Asian Journal of Chemistry; Vol. 23, No. 2 (2011), 676-680

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Antioxidant Activities of The Extracts of *Carya cathayensis Sarg.*

YUAN-HUI LIU[1], JIE-LI LV[2], KE YUAN[1,*] and PING YANG[3]

[1]A Nurturing Station for the State Key Laboratory of Subtropical Silviculture, Zhejiang Agriculture and Forestry University, Lin'an 311300, P.R. China
[2]College of Pharmacy, Xinxiang Medical University, Xinxiang 453003, P.R. China
[3]Zhejiang Provincial Key Lab for Modern Silvicultural Technology, Lin'an 311300, P.R. China

*Corresponding author: Tel: +86 571 63743607; E-mail: yuan_ke001@163.com

(*Received*: 4 March 2010; *Accepted*: 24 September 2010) AJC-9136

With the *Carya cathayensis Sarg.* produced in Lin'an, Zhejiang Province as the research object, this paper determined the content of the total flavonoids and the total phenolic in the *Carya cathayensis Sarg.* extracts of epicarp (CCEE), *Carya cathayensis Sarg.* extracts of leaf (CCEL) and the *Carya cathayensis Sarg.* extracts of tegument (CCET). On this basis, we also researched the ethanol extracts in different part of the *Carya cathayensis Sarg.*, 20 % methanol enrichment fraction of *Carya cathayensis Sarg.* extracts of epicarp (20 % MEF-CSEE), 40 % methanol enrichment fraction of *Carya cathayensis Sarg.* extracts of epicarp (40 %MEF-CSEE), 60 % methanol enrichment fraction of *Carya cathayensis Sarg.* extracts of epicarp (60 % MEF-CSEE) on DPPH radicals scavenging effect. The experiment result shows that the different part of the *Carya cathayensis Sarg.* all contain the flavonoids and the phenolic component, among which the 40 % MEF-CSEE contains the highest amount of total phenolics, the 20 % MEF-CSEE contains the highest amount of total flavonoids. Through the research of antioxidant activity we know that all the parts of the ethanol extracts in the *Carya cathayensis Sarg.* have the effect of scavenging free radicals, among which the antioxidant activity in the 40 % MEF-CSEE part is the strongest. Here, the main components of antioxidant activity should be the flavonoids and the phenolic components and we further know that there is a direct relationship between contents of the flavonoids and the phenolic and the antioxidant activity. The exocarp of *Carya cathayensis Sarg* is a under utilized resource, It might be considered as a potential plant source of antioxidants.The experiment result provided a scientific basis for the further development of its medical effects and the research for the *Carya cathayensis Sarg.* epicarp, its leaf and the tegument.

Key Words: *Carya cathayensis Sarg.*, Extract, Antioxidant activity.

INTRODUCTION

The *Carya cathayensis Sarg.* is Carya plant belonging to Juglandaceae family, mainly produced in the places of Lin'an, Chun'an and An'ji counties[1] in Zhejiang Province, People's Republic of China. When the *Carya cathayensis Sarg.* is not ripe, the outside of its hull is covered with a thick green skin. As it becomes increasingly mature and gets peeled, the outside skin will gradually turns brown-red. The amount of the *Carya cathayensis Sarg.* epicarp is very big when peeled, with nearly 1:1 between the weights of its fruit and its skin. If thrown away, it would be a great waste. So far, the *Carya cathayensis Sarg.* epicarp has not been used fully. According to the literature[2], the leaf, branch and epicarp of *Carya cathayensis Sarg.* all have medicinal uses, having the effect of anti-fever, detoxifcation, scavenging-dampness, intestine-cleaning, worm-killing, *etc*. In recent years, it is used in treating the digestive system diseases, like esophagus cancer and stomach carcinoma and has achieved some good effect[3].

The research of the modern pathology shows that free radicals have much to do with many different illnesses, such as atherosclerosis, liver-related diseases, diabetes, organism-aging and cancers[4], *etc*. The antioxidants can not only eliminate the free radicals to protect the organisms from their attacks, but also can prevent and delay the occurrences of cancers, atherosclerosis, senile dementia and the regressing deseases[5-7], so people begin to do more and more research into the anti-oxidants. These research results show that some synthetical antioxidants like dibutyl-hydroxy-toluene (DBHT), butyl-hydroxy-anisole (BHA) have the different degrees of poisonous and side-effect of causing cancer, so these antioxidants have been stopped to use medically in America and Japan[8,9]. With the stronger awareness of green environment consumption, the antioxidant of the natural plants has become the hot-spot of the medical research and the natural antioxidants are widely used in the industries of food, health-care products and cosmetics[10]. This research found that the *Carya cathayensis Sarg.* plants contain flavonoids, tannins and phenolic compo-nents, which have the medical effect of scavenging free radicals, dilating the blood vessels and improving the cerebral circulation[11], *etc*. Therefore, it is of much significance for us to develop the *Carya cathayensis Sarg.*, getting its antioxidant substances and

using its use of scavenging free radicals to effectively prohibit the oxidation reaction in the foods and organisms. At the same time, this research provided certain experimental basis for the development of the natural antioxidants. Through searches at the foreign medical literature, there have been no reports about the research on the antioxidant activity in extracts of different part of *Carya cathayensis Sarg.* This paper determined the content of toal flavonoids (TF) and toal phenolics (TP) in *Carya cathayensis Sarg.* extracts of epicarp (CCEE), *Carya cathayensis Sarg.* extracts of leaf (CCEL), the *Carya cathayensis Sarg.* extracts of tegument (CCET) and the different enrichment fractions of CCEE and at the same time determined their scavenging abilities on free radicals and thus provided a scientific basis for the further research and development the resource and efficacy of the *Carya cathayensis Sarg.* different part.

