# In Vitro Discovery of Watermelon (*Citrullus Lanatus*) Extract with Moderate Chelating Ability, and Survey of other in Vitro Bioactivities

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## Abstract

*Ferrous chelating ability of aqueous mesocarp extract of watermelon(Citrullus lanatus ) was discovered at the lowest concentration(1%).The aqueous extract of the plant part(mesocarp, the edible portion) displayed moderate chelating ability(55.45,% at 15min) in vitro for ferrous ion($Fe^{2+}$).The extract failed to bind $Fe^{2+}$ at other concentrations(2- 5%).The extract also failed to chelate ferric ion($Fe^{3+}$) in vitro at 1% concentration.This discovery was fortunate in that the aqueous extract of the plant at that concentration (1%) could be strategically targeted for the reduction of iron( ii ) ion in all situations of iron-overload disorders because of its moderate chelating potential.The in vitro inhibition of lipid peroxidation by aqueous extract of watermelon plant parts was in the order:epicarp > endocarp > mesocarp.The aqueous extract of the three parts of the plant showed weak inhibition of lipid peroxidation in vitro(2.58±0.19 to 27.50±1.03 % ) within the concentration range investigated(2 -10%).The aqueous extract of C.lanatus failed to scavenge nitric oxide radical in vitro, but 70 % methanolic extract demonstrated potent in vitro nitric oxide radical scavenging potential at all concentrations investigated(2- 10%).The in vitro antioxidant activity of the aqueous mesocarp was in the region of 60% activity for all the concentrations(2- 10%), and their differences were not significant (P >0.05).The in vitro hydroxyl radical scavenging activity of the endocarp(70% methanol),mesocarp(water extract) and epicarp(70% methanol) of the plant were remarkable ,mostly in the vicinity of 80% activity for most of the concentrations.The in vitro nitric oxide scvaenging potentials of the 70% methanolic extract were 71.3% at 2% concentration and 70.4% at 4% concentration for endocarp and epicarp extracts ,respectively. The 70% methanolic extract of the plant could be exploited for the treatment of inflammatory diseases because of its potent in vitro nitric oxide scavenging capability.*

**Key words**: Phytomedicine; oxidative stress; iron-overload; natural product and toxicology

## *Introduction*

The use of natural products for the prevention and treatment of different pathologies is continuously expanding throughout the world(Mota *et al*., 2009).Medicinal plants are gifts of nature to cure limitless number of diseases among human beings(Beegum and Devi,2003).Desferriosamine(DFO) is a standard iron chelator , that is commonly used for the treatment of iron overload diseases such as thalassemia which binds $Fe^{3+}$ well, but $Fe^{2+}$ poorly(Martell and Smith, 1977).DFO is membrane impermeable, and thus its chelating ability is restricted to the extracellular media , and perhaps the lumen of the endosomal vesicles(Glickstein *et al* .,2005;Lloyd *et al*., 1991). Free radicals such as superoxide , hydroxyl and peroxyl radicals play an important role in oxidative stress related to the pathogenesis of various important diseases(Slater , 1984). Chelation therapy reduces iron-related complications in human and thereby improves the quality of life and overall survival in some diseases such as Thalassemia major(Hebbel *et al*., 1990).

Iron is capable of generating free radicals from peroxides by Fenton reactions, and minimization of the $Fe^{2+}$ concentration in the Fenton reaction affords protection against oxidative damage(Lai *et al.,* 2001) .Water melon (Citrullus lanatus) belongs to the family Curcubitaceae(Erickson *et al.,* 2005).The origin of watermelon is in tropical Africa(Reamakers , 2001).It is a creeping annual plant with large and rounded or oblong fruit(Tindall, 1986).Emulsion obtained from the seed water extract of watermelon is used to cure catarrhal infections, disorders of the bowel, urinary passage and fever (Taiwo *et a.,l* 2008).The plant contains large amount of beta carotene and is a significant natural source of lycopene(Edwards *et al.,* 2003).Watermelon is also rich in citrulline , an effective precursor of L-arginine (Guoyao *et al.,* 1992).Lycopene is a non- provitamin A carotenoid that has up to twice the antioxidant capacity of of beta-carotene in vitro(hm *et al*., 2002; Di Mascio *et al.,* 1989).The mean of lycopene concentration of water melon was 4868ug/100g(Holden *et al*., 1999). Flavonoids have been the subject of several studies because of their antioxidant potential (Valentao *et al.,* 2002).Phenolic compounds are constituents of both edible and non-edible parts of plants (Amarowicz *et al.,* 2004).Nitric oxide is synthesized from L –arginine by the action of nitric oxide synthases(Fitzhugh and Keffer, 2000).

