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# Tyrosinase, elastase, hyaluronidase, inhibitory and antioxidant activity of Sri Lankan medicinal plants for novel cosmeceuticals

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ABSTRACT

The search for natural cosmeceuticals has gained an increasing demand due to its fewer side effects and become more prevalent in cosmetic formulations. Plant sources contain numerous natural compounds which can be used as whitening, anti-aging anti-wrinkle ingredients and also for the treatment of dermatological disorders. Ethanol extracts of 15 Sri Lankan medicinal plants were investigated for their tyrosinase, elastase and hyaluronidase enzyme inhibitory and antioxidant activities for the purpose of identifying anti-aging and skin-whitening ingredients with the potential for use as materials in cosmetics. *Eleocarpus serratus* bark extract showed highest elastase inhibitory activity ($IC_{50}$ 27.27 ± 2.74 μg/mL) while *Curcuma aromatica* rhizomes exhibited marked elastase ($IC_{50}$ 252.7 ± 6.8 μg/mL) and hyaluronidase inhibitory activities (95.0% inhibition at 500 μg/mL). *Artocarpus altilis* and *Artocarpus nobilis* bark extracts are good source of tyrosinase ($IC_{50}$ 27.47 ± 0.45 μg/mL, 53.23 ± 2.65 μg/mL), hyaluronidase (68.59%, 44.78% inhibition at 500 μg/mL), and elastase inhibitors (23.87%, 30.96% inhibition at 500 μg/mL). *Eleocarpus serratus* extracts exhibited highest antioxidant activities compared to other tested plants. Tyrosinase inhibitory activity positively correlates with the Total Flavonoid Content (r = 0.711) and DPPH free radical scavenging activities (r = 0.891). These results suggest that medicinal plants showing biological activities may be potent inhibitors of tyrosinase, elastase and hyaluronidase and could be useful for application in cosmetics. This is the first report of tyrosinase elastase, and hyaluronidase inhibitory activity of ethanol extracts of *A. nobilis* and *E. serratus* bark, leaves and fruit and *M. ferrea* leaves, petals and stamens.

## 1. Introduction

Melanin is the primary determinant of the skin color. It protects the skin from absorbing ultraviolet (UV) radiation by 50% to 75% and scavenges reactive oxygen species (ROS) (Parvez et al., 2007; Tada et al., 2010). Tyrosinase, a copper-containing monooxy-genase, is a key regulatory enzyme that catalyzes melanin synthesis within melanocytes. The major actions of this enzyme are the hydroxylation of L-tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA) and consequent oxidation of L-DOPA to dopaquinone. Dopaquinone is a highly reactive compound that can polymerize spontaneously to form melanin (Prota, 1988). However, overproduction of melanin in the skin may cause hyperpigmentation, melanoma, and could be genotoxic (Brozyna et al., 2007). Therefore, tyrosinase inhibitors have become increasingly important in cosmetics and pharmaceuticals as whitening agents and for the treatment of pigmentory disorders such as melasma, lentigines, and freckles.

Elastin is a highly elastic protein found in connective tissues including skin, lung, and arteries and helps in maintaining tissue configuration after stretch or recoil (Daamen et al., 2007; Debelle and Alix, 1999). Overexposure to UV irradiation and oxidative damage up regulate the expression of elastase, a serine protease which hydrolyzes the dermal elastin fiber network (Braverman and Fonferko, 1982; Kim et al., 2006). This may to lead to reduced skin elasticity and the linearity of dermal elastic fibers, inducing wrinkling and sagging (Kambayashi et al., 2001). There are several research studies that suggest both skin-aging and anti-wrinkle effects are directly associated with degradation of elastin (Akazaki et al., 2002). Recently, a number of studies have investigated the interactions between elastase and its inhibitors such as catechin, epigallocatechin gallate, boswellic acids, and purpurin (Kim et al., 2004; Melzig et al., 2001; Thring et al., 2009; Biswas et al., 2015; Bravo et al., 2016). It has been reported that the

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**Table 1**
Traditional uses of medicinal plants used in this study.

| Plant name | Plant part | Traditional uses |
|---|---|---|
| *Rubia cordifolia* L. | Root | Blood detoxifier, skin disorders like hyperpigmentation, scabies, acne and allergies, eye disorders, anti-inflammatory (Deshkar et al., 2008) |
| *Saussurea lappa* DC | Root | Curing dysentery and ulcer arthritis and expectorant, skin diseases (Law and Salick, 2005) |
| *Curucumaa aromatica* Salisb. | Rhizomes | bruises, sprains and enhancing complexion (Saleem et al., 2011) |
| *Kokoona zeylanica* Thwaites | Bark | Skin diseases, acne, eye diseases and swelling, enhancing complexion (Wijesundara, 2008) |
| *Vetiveria zizanioides* L. | Root | Acne and sores, flavoring agent (Jain, 1991) |
| *Coscinium fenestratum* (Goetgh.) Colebr | Bark | Tastelessness, bleeding piles, cough, wounds, skin diseases, fever (Tushar et al., 2008) |
| *Elaeocarpus serratus* L. | Leaves and bark | Mental disorders, headache, fever, skin diseases, palpitation, infertility (Dennis, 1993) |
| | Fruit | Diarrhea due to its constipating effect (Subrata and Parikh, 1985) |
| *Nymphaea nouchali* Burm. f. | Leaves, root and flowers | Backache, stomach-ache, diabetes, blood disorders, heart troubles, enhancing complexion (James, 2008) |
| *Nymphaea pubescens* Willd. | Whole plant | Blood purifier, diabetes, inflammation (Debnath et al., 2013) |
| *Camellia sinensis* (L.) Kuntze | Leaves | Asthma, angina pectoris, peripheral vascular disease, skin disorders (Sharangi, 2009) |
| *Mesua ferrea* L. | Flowers and leaves | Cough, excessive thirst, perspiration and indigestion, skin disorders blood purifier (Parukutty and Chandra, 1984) |
| *Azadirachta indica* A.Juss. | Leaves | Antifungal, antidiabetic, antibacterial, antiviral, skin diseases (Rahman and Jairajpuri 1993) |
| *Artocarpus nobilis* Thwaites | Fruits | Worm diseases (http://www.instituteofayurveda.org/plants) |
| *Artocarpus altilis* (Parkinson) Fosberg | Leaves | Liver cirrhosis, hypertension and diabetes (Wang et al., 2006) |
| *Artocarpus heterophyllus* Lam. | Pulp and seed | Cooling tonic and pectoral |
| | Leaves and bark | Anemia, asthma, dermatitis, diarrhea (Balbach and Boarim, 1992) |

topical application of specific inhibitors to the surface of human skin may have beneficial effects on UV-irritated and dry skin.

