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Antioxidant, Antibacterial and Tyrosinase Inhibiting Activities of Extracts from *Myristica fragrans* Houtt

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Authors' contributions

This work was carried out in collaboration between all authors. Author SP carried out the experimentation as part of her MSc study and drafted the manuscript. Author HYL managed the design layout of the protocols, and analyses of the study. Author KK initiated the idea and the funding of the project. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the antioxidant, antibacterial and tyrosinase inhibiting activities of the methanolic extracts of the leaf and fruit pericarp from *Myristica fragrans*.
Study Design: *In vitro* assays.
Place and Duration of Study: School of Biosciences, Taylor's University (Jan – December 2013)
Methodology: Total phenolic content (TPC) was assessed using Folin-Ciocalteu's method. The antioxidant activity was evaluated via radical scavenging against 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric ion reducing power (FRP) and ferrous ion chelating (FIC). Antibacterial activity was assessed using disc diffusion on four Gram-positve bacteria: *M. luteus*, *E. faecalis, S. aureus, B. cereus* and three Gram-negative bacteria: *P. aeruginosa*, *E. coli* and *K. pneumoniae*, followed by determination of minimum inhibitory concentration and minimum bactericidal concentration using broth dilution assays. Tyrosinase inhibiting activity was assessed using L-3,4-dihydroxyphenylalanine, L-DOPA as the substrate.
Results: The methanolic leaf extracts exhibited significantly higher TPC 2712-2779 mg gallic

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acidequivalent/100 g in contrast to that obtained from the pericarp. The leaf extracts also exhibited significantly stronger DPPH radical scavenging activity (2962-3787 mg ascorbic acid equivalent/100 g), ferric reducing activity (1383-1653 mg gallic acid equivalent/100 g) and chelating activity, as compared to the pericarp extracts. Leaf extracts were effective against all Gram-positve bacteria tested: *M. luteus*, *E. faecalis*, *S. aureus*, *B. cereus* (minimum inhibitory concentration 250-500 µg/mL; minimum bactericidal concentration 250-500 µg/mL). Studies on the tyrosinase inhibiting properties for applications in preventing food browning or treating hyperpigmentation disorders, showed significantly stronger activities exhibited by the leaf extracts (80-81%) as compared to that shown by pericarp (27-31%).

Conclusion: The present results suggest that nutmeg leaf could be employed as a natural antioxidant, antibacterial and tyrosinase inhibiting agent for applications in pharmaceuticals or in functional foods.

Keywords: Antibacterial; antioxidant; Myristica fragrans; nutmeg; phenolic; tyrosinase inhibition.

1. INTRODUCTION

The role of medicinal plants in promoting health, reducing risks of illness and for treating ailments has garnered increasing interest amongst researchers across the world in seeking evidence to justify the health benefits of these plants. Many diseases are now known to be caused by oxidative stresses and emerging bacterial activities. Consumption of antioxidants is associated with reduced risks of many oxidative diseases such as cardiovascular disorders, diabetes mellitus, cancer and rheumatic arthritis [1]. Antioxidants from natural sources are preferred to synthetics such as butylated hydroxytoluene, butylated hydroxyanisole, tert-butylhydroquinone and propyl gallate due to safety and health effects [2]. Likewise, antimicrobials from plants are also in focus due to concerns on the emerging resistance to antibacterial agents and the safety effects of synthetic antibiotics. It is estimated that infectious diseases are directly responsible for 26% of annual deaths worldwide [3]. There are 1340 plants with defined antimicrobial activities and over 20,000 antimicrobial compounds have been isolated from plants [4]. Tyrosinase inhibitors have important usage for the treatment of skin hyperpigmentation and control of browning process in food industry [5]. Synthetic inhibitors e.g. sulphites, used by food industries have been banned by FDA due to safety reasons [6]. Hence, there is justification to derive antioxidants, antibacterial and tyrosinase inhibiting agents from medicinal plant sources.

