



## Evaluation of cosmeceutical properties of *Talinum triangulare* leaf and stem extracts

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### Abstract

Although skin epidermis possesses an extremely efficient natural antioxidant defense, high production and accumulation of reactive oxygen species (ROS) due to excessive exposure of the skin to ultraviolet (UV) radiation may limit the protective effect that the natural antioxidants offer, leading to cellular aging. The most promising topical treatments incorporate antioxidants and antimicrobial ingredients. Antioxidant potential of leaf and stem extracts of *T. triangulare* were evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH); and antimicrobial activity assay using agar well diffusion technique. DPPH scavenging activity of the extracts indicated by IC<sub>50</sub> (Fifty percent inhibitory concentration) value were compared with butylated hydroxyanisole (BHA), a standard antioxidant. Ethanol extract of the leaf and aqueous extract of the stem showed free radical scavenging activity (IC<sub>50</sub> = 74.01±0.02 µg/cm<sup>3</sup>) and (IC<sub>50</sub> = 88.76±0.03 µg/cm<sup>3</sup>) respectively comparable to BHA (IC<sub>50</sub> = 55.54±0.04 µg/cm<sup>3</sup>) than their respective aqueous (IC<sub>50</sub> = 103.08±0.02 µg/cm<sup>3</sup>) and ethanol (IC<sub>50</sub> = 98.43±0.07 µg/cm<sup>3</sup>) counterparts. Also, the extracts and fractions revealed selective levels of activities against test micro-organisms (*Staphylococcus aureus*, *Vibrio cholera*, *Shigella sp*, *Propionibacterium acnes*, *Aspergillus niger* and *Microsporus sp*). The results of this research indicate the possible industrial applications of *T. triangulare* extracts for the formulation of skincare products.

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## Introduction

The dependency of man on plants for food, drinks, shelter, clothing, equipment, dental care, body care and medicine cannot be over emphasized. Many herbal preparations for healthcare come from plant and even in modern times, plants extracts have formed the basis of many cosmeceuticals (Ajiboye *et al.*, 2017). Plants exhibit a wide range of biological and pharmacological activities such as anti-inflammatory, anti-oxidant, diuretic, laxative, anti-spasmodic, anti-hypertensive and anti-microbial functions. These functions are performed due to chemical constituents comprising sugars, lipids, proteins, vitamins, minerals and phytochemicals (Okwu and Nnamdi, 2008; Nwosu and Val, 2014). Phytochemicals are biologically active, naturally occurring chemical compounds found in all parts of plants, which are useful to humans than those attributed to macronutrients and micronutrients. It is mostly the phytochemicals which are non-nutritive chemicals that have protective or disease preventive properties. It is well known that plants produce these chemicals to protect themselves but over the years, research demonstrates that they can also protect humans against diseases (Nwosu and Val, 2014; Ogboru *et al.*, 2015). Besides functioning as the energy source for animals, plants provide raw materials for many phytochemical-based industries such as pharmaceutical, personal care, flavour and food industries. Plant extracts are known to exert a wide range of beneficial physiological effects which is reflected in their use in traditional medicines (Rachana and Venugopalan, 2014). Some of these plant extracts are incorporated in personal care product formulations like soaps, creams, lotions, pomades, shampoos and tooth pastes because of their curative properties (Barbulova *et al.*, 2014; Igwe, 2015).

Cosmeceuticals are topical cosmetic-pharmaceutical hybrids intended to enhance the beauty of the body through application of ingredients that provide additional health-related functions or benefits to the skins of the users (Dureja *et al.*, 2005). Cosmeceuticals is the fastest growing segment of the

natural personal care industry. Cosmeceuticals are used for nourishing as well as improving the appearance of the skin by delivering nutrients necessary for healthy skin, and are also used as effective agents for treating various dermatologic conditions. Plants have become potential sources for development of new drug entities for cosmeceutical and pharmaceutical applications (Joshi, 2012). Thus, the need to subject *Talinum triangulare* extracts to rigorous chemical analysis for cosmeceutical properties.

*Talinum triangulare* is from the family of Portulacaceae popularly known as waterleaf, a terrestrial perennial deciduous herb which has woody stems and succulent leaves (Herrera, 1991). *T. triangulare* originated from tropical Africa, it is widely cultivated as a medicinal and food crop in West Africa (especially in Nigeria), Asia, and South America (Ezekwe *et al.*, 2013). *T. triangulare* had been implicated medically in the management of cardiovascular diseases like stroke and obesity, and traditionally, it is used as softener of other vegetable species (Okoli *et al.*, 2007). The leaves are crushed and the juice applied on skin to treat measles, skin eruption and burns (Aja *et al.*, 2010a); leaf extract is taken orally for scabies and fresh cuts (Ajibesin, 2012). Aja *et al.* (2010b) reported that both wet and dry leaves contain an appreciable amount of bioactive compounds namely flavonoids, alkaloids, saponins and tannins. A study carried out by Amorim *et al.* (2013) revealed that the hydromethanolic extract of *T. triangulare* exhibited powerful antioxidant activity and inhibited the activity of tyrosinase enzyme.

The leaf extract of *T. triangulare* is potentially active against iron II - induced oxidative stress in the brain and testes of wistar albino rat *in vitro* (Afolabi *et al.*, 2015). Ogbonnaya and Chinedum (2013) reported considerable amounts of proximates, phytochemicals, minerals, vitamins, chlorophyll and anti-oxidant activity but low in carbohydrate and energy value in *T. triangulare* leaf. Studies had found that polysaccharides isolated from *T. triangulare* water extract revealed remarkably different degrees of

antioxidant activities in dose-dependent manners (Liang *et al.*, 2011). Although antioxidant activity of this plant have been evaluated, there is no available literature on the potential of *T. triangulare* extracts as sustainable source of raw materials for the personal care industry despite the preponderance of this plant in Nigeria. Thus, the need to subject *Talinum triangulare* extracts to rigorous chemical analysis for cosmeceutical properties.

