preparation

The fresh tubers of Thao Yai Mom (*T. leontopetaloides* (L.) Kuntze) were collected from Pornudom herbal garden in Sattahip district, Chonburi province, Thailand (Figure 1). The tubers were cleaned, peeled and sliced. Fleshes and peels were sun-dried for two days and then ground into fine particles (37 µm) separately. The extracts were prepared following the scheme shown in Figure 2.



**Figure 1.** Thao Yai Mom (*T. leontopetaloides* (L.) Kuntze) plants (a-b) and tubers (c), cultivated at Pornudom herbal garden, Sattahip District, Chonburi Province, and fresh tubers.

# Screening of Phytochemicals

Qualitative screening of phytochemicals were performed following Godghate et al. [10] and Sonam et al. [11] with modification.

Wagner's reagent test: a few drops of Wagner's reagent solution (1.27 g of  $I_2$  and 2 g of KI dissolved in 100 ml distilled water) were added to 3 ml of sample extract. Observed brownish precipitate indicates the presence of alkaloid.

Frothing test: 3 ml of sample extract was added in a test tube containing 10 ml distilled water and shaken thoroughly for 5 minutes and left stable for 30 minutes. A froth forming indicates the presence of saponin.

Ferric chloride test: diluted sample extract was treated with few drops of 1% FeCl<sub>3</sub> solution. The formation of blackish color indicates the presence of phenol.

Alkaline reagent test: 1 ml of sample extract was treated with 10% NaOH solution. The intense yellow color formation indicates the presence of flavonoid.

Gelatin test: 1 ml of sample extract was treated with 1% gelatin solution then 10 % NaCl solution was added. The forming of white precipitate indicates the presence of tannin.

#### **Total Phenolic Content**

Total phenolic content was determined using Folin-Ciocalteu colorimetric method [12]. Aliquot of 1 ml extract was mixed with 9 ml distilled water in 25 ml-volumetric flask (in triplicates). Folin-Ciocalteu's phenol reagent (1 ml) was added. The content was mixed and allowed to stand for 5 min and 10 ml of 7% Na<sub>2</sub>CO<sub>3</sub> was added. The content volume was adjusted with distilled water to 25 ml, thoroughly mixed and allowed to stand in darkness at room temperature for 90 min. Absorbance was measured at 750 nm. The total phenolic content was expressed as gallic acid equivalent in mg/g dry sample (mg GAE/g).

#### **Total Tannin Content**

Total tannin content was determined using Folin-Ciocalteu reagent as described by Mailoa et al. [13] with modification. Aliquot (0.2 ml) of each sample extract was transferred to a test tube containing 2.5 ml of distilled water then 0.2 ml of Folin-Ciocalteu and 2 ml of 7% Na<sub>2</sub>CO<sub>3</sub> were added. The mixture was shaken well and kept in darkness at room temperature for 90 minutes. Absorbance was measured at 748 nm. The tannin content was expressed as mg of tannic acid equivalent/g dry sample (mg TAE/g).

# **Total Flavonoid Content**

Total flavonoid content was determined following a colorimetric method described by Li et al. [14]. Aliquot (0.5 ml) of extract was transferred into a test tube containing 2 ml distilled water and 0.15 ml of 5% NaNO<sub>2</sub>. After 5 min, 0.15 ml of 10% AlCl<sub>3</sub> was added, thoroughly mixed and allowed to stand for 5 min, then 1 ml of 1 M NaOH was added. The solution was mixed and allowed to stand for 15 min. Absorbance was measured at 415 nm. Total flavonoid content was calculated from a standard quercetin curve and expressed as mg quercetin equivalent/g dry sample (mg QE/g).

#### **Evaluation of Antimicrobial Activity**

Antimicrobial activity of aqueous extracts (100 mg/ml) from Thao Yai Mom flour and peel were determined by broth dilution method previously proposed by Habila et al. [15] with slightly modification, using microbial strains obtained from Thailand Institute of Scientific and Technological Research (TISTR); Enterococcus faecalis TISTR 379, Pseudomonas fluorescens TISTR 904, Aspergillus flavus TISTR 3637 and Escherichia coli, Salmonella sp., Candida lipolytica, Bacillus subtilis, A. niger from Program of Microbiology, Nakhon Pathom Rajabhat University (NPRU), Thailand. The inoculum was cultured in nutrient broth for 24-48 hrs. Then the suspension was adjusted to reach the turbidity of a 0.5 McFarland standard at 600 nm (OD $_{600}$  = 0.08-0.1). Each sample extract 265 µl was introduced into the test tube with 200 µl medium broth. Then, 35 µl suspension of the standardized inoculum was inoculated (positive control was set with sterilized distilled water instead of the extracts and negative control was set with sterilized distilled water instead of the inoculum). The bacterial tubes were incubated at 37°C for 24 hrs while yeast and