The nutmeg *Myristica fragrans* Houtt. (Family: Myristicaceae) has been used since ancient times in Ayurvedic therapy for use as herbal medicine to cure many disorders such as muscle and joint pain relief, indigestion, flatulence, nausea and vomiting. It is a large leafy evergreen plant, indigenous to the Malay Peninsula and Penang but now cultivated in many tropical countries of both the hemispheres [7]. The trees are dioecious i.e. there are separate male, female trees or mix (combination of both female and male in the same trees). However, only the female trees will bear fruits which provide the source of commercial products derivable from this plant. The current commercial products are produced from the seed (called the nutmeg) and mace (the reddish thin layer that surrounds the nutmeg seed): two types of spices (nutmeg and mace spices), essential oils, extracted oleoresins and nutmeg butter. Previous bioactivity studies on nutmeg have primarily focused on the seed and mace, and results indicated antioxidant capacity [8-10], chemopreventive potential [11] and anticariogenic activity [12]. *In vivo* studies using hydroalcoholic extracts of the nutmeg seed showed potential use for anemia, thrombocytopenia and hyprothyroidism treatment [13]. However, studies on the pericarp and the leaf of the nutmeg plant are scarce, although the fruit pericarp is popularly consumed as preserves, jellies, jams and fruit drinks and known locally for its therapeutic effects to cure disorders such as digestion problems, fever, skin diseases and respiratory ailments. The objective of the current study is to investigate the potential use of nutmeg pericarp and leaf in providing antioxidant, antibacterial and tyrosinase inhibiting properties. To the best of our knowledge, these bioactivities from the leaf and fruit pericarp of the nutmeg plant have not been reported.

2. MATERIALS AND METHODS

2.1 Collection of Nutmeg Samples

The nutmeg fruits and leaves were collected from the Ghee Hup Nutmeg Factory in Balik Pulau, Penang, Malaysia. Fruits were collected from the

female trees which yielded two types of fruits, namely female fruit and male fruit, while leaf were collected from the female and mix trees.

2.2 Preparation of Extract

Fresh fruit pericarp and leaf (1.00 g each) were excised, pulverized with liquid nitrogen, homogenized and then extracted with 50 mL of methanol and ethanol respectively continuously for one hour in a rotary orbital shaker. The extracts were filtered under pressure and stored at -20ºC to be used within one week.

2.3 Solvent Efficiency Test

The two solvents methanol and ethanol were compared to determine the more suitable solvent for extracting the total phenolic content (TPC). The Folin-Ciocalteu method was used as routine analysis for determination of TPC.

2.4 Total Phenolic Content (TPC)

TPC in the extract was determined according to the Folin-Ciocalteu procedure employed by Kahkonen et al. [14]. Extract solutions (0.3 mL, in triplicate) were mixed with 1.5 mL of 10% Folin-Ciocalteu's reagent and 1.2 mL of 7.5% sodium carbonate. The mixtures were kept in dark for 30 minutes and the absorbance was read at 765nm. The gallic acid standard curve used was y=0.0125x (R^2=0.9800) where y is the absorbance at 765 nm and x is the concentration of gallic acid in mg/L. TPC was expressed as mg gallic acid equivalent (GAE)/100 g fresh pericarp.

2.5 Antioxidant Activities

2.5.1 DPPH radical scavenging activity

The method described by Miliauskas et al. [15] was employed. Various dilutions of extract solutions (1.0 mL, in triplicate) were added to 2.0mL of DPPH (5.9 mg/100 mL methanol). The mixture was left in dark for 30 minutes. The absorbance was read at 517 nm with methanol as blank. The control consisted of methanol in place of the sample extract. The percentage radical scavenging was calculated as follows: scavenging (%) = ($A_{control} - A_{sample}$) / $A_{control}$ x 100. The results are presented as IC_{50}, the concentration of extract that scavenges 50% of the DPPH radical and also in terms of ascorbic acid equivalent antioxidant capacities (AEAC) which was calculated as follows:

AEAC (mg AA/100g) = IC_{50} (ascorbic acid) / IC_{50} (sample) x 10^5

IC_{50} (ascorbic acid) was determined to be 0.00387 mg/mL.

