JSR Publications

Available Online

J. Sci. Res. **8** (3), 371-380 (2016)

**JOURNAL OF SCIENTIFIC RESEARCH**

www.banglajol.info/index.php/JSR

# Studies on *In Vitro* Antioxidant Activity of Methanolic Extract and Fractions of *Ficus Hispida* Lin. Fruits

**M. S. Hossain, S. Parvin, S. Dutta, M. S. I. Mahbub, M. E. Islam***

Department of Pharmacy, University of Rajshahi, Rajshahi-6205, Bangladesh

Received 8 February 2016, accepted in final revised form 18 June 2016

**Abstract**

The present study was designed to confirm the traditional use of the fruits of *Ficus hispida* Linn. (Moraceae) as an antioxidant agent. Fruits of the plant extracted with methanol and crude methanol extract (CME) were further fractionated with *n*-hexane, chloroform, and ethyl acetate. All the fractions, *n*-hexane (NHF), chloroform (CHF), ethyl acetate (EAF), aqueous (AQF) and CME were preliminary screened for *in vitro* antioxidant activity and total phenolic and total flavonoid content. In DPPH radical scavenging assay, CME exhibited highest scavenging activity ($IC_{50}$ = 11.20 µg/mL) as compared to other fractions. In this assay, $IC_{50}$ of reference standard BHT was 5.10 µg/mL. The reducing power of the samples was in the order as AQF > CME > CHF > EAF > NHF. The results for hydrogen peroxide scavenging activity indicated that CME, EAF and AQF had almost the same scavenging activity except NHF. Total antioxidant capacity of CME and other fractions were ranked as CHF > AQF > CME > EAF > NHF. In the assay of antioxidant constituents (total phenol and total flavonoids content), the CME had highest phenolic and flavonoids content. The results indicate that *Ficus hispida* fruits could be considered as a potential source of natural antioxidant.

*Keywords*: Antioxidant; Free radical; Phenolic content; Flavonoid content.


doi: http://dx.doi.org/10.3329/jsr.v8i3.26711 J. Sci. Res. 8 (3), 371-380 (2016)

## 1. Introduction

The trend in use of alternative and complementary healthcare has prompted scientists to investigate various biological activities of medicinal plants. In ancient literatures, it is mentioned that every plant on this earth is useful for human beings and animals because plant-derived products contain a great diversity of phytochemicals such as phenolic acids, flavonoids, tannins, lignin, and other small compounds. These compounds possess numerous health-related effects such as antibacterial, antimutagenic, anticarcinogenic, antithrombotic, vasodilatory activities etc.

* *Corresponding author:* ekram@ru.ac.bd

Within the human body, millions of chemical reactions are occurring constantly. These processes require oxygen. Reactive oxygen spices (ROS), sometimes called active oxygen species, are various form of activated oxygen, which include free radicals such as superoxide ions ($O_2^-$) and hydroxyl radicals ($OH^{\bullet}$), as well as non-free radical species such as hydrogen peroxide ($H_2O_2$) [1,2]. Free radicals are the compounds generated from normal body processes and also from environmental pollutions. They tend to attack the healthy cells DNA as well as proteins and fats, causing them to deteriorate. Anti-oxidants are compounds that protect cells against the damaging effects of reactive oxygen specious, such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage and finally a number of chronic diseases [3].

*Ficus hispida* which is locally called *Kakdumur* is a small tree and is very common throughout Bangladesh in homestead and village thickets. They are native throughout southwest Asia and the Mediterranean region (from Afghanistan to Portugal), Australia, Thailand, India, Burma and Andaman Islands. Traditionally, different parts of the plant have been used in the treatment of ulcers, psoriasis, anemia, piles jaundice, vitiligo, hemorrhage, diabetes, convulsion, hepatitis, dysentery, biliousness, and as purgative [4-6]. Various scientific works like antineoplastic, cardioprotective, neuroprotective and anti-inflammatory effects have been published to establish the scientific basis of traditional medicinal values attributed to *F. hispida* [7]. But, there is no scientific evidence on antioxidant activity of fruits of this plant. So, this study aims to evaluate the antioxidant activity of fruits extract of *F. hispida* for future investigation toward the finding of new, potent and safe antioxidant compound.

