THE ANTIOXIDANT PROPERTIES OF ORANGE PEEL EXTRACTS

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Abstract
This study investigated the antioxidant properties of ripe and unripe orange peel extracts (OPE). Solvent extraction method was used to extract the phenolic compounds in the ripe and unripe orange peels. Three solvents, methanol, ethanol and hexane were used in the extraction process. The efficiency of the extracting solvents was determined and the total phenolic content (TPC) and total flavonoid content (TFC) of the OPE were evaluated using Folin – Ciocalteu (FC) phenol reagent and colorimetric aluminium chloride method respectively. Also, the antioxidant activity of the OPE was evaluated using 2,2-diphenyl-1-picryhydrazyl (DPPH) scavenging assay and the reducing power capacity.

The results shows that the efficiencies of the solvents for extraction were in the order methanol > ethanol > hexane. The TPC were 290 and 298 mg gallic acid equivalent (mg GAE)/g for the ripe and unripe OPE respectively. Also, the TFC in the unripe OPE were higher than that in the ripe OPE and their TFC ranged from 280 – 637µg of rutin equivalent (µgRE)/g for both the ripe and unripe OPE. The methanolic extracts in both the ripe and unripe OPE exhibited higher reducing power compared to the other extracts. A significant correlation ($r^2 = 0.976$) existed between the TPC of the OPE and inhibition of DPPH scavenging activities. There was also a significant correlation between the DPPH scavenging activity and the TFC which was 0.987.

Keywords: Antioxidant activity, orange peel extracts, total phenolic content, DPPH, reducing power

Introduction

Oranges are one of the most popular citrus fruits in the world. They are available in large quantities in many parts of the world. Other members of this citrus family are tangerine, grape, lemon etc. Orange constitutes about 60% of the total citrus world production. Juices that are extracted from oranges are used in the production of orange fruit juices. After the juice extraction, about 50 - 60%, of the fruit remains as waste consisting mainly of

peels, seeds and membranes (Wilkins *et al*., 2007). Peels represent between 50 to 60% of the total weight of the fruit and remain the primary byproduct. According to the FAO report of 2007, Nigeria produces about 3.325 M tons of citrus annually making her the ninth largest producer of citrus in the world (Alabi *et al*., 2012). In Nigeria, orange and other citrus wastes which are mainly peels are dumped indiscriminately on road sides and side - drains resulting in the blockage of drains and also constitute environmental health hazard to the public. These wastes could serve as a source of economic materials when dried, orange peels have been used as a source of natural feed additives for animals, but this was later stopped because it caused diseases for these animals (Bampidis and Robinson, 2006). Bicu and Mustata (2011) have also used orange peels as raw material for cellulose extraction. Orange peels have also been applied by Shan *et al*. (2012) for the adsorption separation of molybdenum (VI) from an aquatic environment. Other useful products such as ethanol, citric acid, succinic acid, methane have been obtained from processing of orange peels by several researches (Wilkins *et al*., 2007; Rivas *et al*., 2008; Li *et al*., 2010 and Martin *et al*., 2010).

Phenolic compounds are one of the most important groups of natural antioxidants (Artajo *et al*., 2006). They occur in materials of plant origin such as fruits and vegetables, seeds, cereals, olive oils, sweet potato leaves and onion bulbs (Lin and Tang, 2007; John and Shahidi 2010). Antioxidant ability of these polyphenols arises from their high reactivity as hydrogen or electron donors and from the ability of the polyphenol derived radical to stabilise and delocalise the unpaired electron. Studies have shown that the antioxidant activities of plants are closely related with the phenolic compounds that are present in these plants. Several studies have shown good correlation between the total phenolic contents and their antioxidant activities (Sultana et el., 2007; Jonfia-Essien *et al*., 2008; Bubonja-Sonje *et al*., 2011; Osman *et al*., 2004; Stoilova *et al*., 2007, Bozin *et al*., 2008;).

Flavonoids which are phenolic compounds have been identified in orange peel extracts (Sawalha *et al*., 2009; Toledo –Guillen *et al*., 2010). Extracts from orange peels could be channeled into the production of natural antioxidants for enhancing the stability of biodiesel produced from non-edible oils such as Japtropha which is highly susceptible to oxidation. Therefore, the objective of this study is to investigate the antioxidant potential of orange peels from both ripe and unripe oranges which are usually discarded when oranges are utilized. This will go a long way addressing the problems associated with orange peel wastes in the environment.