Hyaluronic acid (HA), also called hyaluronan is widely distributed in Extra Cellular Matrix of soft connective tissues including skin, umbilical cord and synofial fluid (Hanamura et al., 2008). Hyaluronic acid has a unique ability to hold 6000 mL of water in 1 mg of HA and plays an important role in reducing wrinkles, wound healing and keeping the skin smooth and hydrated (Necas et al., 2008; Jegasothy et al., 2014). Enzyme hyaluronidase breakdown HA in to small oligosaccharide molecules by cleaving the N-acetylglucosamidic bonds via a β-elimination process. By catalyzing the hydrolysis of HA, hyaluronidase decreases the viscosity of body fluids and increases the permeability of connective tissues (Stern and Jedrzejas, 2006; Necas et al., 2008). Hyaluronidase inhibitors are therefore effective regulating agents, that maintain the balance between the anabolism and catabolism of HA, and this keep skin moist as well as smooth.

Pollutants, UV radiation, smoke and xenobiotics generate exogenous ROS inside the human body. Reactive oxygen species restrains antioxidant defense mechanisms, resulting severe oxidative stress and contribute to aging, carcinogenesis, inflammation, and cell proliferation etc. Beside these ROS leads to overproduction of elastases, reduction and degeneration of collagen, deposition of glycosaminoglycans, induces melanin biosynthesis, and may induce proliferation of melanocytes (Fisher et al., 2002; Moon et al., 2010; Inomata et al., 2003). These observations suggest that the antioxidants play a vital role by neutralizing ROS in the management of oxidative stress.

Medicinal plants have been extensively used as active ingredients in cosmetics and therapeutics, as beautifying agents and also a remedy for dermatological disorders such as eczema, acne, hyperpigmentation, and photoaging for centuries. These provide a largely unexplored source for the potential development of active ingredients for cosmetic formulations. Hence cosmeceuticals is a growing market segment, driven by the rising desire of people to maintain healthy skin without using chemicals. Sri Lanka holds a treasure of high biodiversity, which has potential applications for use in cosmetic industry (Perera et al., 2016; Samaradivakara et al., 2016). As part of the effort to find new functional ingredients for skin-whitening and anti-aging preparations, 15 less exploited plants grown in Sri Lanka, were investigated evaluating their *in-vitro* anti-tyrosinase, anti-hyaluronidase anti-elastase enzyme inhibitory, and anti oxidant activities, Total Phenolic Content (TPC) and Total Flavanoid Content (TFC).

## 2. Experimental

### 2.1. Chemicals and plant material

Tyrosinase from mushroom, L-3,4-dihydroxyphenylalanine (L-DOPA), elastase from porcine pancreas, kojic acid, ascorbic acid, *N*-Succinyl-Ala-Ala-Ala-*p*-nitroanilide, quercetin, hyaluronidase from bovine testes, hyaluronic acid sodium salt from rooster comb, tannic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-Tripyridyl-s-Triazine (*TPTZ*), *Trolox* (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), Folin-Ciocaltue's reagent and gallic acid were purchased from Sigma-Aldrich Co., Louis, (USA). All chemicals and reagents used in the experiment were analytical grade.

The plants *Rubia cordifolia* L. (root, Rubiaceae), *Saussurea lappa* DC. (root, Asteraceae), *Curcuma aromatica* Salisb. (rhizomes, Zingiberaceae), *Kokoona zeylanica* Thwaites (bark, Celastraceae), *Vetiveria zizanioides* L. (root, Poaceae), *Coscinium fenestratum* (Goetgh.) Colebr (stem, Menispermaceae), *Elaeocarpus serratus* L. (bark, leaves, root, seed, Elaeocarpaceae), *Nymphaea nouchali* Burm. F. (petals, Nymphaeaceae), *Nymphaea pubescens* Willd. (petals, Nymphaeaceae), *Camellia sinensis* (L.) Kuntze (leaves, Theaceae), *Mesua ferrea* L. (leaves, petals, stamens, Calophyllaceae), *Azadirachta indica* A.Juss. (leaves, Meliaceae), *Artocarpus nobils* Thwaites (bark, leaves, fruit, Moraceae), *Artocarpus heterophyllus* Lam. (bark, leaves, fruit, Moraceae), and *Artocarpus altilis* (Parkinson) Fosberg (bark, leaves, fruit, Moraceae) were collected from the same agro-ecological region Western Province in August 2014, Sri Lanka. Plants were selected based on traditional use and literature reference (Table 1) and different plant parts were selected due to variation in bioactivities and phytoceutical composition. Selected plants had been grown under natural growth conditions (rain fall 1600–2500 mm, average temperature 27 °C, soli type: clay loam soil, and humidity: 73%). Taxonomic identification of the plant material was confirmed by the Senior Taxonomist in the Herbal Technology section at Industrial Technology Institute (ITI), Sri Lanka. The voucher specimens were deposited at the Herbal Technology Section at ITI Sri Lanka.

### 2.2. Sample preparation

Plant samples were air-dried at room temperature (27–32 °C) and Relative Humidity 65–70% for 3 days. Fifty grams of coarsely powdered (not less than 0.5 mm) plant materials were soaked in ethanol for overnight and stirred at room temperature for 1 h using a mechanical stirrer. The crude extract was filtered through a ceilite bed and

concentrated under reduced pressure using a rotary evaporator to obtain the ethanol extracts.

### 2.3. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The DPPH free radical scavenging assay was performed according to the method described by Blois (1958) with some modifications. Plant extracts were tested at the assay concentration range of 1–500 μg/mL. DPPH solution (40 μg/mL, 200 μL) was incubated with 100 μL of plant extracts dissolved in methanol at room temperature (25 ± 2 °C) in dark for 10 min. The absorbance was recorded against a blank at the wavelength of 517 nm. Trolox was used as a positive standard. The capacity to scavenge the DPPH radical by 50% ($IC_{50}$) was calculated from the dose effect curves by linear regression and% inhibition was calculated using following equation:

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

Where, $A_{(\text{sample})}$ is the absorbance of the sample extracts and $A_{(\text{control})}$ is the absorbance of the assay using the buffer instead of inhibitor (sample).

### 2.4. Ferric ion reducing antioxidant power (FRAP) assay

FRAP activity was measured according to the method of Benzie and Strain (1996). Plant extracts were tested at the concentration range of 25–250 μg/mL. Briefly, acetate buffer (300 mM, pH 3.6), 2,4,6-tripyridyl-s-triazine (TPTZ) 10 mM in 40 mM HCl, and $FeCl_3{\cdot}6H_2O$ (20 mM) were mixed in the ratio of 10 : 1 : 1 to obtain the working FRAP reagent. The reaction mixture was incubated at 37 °C for 10 min. Plant extracts (50 μL) was mixed with 150 μL of working FRAP solution for 8 min at room temperature. The absorbance of colored product, Ferrous-TPTZ was measured at the wavelength of 593 nm. FRAP activity were determined by a standard curve prepared with trolox ($y = 0.009x + 0.340$; $r^2 = 0.997$Y). The FRAP values of the extracts were expressed as mg/g Trolox equivalent (TE)/g of dry extract.