## 2. Expermintal

### 2.1. *Preparation of plant materials*

The fresh matured fruits of *F. hispida* were collected from Dinajpur, Bangladesh in September, 2013. The fruits were authenticated by Department of Botany, University of Rajshahi and preserved in phytochemistry laboratory, Department of Pharmacy, University of Rajshahi, Bangladesh. The fruits were sliced and air dried for 7 days and finally dried in an oven at 40 - 45$^{o}$C for 36 h. The materials were grinded into coarse powder with the help of a grinder and stored in an air tight container for further use.

### 2.2. *Extraction and fractionation*

About 500 g of the powder was dissolved in methanol at 1:3 ratio for seven days with occasional shaking. The extracts were collected by filtration with cotton and filter paper and evaporated with a rotary evaporator. The extract was then fractioned with n-hexane, chloroform and ethyl acetate using a separating funnel. The several fractions were then air dried.

### 2.3. *Chemicals*

2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Folin ciocalteu reagent (FCR) were collected from Sigma Chemical Company, USA; methanol, n-hexane, chloroform and ethyl acetate were from MERCK, Germany, ascorbic acid, gallic acid and catechin were from Wako Pure Chemicals Ltd., Japan. Other chemicals used, were of analytical grade.

### 2.4. *Estimation of antioxidant phyto-constituents*

#### 2.4.1. *Determination of total phenolic content*

The total phenol content of different extractives was evaluated by the method described by Singleton and Rossi method [8]. 200 µg of each fraction and extract were mixed with 2.5 mL of 10% FCR. After 5 min, 2.0 mL of 7.5 % sodium carbonate solution was added to the above mixture. The absorbance was measured at 760 nm with a spectrophotometer. Gallic acid solutions with concentrations ranging from 20 to 100 µg/mL were used for calibration. A dose response linear regression was generated by using the gallic acid standard absorbance and the levels in the samples were expressed as gallic acid equivalents (mg of GAE/g of extract). The estimation was performed in triplicate, and the results were expressed as mean ± SD.

#### 2.4.2. *Determination of total flavonoid content*

The total flavonoid content was determined by Dewanto *et al*. [9]. 0.5 mL of sample solution containing 40 µg of each sample was mixed with 0.15 mL $NaNO_2$. After 5 min, 0.3 mL $AlCl_3$ was added. After another 5 min, 1 mL NaOH and 0.55 mL distilled water was added to make the final volume 5 mL. The solution was mixed well and absorbance was taken at 510 nm. The total flavonoid content was calculated using standard catechin calibration curve. The results were expressed as milligrams of catechin equivalents (CE) per gram of extract.

### 2.5. *In vitro antioxidant assay*

#### 2.5.1. *DPPH free radical scavenging assay*

The DPPH free radical scavenging assay was carried out according to the method described by Braca *et al*. [10]. The stock solution was prepared by dissolving 5 mg of the extracts (CME, NHF, CHF, EAF, AQF) and standard (BHT) in 1 mL methanol. Then 5, 10, 20, 40, 60 and 80 µg of the sample and standard were diluted with methanol up to 2.5 mL. Then 2.5 mL of 0.008% DPPH solution was added and incubated for 30 min. The control solution was prepared by adding methanol and DPPH solution at 1:1 ratio. The decrease in absorbance at 517 nm was measured after 30 min. The free radical scavenging capacity was expressed as the concentration of the samples required to reduce the original

amount of free radicals by 50 % ($IC_{50}$) and was determined from the curve of % inhibitions plotted against the respective concentration.