Materials and Methods

Plant materials

The ripe orange peels were obtained from sweet oranges purchased at a local market in Benin City, Nigeria, while the unripe orange peels were obtained from unripe oranges harvested at the premises of the

University of Benin, Benin City, Nigeria.

Chemicals

Folin-Ciocalteu (FC) phenol reagent, rutin, methanol, ethanol, hexane, ferric chloride, sodium nitrate, sodium hydroxide, sodium carbonate, 2,2-diphenyl-1-picryhydrazyl (DPPH), phosphate buffer, potassium ferricyanide, trichloroacetic acid, aluminium chloride and gallic acid were purchased from Stanvac Laboratory, Edo State. All chemicals and reagents used in this study were of analytical grade.

Equipment And Apparatus

The following instruments were used: UV vis spectrophotometer, rotary vacuum evaporator, OHAUS, PA213 electronic balance, soxhlet apparatus, and mistral 2000 centrifuge (SANYO **Gallenkamp**).

Preparation Of Orange Peel Extract (OPE)

The orange fruits were washed with water and then manually peeled using a laboratory knife. The orange peels were washed and sun-dried. The dried peels were reduced into a fine powder in a wooden mortar. The fine powder material was made to pass through the 50 mesh sieve and those retained was used for further study. Fifty grams of fine orange peels powder was extracted with 500 mL of solvent (methanol, ethanol and hexane) in a soxhlet apparatus for 5 h. Dry OPE was obtained after evaporation of the solvent. The dry OPE was weighed and the extraction yield in percentage was calculated as

$$\text{Extraction yield (\%)} = \frac{M_{OPE}}{M_S} \times 100$$

where M_{OPE}= mass of the orange peel extract and, M_S= mass of ground peel used for extraction

The dry OPE was stored in amber colored bottles and kept at 4°C in a refrigerator until further analyses.

Determination Of Total Phenolic Content

The total phenolic content (TPC) for each dry OPE was determined using Folin – Ciocalteau (FC) colorimetric method as described by Sun *et al*. (2007). 0.5 mL dry OPE sample was mixed with FC phenol reagent (5mL distilled with water by rate 1:10). The contents were mixed by manual shaking for about 60 second. After 5 min., 4mL aqueous 1 M sodium carbonate was added. The reaction mixture was incubated in the dark at room temperature (28 ± 2°C) for 30 min and the absorbance was measured at 765 nm using a UV-visible spectrophotometer (T70, PG Instrument limited). The total phenolic content was determined using a calibration curve prepared with gallic acid (0, 50, 100, 150, 200 and 250 mg/mL, R^2=0.982) as referenced standard. The values were reported as mg of gallic acid equivalent (GAE) by reference to gallic acid standard curve and the results were expressed as milligrams of GAE per gram dry OPE. All measurements were done in triplicate and the mean value ± standard deviation values are shown in Table 1.

Determination of total flavonoid content

The total flavonoid content (TFC) for each dry OPE was determined using the colorimetric method developed by Sultana *et al*. (2007) with some modification. Briefly, extracts of each orange peel (1 mL containing 0.1 g/mL) were diluted with distilled water (4 mL) in a 10 mL volumetric flask. Initially, 5% $NaNO_2$ solution (0.3 mL) was added to each volumetric flask; at 5 min, 10% $AlCl_3$ (0.3 mL) was added; and at 6 min, 2mL of 1.0 M NaOH was added. After that the volume was made up to 10mL with distilled water. The mixture was shaken vigorously and the absorbance of the reaction mixture was read at 510 nm using a UV-visible spectrophotometer (T70, PG Instrument limited). A calibration curve was prepared using a standard solution of rutin within the range 10 – 100 ppm (R^2=0.9962) and the results were expressed as μg of rutin equivalent per gram ($\mu gRE/g$) of dry OPE. All measurements were done in triplicate and the mean value ± standard deviation values are shown in Table 1.