### 2.5. Oxygen radical absorbance capacity (ORAC) assay

The method of Ou et al. (2001) was modified as follows: The reaction was carried out in 75 mM phosphate buffer (pH 7.4), and the final reaction mixture was 200 μL. Plant extracts (10 μL, varying from 1 to 50 μg/mL) and fluorescein (100 μL; 1.6 μg/mL) solutions were added and mixture was pre incubated for 5 min at 37 °C. Fifty micro liters of AAPH solution was added immediately and decaying of fluorescence was scanned for 35 min at 1 min interval at 37 °C (Ex 494 nm, Em 535 nm). The net area under the curve (AUC) corresponding to a sample was calculated by subtracting the AUC corresponding to the blank. ORAC values were expressed as trolox equivalents by using the standard curve prepared with trolox ($r^2 = 0.996$: $y = 0.349x + 0.845$) The ORAC values of the extracts were expressed as mg/g Trolox equivalent (TE) of dry extract.

### 2.6. Determination of total polyphenolic content

The Total Phenolic Content of plant extracts were determined by using Folin-Ciocalteu reagent following a slightly modified method described by Singleton et al. (1999). A volume of 20 μL of the plant extract (100 μg/mL) was mixed with 110 μL of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and was neutralized with 70 μL of sodium carbonate solution (10%, w/v). The reaction mixture was incubated at room temperature for 30 min. The absorbance of the resulting blue color was measured at the wave length of 765 nm against the sample blank (water). The standard curve of gallic acid, $y = 0.079x + 0.203$($r^2 = 0.986$) was used to determine TPC and expressed as gallic acid equivalent per gram of extract (GAE/g extract).

### 2.7. Total flavonoid content

Total Flavonoid content was determined by the modified $AlCl_3$ method described by Gursoy et al. (2009). Plant extracts dissolved in distilled water (100 μL, 50–500 μg/mL) were separately mixed with 2% $AlCl_3$ in methanol (100 μL). After 10 min incubation at room temperature the absorbance of the reaction mixture was measured at the wavelength of 415 nm against the sample blank (methanol). The standard curve of quercetin, $y = 0.045x + 0.028$ ($r^2 = 0.996$) was used to determine the TFC expressed as mg quercetin equivalent per gram (mg QE/g extract) of extract.

### 2.8. Tyrosinase inhibition assay

Tyrosinase inhibitory assay was performed according to the method previously described by Chiari et al. (2010), using L-DOPA as the substrate. Plant extracts (20 μL, varying from 10 to 500 μg/mL), mushroom tyrosinase aqueous solution (10 μL, 50 units/mL), and phosphate buffer (pH 6.8, 80 μL) were mixed and pre-incubated at 37 °C for 5 min. Then L-DOPA (90 μL, 2 mg/mL) was added. The mixture was then incubated for 20 min at 37 °C. The amount of dopachrome was measured at the wavelength of 475 nm. Kojic acid in dimethylsulfoxide was used as positive control. Phosphate buffer was used as the blank. The percent inhibition of tyrosinase enzyme was calculated using the equation:

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

Where, $A_{(\text{sample})}$ is the absorbance of the sample extracts and $A_{(\text{control})}$ is the absorbance of the assay using the buffer instead of inhibitor (sample).

$IC_{50}$ values were obtained for plants, which showed more than 50% inhibition at 500 μg/mL from the dose-effect curves.

### 2.9. Hyaluronidase inhibition assay

Hyaluronidase inhibitory activity was measured as previously described by Sahasrabudhe and Dedhar (2010) with few modifications. Ten μl of type-1-S bovine testes hyaluronidase (4200 units/mL) dissolved in 0.1 M acetate buffer (pH 3.5) was mixed with 50 μL of plant extract (500 μg/mL) dissolved in 5% dimethylsulfoxide, and incubated in a water bath at 37 °C for 20 min. Twenty μL of 12.5 mM calcium chloride was added to the reaction mixture, and then the mixture was incubated at 37 °C for 10 min. This $Ca^{2+}$ activated hyaluronidase was treated with 50 μL of sodium hyaluronate (12 mg/mL) dissolved in 0.1 M acetate buffer (pH 3.5), and then incubated in a water bath at 37 °C for 40 min. Ten μL of 0.9 M sodium hydroxide and 20 μL of 0.2 M sodium borate were added to the reaction mixture, and then incubated in a boiling water bath for 3 min. After cooling to room temperature, 50 μL of ρ-dimethylaminobenzaldehyde solution (0.25 g of *p*-dimethylaminobenzaldehyde dissolved in 21.88 mL of 100% acetic acid and 3.12 mL of 10N hydrochloric acid) was added to the reaction mixture which was then incubated in a water bath at 37 °C for 10 min. The control group was treated with 50 μL of 5% dimethyl sulfoxide instead of the plant extract. Absorbance was measured at the wave length of 585 nm. The percentage enzyme inhibition was calculated using the following equation:

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

Where, $A_{(\text{sample})}$ is the absorbance of the sample extracts and $A_{(\text{control})}$ is the absorbance of the assay using the buffer instead of inhibitor (sample). Tannic acid is used as a reference standard.

### 2.10. Elastase inhibition assay

Elastase inhibitory activity of plant extract was evaluated *in vitro*

using Porcine pancreatic elastase (PPE) enzyme inhibitory assay as described by Cannel et al. (1988) with some modifications. This assay was performed in 0.2 M Tris-HCL buffer (pH 8.0). Porcine pancreatic elastase was dissolved to make a 100 units stock solution in sterile water. The substrate N-Succinyl-Ala-Ala-Ala-*p*-nitroanilide (AAAPVN) was dissolved in buffer. The plant extracts were incubated with the enzyme for 25 min before adding substrate to begin the reaction. The final reaction mixture (250 µL) contained buffer, 10 µg/mL AAAPVN, 0.001 units PPE and 500 µg/mL of plant extract. Maximum Velocities ($V_{max}$) were taken at a wavelength of 410 nm for 25 min at 30 s intervals and percentage inhibitions were calculated using following equation. Quercetin was used as a positive control. Tris-HCl used as the blank.

$$\% \text{ Inhibition} = [(\text{ maxcontrol} - \text{V maxsample})/\text{V maxcontrol}] \times 100$$

Where $V_{max(sample)}$ is the velocity of the sample extracts and $V_{max(control)}$ is the velocity of the assay using the buffer instead of inhibitor (sample).

$IC_{50}$ values were obtained for plants which showed more than 50% inhibition at 500 µg/mL from the dose-effect curves.

### 2.11. Statistical analysis

Data were presented as means ± standard error (SE). All experiments were performed in triplicates (3n) using 96 well microplate readers (Spectra Max Plus 384, Molecular Devices, USA and Spectra Max-Gemini EM, Molecular Devices Inc, USA). The differences between the different extracts were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference post hoc test with $p = 0.05$. The statistical analysis was performed using SPSS v. 19.0 program.

