Kotchakorn Klangmanee et al /J. Pharm. Sci. & Res. Vol. 11(10), 2019, 3475-3480

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

A rapid phytochemical screening of the effective phenolic antioxidant agents using $FeCl_3$ reagent

Kotchakorn Klangmanee, Anan Athipornchai*
Department of Chemistry and Center of Excellence for Innovation in Chemistry,
Faculty of Science, Burapha University,
Bangsaen, Chonburi 20131 Thailand

Abstract

The beneficial effects of phenolic compounds have been attributed to their antioxidant activity which can protect the human body from free radicals which are believed to be the cause of several human diseases. A rapid instrument-free qualitative method to identify the effective phenolic antioxidant agents using ferric chloride solution has been developed. The differences in the colors of the phenol–Fe^{3+} complexes were used to classify the phenolic compounds. The dark green color of the solutions in different solvents was attributed to the *ortho*-dihydroxy and *ortho*-trihydroxy substitutions on the benzene ring. The UV-Vis spectroscopic technique revealed an absorbance peak of these complexes at 650-700 nm which cannot be used to classify the types of the phenolic antioxidant compounds. The above test can also be used to identify phenolic antioxidant agents which possess a benzoyl moiety on the benzene ring. The results demonstrated that the developed assay could be used as an alternative method to screening the active antioxidant agents in several in food, beverages and natural products samples.

Keywords: Phenolic antioxidant agents, Ferric chloride, Antioxidant activity.

INTRODUCTION

Phenolic compounds have been associated with the health benefits derived from consuming high levels of fruits, vegetables and herbs. These beneficial effects have been attributed to their antioxidant activity which can protect the human body from free radicals which are believed to be the cause of cardiovascular disease, cancer, inflammatory activity and neurodegenerative disorders [1-9]. Phenolic compounds have excellent antioxidant properties due to their ability to donate an electron or hydrogen from phenolic hydroxyl groups. In general the antioxidant activity of phenolic compounds depends on the numbers and substitution pattern of hydroxyl groups in the molecular structure [10]. The structure-activity relationships of the antioxidant activity of phenolic flavonoids isolated from *Pyrethrum tatsienense* were reported by Lin and co-worker [11]. The positions of C-3′and C-4′ in the B ring of flavonoids were all replaced by two hydroxyls, which lead to a significant increase of antioxidant activity. The results indicated that the catechol moiety is the most important active sites because it can be related to the double oxidation mechanisms. After the first hydrogen abstraction, the intramolecular hydrogen bond is formed by semi-quinonoid free radicals with a 4′ hydroxyl and further quinone is formed after the second hydrogen abstraction occurs at the 4′ hydroxyl [11-13]. Therefore, the structural requirement of phenolic compounds considered to be essential for effective antioxidant properties is the presence of an *ortho*-dihydroxy group or catechol moiety in the molecules. This work reports a simple and rapid specifically qualitative method to identify the effective phenolic antioxidant agents using ferric chloride solution.

MATERIAL AND METHODS

Chemicals and Instruments

The analytical grade organic solvents used in this study were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All standard phenolic compounds and ferric chloride ($FeCl_3$) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Colorimetric measurements were recorded using a UV–Visible Specord-210 Plus spectrophotometer.

Solution Preparation

All phenolic compounds were prepared to 10 mM in methanol. 1% Ferric chloride ($FeCl_3$) solution was prepared in six solvents; methanol, ethanol, deionized water, and acetate buffers pH 3.0, 3.6 and 4.0.

Experiment Procedure

A mixture of 1 mL 1% $FeCl_3$ and 1 mL of 10 mM methanolic solution of the phenolic compound was made in a test tube and shaken for 5 minutes. After incubation, the colors of phenolic–Fe^{3+} complexes were observed and visually compared with that of the reference solution. A solution of methanol and 1% $FeCl_3$ (1:1) was used as the reference solution. The above test was confirmed by a UV-Vis spectrophotometer with a full scan mode from 200-900 nm. The results of the phenolic–Fe^{3+} complexes data were compared with the corresponding reference solution.