The antioxidant activity of methanolic fruit extract of C. lanatus has been reported( Jamuna *et al.,* 2011).In addition , the antioxidant activities , total phenolics and flavonoid levels of fermented and unfermented water melon rind(outer layer) have been investigated(Erukainure *et al.,* 2011).Moreover, antioxidant activities, total phenolics and flavonoid contents of the water extract of the plant has been addressed(Tlili *et al.,* 2011).To the best of our knowledge , there is no in vitro chelating ability of any part of the plant to date.Moreover, there is no previous work hitherto on in vitro inhibition of lipid peroxidation and hydroxyl radical scavenging potential of the epicarp ,mesocarp and endocarp extracts of the plant.Also, there is no survey of in vitro nitric oxide radical scavenging potential of the different parts of the plant in a single study .Therefore, our study was designed to address the in vitro bioactivities mentioned above in order to push the frontier of water melon research.

## *Materials and Methods*

### *Chemicals*

Folin-ciocalteu reagent and 1,10-phenanthroline used were products of Merck, Germany. The 2,2-diphenyl -1-picryl hydrazyl used was a product of Sigma-Aldrich , USA.Hydrogen peroxide used was a product of Sigma-Aldrich , Switzerland.

### *Collection of Plant Material*

The first batch of water melon (Citrullus lanatus) was purchased from Igbono market, Osogbo, Osun State, Nigeria on the 16th march, 2010.The second batch of the plant was collected on 16th November, 2012 from Asada Area, Ogbomoso South Local Government,Ogbomoso, Oyo State, Nigeria .The third batch of the plant was purchased from a fruit seller in Sabo market , Ogbomoso, Nigeria in January , 2013. The back of watermelon was washed with distilled water to remove dirt .The fruit was cut with knife , and mesocarp was carefully separated from the epicarp and seed(endocarp).

### *Preparation of Plant Extract*

Five different concentrations(1- 5% w/v) of mesocarp were prepared by scaling down approach in order to minimize the amount of mesocarp and the volume of distilled water used.Under normal condition, 1,2,3,4 and 5 % concentrations(w/v) of mesocarp would be prepared by dissolving 1, 2, 3,4 and 5g of mesocarp in 100ml of distilled water each, affording 1,2, 3, 4 and 5% concentrations(w/v), respectively. In practice, 0.2, 0.3 ,0.4 and 0.5g of mesocarp were separately dissolved in 10ml of distilled water and then centrifuged at 5000 rpm for 5, 10,15 and 20 min time intervals.These afforded five different concentrations(1-5% ,w/v).The supernant obtained after centrifugation was utilized for biochemical assays of interest(In vitro Fe 2+ and Fe 3+ chelating abilities).For other concentrations(2 – 10% w/v). 2 , 4 , 6, 8 and 10 g each of the plant part was soaked in 100ml of the selected solvents , giving 2, 4 , 6 ,8 and 10 % concentration(w/v) ,respectively.

***Biochemical Assays***

***In Vitro $Fe^{2+}$ Chelating Ability Assay***

The in vitro $Fe^{2+}$ chelating ability of plant extract was assayed according to the method of Minnoti and Aust(1958 ) with slight modification( Puntel *et al.,* 2005 ).Briefly , 900 µl of aqueous FeSO4(500 µM) and 150µl of mesocarp extract were incubated for 5min at room temperature.Seventy eight microlitre(78 µl) of 1,10-phenanthroline(0.25 % ,w/v , aqueous) was added.The absobance of the orange colour solution was read at 510nm with a spectrophotometer.The principle of the assay is based on distruption of o-phenanthroline - $Fe^{2+}$ complex in the presence of chelating agent. The in vitro of $Fe^{2+}$ chelating ability of the sample was calculated by using the following formula:

Chelating ability( %) = (Acontrol - A sample)/ Acontrol x 100

where Acontrol = The absorbance of the control(reaction mixture in the absence of sample)($FeSO_4$ alone).
Asample = The absorbance of the reaction mixture(sample,$FeSO_4$ and 1,10-phenanthroline)

***In Vitro $Fe^{3+}$ Chelating Ability Assay***

The in vitro $Fe^{3+}$ chelating ability of the extract was quantitated according to the method of Puntel *et al*(2005) with slight modification in which 500µM $FeCl_3$ solution was replaced by 500µM $FeSO_4$(aqueous).Briefly, 900µl $FeCl_3$ solution(aqueous, 500µM) and 150 µl sample were incubated for 5min , after which 78µl aqueous 1,10-phenathroline was added.The absorbance of the orange colour solution was read at 510nm using a spectrophotometer.

***Calculation***

The in vitro of $Fe^{3+}$ chelating ability of the sample was calculated by using the following formula:
Chelating ability( %) = (Acontrol - A sample)/ Acontrol x 100

where Acontrol = The absorbance of the control(reaction mixture in the absence of sample)($FeCl_3$ alone).
Asample = The absorbance of the reaction mixture(sample,$FeCl_3$ and 1,10-phenanthroline).

***In Vitro Nitric Oxide Radical Scavenging Potential Assay***

The in vitro nitric oxide scavenging activity was estimated according to the method of Marcocci *et al.,* 1994).To 1ml sample, 1ml of sodium nitroprusside(10mM, aqueous) and 1 ml buffer(sodium phosphate buffer,0.2M) were added.The mixture was incubated at room temperature for 150 mins(2hr 30 min) followed by the addition of 01ml Griess reagent.The absorbance of the pink colour solution was read at 540nm on a spectrophotometer.The pink chromophore generated during diazotization of nitrite ions with sulphanilamide and subsequent coupling with N-naphthyl ethylene diamine dihydrochloride (NED) was measured spectrophotometrically at 540nm.

The in vitro NO scavenging activity of the sample was calculated by using the following formula:

Nitric oxide scavenging activity ( %) = (Acontrol - A sample)/ Acontrol x 100

where Acontrol = The absorbance of the control(reaction mixture in the absence of sample).
Asample = The absorbance of the reaction mixture.

***In Vitro Antioxidant Activity (DPPH Based) Assay***

The in vitro antioxidant activity of the sample was quantitated according to the traditional method of Blois(1958).To 1ml of plant extract, 1ml of methanolic solution of 2,2-diphenyl -1-picryl –hydrazyl (DPPH)(0.2mM) was added.The mixture was incubated in the dark for 30min.The absorbance of the yellow colour solution was read at 517nm on a spectrophotometer using distilled as blank.

DPPH scavenged(%) = ($A_{DPPH}$ - Asample ) /$A_{DPPH}$ x 100

### *In Vitro Inhibition of Lipid Peroxidation Assay*

In vitro inhibition of lipid peroxidation was estimated according to the method of Ruberto and Baratta, 2000).In this assay, egg yolk homogenate served as lipid rich medium , and $FeS0_4$ acts as initiator of lipid peroxidation .Briefly , 40µl of plant extract was mixed with 0.25ml 10% egg yolk .This was followed by the addition of 10ul $FeS0_4$( 0.07M ,aqueous).The mixture was incubated at room temperature for 30min.This was followed by the addition of 0.75 ml of glacial acetic acid(5%,v/v aqueous) and 0.75ml of thiobarbituric acid 0.6% in 0.2M NaOH .The mixture was incubated in a boiling water bath($90^0$ C) for 20 min , cooled and centrifuged at 3000rpm.One milliliter(1ml) of the pink colour supernant was read at 532 nm on a spectrophotometer. In vitro inhibition of lipid peroxidation = (Acontol – Asample)/Acontrol X 100

### *In Vitro Hydroxyl Radical Scavenging Activity Assay*

In vitro hydroxyl radical scavenging activity of the extract was performed according to the method of Yu *et al* (2004) .Briefly, 60µl $FeS0_4$(1mM , 90µl aqueous 1,10-phenanthroline(1mM), 2.4ml sodium phosphate buffer(0.2M), 15 µl $H_20_2$(0.17M) and 1.5ml of different concentrations of the plant extracts.The mixture was incubated at $25^0$C for 5min.The absorbance of the reaction mixture was read at 560nm using distilled water as blank.