2.5.2. *Reducing power capacity assay*

The Reducing power of CME and different fractions was evaluated by the method of Oyaizu [11]. 0.25 mL extractives and standard at different concentration (ranging from 6.25 – 100 µg/mL) was mixed with 0.625 mL of sodium buffer (pH-6.6) and 0.625 mL of (1%) potassium ferricyanide and are vortexed well followed by incubation at 50°C for 10 min. Then 0.625 mL of (10%) trichloro acetic acid was added and the mixture was then centrifuged at 3000 rpm for 10 min. 1.8 mL supernatant was withdrawn and mixed with 1.8 mL distilled water and 0.36 mL of (0.1%) ferric chloride was added. The absorbance was taken at 700 nm with a spectrophotometer. A typical blank solution containing the same solution mixture without plant extract or standard and it was incubated under the same condition as the rest of the sample solution. Ascorbic acid was used as positive control. All the tests were run in triplicate and results were reported as mean ± SD.

2.5.3. *Determination of total antioxidant capacity*

The total antioxidant capacity of different fractions was determined by the method described by Prieto *et al.* [12]. Aliquot of sample solution at different concentrations such as 5, 10, 20, 40, 80 and 100 µg/mL were diluted with methanol up to 2 mL. Then 3 mL reaction mixture (containing 0.6 M sulfuric acid, 28 mM sodium phosphate and 1% ammonium molybdate) was added in each test tube and incubated at 95°C for 10 min. The mixture was cooled to room temperature and absorbance was taken at 695 nm. The assays were carried out in triplicate and expressed as mean ± SD. The antioxidant activity was expressed as the absorbance of the sample.

2.5.4. *Hydrogen per-oxide scavenging assay*

The ability of the fruits extract and different fractions to scavenge hydrogen peroxide was assessed by the method of replacement titration [13]. Aliquot of 1.0 mL of 0.1 mM $H_2O_2$ and 1.0 mL of various concentrations of extractives were mixed. Followed by 2 drops of 3% ammonium molybdate, 10 mL of 2M $H_2SO_4$ and 7.0 mL of 1.8 M KI were added. The mixed solution was titrated with 5.09 mM $Na_2S_2O_3$ until yellow colour disappeared. The extent of scavenging of hydrogen peroxide was calculated as:

% Scavenging of Hydrogen Peroxide = $[(V_0\text{-}V_1) / V_0] \times 100$.

Where,

$V_0$ = Volume of $Na_2S_2O_3$ solution used to titrate the control sample in the presence of hydrogen peroxide (without extract),

$V_1$ = Volume of $Na_2S_2O_3$ solution used in the presence of extract.

## 3. Results

### 3.1. *Determination of antioxidant components*

#### 3.1.1. *Total phenolic content*

The total phenolic content of CME of *F. hispida* fruits and various fractions such as NHF, CHF, EAF and AQF determined by Folin-Ciocalteu reagent and calculated on the basis of the standard curve for gallic acid was shown in Table1. The amount of total phenolic content in CME, NHF, CHF, EAF and AQF was 286±1.632, 35.66±0.623, 244.33±1.312, 210.5±1.870, 185.66±5.542 mg of GAEs/gm of extract, respectively. So, the highest phenolic content was found in CME and NHF was considered to be the lowest among all the fractions.

#### 3.1.2. *Total flavonoid content*

Total flavonoid content of CME and various fractions (NHF, CHF, EAF and AQF) were calculated on the basis of standard curve for catechin and are presented in Table 1. The total flavonoid content extracted from *F. hispida* fruits in different solvent systems varied widely ranging from 29.17±3.11 to 181.67±3.11 mg of CEs/gm of extract. Amongst all extractives, the CME showed highest content of flavonoids (181.67±3.11 mg of CEs/gm of extract).