RESULTS AND DISCUSSION

Five groups of sample phenolic compounds were used in the experiment. The phenolic compounds in the first group were studied to establish the effects of the different substitutions of the hydroxyl group on the aromatic ring. By mixing 1% $FeCl_3$ with a 10 mM methanolic solution of the phenolic compound in a test tube, shaking was continued for 5 minutes. The colors of the phenolic–Fe^{3+} complexes (Table 1) were observed and compared with that of the reference solution. From the results, the phenolic compound which has an *ortho*–dihydroxyl group on the aromatic ring (catechol) developed significant color changes to dark green in all solvents. On the other hand, phenolic compounds with only one or two hydroxyl

groups (*meta*- and *para*-substitution) on the benzene ring (phenol, resorcinol and hydroxyquinone) did not develop significant color changes when compared with the corresponding $FeCl_3$ reference solution (yellow solution).

In the second group, the effects of the position of the hydroxyl group in relation to the carboxyl moiety were studied. From the results (Table 2), only salicylic acid developed a dark green color change in all solvents while other hydroxybenzoic acids (*meta*- and *para*-) did not develop significant color changes when compared with the corresponding $FeCl_3$ reference solution. From the results of Groups 1 and 2, phenolic compounds with an *ortho*–dihydroxyl substitution on the benzene ring (catechol moiety) and an *ortho*–hydroxyl substitution on benzoic acid (benzoyl moiety) developed significant color changes. These functional groups of phenolics showed the important role of Fe^{3+} in chelating properties.[29,30] These results were confirmed by UV-Vis spectroscopic technique (Figure 1).

The effects of other substituents on the catechol moiety including formyl, carboxyl, and α,β–unsaturated carboxyl groups were studied (Group 3). The results in Table 3 showed that catechol developed a dark green color in all solvents (methanol, ethanol, water and acetate buffers). The same dark green coloration was also observed in the presence of the formyl, carboxyl, and α,β–unsaturated carboxyl groups and the catechol moiety in water and acetate buffers as the solvents. In the case of protocatechualdehyde, brilliant yellowish green and deep yellow green colors were observed when methanol and ethanol were used as solvents respectively. Protocatechuic acid also developed vivid yellowish green and light yellow green colors respectively while caffeic acid also developed strong yellow green and moderate yellow green colors respectively. All phenolic–Fe^{3+} complexes from Group 3 phenolic compounds gave absorbance at 650-700 nm (Figure 1) but these data were not sufficient to be used for the classification of these phenolic compounds.

Group 4 phenolic compounds such as *ortho*–vanillin, isovanillin and vanillin were studied. The results in Table 4 showed that *ortho*–vanillin (2–hydroxy–3–methoxybenzaldehyde) which has an *ortho*–hydroxyl relative to the formyl moiety developed a dark green color, different from those of vanillin and isovanillin. These results confirmed the importance of the *ortho*–hydroxyformyl group in Fe^{3+} chelation. From the UV-Vis spectroscopic data of these phenolic–Fe^{3+} complexes, only *ortho*–vanillin-Fe^{3+} complex gave a new absorbance peak at 540-600 nm (Figure 2).

TABLE 1: VISUALLY OBSERVATION THE COLOR IN GROUP 1

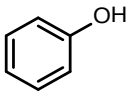
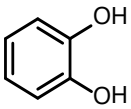
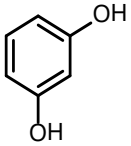
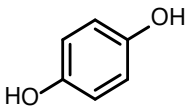
Phenolic compound	1% Ferric chloride reagent					
	MeOH	EtOH	H_2O	Acetate buffer pH 3.0	Acetate buffer pH 3.6	Acetate buffer pH 4.0
Control (MeOH)						
 Phenol						
 Catechol						
 Resorcinol						
 Hydroquinone						