mold were incubated at ambient temperature for 48 hrs. Viable cells (log<sub>10</sub>CFU/ml) were obtained by serial dilution plate count. Antimicrobial activity was retrieved as percentage inhibition calculated as below:

$$P = \left(1 - 10^{-L}\right) \times 100$$

Where:

P = percentage inhibition

L = log reduction of viable cells

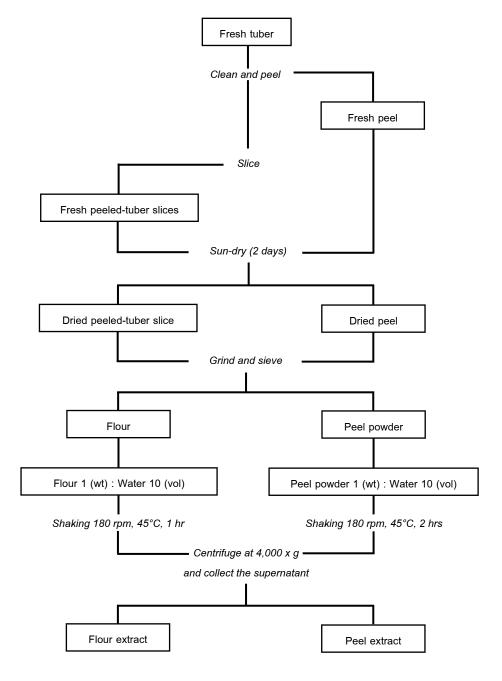


Figure 2. Schematic of extracts preparation.

# **Determination of Minimum Inhibition Concentrations (MIC)**

The lowest concentration of the extracts that inhibited the visible growth of microorganisms were determined by broth dilution method. The 2-fold serial dilution of the extracts from 6.25 to 100 mg dry sample/ml were used. Test tubes with total volume of 500  $\mu$ l, containing 200  $\mu$ l nutrient broth, 260  $\mu$ l extract, 30  $\mu$ l inoculant (1.5 x 10<sup>8</sup> CFU/ml) and 10  $\mu$ l of 0.01% Triphenyl tetrazolium chloride (TTC). Then incubated at 37°C for 24 – 48 hrs. Minimum inhibition concentrations (MIC) were recorded at the lowest concentrations of the extracts showing no colour change of culture broth to pinkish red.

## Results

#### Screening of Phytochemicals

Preliminary results of phytochemical screening of aqueous extracts from Thao Yai Mom flour and peel were shown in Table 1. Different phytochemicals were found in flour and peel. Flour extracts contained alkaloids, saponins, flavonoids and tannins through Wagner's reagent test, frothing test, alkaline reagent test and gelatin test, respectively. These compounds except alkaloids were also found in the peel extracts. The ferric chloride test could not detect phenolic in both extracts.

Table 1. Phytochemical screening of the extracts.

Extracts	Screening of phytochemicals				
	Alkaloid	Saponin	Phenolic	Flavonoid	Tannin
Flour	+	+	-	+	+
Peel	-	+	-	+	+

<sup>+</sup> Present

#### **Total Phenolic, Tannin and Flavonoid Contents**

Total phenolic contents of the aqueous extracts of Thao Yai Mom flour and peel were determined at 2.70 and 8.11 mg GAE/g dry sample, respectively (Table 2). The total phenolic content is remarkably higher in peel than flour. Tannin, a complex phenolic compound found in plants [16-17], was determined in the flour and peel extracts at 2.62 and 8.68 mg TAE/g dry sample, respectively. There was significant difference between flavonoid content of flour and peel extracts. Total flavonoid content of the peel extracts was 20.79 mg QE/g dry sample, about 26 times higher than that of the flour extracts (0.79 mg QE/g dry sample).