2.5.2 Ferric reducing power (FRP) activity

The FRP assay was carried out according to the procedure used by Juntachote and Berghofer [16]. Various dilutions of the extract solutions (1.0mL, in triplicate) were added to 2.5 mL of 0.2M phosphate buffer (pH6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. Then, 2.5 mL of 10% trichloroacetic acid was added. An aliquot of 2.5mL of the mixture was diluted twice with deionized water, before adding 0.5 mL of 0.1% (w/v) $FeCl_3$. The absorbance was read at 700 nm after 30 minutes. The gallic acid standard curve used is$y$=16.516x (R^2=0.9866) where y is the absorbance at 700 nm and x is the concentration of gallic acid in mg/mL. The results were expressed as mg gallic acid equivalent (GAE)/100 g fresh leaves.

2.5.3 Ferrous ion chelating (FIC) activity

The procedure described by Singh and Rajini [17] was followed. Various dilutions of extract solutions (1.0 mL, in triplicate) were added to 1.0 mL of 0.1 mM of $FeSO_4$ and 1.0 mL of 0.25mM ferrozine. The tubes were shaked well and left to stand for 10 minutes. The absorbance was read at 562 nm against blank containing water in place of ferrozine. The control consisted of water in place of extract. The ability of sample to chelate ferrous ion was calculated with the formula:

Chelating activity (%) = (1- $A_{sample}/A_{control}$) x 100

2.6 Antimicrobial Activity

Seven strains of bacteria were obtained from the Research Laboratory, School of Biosciences, Taylor's University. Four Gram-positive bacteria tested were *Micrococcus luteus*, *Enterococcus faecalis ATCC14506*; *Staphylococcus aureus ATCC33862*; *Bacillus cereus ATCC10876* and three Gram-negative bacteria: *Pseudomonas aeruginosa ATCC10145*, *Escherichia coli ATCC10536* and *Kleibsiella pneumoniae ATCC10031.*

2.6.1 Disc diffusion test

Antimicrobial activity was tested using the disc diffusion method as described previously [18]. Briefly, a swab of test microorganism (approximately 1 to $2x10^8$CFU/mL) was spread onto petri plates containing a 4mm depth of Mueller Hinton Agar (MHA). Extracts were dissolved in methanol to a final concentration of 10 mg/mL. Sterile filter paper discs (6mm in diameter) impregnated with 1 mg of plant extract was placed on the cultured plates. The plates were inverted and incubated at 37ºC for 24 hours. Methanol served as negative control. Standard antibiotics streptomycin (10µg) was used as positive control for all bacteria which showed susceptibility, except for *E. faecalis* where Gentamycin was used as the positive control for *E. faecalis*. Antimicrobial activity was indicated by the presence of clear inhibition zones around the discs. The assay was repeated twice and mean of the three experiments was recorded.

2.6.2 Broth dilution assay

The broth dilution assay was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) [19]. The extract was dissolved in 50% DMSO at a concentration of 50 mg/mL which was then diluted with nutrient broth to 1 mg/mL. Extract samples (75 µL) of various concentrations (2-1000 µg/mL) was added into sterile 96-well plates. Bacterial cell suspension (75 µL) corresponding to 1 $x10^6$CFU/mL was added to all wells except those in column 11. Wells of column 11 consisted of 1% DMSO and broth served as controls to check sterility while those in column 12 were filled with nutrient broth and bacterial suspension to check for adequacy of the broth to support bacteria growth. The plates were covered with sterile sealer and incubated at 37ºC for 18-24h. To indicate bacterial growth, 40 µL of 0.2 mg/mL of p-iodonitroterazolium chloride (INT, Sigma) was added to each well and incubated for another 30 min. Inhibition of bacterial growth was visible as a clear colourless well and the presence of growth was detected by the presence of pink-red colour. The lowest concentration showing no colour change was considered as the MIC.

For determination of MBC, a loop of liquid from each well that showed no change in colour was streaked onto Mueller Hinton Agar (MHA) and incubated at 37ºC for 24 h. The lowest concentration that showed no growth was taken as the MBC. Experiments were done in triplicate and repeated twice.

