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Research Article

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF LEAVES, SKIN, FLESH AND SEEDS OF SRI LANKAN VARIETY OF *CUCURBITA MOSCHATA*

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ABSTRACT

Reactive oxygen species are continuously produced by normal physiological activities of the human body and these cause damage to the cell membrane and biomolecules and thereby resulting chronic diseases. Antioxidants are scavengers of such free radicals and could combat oxidative stress. In spite of the advances in modern medicine, plant based remedies are increasingly sort after due to their lesser side effects. The antioxidants capacity and antimicrobial properties of ethyl acetate, acetone and methanol extracts of seeds, leaves, flesh and skin of the Sri Lankan variety of *Cucerbita moschata* were investigate. The ethyl acetate extracts of leaves gave the highest rate of change of reducing power with concentration (0.1625 ml/mg) in Fe^{3+} reducing power assay and the highest hydroxyl radical activity (72.8±3.8%). The acetone extract of leaves gave the highest rate of change of radical scavenging activity with concentration (7.016 ml/mg) during DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Ethyl acetate extract of leaves also showed the highest phenolic content (42.4±2.4 mg (PGE/g)) and highest flavonoid content (40.2±0.6 mg (QE)/g). All extracts of skin and seeds as well as the methanol extract of leaves and acetone extract of flesh showed antibacterial activity against *Bacillus subtilis*. The three extracts of skin and the acetone extract of leaves showed antibacterial activity against the *Escherichia coli*.

KEYWORDS: Antibacterial activity, Antioxidant capacity, *Bacillus subtilis, Cucerbita moschata, Escherichia coli.*

INTRODUCTION

From the beginning of human life plants have been useful for various kinds of purposes; one of the most obvious ones being the use of plants as food. In addition to many species of edible plants being consumed, plants are used as flavors, dyes, aromatic fragrances, lubricants, and medicines. Use of plants for medicinal purposes can be traced back to ancient times as extracts from various plants have been used by primitives to combat and cure various kinds of diseases.[1] There is a tendency to move towards plant based medicines and dedicate more and more research attention towards natural sources for combating many diseases due to the toxicity, side effects, and developing resistance with regard to synthetic medicines.[2] One example of using plants for betterment of human health is the use of food antioxidants to control damaging effects of reactive oxygen species (ROS) on human health. ROS are radical or non-radical oxygen species, which are continuously produced by the normal physiological activities of the human body. An increase in ROS levels or a decrease in cellular antioxidant capacity due to ROS taking over the cellular antioxidant defense system, could lead to oxidative stress, which is the imbalance due to excess ROS or oxidants over the capability of the cell to mount an effective antioxidant response. If ROS are not effectively scavenged by cells, it can lead to many diseases such as heart disease, stroke, arteriosclerosis diabetes, and cancers.[3] Antioxidants are the main defensive substances against these ROS as they delay or inhibit the oxidation of essential biomolecules such as lipid, proteins, carbohydrates and nucleic acid to inhibit the initiation and propagation step leading to the termination of the reaction and delay the oxidation process. Therefore, natural sources of food antioxidants and the consumption of different compounds which have antioxidant activity are of high interest.[4] Most prominent dietary antioxidants are phenols, flavonoids, vitamin C, and carotenoids.

This study involves the determination of antioxidant capacity and antimicrobial activities of acetone, ethyl acetate and methanol extracts of skin, flesh, seeds and leaves of Sri Lankan variety of

Cucerbita moschata. Cucerbita moschata belongs to the family Cucurbitaceae commonly known as pumpkin, native to Central America and Northern South America.[5] There are many varieties of pumpkins in Sri Lankan market and the Sri Lankan variety of *Cucerbita moschata* (Batana) was used in this study.

MATERIALS AND METHODS

Collection of plant material

Pumpkin *C. moschata* fruits and leaves were collected from Chilaw in Puttalam district, Sri Lanka. The fruits and leaves were identified and authenticated at the Department of Plant sciences, University of Colombo. Then pumpkins were washed thoroughly and peels, seeds and flesh were taken separately. All the samples were air dried for two weeks. Dried plant materials were powdered.