# **Antimicrobial Activity**

Viable cells and partial growth inhibition of tested strains were observed (Table 3). Yeast *C. lipolytica* and gram-positive bacteria, *B. subtilis*, *E. faecalis* TISTR 379 and *S. aureus*, showed more sensitivity to the peel extracts with the significant growth inhibition of 99.70%, 76.58%, 72.79% and 69.23%, respectively than those of gram-negative *P. fluorescens* TISTR 904 and *E. coli* which were not inhibited significantly by the peel extracts. However, in this study *Salmonella* sp. was found to be more sensitive

<sup>-</sup> Absent

than *P. fluorescens* TISTR 904 and *E. coli*, and could be inhibited significantly by the peel extracts at 56.92% inhibition. The flour extracts possessed less potential antimicrobial activity than the peel extracts and gave significant inhibitions only on *C. lipolytica* and *E. faecalis* TISTR 379 at 45.86% and 13.55%, respectively. There were no significant inhibitory effects of any extracts on tested fungal strains *A. flavus* TISTR 3637 and *A. niger*. Only microbial strains that were inhibited significantly were selected for further MIC test.

Table 2. Total phenolic, tannin and flavonoid contents of flour and peel extracts from Thao Yai Mom.

Tatal content (man /m dm / comple)	Ext	P	
Total content (mg /g dry sample)	Flour	Peel	Ρ
Phenolic	2.70±0.07	8.11±0.30	0.00
Tannin	2.62±0.30	8.68±1.00	0.00
Flavonoid	0.79±0.09	20.79±3.29	0.00

Significant differences P < 0.05 in bold

**Table 3**. Viable cells (log<sub>10</sub>CFU/ml) and inhibition (%) of untreated, flour-extract and peel-extract treated microbial strains by flour and peel extracts from Thao Yai Mom (100 mg/ml).

Microbial strains (gram type)	Log <sub>10</sub> CFU/ml		Inhibition (%)		
	Untreated	Flour-extract	Peel-	Flour-	Peel-
		treated	extract	extract	extract
			treated	treated	treated
E. coli (-)	8.38 <sup>a</sup> ± 0.12	8.34 <sup>a</sup> ± 0.07	8.33 <sup>a</sup> ± 0.08	7.29 (ns)	10.59 (ns)
P. fluorescens TISTR 904 (-)	$6.09^{\circ} \pm 0.05$	$7.82^a \pm 0.07$	$7.44^{b} \pm 0.06$	n	n
Salmonella sp. (-)	$8.09^a \pm 0.06$	8.01 <sup>a</sup> ± 0.03	$7.73^{b} \pm 0.07$	17.71 (ns)	56.92
B. subtilis (+)	$6.60^{a} \pm 0.06$	$6.59^a \pm 0.05$	$5.97^{b} \pm 0.07$	4.09 (ns)	76.58
E. faecalis TISTR 379 (+)	$9.59^a \pm 0.02$	$9.53^{b} \pm 0.02$	$9.03^{\circ} \pm 0.02$	13.55	72.79
S. aureus (+)	$5.83^{a} \pm 0.07$	$5.87^{a} \pm 0.08$	$5.32^{b} \pm 0.08$	n	69.23
C. lipolytica	$7.60^a \pm 0.11$	$7.33^{b} \pm 0.03$	$5.08^{\circ} \pm 0.09$	45.86	99.70
A. flavus TISTR 3637	$4.53^{a} \pm 0.07$	4.46° ± 0.10	$4.46^a \pm 0.06$	16.12 (ns)	15.20 (ns)
A. niger	$5.30^{b} \pm 0.01$	5.86 <sup>a</sup> ± 0.13	$5.06^{b} \pm 0.03$	n	41.51 (ns)

n = no inhibition

Different letters in superscript indicate significant differences within rows.

Determination of MIC (Table 4) indicated that *S. aureus* was the most sensitive microorganism giving the lowest MIC value (12.5 mg/ml) followed by *E. faecalis* TISTR 379 and *C. lipolytica* (25 mg/ml) and *B. subtilis* (50 mg/ml), respectively. *Salmonella* sp. was observed less sensitive with the highest MIC value (100 mg/ml).

ns = not significantly different between untreated and treated groups at .05 significant level

Table 4. Minimum inhibition concentration (MIC) of the peel extracts against microbial strains.