Table 1: Effect of different solvents on the Yield, Total Phenolic Content (TPC), Total Flavonoid Content and IC_{50} values of both Ripe and Unripe Orange Peel Extract (OPE)

Solvent	Yield (%)		TPC (mgGAE/g)		TFC($\mu gRE/g$)		*Ic50* ($\mu g/mL$)	
	Ripe	Unripe	Ripe	Unripe	Ripe	Unripe	Ripe	Unripe
Methanol	18.3±2.02	5.99±1.2	271± 5.94	294 ± 2.35	603 ± 18.26	629 ± 15.72	1.85	1.9
Ethanol	11.0 ± 1.26	4.2 ± 1.34	290 ± 4.20	298 ± 1.40	620 ± 15.22	637 ± 8.48	4.15	42
Hexane	7.4 ± 0.25	2.4 ± 0.8	167 ± 3.26	193 ± 1.45	280 ± 10.35	320 ± 9.25	n/d	nd

Results are expressed as mean of 3 experiments ± standard deviation
n/d: not detected

The scavenging activity of the OPE was estimated according to the method of Alothman *et al*., (2009). Varied concentrations (2 - 10$\mu g/ml$) of the dry OPE in ethanol were prepared. An aliquot (1mL) of the resulting ethanolic solution was added to 4mL of 0.1mmol/L ethanolic solution of DPPH. The mixture was shaken vigorously and left to stand for 30 min at room temperature in the dark. Absorbance was read using a spectrophotometer at 517nm. A blank probe was also obtained by mixing 3.8mL of 0.1mmol/L ethanolic solution of DPPH and 0.2mL of ethanol. The radical scavenging activity of the extracts expressed as percentage inhibition of DPPH was then calculated according to the following equation:

$$\text{Inhibition of DPPH (\%)} = \left(\frac{A_{blank} - A_{sample}}{A_{blank}}\right) \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all the reagents except

the OPE) . A_{sample} is the absorbance of DPPH solution containing sample solution

The IC_{50} value which is defined as the sample concentration that is needed for 50% inhibition was determined from the plotted graphs of % inhibition of DPPH radical scavenging activity versus concentration of OPE extracts.

Reducing Power

The ability of the OPE to reduce iron (III) was measured according to a modified method described by Arabshahi-Delouee *et al*., (2007). Varied amounts of OPE (0, 1, 2, 3, 5, 7, 9 or 11mg) in 1mL methanol (80%) were mixed with 5mL phosphate buffer (2M, pH 6.6) and 5mL potassium ferricyanide (1%). These mixtures incubated at 50°C for 20 mins. After incubation, 5mL trichloroacetic acid (10%) was added and the mixture was centrifuged at 3,000rpm for 10mins. The supernatant solution (5mL) was mixed with 5mL distilled water and 1mL ferric chloride (0.1%). The absorbance of the pink colour mixture was measured spectrophotometrically at 700nm. Increased absorbance of the mixture indicates increased reducing power.

Results and discussion

Extraction yield, total phenolic content and total flavonoid content

Table 1 shows the extraction yield for both the ripe and unripe OPE using the various solvents. Three different solvents (methanol, ethanol and hexane) were used for the extraction of phenolic compounds from the orange peel. The amount of extractable components expressed as percentage by weight of dried ground orange peels ranged from 7.4 to 18.31% for the ripe peels while that for the unripe orange peels ranged from 2.4 to 5.99%. The maximum amount of OPE from the ripe peels was methanol (18.31%), ethanol (11%) and hexane (7.4%). Similar results were also obtained by Zia-ur-Rehman (2006). Also, methanol gave the maximum amount of extracts for the unripe peels (5.99%), followed by ethanol (4.2%) and hexane (2.4%). Higher yields were obtained for the different solvents used for the extraction of the ripe peels extracts when compared to the unripe peels. This is in agreement with the work done by Li *et al*. (2006), Anagnostopoulou *et al*. (2006), Arabshahi-Delouee (2007) and Razali *et al*. (2012) in which methanol as an extraction solvent gave the highest extraction yield when compared to other solvents.

The total phenolic content (TPC) of the different OPE is shown in Table 1. The TPC for the ripe OPE range from 208 to 290mg GAE/g, while for the unripe OPE, the values ranged from 267 to 298mgGAE/g. The ethanolic extract had the highest total polyphenolic content with 290mgGAE/g and 298mgGAE/g for both the ripe and unripe OPE respectively. This was however followed by the methanolic extracts with 271mgGAE/g and 294mgGAE/g and finally hexane extracts with 208mgGAE/g and 267mgGAE/g for both the ripe and unripe OPE respectively. The results obtained were due to the fact that methanol and ethanol are polar solvents and so can extract more of the phenolic compounds as compared to hexane which is a non-polar

solvent. The phenolic compounds are polar in nature; thereby methanol and ethanol will extract more of the phenolic constituents from the peels than hexane because of their polarity differences. In comparison, the unripe OPE had a greater amount of TPC compared to the ripe OPE. The TPC of the orange peels obtained in our work (167 – 294 mgGAE/g dried OPE) were higher than those reported by Kamran *et al*. (2009) 132.2 – 223.2 mg GAE/g dried matter and much higher than that reported by Lagha-Benamrouche and Madani (2013) 9.603 – 31.623 mgGAE/g. Results obtained by Anagnostopoulou *et al*. (2006) 3.63 – 253 mgGAE/100g dried matter were far less than the results obtained in this work.