Preparation of plant extracts for antioxidant activity

The extraction procedure was carried out according to the previously published procedure.[6] Ten grams of the ground plant materials were mixed with distilled ethyl acetate (50 ml) and acidified using 2% acetic acid (1 ml) and sonicated for 1 hour. The suspensions were centrifuged at 800 rpm for 10 minutes. The supernents were collected in boiling tubes separately and ethyl acetate (50 ml) was added to the pellet. The sonication and above procedure was repeated twice. Then two supernents were combined and evaporated to obtain the extracts. The similar workup was carried out to the remaining pellet with distilled acetone and distilled methanol respectively.

Reducing power assay

The antioxidants cause the reduction of the potassium ferricyanaide (K_3 (Fe (CN_6)) Fe^{3+} to the corresponding potassium ferrocyanide (K_4 (Fe (CN_6)) Fe^{2+}. Initially the yellow colour of the test solution changes to various shades of green and blue due to the reducing power of antioxidants in plant extracts. Final complex shows Prussian blue colour which has higher absorbance maximum at 700 nm. To each newly prepared plant extract (100 µl) distilled water (1.9 ml) was added. The plant extracts in corresponding solvents were mixed with 2.0 ml phosphate buffer (0.2 M, pH6.6) and 2.0 ml of potassium ferricyanide (10mg, ml). The mixture was kept at 50 ^{0}C in water bath for 20 minutes. After becoming room temperature TCA (2.0 ml) was added and centrifuged 3000 rpm for 10 minutes. From the supernatant 2 ml solution was taken out and mixed with distilled water (2.0 ml) and freshly prepared $FeCl_3$ solution (0.4 ml). Absorbance was measured at 700 nm using a UV/Vis spectrophotometer. Blank was prepared in similar manner using 100 µl of distilled water instead of plant extract.[7]

DPPH radical scavenging assay

The radical scavenging activity (RSA %) was tested using previously published procedure. DPPH radical absorbs at 517 nm and gives purple colour to the solution. Deactivating radical causes decolorize the solution and its absorbance decreases. From each plant extract samples, 100 µl was mixed prepared DPPH solution (3.9 ml). The reaction mixtures were shaken well and absorbance of all samples were measured at 517 nm after 15 to 30 minutes incubation in dark at room temperature Blank was prepared by mixing 100 µl of extracts with methanol (3.9 ml). The control was prepared by mixing DPPH (3.9 ml) with methanol (100 µl) and methanol was taken as the blank.[8]

The capacity of antioxidant to scavenge the DPPH radical can be calculated using the following equation.

Equation 1 - Radical scavenging activity (%)

$$\text{Radical scavenging activity (\%)} = \left(\frac{Ac - As}{Ac}\right) \times 100$$

Ac - The absorbance of the control with contains DPPH

As - The absorbance in the presence of plant extract

Hydroxyl scavenging assay

From each plant extract sample (1 mg/ml), 100 µl was mixed with 500 µl of 2-deoxyribose (2.8 mM) in phosphate buffer (50Mm, pH7.4)), premixed ferric chloride (200 µl) and EDTA solution (1:1 v/v) and 100 µl H_2O_2 solution (200 mM). In order to trigger the reaction , 100 µl Ascorbate (300mM) was added and incubated 1 h at 37˚C. From this mixture 0.5 ml was taken out and added TCA (1 ml) and TBA solution (1ml) to the reaction mixture. The mixture was heated in a water bath for 15 minutes. The absorbance was measured at 532 nm using UV/Vis spectrophotometer when the mixture reached to room temperature. The blank was prepared 100 µl of plant extract with methanol instead Fenton reaction mixture. The control sample was prepared by adding the all reagents except the plant extract and the blank was methanol.[9] The hydroxyl scavenging activity was calculated using the Equation 1.

Total phenolic content

An aliquot (200 µl) of the plant extract was mixed with of 2% sodium bicarbonate (4 ml) and was incubated in darkness at room temperature for 2 minutes. Then of the Folin- Ciocalteu (200 µl) reagent was added and the mixture was incubated in darkness at room temperature for 30 minutes. The absorbance of solution was recorded at 765 nm in a spectrophotometer. Blank was prepared in similar

way using 200 µl of methanol instead of Folin-Ciocalteu reagent (200 µl).[10] This procedure was carried out in triplicates for plant extracts.

The total phenolic content was calculated by the calibration curve which was plotted using pyrogallol solution against concentration.