## Materials and methods

### Sample Collection and Identification

*Talinum triangulare* (leaf and stem) were collected in January, 2018, from a garden in Ewet Housing Estate, Uyo, Akwa Ibom State, Nigeria. The plants samples were collected and transferred into polyethelene bags, labelled properly and taken to the laboratory for identification and preparation. The plant materials were identified and authenticated by a Taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Akwa Ibom State. Voucher specimen was deposited at the herbarium with the number, Enengedi, UUH 3545.

### Extraction and Fractionation of Plant Extracts

The method described by Enengedi *et al.* (2019) was used for the extraction and fractionation of the plant materials. The plant materials were extracted by maceration with 80% ethanol for 72 hours (h) at room temperature. Also, wet samples were extracted by maceration with water for 8 h.

The ethanol and aqueous extracts were concentrated in a rotary evaporator at a reduced pressure at 45°C, and the solvent removed completely by evaporation in the water bath. Some amounts of the ethanol extracts were suspended in 200 cm<sup>3</sup> of distilled water and subjected to sequential liquid-liquid extraction with a solvent series of increasing polarity: dichloromethane (DCM), ethyl acetate (EA) and n-butanol (BuOH).

The fractionation was performed until the organic solvent became colourless in 1000 cm<sup>3</sup> glass separatory funnels by mixing 200 cm<sup>3</sup> of solvent with the aqueous phase and the content shaken. The

separatory funnel was supported on a ring clamp, allowing the layers to separate. The pooled fractions: dichloromethane fraction, ethyl acetate fraction, n-butanol fraction and the remaining aqueous fraction were concentrated in a rotary evaporator and evaporated to dryness. Aqueous extracts, parts of the ethanol extract and fractions were stored in a functional refrigerator until used for analysis.

### Determination of phytochemicals

Qualitative determination of phytochemicals in the extracts was carried out using standard phytochemical methods as described by Enengedi *et al.* (2018) while Quantitative Determination of Phytochemicals (Total Phenolics, Total Flavonoids and Total Tannins) were determined using Folin-Ciocalteu phenol, colorimetric method and Folin - Ciocalteu phenol respectively as described elsewhere (Djeridane *et al.*, 2006; Jothy *et al.*, 2011; Tambe and Bhambar, 2014). A set of reference standard solutions of gallic acid (10, 100, 200, 300, 400, 500 and 600 µg/cm<sup>3</sup>), quercetin (10, 100, 200, 300, 400, 500, 600, 700 and 800 µg/cm<sup>3</sup>) and tannic acid (50, 100, 150, 200 and 250 µg/cm<sup>3</sup>) were prepared and the reaction mixture was measured at 760 nm, 510nm and 725 nm respectively using a Jenway 7305 spectrophotometer. The absorbance of each sample was compared with a standard curve plotted from gallic acid, quercetin and tannic acid.

The total phenolic content was calculated from linear regression equation from gallic acid calibration curve (Figure 1), expressed in terms of microgram of gallic acid equivalent per centimeter cubed (µg of GAE/cm<sup>3</sup>) of extract or fraction, total flavonoids content was calculated from linear regression equation from quercetin calibration curve (Figure 2), expressed in terms of microgram of quercetin equivalent per centimeter cubed (µg of QE/cm<sup>3</sup>) of extract or fraction and the tannin content was calculated from linear regression equation from tannic acid calibration curve (Figure 3), expressed in terms of microgram of tannic acid equivalent per centimeter cubed (µg of TAE /cm<sup>3</sup>) of extract or fraction.

### 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Assay

The antioxidant activity of extract or fraction was determined using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay according to the method described by (Hamid *et al.*, 2010). DPPH solution (0.004% w/v) was freshly prepared in methanol and added in each of five vials containing varying concentrations of the extract or their different fractions. The mixture was shaken vigorously and allowed to stand for 30 min at room temperature in the dark. The reduction of the purple colour of DPPH to yellow was determined by measuring the absorbance at 517 nm. The radical scavenging activity of the extract or fraction was calculated using Equation (1):

$$\text{DPPH scavenging effect (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \quad \text{Equation 1}$$

Where A = absorbance at 517 nm.

The commercially known antioxidant, butylated hydroxyanisole (BHA) of the same concentrations and serial dilutions were prepared and used for comparison or as a positive control. The DPPH solution in the absence of extract or fraction was used as control and methanol was used as blank.

### Determination of Antimicrobial Activities of Extracts and Fractions

Bacterial and fungal isolates were obtained from the University of Uyo Health Centre Laboratory, Akwa Ibom State, Nigeria. These isolates were inoculated on selective media (maritol salt agar, saboraaud dextrose agar, dermatophytic test medium, thiosulphate citrate bile sucrose-salt agar, salmonella shigella agar and blood agar) for the isolation of *Staphylococcus aureus*, *Aspergillus niger*, *Microsporus sp*, *Vibrio cholera*, *Shigella sp* and *Propionibacterium acnes* respectively. Colonies that developed were sub-cultured on Nutrient agar and Saboraaud dextrose agar for bacterial and fungal isolates respectively. The antimicrobial activities of crude extracts and fractions were determined using the agar well diffusion technique as described by

(Haruna *et al.*, 2013). Four holes were bored on each plate using a sterile 5mm diameter cork borer at a low temperature. Each hole was for a concentration of the crude extract or fraction.

The holes were filled with their respective concentrations of the extract or fraction. The plates were then kept undisturbed for 15 minutes so as to allow the extract or fraction to diffuse properly and dry to a considerable level before incubation. Plates containing bacterial isolates were incubated for 24 hours at 37°C while plates containing fungi were incubated for 48 hours at 37°C.

The measurements (in millimeters) of the zones of inhibitions of the extracts against the test organisms were measured. The extracts or fractions that were found effective, as antimicrobial agent, were later tested to determine the Minimum Inhibitory Concentration (MIC) values for each micro-organism. The MIC of the extracts and fractions were determined using the tube dilution method described by (Jahan *et al.*, 2011). Turbidity indicated growth of the micro-organism and MIC was regarded as the lowest concentration of the extract or fraction that revealed no visible growth when compared with that of the control tubes.

### Data Analysis

Measurements were done in triplicates and values were expressed as means  $\pm$  standard deviations. Analysis of variance (ANOVA) followed by Duncan's multiple range method were used to compare differences in values. Also, differences in values obtained between *T. triangulare* leaf and *T. triangulare* stem were compared for significance using independent t-test and  $p < 0.05$  were considered to be significant. To enhance data analysis, the Statistical Package for Social Sciences (SPSS, 2013) was used.