The total flavonoid content (TFC) which was also determined for the various OPE is shown in Table 1. Flavonoids are type of phenolic that have been identified in orange peel extracts (Sawalha *et al*., 2009). From the results obtained, TFC for the ripe OPE were 280, 603 and 620 microgram of rutin equivalents per gram (*μgRE/g)* for the hexane, methanolic and ethanolic extracts respectively. Also, for the unripe OPE, the TFC were 320, 629 and 637*μgRE/g* for the hexane, methanolic and ethanolic extracts respectively. Similarly, ethanolic extracts had the highest TPC for both the ripe and unripe OPE.

Antioxidant activity

DPPH free radical scavenging activity

The antioxidant ability of plant extracts is due to their ability to donate hydrogen atoms or electrons and also to capture free radicals. The DPPH analysis was carried out in order to prove the ability of the phenolic components of the OPE to acts as donors of hydrogen atom. The phenolic component reacts with the stable organic nitrogen free radical DPPH (2,2, diphenyl-1-picrylhydrazyl radical) and converts it to 1,1 dihenyl-2-picryl hydrazine due to the hydrogen donating ability at a very rapid rate with discoloration (Soarces *et al*., 2009). Compounds with high antioxidant activity result in a rapid decline in the absorbance of the DPPH (Guimaraes *et al*., 2010).

The methanolic ripe OPE showed the highest scavenging effect at concentrations below 6*μg/ml* whereas at concentrations above 6*μg/mL*, the ethanolic extracts of the ripe OPE becomes significant in inhibiting the DPPH reaching up to 91.4% at concentration of 10*μg/mL* as compared to 87.6% for the methanolic extract. The hexane extract on the other hand showed the least scavenging effect on the DPPH as shown in Figure 1. Similar trends were observed for the unripe OPE as shown in Figure 2. At concentrations below 8*μg/mL* the methanolic extracts is significant in inhibiting DPPH, whereas at higher concentrations the ethanolic extracts becomes significant in inhibiting DPPH reaching up to 92.5% at concentration of 10*μg/mL* as compared to the methanolic extract with a value of 88%. The unripe OPE were found out to have higher antioxidant activity than the ripe OPE and this could be attributed to the higher phenolic contents obtained in the unripe OPE.

The IC_{50} values obtained were 1.85μg/mL and 4.15μg/mL for the methanolic and ethanolic extracts

respectively for the ripe OPE (Figure 1) while 1.9µg/mL and 4.2µg/mL were obtained respectively for those of the unripe OPE as can be seen in Figure 2. The IC_{50} value for the hexane extracts for both OPE were not detected. The IC_{50} values show no significant difference ebetween the methanolic and ethanolic extracts of both the ripe and unripe OPE. The lower the IC_{50} value of