Total flavonoid content

An aliquot (0.5 ml) of all extracts stock solution and each dilution series of quercetin were added separately to test tubes. Then all extracts were added 5% $NaNO_2$ (150 µl). After 5 minutes incubation, 10% $AlCl_3$ (150 µl) was added to the mixture. After 6 minutes 1 M NaOH (1 ml) was added to the mixture, followed by addition of distilled water (1.2 ml). Sample blank for all plant extracts and all concentration of quercetin were prepared in a similar manner by replacing $AlCl_3$ solution with distilled water. The absorbance of all the solutions was measured at 510 nm against appropriate blank.[11] The total flavonoid content was calculated by the calibration curve which was plotted using quercetin solution against concentration.

Antibacterial activity

All plant extracts were tested against pre identified three bacterial strains which were obtained from the pharmacy laboratory of the Faculty of Science, University of Colombo, Sri Lanka. All selected strains were grown in prepared LB (Lysogeny Broth) agar plates and incubated overnight at 37^0C.[12] The bacterial cell suspension was adjusted to the same appearance of 0.5 McFarland standards. A volume of 200 µl of bacterial culture was spread out on LB agar petri plates and it was incubated at 37 °C for 30 minutes. Sterilized filter paper discs with a diameter of 6 mm were separately loaded with 40 µl (4 mg/disc) of each test sample. Sample loaded discs were allowed to dry at room temperature and these dried discs were placed on the bacterial spread plates. A volume of 10 µl (10 µg/disc) of the Ciprofloxacin stock solution (1 mg/ml concentration) as the positive control and a volume of 10 µl of the methanol as the negative control were separately introduced to sterilized filter paper discs. They were also placed on the bacterial spread plates. The plates were inverted and incubated at 37°C overnight and the average diameter of the resultant inhibition zones were measured.[13]

RESULTS AND DISCUSSION

In the reducing power assay there is a direct relationship between the absorbance values and the reducing ability. Therefore, each gradient of Fig.1 has a direct relationship with the reducing power of each plant extract in different solvents. That means if the gradient is higher the reducing ability is also high and *vice versa*. For easy analysis gradient values of each line in the Fig.1 are displayed in Fig.2. According to Fig.2 ethyl acetate extract of leaves gives the highest slope. This means that the ethyl acetate extract of leaves has the highest reducing ability among all the extracts.

In DPPH radical scavenging assay the scavenging activity varies with concentration. The gradients of each line in the Fig.3 are displayed in Fig.4 for easy analysis. According to Fig.4 the acetone extract of leaves gave the highest rate of change of radical scavenging activity with concentration (7.016 ml/mg) during DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay.

The results of hydroxyl radical scavenging activity of different extracts of the plant parts of *C. moschata* are given in Table 1.

Table 1:Hydroxyl radical scavenging activity (%) of plant extracts

Solvent	Fraction	Hydroxyl radical scavenging activity (%)
		Mean ±SD
Acetone	Skin	57.6 ± 0.8
	Flesh	50.1 ± 0.5
	Seeds	27.0 ± 0.3
	Leaves	3.0 ± 1.9
Ethyl acetate	Skin	40.4 ± 1.2
	Flesh	57.1 ± 1
	Seeds	15.4 ± 8.1
	Leaves	72.8 ± 3.8
Methanol	Skin	19.6 ± 3.6
	Flesh	49.2 ± 0.8
	Seeds	15.1 ± 7.1
	Leaves	66.3 ± 0.6

The ethyl acetate extract of leaves has the highest hydroxyl scavenging activity.

Total phenolic content of different extracts of the plant parts of *C. moschata* are given in Table 2.

Table 2:Total phenolic content of each plant extract

Solvent	Fraction	mg(PEG)/g
		Mean ±SD
Acetone	Skin	14.2 ± 0.9
	Flesh	21.1 ± 8.8
	Seeds	38.2 ± 0.9
	Leaves	11.3 ± 0.7
Ethyl acetate	Skin	20.7 ± 0.3
	Flesh	10.4 ± 1.4
	Seeds	14.2 ± 0.2
	Leaves	42.4 ± 2.4
Methanol	Skin	33.4 ± 1.1
	Flesh	10.5 ± 0.2
	Seeds	42 ± 0.7
	Leaves	33.2 ± 1.1

The ethyl acetate extract of leaves was observed with the highest total phenolic content.

Total flavonoid content of different extracts of the plant parts of *C. moschata* are given in Table 3.