Microbial strains	Minimum inhibition concentration (mg/ml)		
Salmonella sp.	100		
B. subtilis	50		
E. faecalis TISTR 379	25		
C. lipolytica	25		
S. aureus	12.5		

#### **Conclusions and Discussion**

Thao Yai Mom (T. leontopetaloides (L.) Kuntze.) flour and peel extracts were evaluated by preliminary methods for the phytochemicals. Saponins, flavonoids and tannins were found existing in both flour and peel extracts while alkaloids were only detected in flour. Another study by Borokini et al. [18] also reported the presence of alkaloid in T. leontopetaloides tuber through Wagner's reagent test. Saponins have been reported existing in T. leontopetaloides tubers which were detected by different methods like foaming/frothing test [4, 18] and high performance thin layer chromatography (HPTLC) [19]. Chemical analysis of T. leontopetaloides peels by Ubwa et al. [5] reported saponin content of the peel was between 31.50 - 35.00 mg/kg. Negative results for phenols tested by ferric chloride were observed in both flour and peel. Previous study reported phenols were detected by ferric chloride test for T. pinnatifida J. R & J. G. Forst tubers extracted with different organic solvents (methanol, acetone, chloroform and isopropyl acetate), but not for aqueous extract [4]. The ferric chloride test is used in qualitative test for simple phenols such as phenolic acids, but some complex phenolics and stilbenes, like p-benzylphenol, trans-diethylstilbestrol, meso-hexestrol, ethyl p-hydroxybenzoate, have been reported as undetected [20]. The addition of potassium ferricyanide to ferric chloride test was done to avoid false negative results of complex phenolics [21]. Simple phenolics with less complex structures, such as catechol and coumarin, shown to have antimicrobial activities [22-23]. In this study, even though potassium ferricyanide was not added to improve the ferric chloride test, phenolics could be evaluated as total phenolic contents by Folin-Ciocalteu colorimetric method.

Alkaline reagent and gelatin test indicated that both flour and peel extracts contained flavonoids and tannins. Shinoda test also gave positive detection for flavonoid in aqueous tuber extracts of *T. pinnatifida* J.R. & J.G. Forst [4]. Tannins were reportedly detected in *T. leontopetaloides* tuber by ferric chloride test in a previous study [18]. Quantitative determination of total phenolic, tannin and flavonoid contents indicated that those were remarkably higher in the extracts from peel (8.11, 8.68 and 20.79 mg/g dry sample, respectively) than flour (2.7, 2.62 and 0.79 mg/g dry sample, respectively). Some studies proposed that *Tacca* tuber and its derived flour have high levels of antinutrients and secondary metabolites. Tuber extracts of *T. pinnatifida* J. R. & J. G. Forst were reported rich in a variety of primary and secondary metabolites especially carbohydrates, glycosides and phenols [4]. The total polyphenols content determined in *Tacca* flour was around 419 mg/100 g dry matter [9] whereas the methanol extract of *Tacca* peel was reported for approximate phenolic content of 4.2 mg (GAE)/g dry weight [24]. The presence of tannin (2.50 mg/100 g) has been previously found in *T. leontopetaloides* L. (Kunze) starch [25] whereas phytochemical

analysis of *T. involucrate* marc showed the presence of tannins at 3.44 mg/100g [2]. The different sample processing methods and extraction solvents could let to the difference of phenolic compound contents. Starch is pure compare to flour due to the slurry and decanting process in which could reduce some water soluble substances. *Tacca* tuber pretreatment can also impact on phenolic content of flour. *Tacca* flour derived from two steps soaking of tubers in water had lower content of total polyphenols, tannins and flavonoids than non-soaking process [9]. The significant difference of flavonoid content of methanol extracts of *T. leontopetaloides* L. Kuntze peel (~ 22.9 mg QE/g dry weight) and tuber (~ 3.37 mg QE/g dry weight) was reported previously by Vu et al. [24].