2.7 Tyrosinase Inhibiting Activity

The method employed by Masuda et al. [20] was followed with a slight modification. A 96-well microtitre plate was used. Each well consisted of 40 µL of test sample (2.5 mg/mL, dissolved in 50% dimetylsulfoxide), 80 µL of a 0.1M potassium phosphate buffer at pH 6.8, 40µL of 0.02 mg/mL of tyrosinase and 40 µL of 2.5 mM of L-DOPA. After 30 minutes, the absorbance was read with a microplate scanning spectrophotometer at 490 nm, with the reference wavelength at 700 nm. Each sample had a blank consisting of all components except L-DOPA. Results were compared with a control consisting of 50% DMSO in place of the sample. Results are expressed as IC_{50}, the concentration of extract in mg/mL that exhibited 50% inhibition. The percentage tyrosinase inhibition was calculated as follows:

$$\text{Tyrosinase inhibition (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

2.8 Statistical Analysis

Data were expressed as mean ± standard deviation (S.D.) Statistical analyses were evaluated by one-way ANOVA followed by Tukey HSD test using IBM SPSS Statistics 19. Values with $P<0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Sampling and Solvent Efficiency Test

As nutmeg trees are dioecious, leaf samples were collected from the female and mix gender trees from Ghee Hup Nutmeg Farm in Penang, due to the abundance of these trees relative to the male trees. In addition, it was noted that the female tree yielded two types of fruits, namely female and male fruit which are easily differentiated from its outer shape. Therefore, both types of fruits were sampled to investigate if there were any significant differences of the total phenolic content and bioactivity due to gender variation. Due to abundance of the female fruits, both ripe and unripe female fruits were collected. However, only ripe male fruits were collected. Hence pericarps were obtained from female fruits (unripe and ripe) and male fruit (ripe). The

pictures of the nutmeg leaf and fruits are shown in Fig. 1.

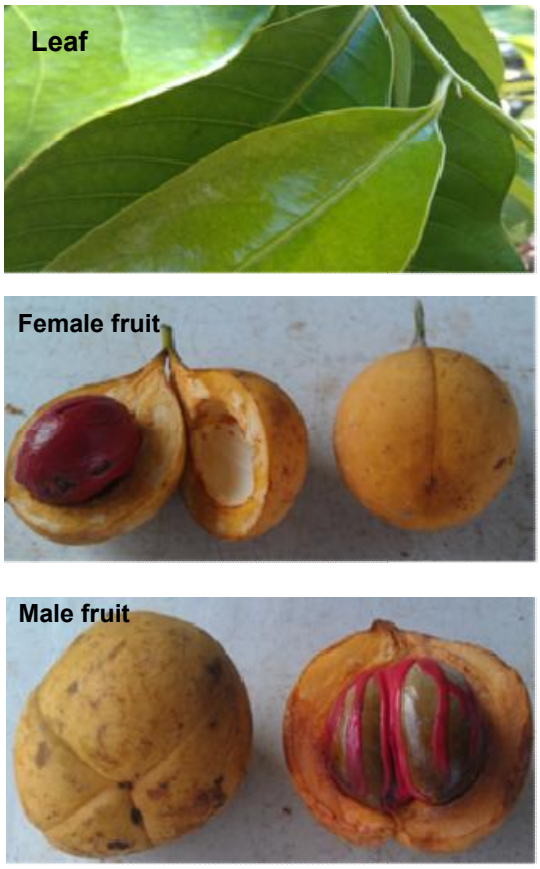

Fig. 1. Leaf, female fruit and male fruit of *Myristica fragrans* Houtt

An initial test to determine the suitable solvent to be used for the extraction was carried out with the pericarp of two types of fruit samples i.e. unripe female fruit and ripe female fruit pericarps. Choice of solvent plays an essential role in the extraction of phenolic compounds. In general, methanol has been reported to be a suitable solvent for the extraction of polyphenols from fresh plants due to its ability to inhibit the action of polyphenol oxidases which could affect the antioxidant activity and to recover the flavonols of low polymerization degree. It has been found that a maximum recovery of phenols required a minimum of 70% methanol in the extraction due to the need to inactivate phenol oxidases which are widely distributed in plants [14]. However, many researchers have reservations on the use of methanol due to its toxicity and ethanol is often preferred if the latter proves as efficient as the former in extractions [14]. Results on the efficiency of methanol and ethanol in the extracting total phenolic content (TPC) from nutmeg pericarp samples are summarized in Table 1. The yield of the TPC as extracted using ethanol was lower by 45.0 mg GAE/100 g and 108.3 mg GAE/100 g in the unripe female fruit pericarp and ripe female fruit pericarp respectively, compared to those extracted using methanol. As methanol proved to be the more efficient solvent in extracting TPC from the nutmeg samples, methanol was therefore subsequently chosen as the extracting solvent on all samples.