Table 1. Total phenolic and flavonoid content of CME and different fractions of *Ficus hispida* fruits.

| Name of sample | Total phenolic content (mg of GAEs/gm of extracts) | Total flavonoid content (mg of CEs/gm of extracts) |
|---|---|---|
| CME | 286±1.632 | 181.67±3.11 |
| NHF | 35.66±0.623 | - |
| CHF | 185.66±5.542 | 100.00±2.04 |
| EAF | 210.5±1.870 | 29.17±3.11 |
| AQF | 244.33±1.312 | 129.17±3.11 |

### 3.2. *In vitro antioxidant assay*

#### 3.2.1. *DPPH free radical scavenging assay*

Fig. 1 showed the dose response curves of DPPH radical scavenging activities of the extractives of *F. hispida* fruits. Among the extractives, at concentration of 80 µg/mL, CME showed the highest DPPH radical scavenging activity (93.6%), followed by AQF (93.13%), EAF (76.33%), CHF (68.12%) and NHF (56.39%). All the test samples could scavenge DPPH free radical in a concentration-dependent manner with $IC_{50}$ values of 11.20, 62.5, 47.25, 33.90 and 13.30µg/mL for CME, NHF, CHF, EAF and AQF, respectively (Fig. 2). The $IC_{50}$ value of standard BHT was 5.10 µg/mL. Therefore, CME

demonstrated strongest scavenging and the NHF showed the weakest scavenging activity on DPPH radical. The results also show that the CME and AQF showed significant DPPH radical scavenging activity when compared with BHT.

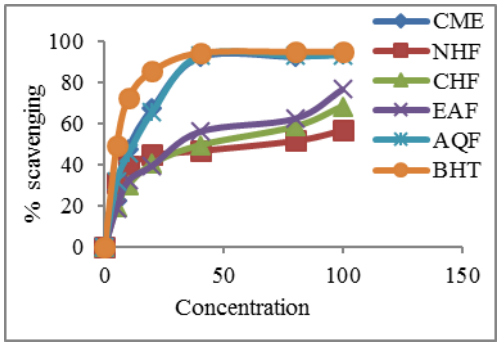


Fig. 1. DPPH radical scavenging activity of CME and different fractions of *F. hispida* fruits with standard (BHT) at different concentrations.

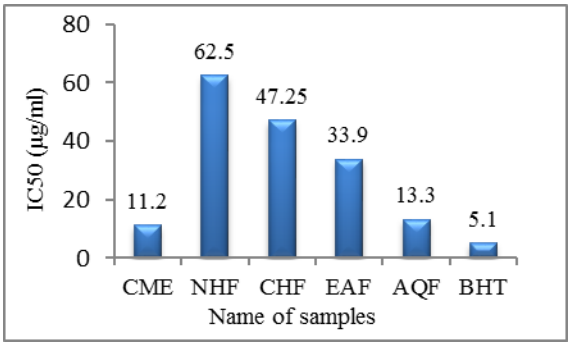


Fig. 2. Comparison of $IC_{50}$ value of CME and different fractions of *F. hispida* fruits with standard BHT.

### 3.2.2. *Reducing power assay*

The presence of reductants i.e. antioxidants caused the concentration of the $Fe^{3+}$/ferric reducing complex to the ferrous form. Therefore, by measuring the formation of Purl's prussian blue at 700 nm, we can monitor the $Fe^{2+}$ concentration; a higher absorbance at 700 nm indicates a higher reducing power. The reducing power of all the samples increased gradually with the increase in concentrations of the samples. The result demonstrated that CME and the fractions exhibited appreciable reducing activity. At 100 µg/mL they were above 0.50 and the order was AQF > CME > CHF > EAF > NHF (Fig. 3).

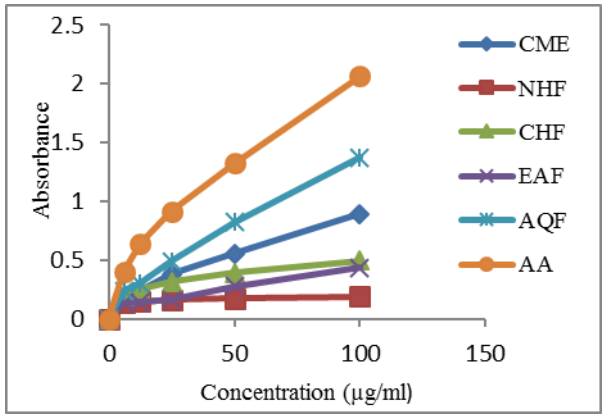


Fig. 3. Reducing power of CME and different fractions of *F. hispida* fruits with standard ascorbic acid.