Antimicrobial activity of peel extracts was significantly higher than that of flour extracts. Significant partial growth inhibitions of C. lipolytica, B. subtilis, E. faecalis TISTR 379, S. aureus and Salmonella sp. by peel extracts were observed at 99.70%, 76.58%, 72.79%, 69.23%, and 56.92% respectively. The flour extracts gave significant growth inhibition only on C. lipolytica at 45.86% and E. faecalis TISTR 379 at 13.55%. These results could be attributed to the different cell surface structures of gram-positive and gramnegative bacteria. Antimicrobial mechanism of phenolic compounds was to inactivate cellular enzymes which was enhanced by permeability of cell membrane [26]. Gram-positive bacteria have a single peptidoglycan layer that does not restrict the membrane penetration, whereas cell surface of gram-negative bacteria is composed of a thick outer layer of phospholipid associated with lipoprotein which is often make the cell membrane resistant to the permeability. Gram-positive Lactobacillus spp. and S. aureus were reported more susceptible to phenolic acids than gram-negative bacteria like E. coli and P. aeruginosa [27]. Disruption of S. aureus cell membrane by phenolic compound (3-p-trans-coumaroyl-2-hydroxyquinic acid) resulted in morphological changes and leakage of intracellular constituents [28]. However, in this study Salmonella sp. was found to be more sensitive than other gram-negative bacteria, and could be inhibited significantly. Moreno et al. [26] reported previously that Salmonella spp. and Shigella sonnei were less resistant to common antibiotics than other gram-negative bacteria isolated from natural sources of water. Antimicrobial activity of T. leontopetaloides L. (Kunze) peels against E. faecalis, S. aureus, E. coli and P. aeruginosa had been reported to be higher than that of other parts like leaves and tubers [24]. There was no significant inhibitory effect of any extracts on A. flavus TISTR 3637 and A. niger. Aspergillus is a filamentous fungi whereas Candida is a single cell fungi that has biofilm forming ability. Evaluation of antifungal activities of some phenolic compounds against filamentous fungi (include Aspergillus) and opportunistic yeasts reported that none of the compounds had activity against the filamentous fungi but showed the distinguished activities against yeasts Candida [29]. Mechanisms of phenolic compounds against Candida were reported include inhibition of enzyme, against biofilm forming, disruption of cell membrane and cell wall [30]. Only microbial strains that were inhibited significantly were selected for MIC test. MIC determination indicated that S. aureus was the most sensitive microorganism giving the lowest MIC value whereas E. coli and Salmonella sp. were observed less sensitive with the highest MIC value. Various phenolic compounds, hydroxycinnamic acid derivatives and phenolic acids (p-coumaric acid, caffeic acid, ferulic acid, sinapic acid, gallic acid); simple phenolics (coumarin and catechol); stilbene (resveratrol); flavonoids (naringenin, quercetin, rutin, catechin) are known to possess antimicrobial properties [23]. It was reported that antimicrobial activity is directly influenced by phenolic contents of yam (Dioscoreaceae) tuber extracts [7]. Antimicrobial action of phenolic compounds is related to inhibition of cellular enzyme activity [26]. Tuber and peel extracts of the plant family Dioscoreaceae have been reported for their phenolic compounds (like flavonoids and tannins) and antimicrobial activity [2, 25, 31-32]. Tannins extracted from some parts of plants such as seeds and barks were found to be antimicrobial against various microorganisms in previous studies [33-37]. This substance has been reported found in *Tacca* tubers and leaves as well [2, 9, 18, 25]. A study by of phytochemicals of medicinal plants including *T. leontopetaloides* indicated that the plant extracts contained various secondary metabolites like alkaloids, tannins, saponins, triterpenes, glycosides and carbohydrates except flavonoids and possessed antibacterial activity against gram-positive and negative bacteria [15]. According to the results, Thao Yai Mom peel could be applicable as natural source of antimicrobial agents and phenolic compound contents might be related to an antimicrobial potential of the extracts.

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## References

- [1] Thaman, R.R. (2016). *Atoll Research Bulletin*. Washington DC: Smithsonian Institution Scholarly Press.
- [2] Bosha, J.A.; Anaga, A.O., & Asuzu, I.U. (2015, January). Chemical composition of the marc of a wild tropical plant *Tacca involucrata* (Schumach and Thonn, 1827). *Food and Nutrition Sciences*, 6(1), 135-140.
- [3] Caddick, L.R.; Rudall, P.J.; Wilkin, P.; Hedderson, T.A.J., & Chase, M.W. (2002, March).
  Phylogenetics of Dioscoreales based on combined analyses of morphological and molecular data. Botanical Journal of the Linnean Society, 138(2), 123-144.
- [4] Jagtap, S., & Satpute, R. (2014, January). Phytochemical screening and antioxidant activity of tuber extracts of *Tacca pinnatifida*. *International Journal of Recent Trends in Science and Technology*, 9(3), 389-396.
- [5] Ubwa, S.T.; Anhwange, B.A., & Chia, J.T. (2011, October). Chemical analysis of *Tacca leontopetaloides* peels. *American Journal of Food Technology*, 6(10), 932-938.
- [6] Kelmanson, J. E.; Jäger, A.K., & van Staden, J. (2000, March). Zulu medicinal plants with antibacterial activity. *Journal of Ethnopharmacology*, 69(3), 241-246.
- [7] Sonibare, M.A., & Abegunde, R.B. (2012, March). In vitro antimicrobial and antioxidant analysis of *Dioscorea dumetorum* (Kunth) Pax and *Dioscorea hirtiflora* (Linn.) and their bioactive metabolites from Nigeria. *Journal of Applied Biosciences*, 51, 3583-3590.