TABLE 2: VISUALLY OBSERVATION THE COLOR IN GROUP 2

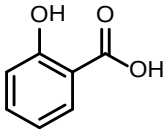
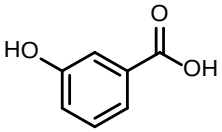
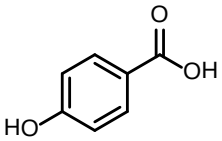
Phenolic compound	1% Ferric chloride reagent					
	MeOH	EtOH	H_2O	Acetate buffer pH 3.0	Acetate buffer pH 3.6	Acetate buffer pH 4.0
Control (MeOH)						
 Salicylic acid						
 *m*-Hydroxybenzoic acid						
 *p*-Hydroxybenzoic acid						

TABLE 3: VISUALLY OBSERVATION THE COLOR IN GROUP 3

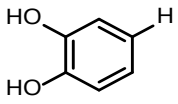
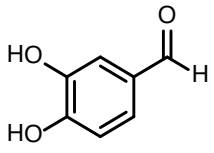
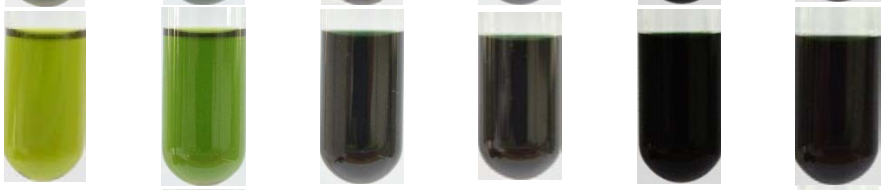
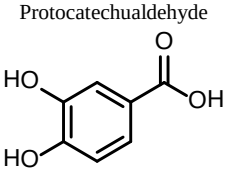
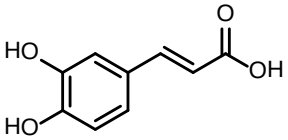
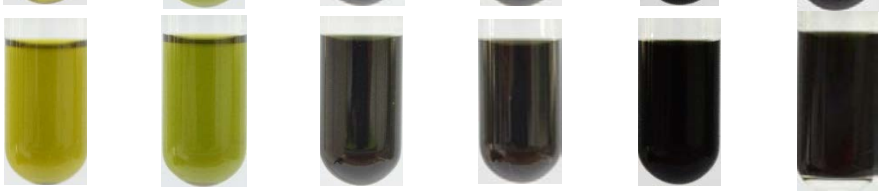
Phenolic compound	1% Ferric chloride reagent					
	MeOH	EtOH	H_2O	Acetate buffer pH 3.0	Acetate buffer pH 3.6	Acetate buffer pH 4.0
Control (MeOH)						
 Catechol						
 Protocatechualdehyde						
 Protocatechuic acid						
 Caffeic acid						

TABLE 4: VISUALLY OBSERVATION THE COLOR IN GROUP 4

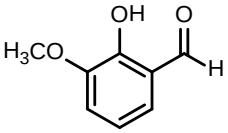
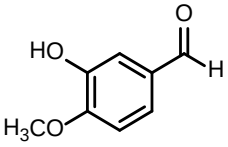
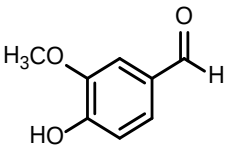
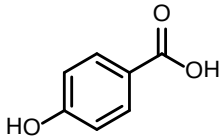
Phenolic compound	1% Ferric chloride reagent					
	MeOH	EtOH	H_2O	Acetate buffer pH 3.0	Acetate buffer pH 3.6	Acetate buffer pH 4.0
Control (MeOH)						
 *o*-Vanillin						
 Isovanillin						
 Vanillin						

TABLE 5: VISUALLY OBSERVATION THE COLOR IN GROUP 5

Phenolic compound	1% Ferric chloride reagent					
	MeOH	EtOH	H_2O	Acetate buffer pH 3.0	Acetate buffer pH 3.6	Acetate buffer pH 4.0
Control (MeOH)						
 *p*-Hydroxybenzoic acid						
Protocatechuic acid						
Gallic acid						