### Results

The phytochemical screening of the ethanol and aqueous extracts of *T. triangulare* leaf and stem are presented on Tables 1.

**Table 1.** Phytochemical screening of ethanol and aqueous extracts of *T. triangulare* leaf and stem.

Phytochemical	Ethanol sample		Aqueous sample	
	T.t (L)	T.t (S)	T.t (L)	T.t (S)
Flavonoids	-	++	++	++
Phenols	+	+++	++	+
Tannins	+	++	-	-
Saponins	+++	+	+++	+
Alkaloids	+	++	+	+
Cardiac glycosides	++	++	+	+
Anthraquinones	++	-	-	-
Terpenoids	-	+++	++	+

\*T. t (L) = *T. triangulare* leaf and T. t (S) = *T. triangulare* stem

+ = present in low intensity, ++ = present in moderate intensity,

+++ = present in high intensity, - = absent.

Total phenolic, total flavonoid and total tannin contents as shown in Table 2 were calculated from their respective calibration curves (Figures 1, 2 and 3) respectively.

DPPH free radical assay and the scavenging activity as indicated by IC<sub>50</sub> value of aqueous extracts, ethanol extracts and the various fractions of *T. triangulare* leaf and stem as compared with BHA, a known antioxidant is shown in Table 3. Fifty percent inhibitory concentration (IC<sub>50</sub>) was calculated from linear regression equation of DPPH scavenging activity of BHA, plants' extracts and fractions (Figures 4, 5, 6 and 7). The results of the antimicrobial activities of the ethanol extract, dichloromethane

fraction, ethyl acetate fraction, n-butanol fraction, aqueous fraction and aqueous extract of *T. triangulare* leaf and stem against the test organisms namely: *S. aureus*, *V. cholera*, *Shigella sp*, *P. acnes*, *A. niger* and *Microsporus sp* are shown in Tables 4 and 5 respectively. The zones of inhibition of growth of the micro-organisms are functions of relative antimicrobial activity of the extracts or fractions. The extracts and fractions of the leaf revealed selective levels of activities against the micro-organisms.

**Table 2.** Total phenolic content (TPC), total flavonoid content (TFC) and total tannin content (TTC) of *T. triangulare* leaf, stem and their respective fractions.

Sample	Leaf			Stem		
	TPC (µg GAE/cm <sup>3</sup> )	TFC (µg QE/cm <sup>3</sup> )	TTC (µg TAE/cm <sup>3</sup> )	TPC (µg GAE/cm <sup>3</sup> )	TFC (µg QE/cm <sup>3</sup> )	TTC (µg TAE/cm <sup>3</sup> )
T. t	66.83± 0.03 <sup>a</sup>	59.83± 0.02 <sup>b</sup>	106.53± 0.01 <sup>b</sup>	29.06± 0.03 <sup>b</sup>	25.33± 0.04 <sup>b</sup>	99.09± 0.01 <sup>a</sup>
T.t DCM	24.61± 0.02 <sup>c</sup>	27.00± 0.03 <sup>c</sup>	115.04± 0.02 <sup>a</sup>	4.61± 2.06 <sup>d</sup>	5.33± 0.04 <sup>e</sup>	59.51± 0.05 <sup>c</sup>
T. t EA	15.72± 0.02 <sup>d</sup>	158.17± 0.04 <sup>a</sup>	61.00± 2.04 <sup>d</sup>	84.61± 0.05 <sup>a</sup>	52.00± 0.03 <sup>a</sup>	95.89± 4.02 <sup>b</sup>
T.t BuOH	47.94± 0.03 <sup>b</sup>	23.67± 0.02 <sup>d</sup>	59.08± 3.03 <sup>d</sup>	4.61± 4.05 <sup>d</sup>	0.33± 0.04 <sup>f</sup>	58.66± 4.01 <sup>c</sup>
T. t AqF	15.17± 0.03 <sup>d</sup>	0.33± 0.02 <sup>f</sup>	58.66± 7.03 <sup>d</sup>	2.39± 0.02 <sup>e</sup>	10.33± 0.04 <sup>d</sup>	58.87± 3.02 <sup>c</sup>
T. t AqE	16.83± 0.03 <sup>d</sup>	10.33± 0.01 <sup>e</sup>	100.79± 0.04 <sup>c</sup>	11.28± 0.01 <sup>c</sup>	23.67± 0.04 <sup>c</sup>	95.68± 3.02 <sup>b</sup>

\* Same letters along the column means not significantly different ( $p > 0.05$ ) while different letters along the column means significantly different ( $p < 0.05$ ).

T. t = *Talinum triangulare* ethanol extract, T. t DCM = dichloromethane fraction, T. t EA = ethyl acetate fraction, T. t BuOH = n-butanol fraction, T. t AqF = aqueous fraction from the T. t, T. t AqE = *Talinum triangulare* aqueous extract.

µg GAE/cm<sup>3</sup> = microgram of gallic acid equivalent per centimeter cubed of extract, µg QE/cm<sup>3</sup> = microgram of quercetin equivalent per centimeter cubed of extract, µg TAE/cm<sup>3</sup> = microgram of tannic acid equivalent per centimeter cubed of extract.

## Discussion

Phytochemical screening is very useful in the evaluation of some active biological components of some vegetables and medicinal plants (Enengedi *et al.*, 2018). Phenols, flavonoids, tannins, saponins, alkaloids, cardiac glycosides, anthraquinones and terpenoids were found to be present in all or some of the ethanol and aqueous extracts screened for phytochemicals. Some plants are important source of natural antioxidants that have been shown to reduce the risk and progression of certain acute and chronic diseases such as cancer, heart diseases and stroke by

scavenging free radicals which are implicated in the pathogenesis of many diseases (Kavitha and Indira, 2016). Also, some of these phytochemicals in the form of plant extracts are incorporated in personal care product formulations, such as, soaps, creams, lotions, pomades and shampoos, because of their curative properties (Barbulova *et al.*, 2014; Igwe, 2015).