3.2 Total Phenolic Content (TPC)

Phenolic compounds are the most widespread secondary metabolites in the plant kingdom and have received much attention as potential natural antioxidants in terms of their abilities to act as efficient radical scavengers and metal chelators as well as many other health benefits [6,13,15,21]. In this study, the total phenolic contents (TPC) were determined using the Folin-Ciocalteu reagent. Results were expressed as mg gallic acid equivalent (GAE) per 100 g leaves. In the determination of TPC using Folin-Ciocalteu method, the standard graph of TPC determination using gallic acid gives the equation $y=0.0125x$; $R^2=0.9800$, where y is the absorbance at 765nm and x is the concentration of gallic acid in mg/L.

The TPC of methanolic extracts of the leaf and pericarp is summarized in Table 2. The leaf showed significantly higher TPC (2713–2779 mg GAE/100 g) than those of the pericarp (138–153 mg GAE/100 g). There was no significant difference in the TPC between the male and female counterparts.

The higher TPC may suggest better health benefits derivable from the nutmeg leaf extract compared to that from the pericarp. At present, both nutmeg pericarp and leaf are used in making drinks and snacks: the former as juice, jellies, jam while the latter as tea drink.

While reports on nutmeg leaf and pericarp were scarce, there have been few studies on the phytochemicals in the nutmeg seed, which indicated the presence of pine, sabincene, camphene, myristin, elemicin, isoelemicin, eugenol, isoeugenol, methoxyeugenol, safrole, diametric phenylpropanoids, lignas, neolignas, licarin-B, dehydrodiisoeugenol, malabaricone B,

Table 1. Solvent efficiency of methanol and ethanol in the extraction of nutmeg pericarp

Sample	Total phenolic content, TPC (mg of GAE*/100 g)	
	Methanol as extracting solvent	Ethanol as extracting solvent
Unripe female fruit pericarp	337.5±12.0	292.5±25.8
Ripe female fruit pericarp	375.7±17.7	267.4±46.0

*Data are mean±standard deviation of triplicate readings from two batches of fruits sampling (n=6) *GAE: Gallic acid equivalent*

malabaricone C, β-sitosterol and daucosterol [10,22]. Nevertheless, studies have indicated that the phytochemicals and bioactivities on the leaf and pericarp may differ vastly from those in the seed. For example, the antioxidant analysis on different parts of *Carica papaya* showed that the antioxidant activities were significantly higher in the leaf as compared to those in the ripe and unripe fruit and seeds [23].

Table 2. Total phenolic content (TPC) of extracts

Sample	TPC (mgGAE/100 g)
Leaf (from female tree)	2779.4±271.7[a]
Leaf (from mix tree)	2712.7±156.0[a]
Ripe male fruit pericarp	152.7±12.3[b]
Ripe female fruit pericarp	145.8±18.0[b]
Unripe female fruit pericarp	137.8±27.1[b]

Data are mean ± S.D. from three batches of sampling and in triplicate measurement per assay (n=9).Superscipts (a,b) showed significant differences (P<0.05) using ANOVA followed by Tukey HSD test

3.3 Antioxidant Activities

The antioxidant activities were measured by three different methods: DPPH radical scavenging, ferric ion reducing power (FRP) and ferrous ion chelating (FIC). The DPPH radical scavenging activity has been widely used to evaluate the antiradical activities of various samples [24]. In the DPPH method, the antioxidant activity was measured by the decrease in absorbance at 517nm as the DPPH radical received an electron or hydrogen from an antioxidant compound to be a stable diamagnetic molecule [1]. The antioxidant activity was expressed as IC_{50} and AEAC (ascorbic acid equivalent antioxidant capacity) [17]. The lower the IC_{50} and the higher the AEAC value, the greater was the antioxidant activity. The FRP method measured the ability of an antioxidant to donate electron to Fe (III) resulting in the reduction of Fe^{3+}/ ferricyanide complex to ferrous ion Fe (II) complex, which could be monitored by the formation of Perl's Prussian blue at 700 nm [16]. The higher the FRP value, the greater was the reducing power, thus the greater the antioxidant activity. The results of the DPPH radical scavenging and ferric ion reducing power (FRP) assays are summarized in Table 3.