- [8] Farombi, E.O.; Britton, G., & Emerole, G.O. (2000, July). Evaluation of the antioxidant and partial characterisation of extracts from browned yam flour diet. Food Research International, 33(6), 493-499.
- [9] Ndouyang, C.J.; Njintang, N.Y.; Facho, B.; Scher, J., & Mbofung, C.M.F. (2015, January). Effect of processing method on the antinutrient content of *Tacca leontopetaloides* (L.) Kuntze flour. *British Journal of Applied Science & Technology*, 5(3), 258-269.
- [10] Godghate, A.; Sawant, R., & Sutar, A. (2012). Phytochemical analysis of ethanolic extract of roots of *Carrisa carandus* Linn. *Rasayan Journal of Chemistry*, *5*(4), 456-459.
- [11] Sonam, M.; Singh, R.P., & Pooja, S. (2017). Phytochemical Screening and TLC Profiling of Various Extracts of Reinwardtia indica. International Journal of Pharmacognosy and Phytochemical Research, 9(4), 523-527.
- [12] Yi, B.; Hu, L.; Mei, W.; Zhou, K.; Wang, H.; Luo, Y.; Wei, X., & Dai, H. (2011, December).

  Antioxidant phenolic compounds of cassava (*Manihot esculenta*) from Hainan. *Molecules*, 16(12), 10157-10167.
- [13] Mailoa, M.N.; Mahendradatta, M.; Laga, A., & Djide, N. (2014, January). Antimicrobial activities of tannins extract from guava leaves (*Psidium guajava* L.) on pathogens microbial. *International Journal of Scientific & Technology Research*, 3(1), 236-241.
- [14] Li, Y.; Ma, D.; Sun, D.; Wang, C.; Zhang, J.; Xie, Y., & Guo, T. (2015, August). Total phenolic, flavonoid content, and antioxidant activity of flour, noodles, and steamed bread made from different colored wheat grains by three milling methods. *The Crop Journal*, 3(4), 328-334.
- [15] Habila, J.D.; Bello, I.A.; Dzikwe, A.A.; Ladan, Z., & Sabiu, M. (2011). Comparative evaluation of phytochemicals, antioxidant and antimicrobial activity of four medicinal plants native to Northern Nigeria. Australian Journal of Basic and Applied Sciences, 5(5), 537-543.
- [16] Hans-Walter, H., & Piechulla, B. (2011). *Plant Biochemistry*. 4th ed. San Diego: Academic Press.
- [17] Trugo, L.C.; von Baer, D., & von Baer, E. (2003). *Encyclopedia of Food Sciences and Nutrition*. 2nd ed. Oxford: Academic Press.
- [18] Borokini, I., & Ayodele, A. (2012, January). Phytochemical screening of *Tacca Leontopetaloides* (L.) Kuntze collected from four geographical locations in Nigeria. Indian Journal of Medical Biochemistry, 2(4), 97-102.
- [19] Jagtap, S., & Satpute, R. (2015). Phytochemical screening, antioxidant, antimicrobial and quantitative multi-elemental analysis of *Peristylus densus* (Lindl.) Santapau and Kapadia. *Journal* of Academia and Industrial Research, 3(10), 511-519.
- [20] Soloway, S., & Wilen, S.H. (1952, June). Improved ferric chloride test for phenols. *Analytical Chemistry*, 24(6), 979-983.
- [21] MacWilliam, I.C., & Wenn, R.V. (1972, July-August). Interpretation of colour tests for polyphenols and melanoidins. *Journal of The Institute of Brewing*, 78(4), 309-309.