The rich phytochemical profile of the ethanol and aqueous extracts of *T. triangulare* is an indication of their ability to serve as sources of therapeutic ingredients in cosmeceuticals.

**Table 3.** IC<sub>50</sub> values of *T. triangulare* leaf and stem.

Scavenging activity	IC <sub>50</sub> value (µg/cm <sup>3</sup> ) leaf	IC <sub>50</sub> value (µg/cm <sup>3</sup> ) stem
BHA	55.54±0.04 <sup>f</sup>	55.54±0.03 <sup>f</sup>
Ethanol extract	74.01±0.02 <sup>e</sup>	98.43±0.07 <sup>b</sup>
Aqueous extract	103.08±0.02 <sup>d</sup>	88.76±0.03 <sup>d</sup>
Dichloromethane fraction	131.58±0.02 <sup>a</sup>	91.43±0.04 <sup>c</sup>
Ethyl acetate fraction	106.47±3.05 <sup>c</sup>	84.97±0.02 <sup>e</sup>
n-butanol fraction	109.88±9.02 <sup>c</sup>	88.90±7.01 <sup>d</sup>
Aqueous fractions	124.64±0.04 <sup>b</sup>	111.27±0.02 <sup>a</sup>

\*Same letters along the column means not significantly different ( $p > 0.05$ ) while different letters along the column means significantly different ( $p < 0.05$ ).

**Table 4.** Mean inhibitory zone diameter (mm) of different concentrations of ethanol extract, fractions and aqueous extracts of *T. triangulare* leaf [T. t (L)] against micro-organisms.

Micro-organism	Concentration (mg/cm <sup>3</sup> ) of extract/ fraction																							
	T. t (L)				T. t (L) DCM				T. t (L) EA				T. t (L) BuOH				T. t (L) AqF				T. t (L) AqE			
	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-	-	-	-	-	-	8	-	-	-	-	-	-	-	-	-	-	-
<i>Vibrio cholera</i>	-	-	-	-	-	-	-	-	10	-	-	-	-	-	-	-	19	16	12	10	21	18	15	12
<i>Shigella sp</i>	15	12	10	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Propionibacterium acnes</i>	-	-	-	-	-	-	-	-	10	8	7	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Aspergillus niger</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Microsporus sp</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

\*T. t (L) = *Talinum triangulare* ethanol leaf extract, T. t (L) DCM = dichloromethane fraction, T. t (L) EA = ethyl acetate fraction, T. t (L) BuOH = n-butanol fraction, T. t (L) AqF = aqueous fraction from the T. t (L), T. t (L) AqE = *Talinum triangulare* aqueous leaf extract.

Phenolics have been known to possess a capacity to scavenge free radicals and are always associated with strong antioxidant properties (Aminudin *et al.*, 2015), and are also potent antimicrobial compounds (Cowan, 1999). A higher phenolic contents in *T.*

*triangulare* leaf than *T. triangulare* stem was observed. This is supported by the report of Enengedi *et al.* (2019), in which higher concentrations of phenolics was recorded in the leaf than the bark of *Dacyrodes edulis* from two locations. *T. triangulare*

leaf extract would be a better scavenger of free radicals caused by repeated exposure of the skin to UV radiation. Extracts with higher total phenolic contents can be better ingredients in cosmetic formulations for post-sun skin care (Enengedi *et al.*, 2019). Significantly higher total flavonoid contents

( $p < 0.05$ ) was observed in the ethanol extracts of the samples than the aqueous extracts, with ethyl acetate fraction having the highest content. This reveals that flavonoids of these extracts were best fractionated with moderately-polar and non-polar solvents.

**Table 5.** Mean inhibitory zone diameter (mm) of different concentrations of ethanol extract, fractions and aqueous extracts of *T. triangulare* stem against micro-organisms.

Micro-organism	Concentration (mg/cm <sup>3</sup> ) of extract/ fraction																							
	T. t (S)				T. t (S) DCM				T. t (S) EA				T. t (S) BuOH				T. t (S) AqF				T. t (S) AqE			
	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25	100	75	50	25
<i>Staphylococcus aureus</i>	-	-	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Vibrio cholera</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	20	15	12	10	21	18	15	12
<i>Shigella sp</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Propionibacterium acnes</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Aspergillus niger</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Microsporus sp</i>	-	-	-	-	-	-	-	-	-	-	-	-	13	11	9	7	-	-	-	-	-	-	-	-

\*T. t (S) = *Talinum triangulare* ethanol stem extract, T. t (S) DCM = dichloromethane fraction, T. t (S) EA = ethyl acetate fraction, T. t (S) BuOH = n-butanol fraction, T. t (S) AqF = aqueous fraction from the T. t (S), T. t (S) AqE = *Talinum triangulare* aqueous stem extract.

The Minimum inhibitory concentration (MIC) for the extracts and fractions of *T. triangulare* leaf and stem that were found effective are shown in Table 6 and 7 respectively.

**Table 6.** Minimum inhibitory concentration (MIC) for T. t (L) extracts and fractions.

Micro-organism	MIC (mg/cm <sup>3</sup> )					
	T. t (L)	T. t (L) DCM	T. t (L) EA	T. t (L) BuOH	T. t (L) AqF	T. t (L) AqE
<i>Staphylococcus aureus</i>	-	-	-	95	-	-
<i>Vibrio cholera</i>	-	-	80	-	10	20
<i>Shigella sp</i>	20	-	-	-	-	-
<i>Propionibacterium acnes</i>	-	-	45	-	-	-
<i>Aspergillus niger</i>	45	-	-	-	10	20
<i>Microsporus sp</i>	-	-	65	10	-	-

\*T. t (L) = *Talinum triangulare* ethanol leaf extract, T. t (L) DCM = dichloromethane fraction, T. t (L) EA = ethyl acetate fraction, T. t (L) BuOH = n-butanol fraction, T. t (L) AqF = aqueous fraction from the T. t (L), T. t (L) AqE = *Talinum triangulare* aqueous leaf extract.