3.2.3. *Total antioxidant capacity assay*

Total antioxidant capacity of CME and different fractions was ranked as CHF > AQF > CME > EAF > NHF. All the samples showed a concentration dependent increase in antioxidant activity. Though CHF showed the total highest antioxidant capacity but AQF and CME also had almost the same capacity. The difference in the amount of antioxidant of these samples may be attributed to the differences in the amount and kind of antioxidant compounds in them. The result was shown in Fig. 4.

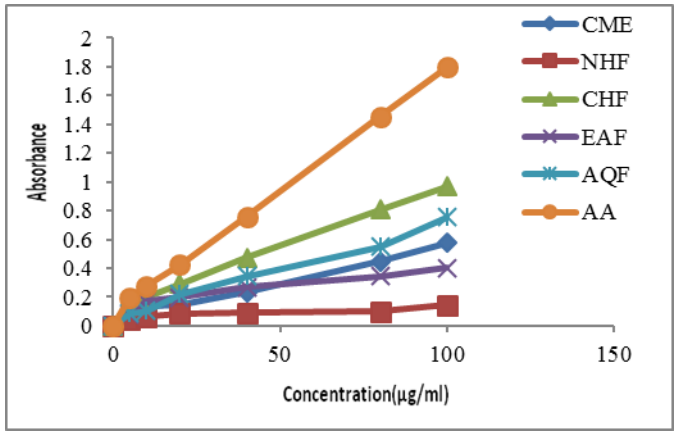


Fig. 4. Total antioxidant activity of CME and different fractions of *F. hispida* fruits with standard ascorbic acid.

### 3.2.4. *Hydrogen peroxide scavenging assay*

The hydrogen peroxide scavenging activity of the extract is directly related with their reducing property. The extractives were capable of scavenging hydrogen peroxide in a concentration dependent manner. The results shown in Fig. 5, indicates that CME, EAF and AQF had almost the same scavenging activity (ranging from 70% to 72.5%) at concentration of 200 µg/mL. Moreover, compared with positive control, less activity was observed by NHF.

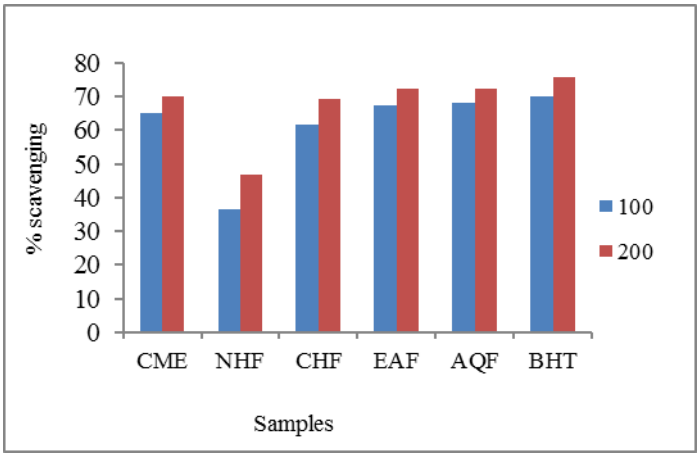


Fig. 5. Hydrogen peroxide scavenging activity of CME and different fractions with standard BHT.