In vitro hydroxyl radical scavenging activity (%)=Acontrol - Asample)/Acontrol X 100

Where Acontrol = Absorbance of the reaction mixture in the absence of sample

Asample = Absorbance of sample in the presence of other reagents in the reaction mixture.

### *Total phenolic content assay*

The phenolic content of the sample was determined according to the method of Taga *et al* (1974).To 0.1ml of sample, 2ml of sodium carbonate solution (0.2% w/v) was added , followed by the addition of 0.1ml of Folin-Ciocalteu reagent(10% , v/v).The mixture was incubated for 10 min. The absorbance of the blue colour solution was read at 480nm .The concentration of total phenolic(mg/ml) in the extract was extrapolated from pyrocatechol calibration curve.

### *Total flavonoid content assay*

The flavonoid content of the sample was determined according to the method of Lamaison and Carnet(1990 ).To 0.5ml sample , 0.5ml of 70% $AlCl_3.6H_20$ (2%) was added and the mixture incubated for 10min . The absobance of the yellow colour solution was read at 430nm after 10min on a spectrophotometer using distilled water as blank.The total flavonoid concentration(mg/ml) of the extract was obtained from a calibration curve using quercetin as a standard flavonoid.

## *Results*

***Table 1: Changes in levels of In vitro chelating ability of aqueous mesocarp extract of watermelon(Citrullus lanatus ) from Sabo market in Ogbomoso, Nigeria ( January, 2013)***

| Conc (%, w/v) | $Fe^{2+}$ chelating ability(%) | | | | $Fe^{3+}$ chelating ability(%) | | | |
|---|---|---|---|---|---|---|---|---|
| | 5min | 10min | 15min | 20min | 5min | 10min | 15min | 20min |
| 1 | 47.80±1.60 | 49.8±2.68 | 55.40±0.75 | 51.10±3.93 | -31.4±0.71 | -64.00±1.71 | -42.5±0.70 | -17.50±2.54 |
| 2 | -13.40±0.96 | -19.5±0.69 | -35.6±0.62 | -40.0±1.32 | 8.36 ±1.12 | 5.02±1.19 | 26.30±0.79 | 24.40±0.65 |
| 3 | -12.60±1.10 | 4.80±0.90 | -4.86±1.30 | -39.8±1.13 | -9.89± 1.84 | 1.60±0.84 | -12.3±0.79 | 12.10±0.60 |
| 4 | 18.70±0.58 | 34.60 ±0.99 | 18.80±0.62 | 33.0± 0.68 | -52.0± 1.45 | -53.3 ±0.49 | -35.1±1.17 | -39.3±1.07 |
| 5 | 19.9± 0.72 | 10.2 ±0.51 | 18.80± 0.62 | 28.9±1.00 | -24.1± 0.03 | -27.60±2.60 | -39.3±1.07 | -33.4±0.87 |

*The values are mean ± SD of 5 analyses per concentration*

At 1% concentration ,the aqueous mesocarp extract of the plant showed maximum $Fe^{2+}$ chelating ability(55.40 %) at 15min in vitro. The $Fe^{2+}$ chelation of the extract decreased from 15min to 20 min(55.40±0.75 to 51.10± 3.93 %) and the difference was not significant(P>0.05).

At 2% concentration , the extract failed to chelate $Fe^{2+}$ in vitro( -40.00,1.32 to -13.4 ±0.96 %).At 3% concentration , the extract at 10min showed poor $Fe^{2+}$ chelation ability(4.80±0.90%). At other time intervals(5, 10 and 15min),3% extract failed to chelate $Fe^{2+}$ in vitro.At 4 and 5% concentrations, the extracts exhibited weak $Fe^{2+}$chelation at all the time intervals(5, 10, 15 and 20min). At 2% concentration , the extract showed weak chelation for $Fe^{3+}$ at all the time intervals(5 – 20min).At 4, and 5% concentrations , the extract lacked $Fe^{3+}$chelating ability in vitro**(Table 1).**