## 3. Results and discussion

New and emerging trends of natural cosmetic products encourage researchers to find new cosmeceuticals. According to Persistence Market Research and Grand View Research, current global natural and organic personal care products market is valued about US$ 11 billion and expected to reach US$ 22 billion by 2022. Hence, Plant extracts have an increase global demand that can be used for de-pigmenting, anti-wrinkle, and other cosmeceutical purposes because of low incidence of side effects and compatible with all skin types (Ashawat et al., 2008). In the current investigation tyrosinase, elastase, hyaluronidase inhibitory and antioxidant activities of Sri Lankan medicinal plants were evaluated for their use in skin whitening, aging and skin health.

### 3.1. Antioxidant capacities of selected medicinal plants

DPPH free radical scavenging activities of different medicinal plants are depicted in Tables 2 and 3. The effective concentration to reduce the DPPH radical to 50% ($IC_{50}$) was determined by plotting linear regression curve of DPPH activity versus ratio of sample concentration to DPPH as previously reported (Marxen et al., 2007). The extracts of all the tested medicinal plant materials possessed DPPH free radical scavenging properties, but to varying degrees, ranging from $IC_{50}$ 3.31 ± 0.05 to 1348.68 ± 77.70 µg/mL. The highest scavenging activity was showed by ethanolic extract of *E. serratus* bark ($IC_{50}$ 3.31 ± 0.05 µg/mL) followed by *C. sinensis* ($IC_{50}$ 9.96 ± 0.12 µg/mL) and *E. serratus* leaves ($IC_{50}$ 15.81 ± 1.91 µg/mL) in comparison with the positive control trolox ($IC_{50}$ 5.29 ± 0.04 µg/mL). Ethanolic extracts of *A. nobilis* bark and leaves exhibited DPPH radical scavenging activity ($IC_{50}$ 30.52 ± 1.16, 30.76 ± 0.57 µg/mL) comparable with that of *A. heterophyllus* and *A. altilis* bark and leaves, but *A. nobilis, A. heterophyllus,* and *A. altilis* fruits investigated in the present study was found to be lower than that of leaves and bark of *Artocarpus* species (Table 3). The ethanolic extracts, of *M. ferrea* leaves ($IC_{50}$ 62.69 ± 0.41 µg/mL) showed good scavenging activity as compared with those of petals and stamens ($IC_{50}$ 146.96 ± 6.47, 90.23 ± 2.11 µg/mL).

The rest of the medicinal plant ethanolic extracts, exhibited good scavenging activity but lower than the positive control trolox. It has well established that free radical scavenging activity of plant extracts is mainly due to phenolic compounds.

The ability of the tested plant extracts to deviate the mechanism of Fenton reaction by chelating the metal ions, such as $Fe^{2+}$ and $Cu^{2+}$, which is responsible to convert the hydrogen peroxide to hydroxyl radical on the skin, can be measured using FRAP assay (Benzie and Szeto, 1991). *Eleocarpus serratus* bark showed the highest FRAP value of 7155.55 ± 25.66 mg TE/g of extract and significantly higher than *E. serratus* leaves and fruit. *Nymphaea nouchali* exhibited FRAP value of 4057.77 ± 130.63 mg TE/g of extract followed by *C. sinensis, K. zeylanica. Artocarpus nobilis,* and *A. heterophyllus* leaves exhibited similar FRAP activity but it was less than the *A. nobilis* bark and higher than the *A. heterophyllus* bark. *Artocarpus heterophyllus* fruit showed the lowest FRAP activity which was 6.67 ± 0.74 mg TE/g of extract.

Oxygen Radical Absorbance Capacity of various plant extracts is given in Table 2. The ORAC values were determined as Trolox equivalents (TE). Among tested medicinal plants, extracts of *E. serratus* bark and *C. sinensis* leaves exhibited the highest ORAC values (1771 ± 47 and 1676 ± 39 mg TE/g of extract) followed by *C. fenestratum* bark, *C. aromatica* rhizomes, *R. cordifolia* roots, and *N. nouchali* petals (1628 ± 145, 1532 ± 95, 1501 ± 63, and 1469 ± 32 mg TE/g of extract), respectively. Among the tested *Artocarpus* species leaves showed significant ORAC values compared with those of barks and fruit (Table 3).

In the present study ethanolic extracts of *E. serratus* bark, leaves, and C. *sinensis* leaves showed striking DPPH free radical scavenging, FRAP and ORAC activities. These high antioxidant capacities are mainly due to the catechins and other polyphenols such as bisfiavanols, theaflavins, and thearubigens found in *C. sinensis* leaves (Dufresne and Farnworth, 2001). Several flavonoid compounds, myricitrin, mearnsetin, 3-*O*-β-D-glucopyranoside, and mearnsitrin have been isolated from *E. serratus* leaves may be responsible for the DPPH free radical scavenging, FRAP and ORAC activities detected in this study (Jayasinghe et al., 2012).

### 3.2. Total phenolic and flavonoid content of selected medicinal plants

Phenolic compounds are known to be effective antioxidants. They act as radical scavengers, metal chelators and the largest groups of tyrosinase inhibitors to date. Several polyphenols act as alternative substrates to tyrosinase, and they could be mistakenly classified as tyrosinase inhibitors (Lin et al., 2008).

The present study identified the TPC in plant extracts of selected medicinal plants using the Folin–Ciocalteu method. The phenolic content of plant extracts varied from 255.35 ± 0.90 mg GAE/g of extract to 5.91 ± 1.74 mg GAE/g of extract, which represents a variation of about 40 fold (Table 2 and 3). Bark extract of *A. nobilis* showed the highest phenolic content (255.35 ± 0.90 mg GAE/g of extract) followed by *M. ferrea* leaves (215.46 ± 2.92 mg GAE/g), *E. serratus* bark (175.78 ± 8.89 mg GAE/g of extract) and *N. nouchali* (113.50 ± 0.29 mg GAE/g of extract). The lowest TPC was exhibited by *A. nobilis* fruit (5.91 ± 1.74 mg GAE/g of extract). However, *A. heterophyllus* bark (20.21 ± 2.55 mg GAE/g of extract), leaf (22.95 ± 5.20 mg GAE/g of extract), and fruit (21.65 ± 1.32 mg GAE/g of extract) exhibited statistically similar ($p < 0.05$) TPC values.