The order of the samples in the antioxidant activities was similar to that for TPC i.e. leaf (AEAC 2962-3787) >> pericarp (AEAC 307-416). Strong correlations of TPC with AEAC and with FRP values have been reported previously in many plant samples [16,18] which indicate that the scavenging and reducing power of the nutmeg leaf extracts are due to phenolic compounds. Table 3 also shows that extract from the leaf of female tree possessed significantly higher DPPH scavenging and reducing activities, as compared to the extract from the leaf of mix tree. This indicates a higher potential use of the female trees and coincidentally, the female trees are more abundant in the farm compared to the number of male and mix trees.

It can be observed that the antioxidant properties of the leaf extracts are higher when compared with some of the reported high antioxidant properties of several other medicinal fruits, for example, fruit extracts of *Rubus ellipticus* which recorded a 28.3% scavenging [25] and ethanolic root extract of *Curculigo orchioides* ('Golden eye' grass) which showed 37.1% scavenging [26]. In this study, we found that the nutmeg leaf extract showed relatively higher scavenging activities i.e. 57-64%. Hence, the nutmeg leaf extract could be potentially developed for antioxidant properties, for example for use in making tea drinks. Fe (II) ions are known to accelerate lipid peroxidation by breaking down hydrogen peroxide and lipid peroxides to reactive hydroxyl radicals via the Fenton reaction, leading to occurrence of many diseases [16].

Hence reduction of Fe (II) ions is favorable as this would protect against oxidative damage. It was reported that chelating agents that form σ-bonds with a metal, are effective as secondary antioxidants since they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion [25]. In this assay, ferrozine acts as

Table 3. DPPH scavenging activity (expressed in IC_{50} and AEAC) and ferric reducing power (FRP) of extracts

Sample	IC_{50} (mg/ml)	AEAC (mg AA/100 g)	FRP(mg GAE/100 g)
Leaf (from female tree)	0.10±0.01	3787±304[a]	1653±65[a]
Leaf (from mix tree)	0.13±0.00	2962±84[b]	1383±20[b]
Ripe male fruit pericarp	0.93±0.05	416±23[c]	128±2[c]
Ripe female fruit pericarp	1.02±0.10	384±39[c]	128±6[c]
Unripe female fruit pericarp	1.26±0.05	307±12[d]	119±2[d]

Data are mean ± S.D. from three batches of sampling and in triplicate measurement per assay (n=9). For each column, values followed by the same letters (a, b, c, d) are not statistically different (P>0.05)

a chelating agent and forms purple complex ion with Fe (II) giving maximum absorbance at 562nm. A decrease in absorbance would indicate a stronger chelating activity in the test sample that competes with ferrozine for Fe (II). Hence, samples with strong chelating activity will show large decreases in absorbance at 562 nm [16]. The chelating activity is summarized in Fig. 2.

Fig. 2 shows the chelating activity of the extracts. Higher chelating activities were found in the leaf extracts, as compared to those of the pericarp extracts. At the average concentration of 5.4 mg/mL, the leaf extract showed 56.6-65.6% chelating activity. The pericarp extracts except for the unripe female fruit pericarp, recorded very low chelating activity.The results indicate the phenolic compounds in the leaf are potent ion chelators that may serve as an antioxidant.

3.4 Antibacterial Activities

A total of seven bacteria were selected for the study i.e. four Gram-positive bacteria (*M. luteus*, *E. faecalis*, *S. aureus* and *B. cereus*) and three Gram-negative bacteria (*P. aeruginosa*, *E. coli* and *K. pneumoniae*. Gentamycin and Streptomycin were used as the positive controls. An initial susceptibility test was conducted using disc diffusion method. The antimicrobial activity was assessed by the size of inhibition zone diameter as compared to size diameter shown by the respective positive control (Table 4). The strength of inhibition can be categorized as strong for inhibition ≥70%, moderate for inhibition 50-69%, or weak for inhibition <50%. The results are summarized in Table 4. The leaf extracts showed inhibition against all the Gram-positive bacteria with strong inhibition on *E. faecalis* (85-100%, as compared to positive control Gentamycin) and *S. aureus* (66-100% as compared to positive control Streptomycin), moderate inhibition against *B. cereus* (50-57%) and weak inhibition against *M. luteus* (34-50%). The leaf extracts showed inhibition against all the Gram-positive bacteria with strong inhibition on *E. faecalis* (85-100%, as compared to positive control Gentamycin) and *S. aureus* (66-100% as compared to positive control Streptomycin), moderate inhibition against *B. cereus* (50-57%) and weak inhibition against *M. luteus* (34-50%).