***Table 2: Changes in levels of antioxidant activity ( DPPH based) of aqueous extract of water Melon in march, 2010 from Igbono market, Osogbo, Nigeria***

| CONC(%,w/v) | EPICARP(%) | MESOCARP(%) | ENDOCARP(%) |
|---|---|---|---|
| **2** | 12.77±1.06 | 74.25 ± 1.39 | 23.96 ± 1.76 |
| **4** | 9.67 ±0.78 | 63.62 ± 1.39 | 20.21 ± 1.30 |
| **6** | 8.51 ±1.06 | 56.77 ± 1.86 | 18.30 ± 1.39 |
| **8** | 7.23 ±0.82 | 54.53 ± 1.38 | 17.25 ±1.37 |
| **10** | 5.32 ±1.96 | 44.26 ±1.43 | 15.96 ± 1.07 |

The order of DPPH radical scavenging activity in vitro for the aqueous extract of water melon plant parts was : Mesocarp >Endocarp >Epicarp for all the concentrations investigated(2- 10%).There was a concentration dependent decrease in antioxidant activity as the concentration of the extract increased.The aqueous mesocarp extract demonstrated high antioxidant activity in vitro(74.25%) at 2% concentration , while the aqueous extracts of epicarp and endocarp exhibited weak antioxidant activities in vitro(5.32% at 10% concentration and 15.96% at 10% concentration ,respectively.

***Table 3: Changes in the levels of in vitro inhibition of lipid peroxidation in aqueous extract of water melon in march, 2010 from Igbono market, Osogbo, Nigeria***

| CONC(%,w/v) | EPICARP(%) | MESOCARP(%) | ENDOCARP(%) |
|---|---|---|---|
| **2** | 27.50 ±1.03 | 10.63 ± 0.39 | 11.90±0.17 |
| **4** | 26.53 ± 0.19 | 6.93 ± 1.46 | 11.50 ±0.26 |
| **6** | 25.98 ±0.17 | 4.65 ± 0.44 | 10.95 ± 0.34 |
| **8** | 24.58 ±2.29 | 3.58 ±0.72 | 10.15 ±0.17 |
| **10** | 24.80 ±0.14 | 2.58 ±0.19 | 9.73 ± 0.27 |

The in vitro inhibition of lipid peroxidation for the parts of the plant was weak.The epicarp extract displayed maximum anti-lipid peroxidative activity in vitro at the lowest concentration(2%, w/v).The in vitro inhibition of lipid peroxidation followed the order:epicarp > endocarp >mesocarp for all the concentrations investigated**(Table 3**)

***Table 4 :Changes in levels ot total phenolics content of aqueous extract of watermelon in march, 2010 from Igbono market,Osogbo, Nigeria***

| CONC(%,w/v) | EPICARP(%) | MESOCARP(%) | ENDOCARP(%) |
|---|---|---|---|
| **2** | 3.98 ± 0.58 | 4.16 ± 0.09 | 11.46 ± 0.06 |
| **4** | 4.70 ± 0.48 | 3.86 ± 0.49 | 12.00 ± 0.53 |
| **6** | 7.14 ± 0.62 | 4.92 ± 0.58 | 13.28 ± 1.19 |
| **8** | 8.98 ± 0.90 | 7.10 ± 0.65 | 13.28 ±1.19 |
| **10** | 10.09 ± 0.84 | 8.78 ±0.80 | 17.88 ± 0.11 |

The aqueous endocarp extract of watermelon was rich in total phenolics than the aqueous mesocarp and epicarp at high concentrations of the extract(6 - 10%,w/v). The total phenolic concent of the extract at 10% concentration was significantly higher than the epicarp extract at 2% concentration($P < 0.05$)**(Table 4).**

***Table 5: Changes in levels of total flavonoid content of aqueous extract of water melon in march, 2010 fromIgbono market,Osogbo, Nigeria***

| CONC(%,w/v) | EPICARP(mg/ml) | MESOCARP(mg/ml) | ENDOCARP(mg/ml ) |
|---|---|---|---|
| **2** | 1.04 ±0.05 | 0.40 ± 0.01 | 0.92 ± 0.04 |
| **4** | 1.13 ±0.03 | 0.56± 0.06 | 1.12 ± 0.11 |
| **6** | 1.30±0.00 | 0.60± 0.00 | 1.24 ± 0.05 |
| **8** | 1.28±0.05 | 0.60±0.00 | 1.30 ± 0.12 |
| **10** | 1.28 ±0.04 | 0.74 ±0.05 | 1.26 ± 0.05 |