Table 3:Total flavonoid content of each plant extract

Solvent	Fraction	mg(QE)/g
		Mean ±SD
Acetone	Skin	11 ± 1.2
	Flesh	22.7 ± 6.7
	Seeds	27.1 ± 0.8
	Leaves	21.2 ± 0.5
Ethyl acetate	Skin	20.4 ± 10.8
	Flesh	29 ± 1.8
	Seeds	39.8 ± 2.5
	Leaves	40.2 ± 0.6
Methanol	Skin	12.6 ± 0.6
	Flesh	16.5 ± 0.6
	Seeds	17 ± 4.5
	Leaves	25.0 ± 1

Hereto the highest flavonoid content is resulted in ethyl acetate extract of leaves.

Table 4:Average diameter of inhibition zones for different plant extracts in the agar well anti-bacterial assay

Fraction	Solvant	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*
Skin	Ethyl acetate	11.7 ± 0.9	-	18 ± 1.1
	Acetone	11 ± 0.0	-	17.3 ± 1.9
	Methanol	11 ±0.0	18.9 ± 2.8	23.7 ± 4.7
Seed	Ethyl acetate	12 ± 1.2	-	-
	Acetone	11.6 ±2.2	-	-
	Methanol	11 ± 1.4	-	-
Leaves	Ethyl acetate	-	-	-
	Acetone	-	-	15.4 ± 1.6
	Methanol	15.5 ± 0.7	11.1 ± 0.2	-
flesh	Ethyl acetate	-	-	-
	Acetone	12.3 ± 2.2	-	-
	Methanol	-	-	-

All three solvents extracts of skin and seeds as well as methanol extract leaves and acetone extract of flesh showed Inhibitory activity against *B. subtilis*. Methanol extracts of skin and leaves showed Significant inhibition activity against *S. aureus*. The three solvent extracts of skin and acetone extract of leaves showed inhibitory activity against the *E.coli*. The highest inhibition zone (23.7±4.7) found against

E.coli was observed from the methanolic extract of skin.

DISCUSSION

The main objective of this study was to investigate the antioxidant and antibacterial activity of the plant parts of the Srilankan verity of *C.moschata*. In reducing power assay, the reduction of Fe^{3+} to Fe^{2+} is due to the presence of antioxidants in the sample. The yellow colour of the test sample changes to blue depending on the amount of antioxidants and increase in absorbance at 700 nm indicates an increase in reductive ability. Free radicals donate electron to Fe^{3+} and become more stable products by terminating the free radical initiated chain reaction.[9] The reducing capacity of compounds can be considered as a measure of the antioxidant power of the sample extract. In this study, ethyl acetate extract of leaves showed highest reducing potential when compared to other solvent extracts of *C. moschata.*

The electron donation ability of plant extracts is measured in DPPH assay. The DPPH radical donates electrons and hence the purple coloured DPPH solution change to yellow coloured compound diphenylpicryl hydrazine. The acetone extract of leaves gave the highest rate of change of radical scavenging activity with concentration (7.016 ml/mg) during DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay.[9]

Hydroxyl radicals are highly reactive oxygen species which target the cell membrane phospholipids and damage the cell. These hydroxyl radicals are capable of damaging bio molecules. In this method hydroxyl radicals were generated by the reaction of H_2O_2 and the ferrous. These hydroxyl radicals reacted with 2-deoxyribose and formed a malonaldehyde which gave the red colour with TBA.[9] The low intensity of red colour depicted the highest hydroxyl scavenging capacity of the plant extract. Ethyl acetate extract of leaves showed the highest hydroxyl scavenging capacity and prevented the degradation of 2-deoxyribose with low intensity of red colour.

Phenolic compounds are secondary metabolites and they are very important as free radical scavengers because their hydroxyl groups confer scavenging ability.[14] In this study total phenolic content of the different solvents extraction of seeds, leaves, flesh and skin of *C. moschata* was determined by Folin-Ciocalteu method. Phenolic compounds of plant extracts reduce the oxidizing agent phosphomolybdatein Folin-Ciocalteu reagent under alkaline condition.[9] A blue colour complex is formed with a λ_{max}650 nm. Flavonoids and flavonoids derivatives show highly scavenging activity towards the free radicals. Total flavonoid content was quantified by the complexation with $AlCl_3$. In this study ethyl acetate extract of leaves showed the highest value of the total phenolic content and total flavonoids content. The results of this study suggested that there is a significant correlation between antioxidant capacity and the contents of phenolics and flavonoids of plant extracts.