- [22] Cowan, M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12, 564-582.
- [23] Maddox, C.E.; Laur, L.M., & Tian, L. (2010, January). Antibacterial activity of phenolic compounds against the phytopathogen *Xylella fastidiosa*. *Current Microbiology*, *60*(1), 53-58.
- [24] Vu, Q.T.H.; Le, P.T.K.; Vo, H.P.H.; Nguyen, T.T., & Nguyen, T.K.M. (2017, September). Characteristics of *Tacca leontopetaloides* L. Kuntze collected from an Giang in Vietnam. *AIP Conference Proceedings*. Retrieved January 9, 2019, from https://www.researchgate.net/publication/319865480\_Characteristics\_of\_Tacca\_leontopetaloides\_L\_Kuntze\_collected\_from\_An\_Giang\_in\_Vietnam
- [25] Ogbonna, A.I.; Adepoju, S.O.; Ogbonna, C.I.C.; Yakubu, T.; Itelima, J.U., & Dajin, V.Y. (2017, January). Root tuber of *Tacca leontopetaloides* L. (kunze) for food and nutritional security. *Microbiology: Current Research*, 1(1), 5-11.
- [26] Moreno, S.; Scheyer, T.; Romano, C.S., & Vojnov, A.A. (2006, February). Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. *Free Radical Research*, 40(2), 223-231.
- [27] Cetin-Karaca, H. (2011). Evaluation of natural antimicrobial phenolic compounds against foodborne pathogens. Thesis, M.S. (College of Agriculture). Kentucky: Graduate school University of Kentucky.
- [28] Wu, Y; Bai, J,: Zhong, K.; Huang, Y.; Qi, H.; Jiang, Y., & Gao, H. (2016). Antibacterial activity and membrane-disruptive mechanism of 3-p-trans-coumaroyl-2-hydroxyquinic acid, a novel phenolic compound from pine needles of cedrus deodara, against *Staphylococcus aureus*. *Molecules*, 21, 1-12.
- [29] Latté, K.P., & Kolodziej, H. (2000). Antifungal effects of hydrolysable tannins and related compounds on dermatophytes, mould fungi and yeasts. Zeitschrift fur Naturforschung C, 55(5-6), 467-72.
- [30] Teodoro, G.R.; Ellepola, K.; Seneviratne, C.J., & Koga-Ito, C.Y. (2015). Potential use of phenolic acids as anti-candida agents: a review. Frontiers in Microbiology, 6, 1-10.
- [31] Poonia, S.; Singh, T.S., & Tsering, D.C. (2014, July). Antibiotic Susceptibility Profile of bacteria isolated from natural sources of water from rural areas of east Sikkim. *Indian Journal of Community Medicine*, 39(3), 156-160.
- [32] Adeosun, O.M.; Arotupin, D.J.; Toba, O.A., & Adebayo, A.A. (2016, January-February).
  Antibacterial activities and phytochemical properties of extracts of *Dioscorea bulbifera* Linn (Air Potatoe) tubers and peels against some pathogenic bacteria. *The Journal of Phytopharmacology*, 5(1), 20-26.
- [33] Akiyama, H.; Fujii, K.; Yamasaki, O.; Oono, T., & Iwatsuki, K. (2001, October). Antibacterial action of several tannins against *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy*, 48(4), 487-491.

- [34] Amarowicz, R.; Dykes, G.A., & Pegg, R.B. (2008, February). Antibacterial activity of tannin constituents from *Phaseolus vulgaris*, *Fagoypyrum esculentum*, *Corylus avellana* and *Juglans nigra*. *Fitoterapia*, 79(3), 217-219.
- [35] Fiori, G.M.L.; Fachin, A.L.; Correa, V.S.C.; Bertoni, B.W.; Giuliatti, S.; Amui, S.F.; França, S.C., & Pereira, A.M.S. (2013, November). Antimicrobial activity and rates of tannins in *Stryphnodendron adstringens* Mart. accessions collected in the Brazilian Cerrado. *American Journal of Political Science*, *4*(11), 2193-2198.
- [36] Joseph, N.; Mirelle, A.F.R.; Matchawe, C.; Patrice, D.N., & Josaphat, N. (2016, July-August).

  Evaluation of the antimicrobial activity of tannin extracted from the barks of *Erythrophleum guineensis* (Caesalpiniaceae). *Journal of Pharmacognosy and Phytochemistry*, *5*(4), 287-291.
- [37] Wafa, N.; Sofiane, G., & Mouhamed, K. (2016, May-June). The antioxidant and antimicrobial activities of flavonoids and tannins extracted from Phlomis bovei De Noé. *European Journal of Experimental Biology*, 6(3), 55-61.