The total phenolic concentration of the mesocarp was almost half of the epicarp, and endocarp. The total phenolic concentration of the epicarp and endocarp was almost the same**(Table 5)**

***Table 6:Observable changes in some vitro bioactivies (water extract) of mesocarp November , 2012***

| Conc | Antioxidant activity(DPPH based) (%) | Nitric oxide radical scavenging activity (%) | Hydroxyl radical scavenging activity (%) |
|---|---|---|---|
| **2** | 66.00 ± 1.70 | -13.00 ± 3.77 | 89.00 ± .81 |
| **4** | 67.00 ± 1.40 | -5.67 ± 6.44 | 86.00 ± 1.00 |
| **6** | 68.00 ± 0.37 | -2.19 ± 13.9 | 82.00 ± 1.3 |
| **8** | 68.00 ± 0.82 | -10.80 ± 24.7 | 81.00 ± 0.52 |
| **10** | 67.00 ± 0.94 | -13.7 ± 13.6 | 73.00 ± 0.49 |

The aqueous mesocarp extract of the plant lacked nitric oxide scavenging activity at all concentrations(2-10%).However, the plant part showed potent antioxidant and hydroxyl radical scavenging activities at all concentrations**(Table 6).**

**Table 7:Observable changes in some in vitro bioactivities(70% methanolic extract of epicarp) November 2012**

| Conc | Antioxidant activity(DPPH based) (%) | Nitric oxide radical scavenging activity (%) | Hydroxyl radical scavenging activity (%) |
|---|---|---|---|
| **2** | 82.00 ± 0.33 | 61.80 ± 8.96 | 87.00 ± 2.00 |
| **4** | 86.00 ± 0.14 | 70.40 ± 1.98 | 88.00 ± 1.50 |
| **6** | 76.00 ± 1.30 | 66.50 ± 3.10 | 86.00 ±1.80 |
| **8** | 79.00 ±0.16 | 61.30 ± 4.96 | 85.00 ±1.10 |
| **10** | 82.00± 1.20 | 67.80 ± 7.63 | 81.00 ± 0.66 |

The 70% methanolic epicarp extract of the plant displayed potent antioxidant , nitric oxide and hydroxyl radical scavenging activities at all concentrations investigated in vitro **(Table 7).**

***Table 8:Observable changes in some in vitro bioactivities(70 % methanolic extract) of endocarp November , 2012***

| Conc | Antioxidant activity(DPPH based) (%) | Nitric oxide radical scavenging activity (%) | Hydroxyl radical scavenging activity (%) |
|---|---|---|---|
| **2** | 91.00 ± 0.82 | 71.30 ± 3.88 | 88.00 ± 0.61 |
| **4** | 90.00 ± 0.83 | 68.70 ± 2.92 | 89.00 ± 0.39 |
| **6** | 91.00 ± 0.68 | 69.10 ± 2.46 | 91.00 ± 1.20 |
| **8** | 91.00 ± 0.78 | 65.00 ± 3.89 | 88.00 ± 0.73 |
| **10** | 89.00 ± 1.20 | 67.00 ± 1.39 | 87.00 ± 0.81 |

The in vitro hydroxyl scavenging and antioxidant activities of 70% methanolic endocarp of the plant were excellent at all concentrations.These two parameters were not concentration dependent.Their values were approximately constant.The value of hydroxyl radical scavenging activities at 6% concentration for the 70% methanolic endocarp extract was the same with the antioxidant activity of the plant at the same concentration(91.0% activity).The 70% methanolic endocarp extract also showed good nitric oxide scavenging activity all concentrations(2-10%).The antioxidant activities of the plant part(endocarp extract) in 70% methanol was significantly higher than the nitric oxide scavenging potential at all concentrations($P<0.05$)**(Table8).**