This is supported by the result of (Stankovic *et al.*, 2014), in which highest concentrations of flavonoids were recorded in ethyl acetate, acetone and petroleum ether leaves extracts of *Cornus mas* L. Also, Brum *et*

*al.* (2013) observed a higher total flavonoid contents in ethyl acetate fraction of leaves of *Vitex megapotamica* than the crude extract. Since flavonoids are well known for their antioxidant

activity, extracts in this work can have potent antioxidant property and if incorporated into personal care products can scavenge reactive oxygen species on the skin, thereby preventing, delaying and remedying oxidative stress-mediated extrinsic aging

(photoaging), skin hyper-pigmentation and skin diseases. There was significant difference in the total tannin contents of the ethanol and aqueous extracts of the leaf and stem of *T. triangulare*.

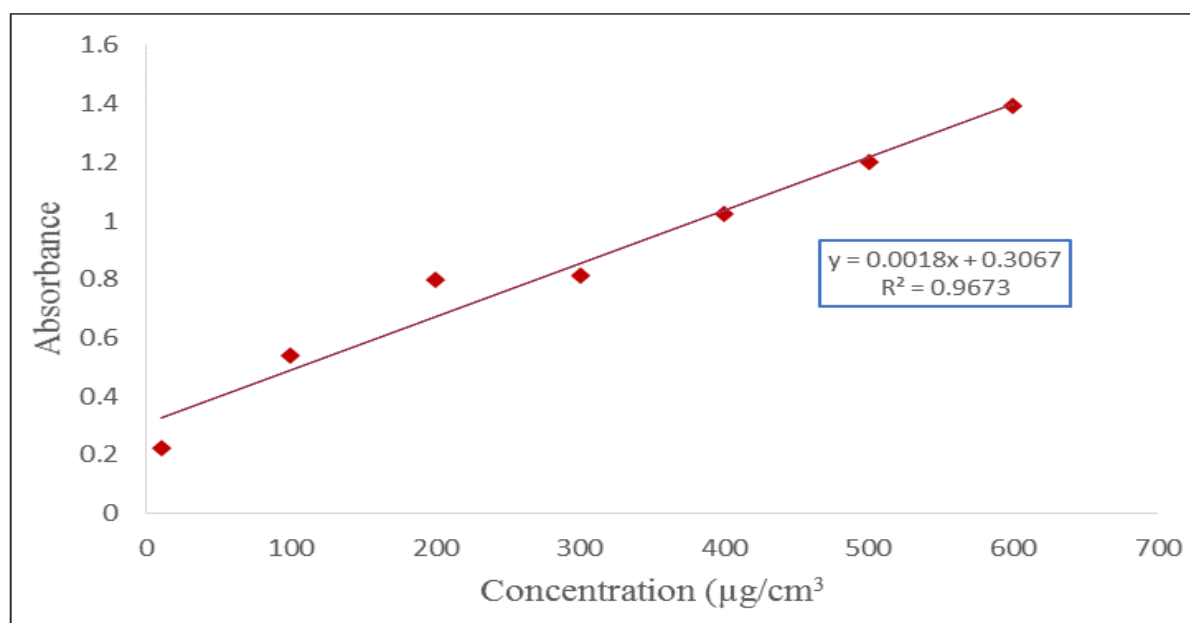
**Table 7.** Minimum inhibitory concentration (MIC) for T. t (S) extracts and fractions.

Micro-organism	MIC (mg/cm <sup>3</sup> )					
	T. t (S)	T. t (S) DCM	T. t (S) EA	T. t (S) BuOH	T. t (S) AqF	T. t (S) AqE
<i>Staphylococcus aureus</i>	-	80	-	-	-	-
<i>Vibrio cholera</i>	-	-	-	-	20	-
<i>Shigella sp</i>	-	-	-	-	-	-
<i>Propionibacterium acnes</i>	-	-	-	-	-	-
<i>Aspergillus niger</i>	-	-	-	-	-	-
<i>Microsporus sp</i>	-	-	-	20	-	-

\*T. t (S) = *Talinum triangulare* ethanol stem extract, T. t (S) DCM = dichloromethane fraction, T. t (S) EA = ethyl acetate fraction, T. t (S) BuOH = n-butanol fraction, T. t (S) AqF = aqueous fraction from the T. t (S), T. t (S) AqE = *Talinum triangulare* aqueous stem extract.

The high total tannin contents observed in these extracts and fractions reveal that, these extracts or fractions, if incorporated into personal care products can exhibit skin tightening effect and prevent infections, leading to healthy skin. Tannins are known

to hasten the healing of wounds and inflamed mucous membranes. Tannins not only heal burns and stop bleeding, but they also stop infection while they continue to heal the wound internally (Enengedi *et al.*, 2018).



**Fig. 1.** Calibration curve for Gallic acid.



The ability of tannins to form a protective layer over the exposed tissue keeps the wound from being infected even more (Ashok and Upadhyaya, 2012). Tannins have been used for immediate relief of sore throats, diarrhea, dysentery, hemorrhage, fatigue and skin ulcers. Tannins can cause regression of tumors that are already present in tissue, but if used

excessively over time, they can cause tumors in healthy tissue (Ashok and Upadhyaya, 2012). The  $IC_{50}$  value is the concentration ( $\mu\text{g}/\text{cm}^3$ ) of extract/fraction/standard that causes a decrease in the initial amount of DPPH radicals by Fifty percent (50%). Lower  $IC_{50}$  value indicates higher antioxidant capacity.