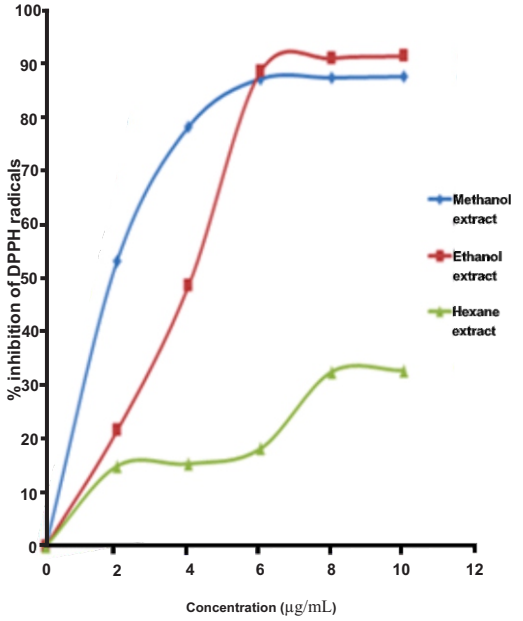

Fig. 1. DDPH Scavenging activity of various ripe OPE

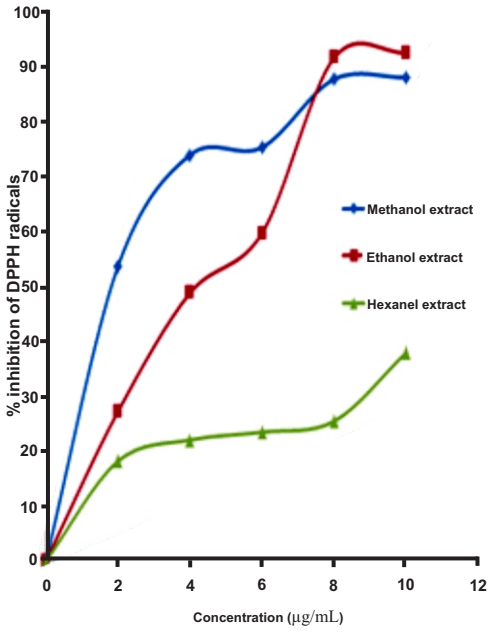

Fig. 2. DDPH Scavenging activity of various unripe OPE

the OPE, the more effective it will be for the inhibition of DPPH. The methanolic extract for both the ripe and unripe OPE is more potent and can scavenge most free radicals because it has the least IC_{50} value. The reducing power is often used as an indicator of electron donating activity, which is an important mechanism for testing the antioxidative action of phenolics (Terpinc et al., 2012). For the measurement of reductive ability, the transformation of ferric ion (Fe^{3+}) to the ferrous form (Fe^{2+}) was investigated using the potassium ferricyanide reduction method. The reduction of ferric ion in will result in the formation of blue black product, whose absorbance is read at 700 nm. For this investigation, it was observed that the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each compound. An increase in the absorbance indicated an increase in the reducing capacity due to an increase in the formation of the Prussian blue complex (Soarces et al., 2009). For the ripe OPE, the

methanolic extract was the most potent reducing agent at concentrations below 1.7 mg/mL, whereas at concentrations above this, the ethanolic extracts becomes more potent as can be seen in Figure 3. The reducing power of the methanolic extracts for the unripe OPE was more effective than the ethanolic extracts at the various extract concentrations under investigation (Figure 4). In both cases, the hexane extracts exhibited the least ferric reducing activity as shown in Figures 3 and 4. It was also observed that the reducing power of the hexane extracts of both the ripe and unripe OPE starts decreasing at concentrations

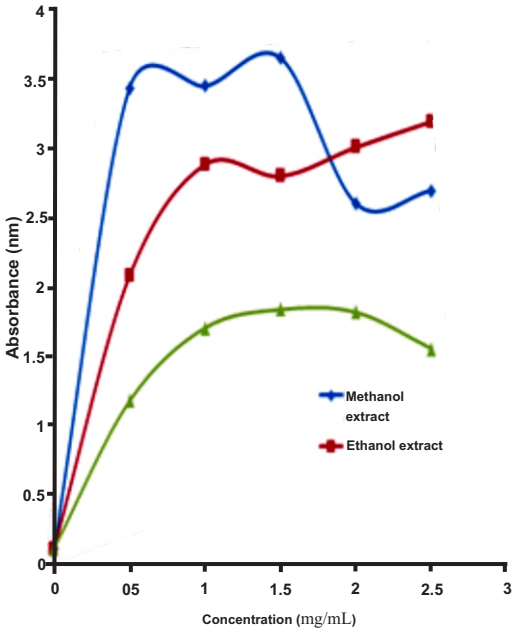

Fig. 3. Reducing power of difficult concentrations of ripe OPE

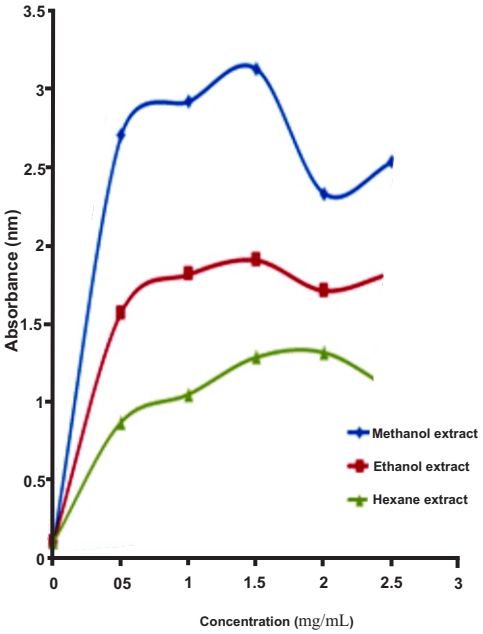

Fig. 4. Reducing power of difficult concentrations of unripe OPE

above 2 mg/mL as can be seen from Figures 3 and 4. The values obtained revealed that all the samples had the capacity to reduce iron (III) ions and had electron donor properties for neutralizing free radicals by forming stable products.