## *Discussion*

In our past work , we reported for the first time the chelating ability of aqueous extract of African walnut(Tetracarpidium conophorum) with remarkable activtity (97.38%) at the lowest concentration(2%)(Olabinri *et al.,* 2010). *I*n the present work , we discovered moderately chelatable aqueous extract of water melon(*Citrullus lanatus*) at the lowest concentration (1%) for ferrous ion ($Fe^{2+}$ ) with maximum activity(55.4% ) at 15min centrifugation. The aqueous of C.lanatus lacked chelating ability for ferric ion $Fe^{3+}$ at 1% concentration in vitro**.**Natural products have the potential to be developed into new drugs for the treatment of various diseases(Chen *et al* ., 2009).If any drug scavenges the hydroxyl radical it may either scavenge the radical or may chelate $Fe^{2+}$ ion making it unavailable for the Fenton reaction(Manian *et al.* 2008).The ability of phenolic comounds to chelate metal ions depend on the availability of properly oriented functional groups(Van -Acker *et al.,* 1996) .Many polyphenolic compounds may chelate iron (Rice-Evans *et al.,* 1993 ).Polyphenols are known to decrease oxidative stress induced by transition metal ions such as iron due to its chelating effect(Brown *et al.,* 1996).Bidentate ligands are more powerful scavengers of metal cations than monodentate ligands (Hider *et al.,* 2001).When a phenolic group is conjugated with a carbohydrate , as in naturally occurring phenolic glycosides , it can no longer bind metals(Hider *et al*., 2001).

Antioxidation is an extremely significant activity which can be used as a preventive agent against diseases(Motawi et al 2011).Phenolic compounds are the most active natural antioxidants in plants(Bors *et al.,* 2001).They are very important plant constituents because their hydroxyl groups which confer scavenging ability(Yildrim *et al.,* 2000) and because of the reactivity of the phenolic moiety(Wanasundara and Shadidi, 1998). In this work, the antioxidant activity of watermelon was higher in the epicarp(rind, outer layer ) than the flesh(mesocarp), consistent with the finding of Cui- ping (2011). Also, the free radical scavenging and antioxidant activities of plant extract may be attributed to the presence of phenolic compounds(Naskar *et al.,* 2010). The use of DPPH radicals provides an easy, rapid and convenient method to evaluate the antioxidant and radical scavenging( Soares *et al.,* 1997).The method is a sensitive way to survey the antioxidant activity of a specific compound or plant extracts(Koleva *et al.,* 2002).This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug(Hepsibha et al 2010). DPPH radical is commonly used for fast evaluation of antioxidant activity because of its stability in the radical form and simplicity of the assay(Bozin *et al.,* 2008).The degree of discolouration indicates the scavenging potentials of the extract (Bhujan *et al.,* 2009).Plant phenolic compounds are strong metal chelators(Van -Acker *et al.,* 1996).

The antioxidant activity depends on the type and polarity of extracting solvent(Meyer *et al.,* 1998).The best known antioxidants are phenolic compounds and flavonoids (Kessler *et al.,* 2003 ) exhibiting extensive free radical scavenging activities through their reactivity as hydrogen or electron donating agents and metal ion chelating properties( Rice -Evans *et al.,* 1993 ).The major phytochemicals responsible for the antioxidant capacity most likely can be accounted for by the flavonoid compounds which are known as secondary metabolites(Prior *et al.* 2005).Phenolic antioxidants inhibit lipid peroxidation and the activity depends on the structure of the molecules, the number and position of the hydroxyl group in the molecule(Millic *et al.,* 1998).Compunds such as flavonoids are responsible for the inhibition of lipid peroxidation effect in plants(Das and Pereira, 1990). The absorbance of the chromophore formed during diazotization of the nitrite with naphthyl ethylene diamine dihydrochloride is used as a marker of for nitric oxide scavenging activity(Mukherjee , 1989).Scavengers of nitric oxide radical compete with oxygen leading to reduced production of nitrite(Marcocci *et al.,* 1994).

In the present study, 70% methanolic endocarp extract of Water melon in the present study , displayed potent in vitro nitric oxide scavenging potential at all concentrations investigated with maximum activity ( 71.3% ) at the lowest concentration(2%).

However, the value observed in this work was lower than that of fermented water melon rind(99.34%) of the plant at 75ug/ml reported by Erukainure *et al* (2011). Consumption of lipid peroxidation products are thought to be associated with various diseases(Sinclair , 1990).

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