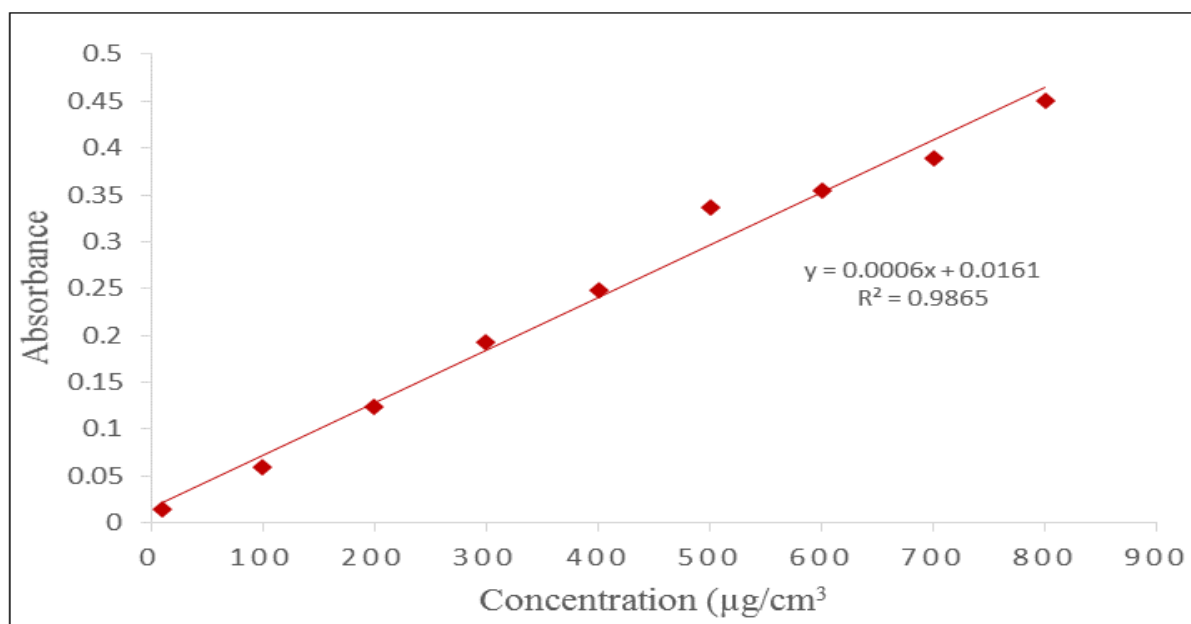


Fig. 2. Calibration curve for Quercetin.

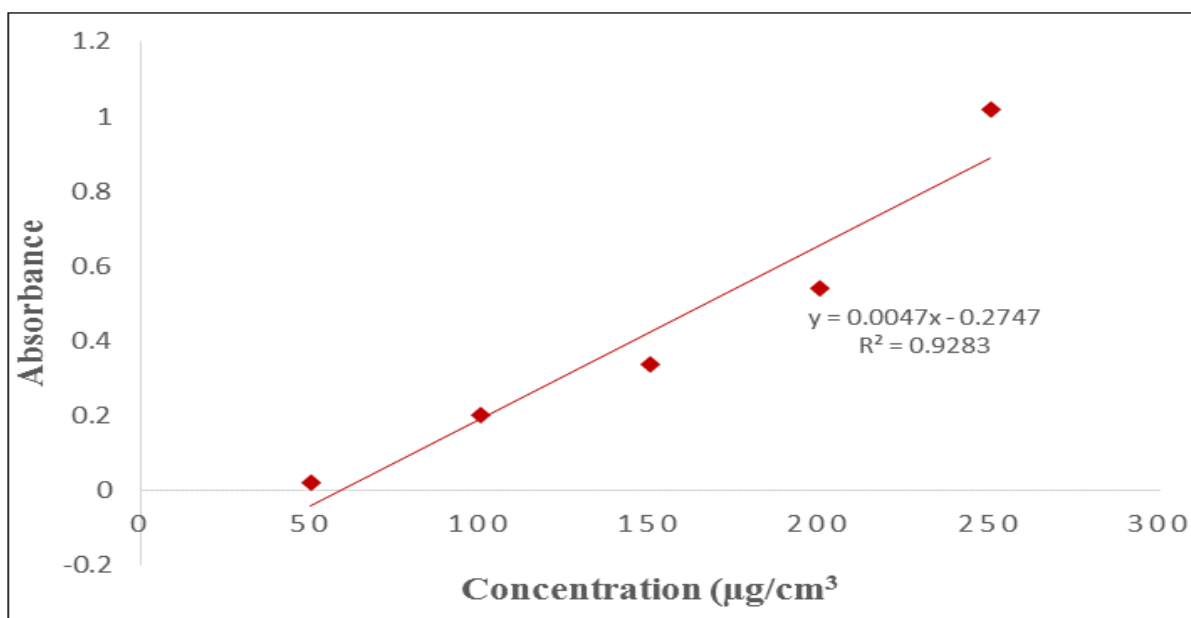


Fig. 3. Calibration curve for Tannic acid.

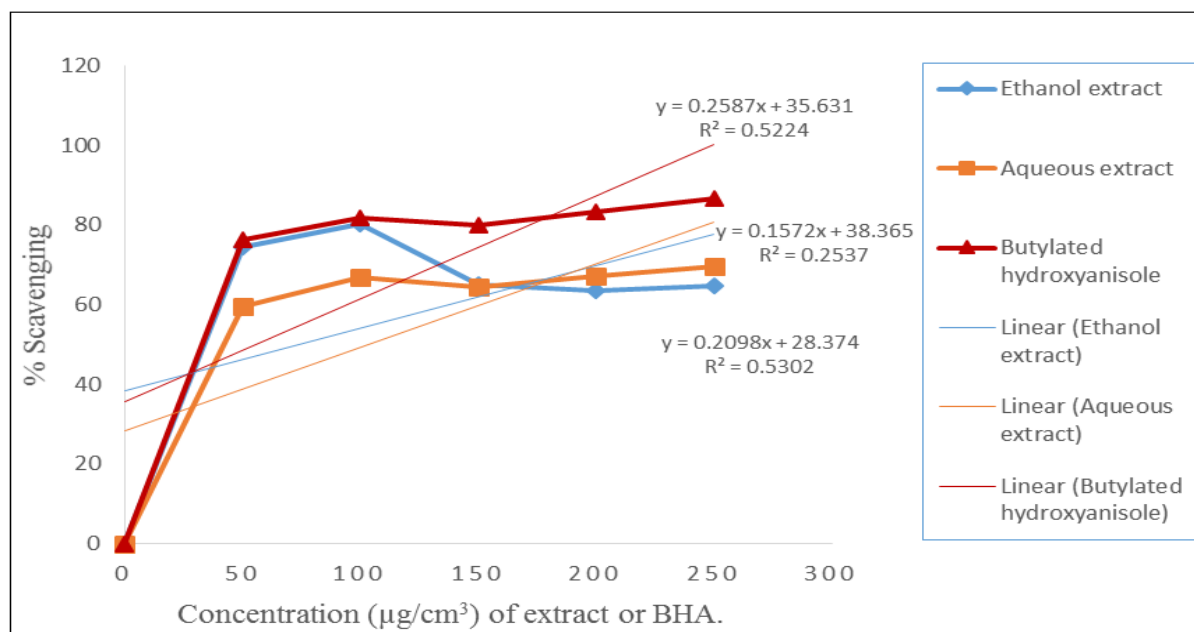
The lower  $IC_{50}$  value of ethanol extract of *T. triangulare* leaf ( $IC_{50} = 74.01 \pm 0.02 \mu\text{g}/\text{cm}^3$ ) than  $IC_{50}$  value of ethanol extract of *T. triangulare* stem ( $IC_{50} =$