Correlation between total phenolic content and antioxidant activities

Correlation analyses between the TPC, DPPH scavenging activity and FRAP assay for the various OPE were calculated using linear regression analyses. Results showed significant correlation between TPC and DPPH radical scavenging activity ($r^2 = 0.976$) as shown in Figure 5. A positive correlation ($r^2 = 0.9687$) was also evident between TFC and DPPH radical scavenging activity as shown in Figure 6. Several studies have also resulted in positive correlation between total phenolics and antio-

xidant activities of plant extracts. (Li *et al*., 2008; Ghaseme *et al*., 2009; Razali *et al*., 2012). It is evident that the presence of phenolic in the OPE contributed to the antioxidant activities of the extracts. The results indicate that the presence of phenolic compounds in the extracts contribute significantly to antioxidant activities of the samples.

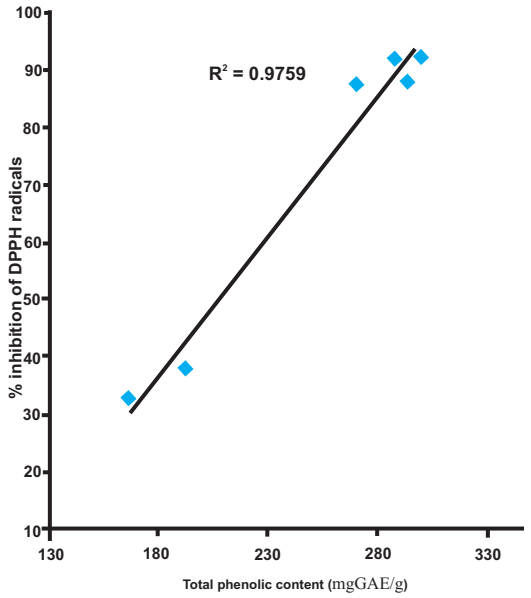

Fig. 5. Correlation between scavenging activity and total phenolic content

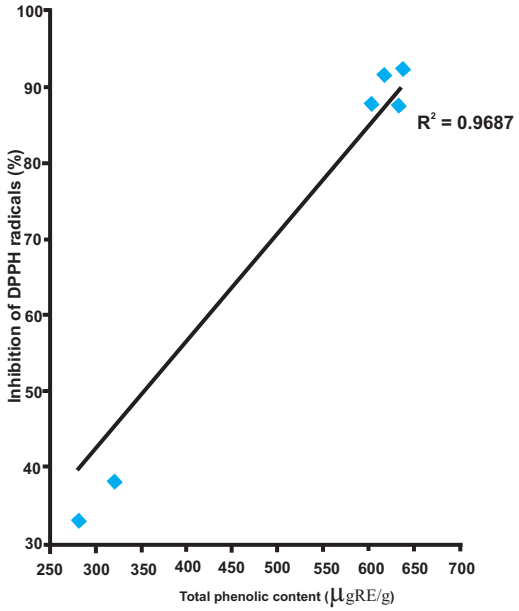

Fig. 6. Correlation between DPPH scavenging activity and total flavonoid content

Conclusion

The total phenolic content of both ripe and unripe orange peel extracts is affected by the type of solvent used for extraction. Methanol was found to be the best solvent for extraction giving an extraction yield of 18.39 and 5.99% for the ripe and unripe peels respectively. The unripe orange peel extracts had a higher total phenolic content, with the ethanolic extract having a higher total phenolic content of 298 and 290mg GAE/g for the unripe and ripe orange peel respectively. The total flavonoid content was also observed to be higher in the ethanolic extracts of both the ripe and unripe OPE, with the unripe OPE having the highest of 637 µgRE/g of OPE. A significant correlation was also established for the total phenolic content and the DPPH radical scavenging activity.

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