CONCLUSION

Dietary antioxidants play an important role in preventing of many diseases in which radicals are implicated including the combating of aging. These results highlighted that the leaves of *C. moschata* has prominent antioxidant capacity and may be used in preventing radical initiated diseases. Furthermore results show that non-utilized parts of *C. moschata* such as skin, seeds and leaves have considerable antibacterial activity against *B. subtilis, S.aureus* and *E.coli.*

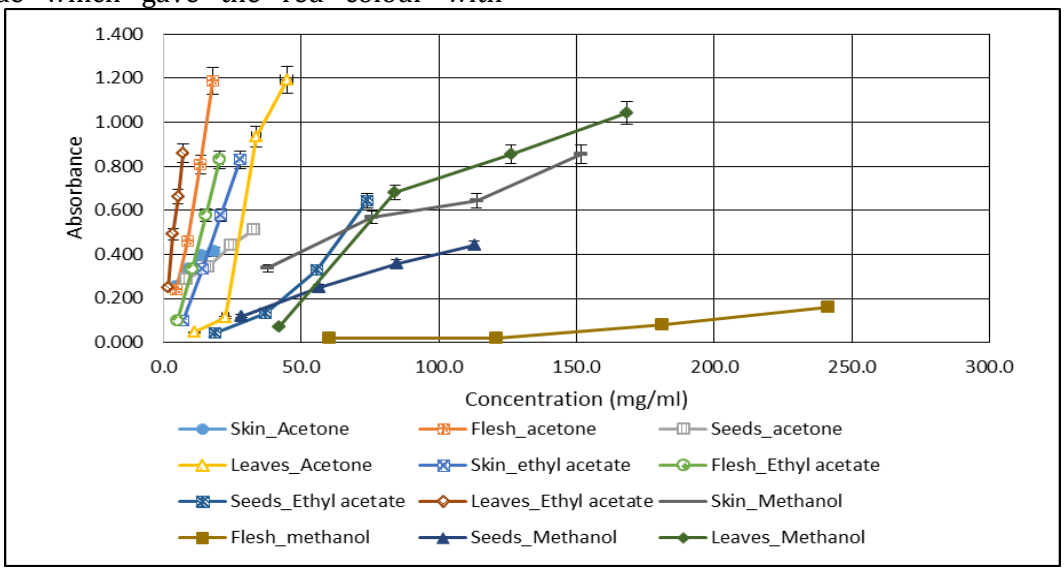

Figure 1: Absorbance *vs.* concentration for different plant extracts in different solvents

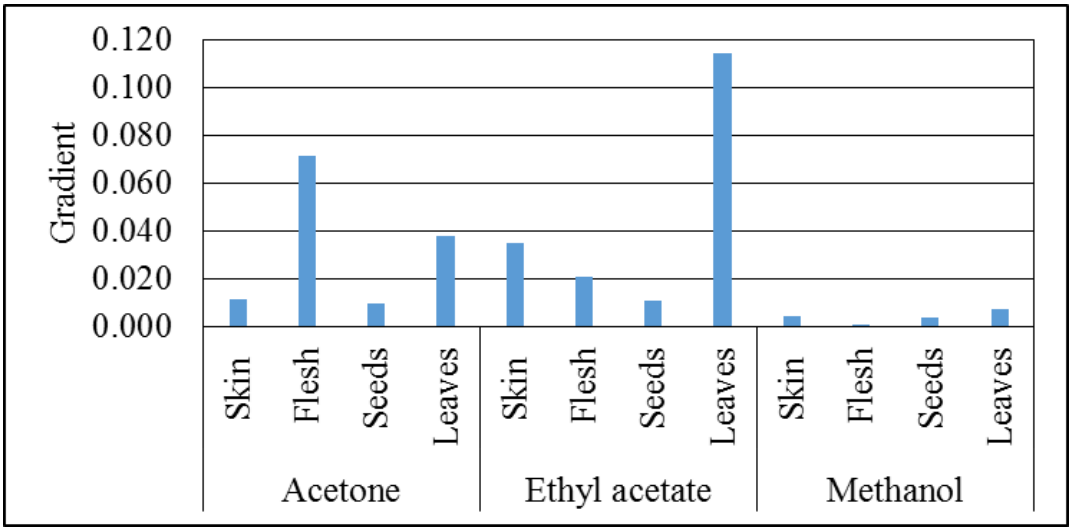

Figure 2: Slopes of lines in Figure 1 – Representing rate of change of reducing power with concentration