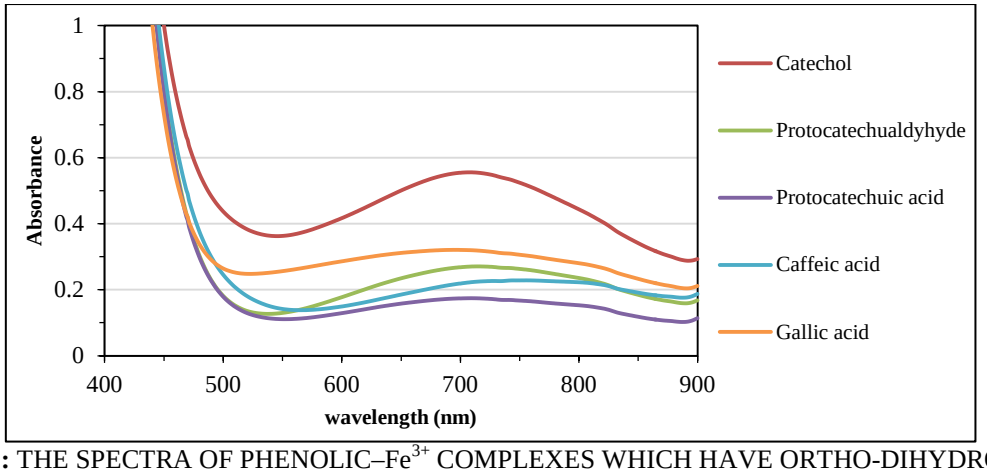


FIGURE 1: THE SPECTRA OF PHENOLIC–Fe^{3+} COMPLEXES WHICH HAVE ORTHO-DIHYDROXY AND ORTHO-TRIHYDROXY SUBSTITUTIONS ON AROMATIC RING

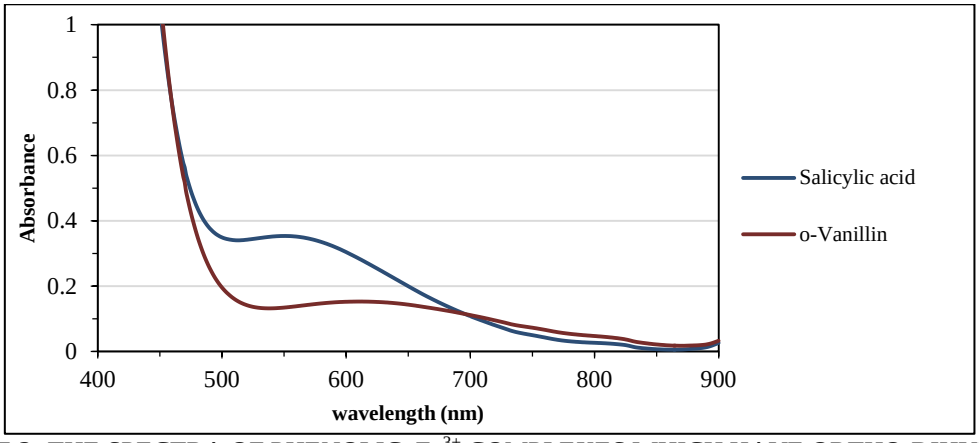


FIGURE 2: THE SPECTRA OF PHENOLIC–Fe^{3+} COMPLEXES WHICH HAVE ORTHO-DIHYDROXY SUBSTITUTION ON THE BENZOYL MOIETY

The influence of the number of hydroxyl groups on the colors of the phenolic–Fe^{3+} complexes was studied (Group 5). The results in Table 5 showed that *para*–hydroxybenzoic acid- Fe^{3+} complex gave the same color as that of the reference solution while protocatechuic acid and gallic acid gave dark green color solutions in water and acetate buffers. In addition, these phenolics developed yellowish green colors in ethanol and methanol, different from those of water and acetate buffers as the solvents. The UV-Vis spectral data of the protocatechuic acid-Fe^{3+} and gallic acid- Fe^{3+} complexes revealed the same new absorbance peak at 650-700 nm (Figure 1) which was not sufficient to be used to classify the types of the phenolic compounds. On the other hand, these phenolics were also classified by visually observation the colors in the methanol or ethanol as the solvents.