## 4. Discussion

Free radicals are continuously generated and damaging body molecules. Thus can accelerate the tissue damage and lead to disease condition like cardiac diseases, neurodegenerative diseases, cancer, etc. [14]. A substance may act as an antioxidant due to its ability to reduce ROS, by donating hydrogen atoms [15]. The phenolic compounds in the extractives may contribute to antioxidant activity, because they have direct antioxidant properties due to the presence of hydroxyl groups, which can act as $H_2$ donors [16]. The total phenolic content of extract and fractions were evaluated by using Folin Ciocaltue Reagent and were expressed as gallic acid equivalents. Among them CME shows the highest content whereas CHF, EAF and AQF shows a considerable amount of phenolic content which may contribute to their antioxidant activity.

With phenols, flavonoids are the natural compounds responsible for antioxidant activity due to their inhibition of hydrolytic and oxidative enzymes [17,18]. In many studies, flavonoids have shown to possess innumerous biological activities from which the antioxidant properties are the best-described [19-21]. The result was calculated from the standard catechin calibration curve. Like phenolic content, CME shows the height content of flavonoids and other fraction shows a considerable content. From the phenolic and

flavonoid content, the antioxidant activity of the extract and fractions were anticipated and finally confirmed by several tests including DPPH radical scavenging test, reducing power capacity test, total antioxidant capacity test and hydrogen peroxide scavenging test.

The model method of scavenging the stable DPPH radical is widely used to evaluate the free radical scavenging ability of various samples [23]. DPPH is stable nitrogen centred free radical the colour of which changes from violet to yellow upon reduction by either the process of hydrogen or electron donation. Substances which are able to perform this reaction can be considered as antioxidants and, therefore, radical scavengers [24]. The test was carried out at concentration of 80 µg/mL. CME shows the highest DPPH radical scavenging activity with $IC_{50}$ value of 11.20 whereas standard BHT shows an $IC_{50}$ value of 5.10 µg/mL. The lowest scavenging activity was shown by NHF with $IC_{50}$ value of 62.5 µg/mL.

The reducing properties are generally associated with the presence of reductants, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom. The test is based mainly on the conversion of $Fe^{3+}$ into $Fe^{2+}$ and formation of Purl´s prussian blue. Test shows an increase in reducing power with a gradual increase in concentration of the extract and fractions. CME and different fractions show appreciable reducing activities.

The phosphomolybdenum method was used to evaluate the total antioxidant capacity and is based on the reduction of Mo (V1) to Mo (v) by the antioxidant compound and the formation of green phosphate/ Mo (v) complex. The high absorbance values indicated that the samples possessed significant antioxidant activity. In this test CHF and AQF shows highest absorbance value as well as antioxidant capacity.

Hydrogen peroxide itself is not very reactive but it can sometimes be toxic to cells, since it may give rise to hydroxyl radicals inside the cell [25,26]. Hydrogen peroxide is unique in that it can be converted to the highly damaging hydroxyl radical or be catalyzed and excreted harmlessly as water. CME, AQF and EAF show same scavenging of hydrogen peroxide radical which can prove their reducing property as well as antioxidant activity.

So the present results suggest that all the tested fruit extracts have moderate to potent antioxidant activity. Since a variety of constituents are present in the extracts studied, it becomes difficult to ascribe the antioxidant properties selectively to any one group of constituents without further studies, which are beyond the scope of this research.

## 5. Conclusion

It can be concluded that, the fruit extract of *Ficus hispida* shows a moderate to potent antioxidant activity and can be a potential candidate for further research to investigate the specific compound responsible for the activity which can be used as drug to treat diseases caused by free radicals.

**Acknowledgment**

This study was partially funded to M.S. Hossain by National Science and Technology Fellowship 2013-2014, Ministry of Science and Technology, Government of the People's Republic of Bangladesh (Ref. No. 39.012.002.01.03.019.2013-14).