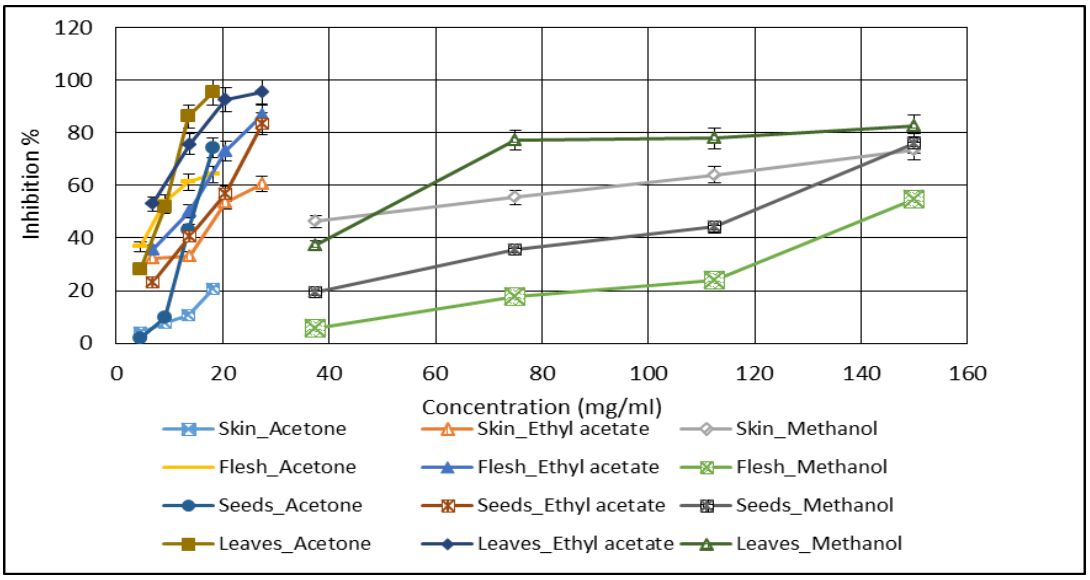

Figure 3: Rate of change of radical scavenging activity with concentration

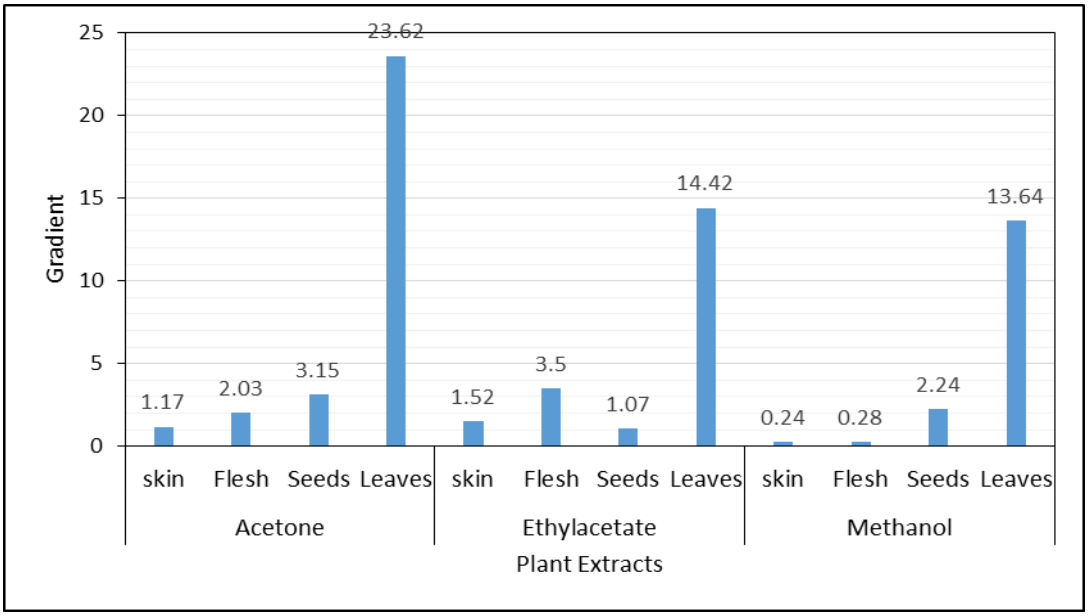

Figure 4: Rate of change of radical scavenging activity with concentration (gradients of Fig.3) of each plant extract

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Conflict of interest: None Declared

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