On the other hand, the UV-Vis spectroscopic technique could be used to classify and identify the type of phenolic compounds with two functional groups including *ortho*–hydroxybenzoyl and *ortho*–dihydroxyl (catechol moiety) groups. These results shown in the Figures 1 and 2.

CONCLUSION

A simple and rapid qualitative test method to identify the effective phenolic compounds based on visual observation of colors has been developed. Phenolic compounds bearing a catechol and an *ortho*-hydroxybenzoyl moiety developed most intense colors. These phenolic agents considered to be essential for effective antioxidant properties. Therefore, the developed assay could be used as an alternative method to screening the active antioxidant agents in several in food, beverages and natural products samples.

Acknowledgements

Authors to thank Natchanon Taprab, the Project for the Promotion of Science and Mathematics Talented Teachers (PSMT) Scholarship in the management of the Institute for the Promotion of Teaching Science and Technology (IPST), the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Department of Chemistry, and the Science Innovation Facility (SIF, SIF-IN-610551), Faculty of Science, Burapha University for providing research facilities. Special thanks to Dr. Ronald Beckett and Associate Professor Surachai Nimgirawath for his comments and grammatical suggestions.

Conflict of interest statement

We declare that we have no conflict of interest.

REFERENCES

1. Rice-Evans CA, Miller, N.J., Paganga, G. Antioxidant properties of phenolic compounds. Trends in Plant Science. 1997, 2: 152–159.
2. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sciences. 2004, 74: 2157–2184.
3. Moein MR, Moein S, Ahmadizadeh S. Radical scavenging and reducing power of *Salvia mirzayanii* subfractions. Molecules. 2008, 13: 2804–2813.
4. Sachdev S, Davies KJA. Production, detection, and adaptive responses to free radicals in exercise. Free Radical Biology and Medicine. 2008, 44: 215–223.
5. Bhuiyan MAR, Hoque MZ, Hossain SJ. Free radical scavenging activities of *Zizyphus mauritiana*. World Journal of Agricultural Sciences. 2009, 5: 318–322.
6. Mimica-Dukic N, Bugarin D, Grbović S, Mitić-Ćulafić D, Vuković-Gačić B, Orčić D. Essential oil of *Myrtus communis* L. as a potential antioxidant and antimutagenic agents. Molecules. 2010, 15: 2759–2770.
7. Sen S, Chakraborty R, Sridhar C, Reddy YSR, De B. Free radicals, antioxidants, diseases and phytomedicine: Current status and future prospect. International Journal of Pharmaceutical Sciences Review and Research. 2010, 3: 91–100.
8. Craft BD, Kerrihard AL, Amarowicz R, Pegg RB. Phenol-based antioxidants and the in vitro methods used for their assessment. Comprehensive Reviews in Food Science and Food Safety. 2012, 11: 148–173.
9. Okoh SO, Asekun OT, Familoni OB, Afolayan AJ. Antioxidant and free radical scavenging capacity of seed and shell essential oils extracted from *Abrus precatorius* (L). Antioxidants. 2014, 3: 278–287.
10. Bendary E, Francis RR, Ali HMG, Sarwat MI, Hady SE. Antioxidant and structure–activity relationships (SARs) of some phenolic and anilines compounds. Annals of Agricultural Science. 2013, 58: 173–181.
11. Lin CZ, Zhu CC, Hu M, Wu AZ, Bairu ZD, Kangsa SQ. Structure-activity relationships of antioxidant activity in vitro about flavonoids isolated from *Pyrethrum tatsienese*. Journal of Intercultural Ethnopharmacology. 2014, 3: 123–127.
12. Amić D, Davidović-Amić D, Bešlo D, Trinajstić N. Structure-radical scavenging activity relationships of flavonoids. Croatica Chemica Acta. 2003, 76: 55–61.
13. Rösch D, Bergmann M, Knorr D, Kroh LW. Structure-antioxidant efficiency relationships of phenolic compounds and their contribution to the antioxidant activity of sea buckthorn juice. Journal of Agricultural and Food Chemistry. 2003, 51: 4233–4239.
14.