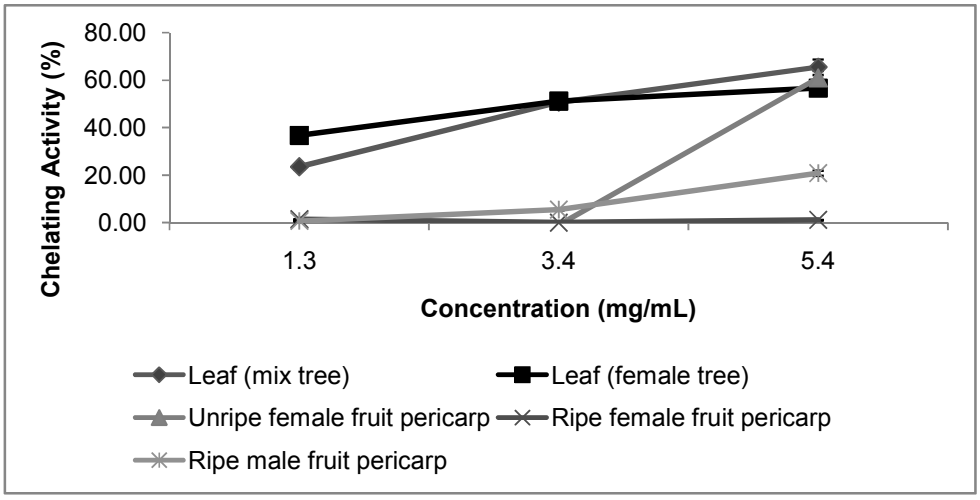

Fig. 2. Chelating activity (%) of extracts

The pericarp extracts showed weak inhibition against *B. cereus* (43-45%). All extracts did not show inhibition against Gram-negative bacteria. The insensitivity of the extracts against Gram-negative bacteria is presumably due to the impermeable nature of the outer membrane of the bacteria. These findings are consistent with the observations in many previous reports on medicinal plants which show activity against Gram-positive while non-activity against Gram-negative bacteria [18].

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the leaf extracts against the Gram-positive bacteria were recorded to be 250-500 µg/mL (Table 5). Comparisons on these MIC values were made with reference to previous reports of antibacterial activity by various medicinal plants across the globe. It is noted that while some authors consider values of 250 µg/mL as strong antibacterial activity, others use a stricter endpoint criteria, in which crude extracts with MIC values less than 100 µg/mL were worthy for further study [27]. The antibacterial activity of the nutmeg leaf extracts (MIC 250-500 ug/mL) may thus be considered as weak to moderate, in comparison with the MIC values of 70-110 µg/mL in some potential Jordanian medicinal plants [28] and 8-250 µg/mL in potential African medicinal plants [26]. Nevertheless, it is noteworthy to observe that many medicinal plants do not exhibit any antibacterial at all. As reported by Boarchard et al. [29] who tested on 597 plant extracts, only 24% were found to exhibit some antibacterial activity; and some of which inhibited only one or two bacteria that were tested. It is also noteworthy to state the strong inhibition exhibited by the nutmeg leaf extract against *E. faecalis* indicates a strong potential of the leaf extract for application as an antibacterial agent against this bacteria, hence, for related treatment such as urinary tract infection or diarrhoea ailments, which justifies the medicinal uses of the plant.

3.5 Tyrosinase Inhibiting Activity

Tyrosinase inhibitors are chemical agents capable of reducing enzymatic reactions such as food browning and melanisation of human skin [30]. In this study, tyrosinase inhibiting activities were evaluated at concentrations of 0.5 mg/mL, 1.25 mg/mL and 2.5 mg/mL with quercetin and kojic acid as standards. The results of tyrosinase inhibition activity of the nutmeg leaves and pericarps are expressed as IC_{50} and percentage inhibition at 2.5 mg/mL as shown in Table 6.