Flavonoids are among the most numerous and best-studied polyphenols, that is, benzo-γ-pyrone derivatives consisting of phenolic and pyrene rings. Different classes of flavonoid are distinguished by additional oxygen-heterocyclic rings, by positional differences of the B ring, and by hydroxyl, methyl, isoprenoid, and methoxy groups distributed in

**Table 2**
Antioxidant capacities and Total Phenol and Flavonoid Contents of the medicinal plants.

| Name of the Plant | DPPH $IC_{50}$ (μg/ml) | FRAP (mg TE/g extract)[1] | TPC (mg GAE/g extract)[2] | ORAC (mg TE/g extract)[1] | TFC (mg QE/g extract)[3] |
|---|---|---|---|---|---|
| *Rubia cordifolia* (R) | 84.70 ± 2.06$^{ah}$ | 953.3 ± 6.8$^{a}$ | 61.47 ± 2.23$^{af}$ | 1501 ± 63$^{af}$ | 9.07 ± 0.39$^{a}$ |
| *Saussurea lappa* (R) | 176.7 ± 4.8$^{b}$ | 288.9 ± 65.9$^{a}$ | 20.25 ± 4.44$^{b}$ | 453.6 ± 159.5$^{bcd}$ | 2.09 ± 0.02$^{b}$ |
| *Curucumaa aromatica* (Rh) | 50.90 ± 1.12$^{c}$ | 600.6 ± 6.8$^{a}$ | 76.28 ± 4.69fg | 1532 ± 95$^{af}$ | 11.8 ± 1.11$^{c}$ |
| *Kokoona zeylanica* (B) | 43.58 ± 4.50$^{c}$ | 1137 ± 17$^{ab}$ | 21.98 ± 2.27c | 737.1 ± 31.8$^{cd}$ | 20.4 ± 0.6$^{d}$ |
| *Vetiveria zizanioides* (R) | 147.4 ± 5.6$^{d}$ | 375.1 ± 10.4$^{a}$ | 12.91 ± 1.01$^{bc}$ | 472.8 ± 161.1$^{bcd}$ | 4.51 ± 0.07$^{ef}$ |
| *Coscinium fenestratum* (B) | 216.4 ± 3.83$^{e}$ | 244.4 ± 29.2$^{a}$ | 48.48 ± 1.20ae | 1628 ± 145$^{af}$ | 17.9 ± 0.2$^{g}$ |
| *Elaeocarpus serratus* (L) | 15.81 ± 1.91$^{f}$ | 3373 ± 123$^{c}$ | 64.72 ± 1.92af | 721.1 ± 38.9$^{bcd}$ | 12.1 ± 0.3$^{c}$ |
| *Elaeocarpus serratus* (B) | 3.31 ± 0.05$^{f}$ | 7155 ± 25$^{d}$ | 175.9 ± 8.9j | 1771 ± 47$^{f}$ | 4.46 ± 0.26e$^{f}$ |
| *Elaeocarpus serratus* (F) | 68.93 ± 2.80$^{h}$ | 755.5 ± 33.5$^{a}$ | 8.75 ± 0.36$^{bc}$ | 482.3 ± 55.1$^{bc}$ | 5.29 ± 0.12$^{fi}$ |
| *Elaeocarpus serratus* (S) | 77.30 ± 2.51$^{h}$ | 397.2 ± 24.9$^{a}$ | 8.60 ± 1.32$^{bc}$ | 816.6 ± 38.9$^{d}$ | 2.73 ± 0.14$^{be}$ |
| *Nymphaea nouchali* (P) | 31.27 ± 1.56$^{c}$ | 4057 ± 130$^{c}$ | 113.5 ± 0.3$^{i}$ | 1469 ± 32$^{af}$ | 1.58 ± 0.10$^{b}$ |
| *Nymphaea pubescens* (P) | 57.91 ± 0.82$^{i}$ | 828.1 ± 69.4$^{a}$ | 39.11 ± 3.56$^{e}$ | 1341 ± 165$^{a}$ | 8.50 ± 0.18$^{a}$ |
| *Camellia sinensis* (L) | 9.96 ± 0.12$^{f}$ | 2161 ± 62$^{b}$ | 93.45 ± 5.75$^{hi}$ | 1676 ± 39$^{f}$ | 15.2 ± 0.3$^{h}$ |
| *Mesua ferrea* (L) | 62.69 ± 0.41$^{i}$ | 7162 ± 702$^{d}$ | 215.5 ± 2.9$^{k}$ | 103.5 ± 11.5$^{e}$ | 16.6 ± 0.1$^{gh}$ |
| *Mesua ferrea* (P) | 146.9 ± 6.4$^{d}$ | 308.1 ± 14.8$^{a}$ | 92.66 ± 2.92$^{h}$ | 122.6 ± 22.3$^{e}$ | 7.11 ± 0.10$^{ai}$ |
| *Mesua ferrea* (St) | 90.23 ± 2.11$^{a}$ | 321.5 ± 7.3$^{a}$ | 93.45 ± 2.92$^{h}$ | 116.2 ± 15.9$^{e}$ | 11.6 ± 0.4$^{c}$ |
| *Azadirachta indica* (L) | 532.5 ± 28.9$^{j}$ | 161.1 ± 25.1$^{a}$ | 52.45 ± 4.45a$^{e}$ | 475.9 ± 37.7$^{b}$ | 10.7 ± 0.1 $^{c}$ |
| Trolox | 5.29 ± 0.04$^{f}$ | – | – | – | – |

(L)- Leaf, B-bark, (S)- seed, (P)-Petals, (St)- Stamens, (R)- Roots, (Rh)- Rhizomes, (F)-Fruit. DPPH: 2,2-diphenyl-2-picryl-hydrazyl free radical scavenging activity, FRAP: Ferric reducing antioxidant power, ORAC: Oxygen radical absorbance capacity, TPC: Total Phenolic Content, TFC: Total Flavonoid Content. Data are given as mean ± SD ($n$ = 3). Means followed by the same letter are not significantly different at $p < 0.05$.

[1] GAE- trolox-equivalent.

[2] TE- gallic acid equivalent.

[3] QE- quercetin equivalent.

different patterns about the rings. The structure of flavonoid is also in principle compatible with the roles of both substrates and (presumably competitive) inhibitors of tyrosinase (Chang 2009).

The highest flavonoid content was found in the bark extract of *K. zeylanica* (20.36 ± 0.64 mg QE/g extract). *Artocarpus nobilis* bark (5.69 ± 0.40 mg QE/g extract) and *A. nobilis* leaves (5.45 ± 0.22 mg QE/g extract) exhibited statistically similar TFC values, but lower compared with *A. nobilis* fruit extract (10.47 ± 0.01 mg QE/g extract). Bark extracts of *A. heterophyllus* and *A. altilis* showed lowest flavonoid content (Table 3). Previous research studies have reported isolation of many flavonoid compounds from *Artocarpus* species such as flavanones, flavan-3-ol, prenyl flavon, pyranoflavone, and oxepinoflavone (Nomura et al., 1998; Wang et al., 2004). Several phenolic compounds have also been isolated from *M. ferrea, K zeylanica, C. sinensis,* and *R. cordifolia* in previous research studies (Verotta et al., 2004; Gamlath and Gunatilaka, 1988; Lin et al., 2003; Cai et al., 2004). This further confirms the presence of flavonoids and polyphenols in the plant extracts studied in this study.