$98.43 \pm 0.07 \mu\text{g}/\text{cm}^3$ ) clearly indicates that, the higher amount of phenolics, flavonoids and tannin compounds in the leaf than the stem, had contributed

to the stronger radical scavenging ability of the ethanol leaf extract than the stem extract. Aqueous extract of *T. triangulare* stem revealed a stronger antioxidant activity ( $IC_{50} = 88.76 \pm 0.03 \mu\text{g}/\text{cm}^3$ ) than aqueous extract of *T. triangulare* leaf ( $IC_{50} = 103.08 \pm 0.02 \mu\text{g}/\text{cm}^3$ ) even though the aqueous extract of the leaf had a higher total phenolic content than the aqueous extract of the stem. This implies

that antioxidant compounds other than phenolics (such as flavonoids and tannins) were also involved in the inhibition of the DPPH radicals.

This result is supported by the report of Okolie *et al.* (2016), in which the antioxidant potential of two different varieties of banana peels was investigated using ethanol and methanol as extracting solvents.



**Fig. 4.** DPPH scavenging activity of *T. triangulare* leaf extracts and BHA.

The results revealed that ethanol extracts had higher phenolics and flavonoid contents compared to the methanol extracts of the same banana varieties. However, the methanol extracts exhibited higher DPPH antioxidants activity compared to ethanol extracts. Also, Kahkonen *et al.* (1999) reported that apple extracts (two varieties) revealed strong antioxidant activity even though their total phenolic contents were low. Ethanol extract of *T. triangulare* leaf had the best antioxidant activity among the extracts of *T. triangulare* (leaf and stem) and fractions. It also exhibited free radical scavenging activity ( $IC_{50} = 74.01 \pm 0.02 \mu\text{g}/\text{cm}^3$ ) close to that of the standard antioxidant, butylated hydroxyanisole (BHA) ( $IC_{50} = 55.54 \pm 0.04 \mu\text{g}/\text{cm}^3$ ). Other components like polysaccharides of *T. triangulare* leaf might also have contributed to the scavenging activity of *T. triangulare* leaf. Studies have shown that polysaccharides isolated from *T. triangulare*

water extract revealed remarkably different degrees of antioxidant activities in dose-dependent manners (Liang *et al.*, 2011). With the antioxidant activity of *T. triangulare* observed in this research, these extracts, especially the ethanol extract of *T. triangulare* leaf, can be used to prepare skin lightening personal care products. Research by Amorim *et al.* (2013) reported that hydromethanolic extract of *T. triangulare* leaf stem exhibited powerful antioxidant activity and inhibited the activity of tyrosinase enzyme. Tyrosinase is known to be the key enzyme in melanin biosynthesis. Over-activity of this enzyme leads to overproduction of melanin leading to hyper-pigmentation of the skin, age spots and freckles (Aminudin *et al.*, 2015; Mapunya *et al.*, 2011; Lall and Kishore, 2014). Hyper-pigmentation can also be caused by excessive exposure to UV light and drug reaction and also occurs during aging (Aminudin *et al.*, 2015).

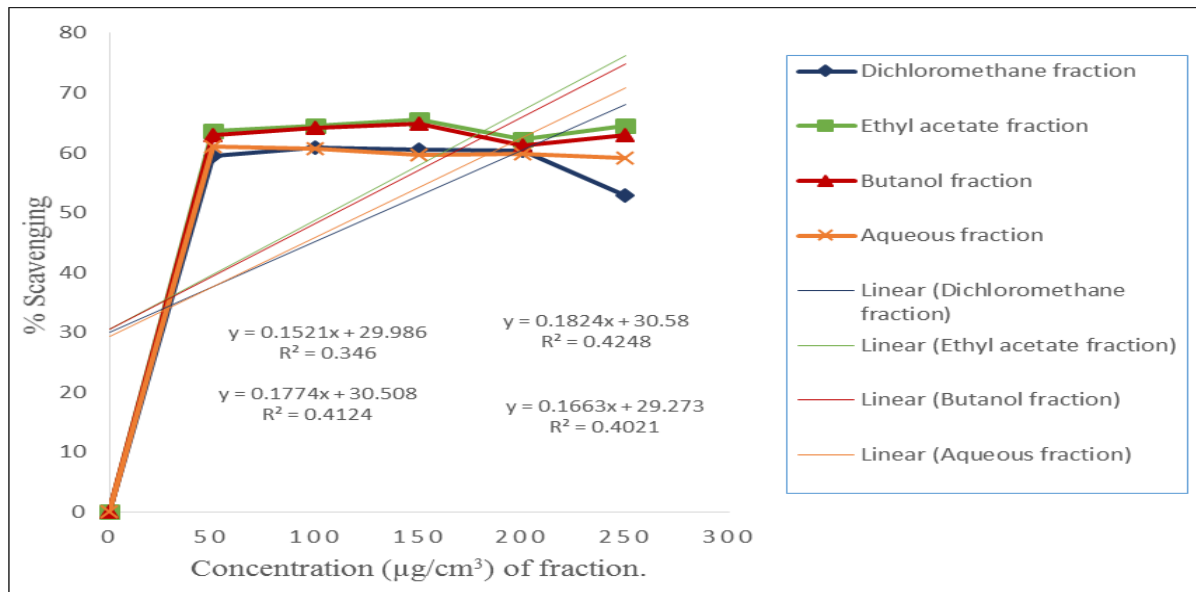


Fig. 5. DPPH scavenging activity of *T. triangulare* leaf fractions.