In the tyrosinase inhibition assay, leaf extracts showed significantly greater tyrosinase inhibiting activity at the concentration of 2.5mg/mL (80-81%) as compared to those from the pericarp extracts (27-31%).The IC_{50} values of leaf extracts are 0.47-0.53mg/mL, which are comparable with the IC_{50} of the positive control, quercetin. Quercetin has been established to be a potent tyrosinase inhibitor (Chang, 2009). The promising potential of tyrosinase inhibiting activity as shown by nutmeg female leaf is comparable with those reported by Khazaeli et al., (2009) [31] on some medicinal plant extracts (*Quercus infectoria* and *Terminalia chebula*)

Table 4. Antibacterial activity: inhibition zone diameter and percentage inhibition

Sample	Inhibition zone diameter in mm[b]/1 mg per disc (% inhibition compared to positive control)						
	ML[a]	EF[a]	SA[a]	BC[a]	PA[a]	EC[a]	KP[a]
Leaf (mix tree)	8.3(34%)	9.0 (85%)	20.3 (100%)	11.3 (57%)	n.d	n.d	n.d
Leaf (female tree)	12.3 (50%)	11.0 (>100%)	13.3 (66%)	10.0 (50%)	n.d	n.d	n.d
Unripe female fruit pericarp	n.d	n.d	n.d	9.0 (45%)	n.d	n.d	n.d
Ripe female fruit pericarp	n.d	n.d	n.d	9.0 (45%)	n.d	n.d	n.d
Ripe male fruit pericarp	n.d	n.d	n.d	8.6 (43%)	n.d	n.d	n.d
Streptomycin*	24.6	n.d	20.3	20.0	17.3	19.3	21.3
Gentamycin*	n.t	10.6	n.t	n.t	n.t	n.t	n.t

[a]Microorganisms: ML, Micrococcus luteus; EF, Enterococcus faecalis; SA, Staphylococcus aureus; BC, Bacillus cereus; PA, Pseudomonas aeruginosa; EC, Escherichia coli; KP, Klebsiella pneumoniae. [b]Each value is the mean of triplicate measurements of the inhibition zone diameter (mm) including the diameter of disc (6mm). indicates positve controls; 'n.d' indicates not detected and 'n.t' indicates not tested*

Table 5. Minimum inhibitory concentration (MIC in µg/ml) and minimum bactericidal concentration (MBC in µg/ml)

Sample	*Bacteria*			
	M. luteus	***E. faecalis***	***S. aureus***	***B. cereus***
Minimum Inhibitory concentration (MIC, µg/ml)				
Leaf (mix tree)	250	250	500	500
Leaf (female tree)	250	250	500	250
Minimum bactericidal concentration (MBC, µg/ml)				
Leaf (mix tree)	250	250	500	500
Leaf (female tree)	250	250	500	250

Table 6. Tyrosinase inhibiting activity of extracts

Sample	IC_{50} (mg/mL)	% Inhibition at 2.5mg/mL
Leaf (mix tree)	0.47±0.04	$80.6±1.8^{a}$
Leaf (female tree)	0.53±0.02	$81.7±0.5^{a}$
Unripe female fruit pericarp	8.95±1.50	$27.8±1.1^{b}$
Ripe female fruit pericarp	6.06±0.08	$31.7±0.3^{b}$
Ripe male fruit pericarp	6.09±0.15	$29.6±0.3^{b}$
Kojic acid*	0.26 ±0.07	$94.1±0.5^{a}$
Quercetin*	0.43±0.01	$87.3±0.4^{a}$

*Data are mean ± S.D. Triplicate measurements were conducted in each assay. *indicates positive controls. Superscipts (a,b) showed significant difference (P<0.05)*

which displayed an IC_{50} of 0.10-0.19 mg/mL and similar trend of dose-dependent manner of inhibition. Hence, nutmeg leaf can be apotential natural source for tyrosinase inhibitors.

4. CONCLUSION

The present results suggest that nutmeg leaf has potential as natural antioxidant, antibacterial and tyrosinase inhibiting agent, which is worthy of further evaluations for applications in development of pharmaceuticals, preservation of foods and functional foods.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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