### 3.3. Tyrosinase inhibitory activity of selected medicinal plants

The inhibitory effects of 15 medicinal plants on tyrosinase activity were investigated. Among tested 15 medicinal plants 8 plants (*R. cordifolia, E. serratus, N. nouchali, C. sinensis, V. zizanioides, A. heterophyllus. A. nobilis,* and *A. altilis)* showed tyrosinase inhibitory activity at 500 μg/mL (Table 4). In this assay, *E. serratus* bark extract showed 50.87% inhibition at the concentration of 500 μg/mL. *Artocarpus nobilis, A. altilis,* and *A. heretophyllus* bark extracts exhibited 100% inhibition of tyrosinase at a final concentration of 500 μg/mL. $IC_{50}$ values were calculated for *Artocarpus* extracts, which showed high inhibitory activities against tyrosinase in the initial screening. Except ethanolic extracts of *Artocarpus* leaves other extracts showed dose dependent activity. *Artocarpus altilis* bark extract exhibited the highest tyrosinase inhibitory activity ($IC_{50}$ 27.47 ± 0.45 μg/mL) followed by *A. nobilis* bark extract ($IC_{50}$ 53.23 ± 2.65 μg/mL) and it was better than the positive standards kojic acid ($IC_{50}$ 76.61 ± 0.80 μg/mL) (Table 5). Although the *A. nobilis* and *A. heterophyllus* fruit extracts showed less tyrosinase inhibitory activity having $IC_{50}$ values of 2124.69 ± 106.35 and 1196.82 ± 138.03 μg/mL respectively, *A. altilis* fruit extract exhibited good tyrosinase inhibitory activity 173.85 ± 3.85 μg/mL

**Table 3**
Antioxidant capacities and Total Phenol and Flavonoid contents of *Artocarpus nobilis, Artocarpus heterophyllus, Artocarpus altilis.*

| Name of the Plant | DPPH $IC_{50}$ (μg/mL) | FRAP (mg TE/g of extract)[1] | TPC (mg GAE/g of extract)[2] | ORAC (mg TE/g extract)[1] | TFC (mg QE/g extract)[3] |
|---|---|---|---|---|---|
| *Artocarpus nobilis* (B) | 30.52 ± 1.16$^{a}$ | 1664 ± 87$^{a}$ | 255.4 ± 0.9$^{a}$ | 833.5 ± 55.1$^{a}$ | 5.69 ± 0.40$^{a}$ |
| *Artocarpus nobilis* (L) | 30.76 ± 0.57$^{a}$ | 772.2 ± 33.1$^{b}$ | 30.45 ± 2.55$^{d}$ | 1201 ± 222$^{b}$ | 5.45 ± 0.22$^{a}$ |
| *Artocarpus nobilis* (F) | 1340 ± 25$^{c}$ | 95.93 ± 1.33$^{c}$ | 5.91 ± 1.74$^{b}$ | 195.1 ± 5.5d$^{e}$ | 10.47 ± 0.01$^{b}$ |
| *Artocarpus altilis* (B) | 476.3 ± 8.7$^{d}$ | 97.41 ± 10.27$^{c}$ | 15.66 ± 1.26$^{bc}$ | 342.3 ± 31.8de$^{f}$ | 1.38 ± 0.01$^{c}$ |
| *Artocarpus altilis* (L) | 30.72 ± 0.85$^{a}$ | 234.8 ± 1.6$^{d}$ | 10.11 ± 0.29$^{b}$ | 673.4 ± 33.5$^{c}$ | 3.07 ± 0.37$^{d}$ |
| *Artocarpus altilis* (F) | 1348 ± 77$^{c}$ | 254.8 ± 2.9$^{d}$ | 14.18 ± 2.63$^{bc}$ | 374.1 ± 31.4$^{ef}$ | 4.71 ± 0.05$^{a}$ |
| *Artocarpus heterophyllus* (B) | 46.71 ± 0.38$^{a}$ | 67.78 ± 3.33$^{c}$ | 20.21 ± 2.55$^{cd}$ | 176.7 ± 25.3$^{d}$ | 1.18 ± 0.03$^{c}$ |
| *Artocarpus heterophyllus* (L) | 30.76 ± 0.57$^{a}$ | 778.2 ± 27.4$^{b}$ | 22.95 ± 5.20$^{cd}$ | 418.7 ± 36.7$^{f}$ | 2.78 ± 0.16$^{d}$ |
| *Artocarpus heterophyllus* (F) | 578.7 ± 4.8$^{d}$ | 6.67 ± 0.74$^{e}$ | 21.65 ± 1.32$^{cd}$ | 186.2 ± 11.1$^{de}$ | 6.69 ± 0.15$^{e}$ |
| Trolox | 5.29 ± 0.04$^{b}$ | – | – | – | – |

(L)- Leaf, B-bark, (F)-Fruit. DPPH: 2,2-diphenyl-2-picryl-hydrazyl free radical scavenging activity, FRAP: Ferric reducing antioxidant power, ORAC: Oxygen radical absorbance capacity, TPC: Total Phenolic Content, TFC: Total Flavonoid Content. Data are given as mean ± SD ($n$ = 3). Means followed by the same letter are not significantly different at $p < 0.05$.

[1] GAE- trolox-equivalent.

[2] TE- gallic acid equivalent.

[3] QE- quercetin equivalent.

**Table 4**
Tyrosinase, elastase, hyaluronidase inhibitory activity of the medicinal plants.

| Name of the Plant | Tyrosinase $IC_{50}$ (μg/ml)/inhibition (%)[1] | Elastase $IC_{50}$ (μg/ml)/inhibition (%)[1] | Hyaluronidase Inhibition (%)[1] |
|---|---|---|---|
| *Rubia cordifolia* (R) | 20.94[1] | 17.18[1] | NI |
| *Saussurea lappa* (R) | NI | 21.9[1] | NI |
| *Curucumaa aromatica* (Rh) | NI | 252.7 ± 6.8[a] | 95.02[1] |
| *Kokoona zeylanica* (B) | NI | 20.67[1] | NI |
| *Vetiveria zizanioides* (R) | 29.13[1] | 20.67[1] | NI |
| *Coscinium fenestratum* (B) | NI | 22.85[1] | NI |
| *Elaeocarpus serratus* (L) | 20.29[1] | 20.2[1] | NI |
| *Elaeocarpus serratus* (B) | 50.87[1] | 27.27 ± 2.74[b] | 38.32[1] |
| *Elaeocarpus serratus* (F) | 7.05[1] | 13.32[1] | NI |
| *Elaeocarpus serratus* (S) | NI | 16.43[1] | NI |
| *Nymphaea nouchali* (P) | 30.09[1] | NI | NI |
| *Nymphaea pubescens* (P) | 22.33[1] | NI | NI |
| *Camellia sinensis* (L) | 11.24[1] | 26.8[1] | 74.85[1] |
| *Mesua ferrea* (L) | NI | NI | NI |
| *Mesua ferrea* (P) | NI | NI | NI |
| *Mesua ferrea* (St) | NI | 21.21[1] | 34.82[1] |
| *Azadirachta indica* (L) | NI | NI | 65.64[1] |
| Kojic acid | 76.61 ± 0.80 | – | – |

(L)- Leaf, B-bark, (S)- seed, (P)-Petals, (St)- Stamens, (R)- Roots, (Rh)- Rhizomes, (F)-Fruit. Data are given as mean ± SD ($n$ = 3). Data are given as mean ± SD (n = 3). Means followed by the same letter are not significantly different at $p < 0.05$.
[1] -% inhibition at 500 μg/mL, NI-no inhibition at 500 μg/mL

(Fig. 1).