The zones of inhibition of growth of the microorganisms are functions of relative antimicrobial activity of the extracts or fractions. The extracts and fractions of the leaf revealed selective levels of activities against the microorganisms. The effectiveness of some of the extracts and fractions of

*T. triangulare* on the tested isolates can be due to the high phenolics content of the extracts and fractions of *T. triangulare* leaf and stem.

Phenolic compounds are synthesized by plants for defense mechanisms (Cowan, 1999).

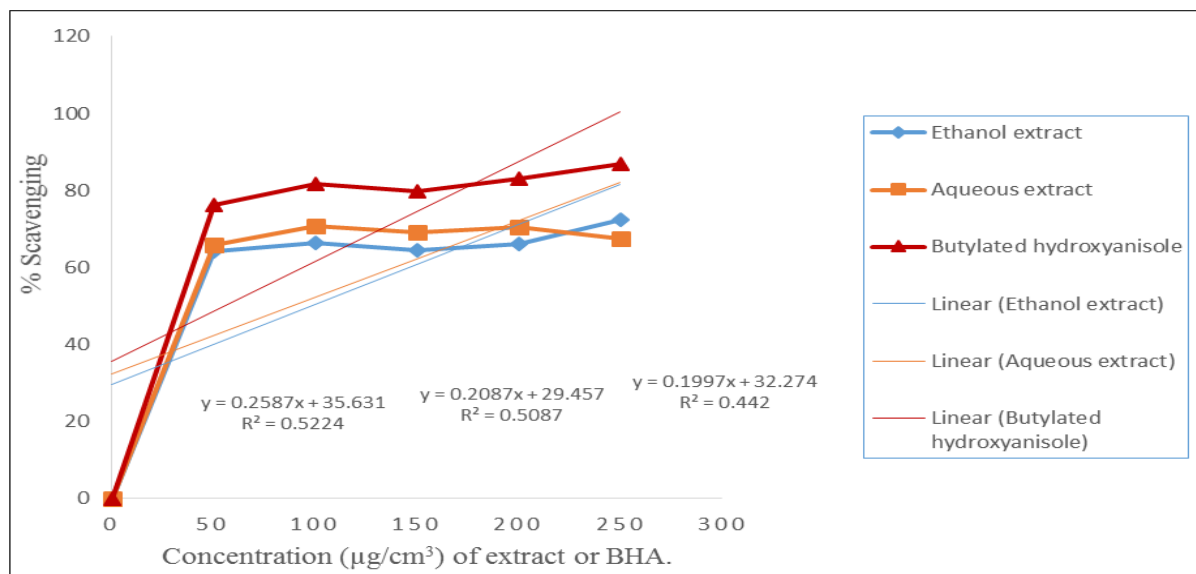


Fig. 6. DPPH scavenging activity of *T. triangulare* stem extracts and BHA.

They can act by interacting with the microorganism's cell membrane or cell wall, leading to changes in membrane permeability, and resulting in cell destruction. Phenolics can also penetrate into bacterial cells and promote the coagulation of their content. In another way, phenolic compounds as

natural antimicrobials could improve the shelf life of different products, inhibiting the growth of pathogenic microorganisms. *T. triangulare* leaf extract and fractions reveal a better antimicrobial activity than the stem extract and fractions.

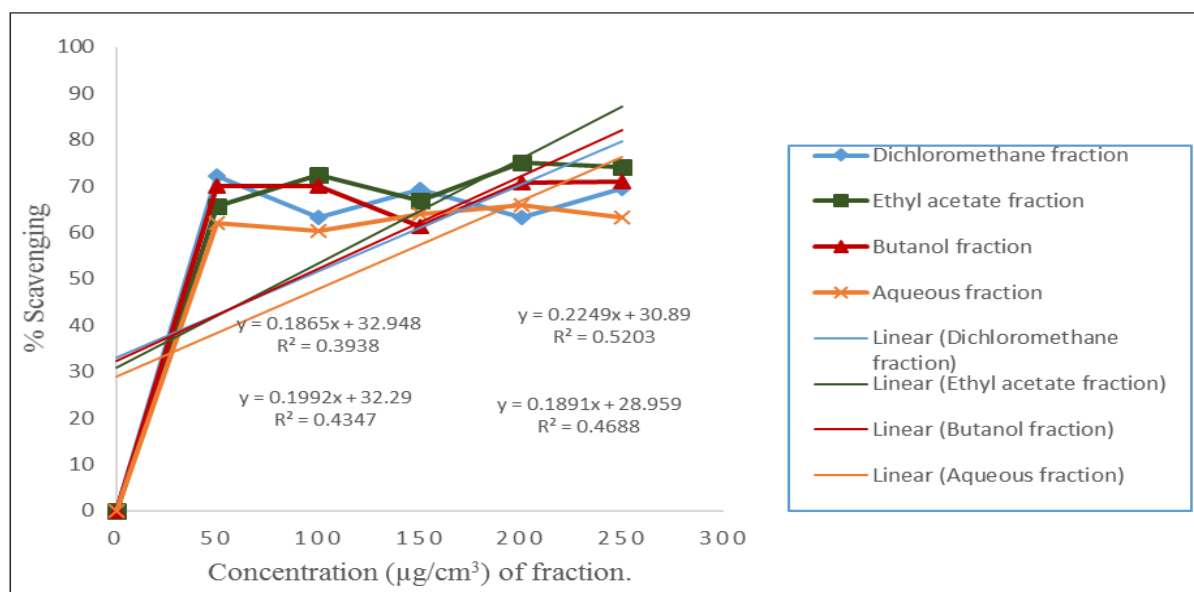


Fig. 7. DPPH scavenging activity of *T. triangulare* stem fractions.

### Conclusion

The rich phytochemical profile of the ethanol and aqueous extracts of *T. triangulare* is an indication of their ability to serve as sources of therapeutic ingredients in cosmeceuticals. With the antioxidant activity of *T. triangulare* observed in this research, these extracts, especially the ethanol extract of *T. triangulare* leaf, can be used to prepare skin lightening personal care products. This could eliminate hyper-pigmentation of the skin caused by excessive exposure to UV light and aging. *T. triangulare* leaf extract and fractions revealed a better antimicrobial activity than the stem extract and fractions.

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