**References**

1. B. Halliwell, Biochem. Soc. Symp. **61**, 73 (1995). http://dx.doi.org/10.1042/bss0610073
2. G. L. Squadriato and W. A. Pelor, Biol. Med. **25**, 392 (1998).
3. B. Uday, Curr. Sci. 77, 658 (1999).
4. P. Kanika, S.M.K. Pandit, and A. M. Bhagwat, Int. J. Pharm. Sci. Res. **3**, 188 (2011).
5. P. L. Ephraim, M. P. Helena, D. P. Alison, and A. N. Robert, J. Ethnopharmacol. **119**, 195 (2008). http://dx.doi.org/10.1016/j.jep.2008.06.025
6. M. Shahriar, M. S. Islam, S. Parvin, and S. Hoque, J. Sci. Res. **5(2)**, 393 (2013). http://dx.doi.org/10.3329/jsr.v5i2.11988
7. B. Pratumvinit, T. Srisapoomi, P. Worawattananon, N. Opartkiattikul, W. Jiratchariyakul, and T. Kummalue, J. Med. Plant. Res. **3**, 255 (2009).
8. V. L Singleton, J. A. Rossi, Am. J. Enolol. Viticul. **16**, 144 (1965).
9. V. W. Dewanto, K. K. Adom, and R. H. Liu, J. Agri. Food. Chem. **50**, 3010 (2002). http://dx.doi.org/10.1021/jf0255937
10. A. Braca, N. D. Tommasi, L. D. Bari, C. Pizza, M. Politi, and I. Morelli, J. Nat. Prod. **64**, 892 (2001). http://dx.doi.org/10.1021/np0100845
11. K. Oyaizu, Jpn. J. Nut. **44**, 307 (1986). http://dx.doi.org/10.5264/eiyogakuzashi.44.307
12. P. Prieto, M. Pineda, and M. Aguilar, Analyt. Biochem. **269**, 337 (1999). http://dx.doi.org/10.1006/abio.1999.4019
13. X. Y. Zhang, Beijing China Sci. Press. 275 (2000).
14. B. Halliwell and J. M. Gutteridge, Free Radicals in Biology and Medicine (Oxford University Press, 1998).
15. G. K. Jayaprakasha, R. P. Singh, and K. Sakariah, Food Chem. **73**, 285 (2001). http://dx.doi.org/10.1016/S0308-8146(00)00298-3
16. I. E. Dreosti, Nutrition **16**, 692 (2000). http://dx.doi.org/10.1016/S0899-9007(00)00304-X
17. C. A. Rice-Evans, N. J. Miller, and G. Paganga, Trends Plant Sci. **4**, 304 (1996).
18. G. Di Carlo, N. Mascolo, A. A. Izzo, and F. Capasso, Life Sci. **65**, 337 (1999). http://dx.doi.org/10.1016/S0024-3205(99)00120-4
19. E. J. Middleton and C. T. Kandaswami, Thera. Curr. Pharmacol. Rev. 52 (2000).
20. E. Rice, Curr. Med. Chem. **8**, 797 (2001). http://dx.doi.org/10.2174/0929867013373011
21. M. M. Silva, M. R. Santos, G. Caroco, R. Rocha, G. Justino, and L. Mira, Free Redical Res. **36**, 1219 (2002). http://dx.doi.org/10.1080/198-1071576021000016472
22. J. Nusrat, P. Shahnaj, D. Nandita, I. Saiful, and I. Ekramul, J. Acu. Med. **4**, 103 (2014).
23. I. Koleva, T. A. Beek, J. P. H. Linssen, D. Groot, and L. N. Evstatieva, Phytochem. Ana. **13**, 8 (2001). http://dx.doi.org/10.1002/pca.611
24. A. A. Dehpour, M. A. Ebrahimzadeh, S. F. Nabavi, and S. M. Nabavi, Gras. Aceites. **60**, 405 (2009). http://dx.doi.org/10.3989/gya.010109
25. B. Halliwell, Am. J. Med. **91**, 14 (1991). http://dx.doi.org/10.1016/S0002-9343(91)80142-9
26. S. Keser, S. Celik, S. Turkoglu, Ö. Yilmaz, and I. Turkoglu, Chem. J. **2(1)**, 14 (2012).