Flavonoids, stilbenes, triterpenes, and sterols have isolated from several *Artocarpus* species which are known to be strong tyrosinase inhibitors (Lin et al., 1995; Likhitwitayawuid and Sritularak, 2001). Previous studies have shown the presence of tyrosinase inhibitors such as norartocarpetin, artocarpanone, and artocarpesin in *A. heterophyllus* (Zheng et al., 2008). However, there are no reports to confer evaluation of tyrosinase inhibitory activity of *A. nobilis* and comparison of different plant parts of *Artocarpus* species tested in this study.

### 3.4. Elastase and hyaluronidase inhibitory activity of selected medicinal plants

The inhibitory effects of 15 medicinal plants on elastase activity were investigated. Of these 15 plants, *N. nouchali, M. ferra* leaves and petal extracts did not inhibit porcine pancreatic PPE activity. Inhibition of PPE by the other 15 plant extracts in the initial screening is shown in Table 3. In this assay, most of the plant extracts showed about 20% inhibition at the concentration of 500 mg/mL. *Eleocapus seratus* bark and *C. aromatica* rhizome extracts exhibited 100% inhibition of elastase activity at a final concentration of 500 mg/mL. In order to determine $IC_{50}$ values of these two plant extracts showing high biological activities, linear regression curve of elastase inhibitory activity versus ratio of sample concentration to elastase were plotted. *Eleocarpus serratus* had a far greater inhibitory effect on PPE activity, with $IC_{50}$ value of 27.27 ± 2.74 μg/mL and significantly higher than the positive control quecertin ($IC_{50}$ 221.69 ± 5.24 μg/mL) (Fig. 2). *Curcuma aromatica* had an $IC_{50}$ value of 252.72 ± 6.87 μg/mL and similar to quecertin (Table 4).

*Curcuma aromatic*a exhibited best hyaluronidase inhibition activity (95%) which is comparable with positive standard tannic acid (90.25% at 500 μg/mL). Ethanolic extracts of *A. indica, C sinensis, E. serratus* bark, *M. ferrea* stamens showed 65.64%, 74.85%, 38.32%, and 34.82% hyaluronidase inhibitory activity, respectively, at the concentration of 500 μg/mL while other tested plant extracts showed no inhibition at the concentration of 500 μg/mL (Fig. 3). No previous studies are reported on *in vitro* elastase and hyaluronidase activities of these plant extract except *C. sinensis* (Thring et al., 2009). However, in a previous study methanolic extract of *C. longa*, a related species to *C. aromatica* has exhibited elastase inhibitory activity having $IC_{50}$ value of 398.4 mg/mL (Lee et al., 1999). Further it was reported polyphenols present in the plants are known to be strong elastase and hyaluronidase inhibitors (Karim et al., 2014). Therefore these activities could be due to the polyphenols present in this plant species.

The results of this study suggest that tyrosinase inhibitory activity positively correlates with the TFC (r = 0.711) and DPPH free radical scavenging activities (r = 0.891) suggesting that flavonoid compounds present in these plants are responsible for the tyrosinase inhibitory

**Table 5**
Tyrosinase, elastase, hyaluronidase inhibitory activity of *Artocarpus nobilis, Artocarpus heterophyllus, Artocarpus altilis*.

| Name of the Plant | Tyrosinase $IC_{50}$ (μg/ml)/inhibition (%)[1] | Elastase $IC_{50}$ (μg/ml)/inhibition (%)[1] | Hyaluronidase Inhibition (%)[1] |
|---|---|---|---|
| *Artocarpus nobilis* (B) | 53.23 ± 2.65[a] | 30.96[x] | 44.78[x] |
| *Artocarpus nobilis* (L) | 51.53[1] | NI | NI |
| *Artocarpus nobilis* (F) | 2124 ± 106[g] | NI | NI |
| *Artocarpus altilis* (B) | 27.47 ± 0.45[d] | 23.87[1] | 68.59[1] |
| *Artocarpus altilis* (L) | 52.43[1] | NI | NI |
| *Artocarpus altilis* (F) | 173.9 ± 3.9[e] | NI | NI |
| *Artocarpus heterophyllus* (B) | 125.2 ± 6.8[c] | NI | 52.68[1] |
| *Artocarpus heterophyllus* (L) | 45.24[1] | NI | NI |
| *Artocarpus heterophyllus* (F) | 1196 ± 138[f] | NI | NI |
| Kojic acid | 76.61 ± 0.80[b] | NI | – |
| Ascorbic acid | 69.33 ± 2.56[ab] | NI | – |
| Tannic acid | – | – | 90.25[1] |
| Quecertin | – | 221.69 ± 5.52 | – |

(L)- Leaf, B-bark, (F)-Fruit. Data are given as mean ± SD ($n$ = 3). The data marked with the different letters of each sample category share significant differences at $p < 0.05$.
[1] -% inhibition at 500 μg/mL, NI-no inhibition at 500 μg/mL

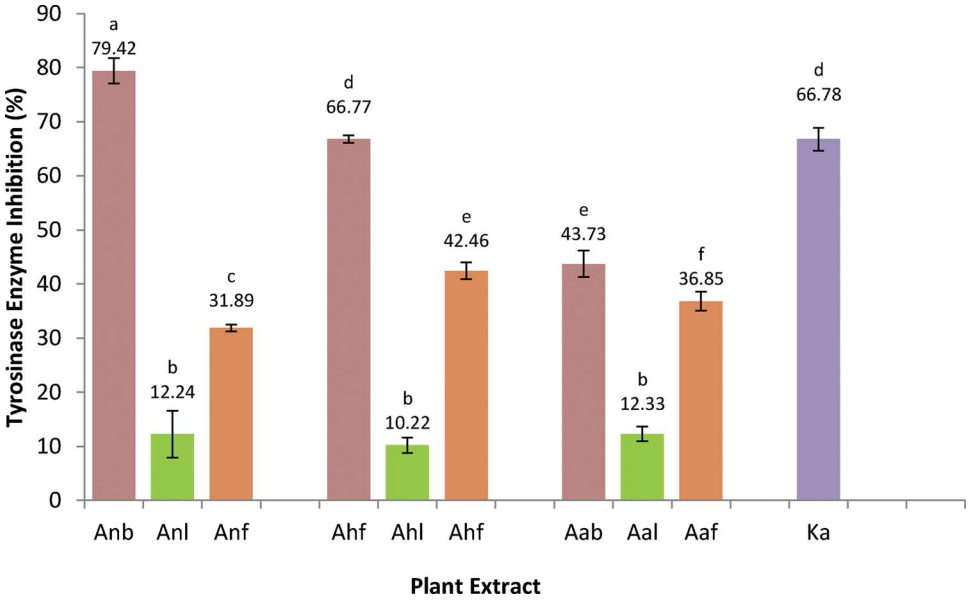


**Fig. 1.** Tyrosinase enzyme inhibitory activity of total ethanol extracts of *Artocarpus* species at 100 µg/mL assay concentration.
Data represented as mean ± SE (n = 3). Means followed by the same letter are not significantly different at $p < 0.05$. Anb: *Artocarpus nobilis* bark, Anl: *Artocarpus nobilis* leaf, Anf: *Artocarpus nobilis* fruit, Ahb: *Artocarpus heterophyllus* bark, Ahl: *Artocarpus heterophyllus* leaf, Ahf: *Artocarpus heterophyllus* fruit, Aab: *Artocarpus altilis* bark, aal:*Artocarpus altilis* leaf, aaf:*Artocarpus altilis* fruit, and Ka: Kojic acid

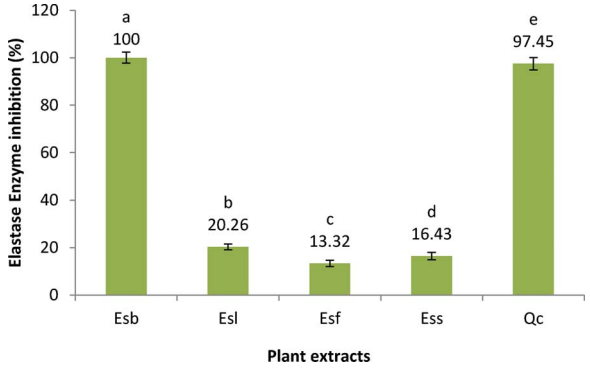


**Fig. 2.** Elastase enzyme inhibitory activity of total ethanol extracts of *Elaeocarpus serratus* at 500 µg/mL assay concentration.
Data represented as mean ± SE (n = 3). Means followed by the same letter are not significantly different at $p < 0.05$. Esb: *Elaeocarpus serratus* bark, Esl: *Elaeocarpus serratus* leaf, Esf: *Elaeocarpus serratus* fruit, Ess: *Elaeocarpus serratus* seed, and Qc: quecertin

activities of the species. Tyrosinase inhibitory activity negatively correlates with the ORAC and FRAP activities of the extracts, suggesting that when the ORAC and FRAP activities are higher, the inhibitory concentration will be lower. There were no significant correlation was found between TPC ($p > 0.05$, $r = -0.3215$), ORAC ($r = -0.3682$), and FRAP activities ($r = -0.3119$). This suggests that other non-phenolic components might contribute to DPPH free radical scavenging activity (Rumbaoa et al., 2009).

## 4. Conclusion

From all these results, it is evident that ethanolic extracts of barks *A. nobilis, A. heterophyllus,* and *A. altilis* are rich sources of tyrosinase inhibitory bio active compounds due to its ability to inhibit tyrosinase enzyme. These extracts are also good sources of hyaluronidase inhibitors and as well as anti-oxidants. *Artocarpus nobilis, A. altilis* showed better tyrosinase inhibitory activity compared to the positive control kojic acid. *Eleocarpus serratus* bark showed highest elastase inhibitory activity and DPPH free radical scavenging activities. Ethanolic extract of *C. aromatica* rhizomes was found to be more active against

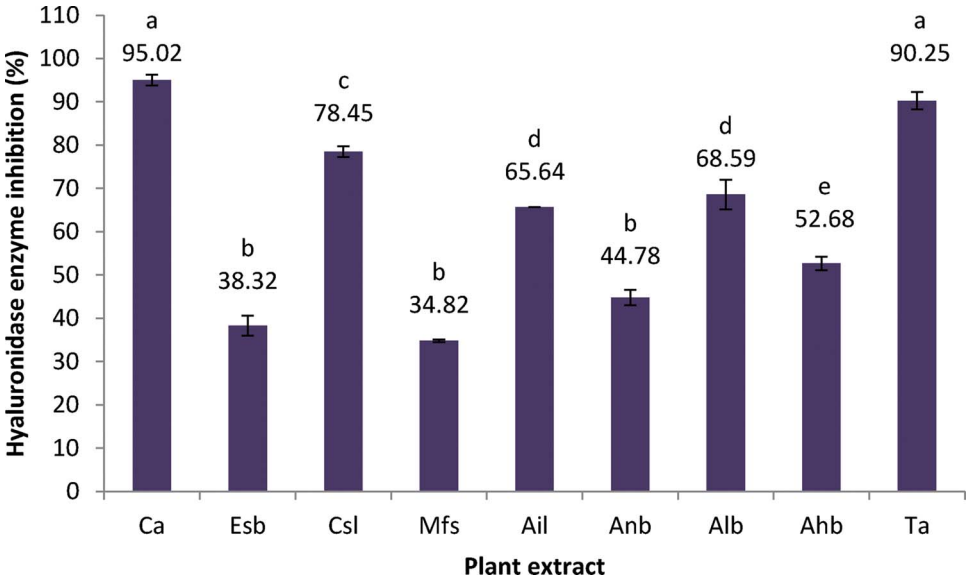


**Fig. 3.** Hyaluronidase enzyme inhibitory activity of total ethanol extracts of medicinal plants at 500 µg/mL assay concentration.
Data represented as mean ± SE (n = 3). Means followed by the same letter are not significantly different at $p < 0.05$. Ca: *Curucumaa aromatica* rhizomes, Esb: *Elaeocarpus serratus* bark, Csl: *Camellia sinensis* leaves, Mfs: *Mesua ferrea* stamens, Ail: *Azadirachta indica* leaves, Anb: *Artocarpus nobilis* bark, Alb: *Artocarpus altilis* bark, Ahb: *Artocarpus heterophyllus* bark, and Ta: Tannic acid

hyaluronidase compared to other tested plant extracts. The bioactivities, exhibited by these plant extracts reflect the use of extracts as active agents in cosmeceuticals which might used to treat various skin disorders such as hyper-pigmentation, to obtain lighter skin complexion, wrinkling, pre-mature aging, and biological aging.

It could be concluded that these findings may provide some guidance for the cosmetic industry to design and synthesize potential multi target cosmeceuticals.

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