EXPERIMENTAL

Carya cathayensis Sarg. epicarp, *Carya cathayensis Sarg.* leaf, *Carya cathayensis Sarg.* tegument, picked from Lin'an, Zhejiang Province and all these samples are identified by Prof. Lu-huan Lou from Zhejiang Agriculture and Forestry University. Put the epicarps in the electric blast-drying box with constant temperature and drying at 40 ºC, while the leaves and tegument should be kept dry naturally and then make them into powder respectively and make them through the 40-hole sieve to be stored in the cold for future use.

Infinite M 200 Universal Microplate Spectrophotometer (Swiss Tecan company, Swiss) was used to measure the absorbance (DPPH) and UV-2102 PCS UV-vis spectrophotometer (Shanghai Unica Company Ltd., China) was used to measure the absorbance of chromogenic method (Rutin and gallic acid), A KQ2200DE ultrasonic cleaning instrument (Kunshan Shumei Ultrasonic Instrument Co., China) was used for extraction, A R201B rotary evaporator (Shanghai Shen Sheng Biotechnology Co., Ltd.) was used for concentration.

1,1-Diphenyl-2-picryl-hydrazyl (DPPH, Sigma-Aldrich Company), Diaion HP-20 adsorption resin (Mitsubishi Chemical Industry Ltd.), standard substance of rutin and gallic acid (Testing Station of China Leechdom and Biological Products) and other reagents were all of analytical grade.

Preparation of extracts

Preparation of CCEE,CCEL and CCET: Weigh respectively 25 g of the dry *Carya cathayensis Sarg.* epicarp, *Carya cathayensis Sarg.* leaf, *Carya cathayensis Sarg.* tegument, extract twice by using 95 % of the ethanol supersonically, each for 0.5 h. After concentration the extracts by vacuum into dry powder, weigh accurate 5 mg of each sample and then dissolved them with 95 % ethanol in 10 mL and then prepare them, respectively to be solutions of different concentration of 0.5, 0.25, 0.125, 0.0625, 0.03125 mg mL^{-1}.

Preparation of different enrichment fraction in CCEC: Dry the epicarp in the shade and put them in the container. Then extract twice by 70 % acetone soakage for 5 h each time. After concentration the extracts, dissolve them in water and put them through the big-hole resin column chromatography and elute successive them by water, methanol of 20, 40, 60 and 70 % acetone elution system. After concentration the elution solution become dry powder by vacuum and thus get each enrichment fraction. Weigh respectively 5 mg dry powder of different methanol part 20, 40 and 60 % and dissolve them by 95 % of the ethanol and turn them to measuring flask of 10 mL. And then prepare them respectively into the solutions of different concentration of 0.5, 0.25, 0.125, 0.0625, 0.03125 mg mL^{-1} and store them for future use.

Preparation of the storage solution of DPPH: Weigh precisely a certain amount of DPPH reagent in the small flask and dissolve it by 95 % ethanol and prepare them into the DPPH storage solution of 0.059 mg mL^{-1} and then put it into the deep freeze for later use.

Content determination of total flavonoids and total phenolics in extracts

Determinations of total flavonoids contents: This paper uses $NaNO_2$-$Al(NO_3)_3$ chromogenic method to determine the total flavonoids content in the ethanol extracts[12]. Weigh precise 12.8 mg standard sample of rutin with constant weight, then add 60 % ethanol solution and dissolve it into the measuring flask of 100 mL and shake it evenly to become the contrast solution of 0.128 mg mL^{-1}. Take respectively the above solution of standard sample of rutin 0, 1.0, 2.0, 3.0, 4.0, 5.0 mL and put them into the measuring flask of 10 mL and add 60 % ethanol solution to 5 mL, then add 0.3 mL 5 % $NaNO_2$ solution and shake evenly and lay it alone for 5 min and then add again, respectively 0.3 mL of 10 % $Al(NO_3)_3$ solution and shake evenly again. After 5 min, add again 4 mL of 1 mol/L NaOH solution and shake it, then dissolve it by 60 % ethanol solution and dissolve it to graduation. 10 min later, determine the absorbency at 510 nm by using the reagent as a blank reference. Use the concentration (X) of rutin as the abscissa, with absorbency (Y) as the vertical coordinate to make the standard curve and using the least square method to make the linear regression and get the regression equation $Y = 8.969X + 0.0208$, $R^2 = 0.9994$. Take respectively a certain amount of sample solution into the measuring flask and according to the above method of drawing the standard curve of tutin, determine its absorbency and calculate the total total flavonoids in the samples.

Determination of total phenolics contents: This paper uses Folin-Ciocalteu[13] method to determine the total phenolics content. Weigh precisely 12.4 mg standard sample of gallic acid. Using distilled water to dissolve to the measuring flask of 50 mL and make it into the contrast solution with the concentration of 0.248 mg mL^{-1}. Take respectively the contrast solution of 0, 0.10, 0.20, 0.30, 0.40, 0.50, 0.6 mL and put them into the 10 mL the measuring flask, add distilled water and dissolve it to 5 mL and add again 0.5 mL of Folin-Ciocalteu reagents and use 5 % waterless Na_2CO_3 solution and dissolve it to the measuring flask of 10 mL, leave them in the shade for 3 h and determine the absorbency at 765 nm by using the reagent as a blank reference. Use the concentration (X) of gallic acid as the abscissa, with absorbency (Y) as the vertical coordinate to make the standard curve and get the regression equation $Y = 81.098X + 0.1023$, $R^2 = 0.9989$. Take respectively a certain amount of sample solution into the measuring flask and according to the above method of drawing the standard curve of gallic acid, determine its absorbency and calculate the total total phenolics in the samples.

Determination of antioxidant activity

The determining principle[14]: DPPH• (1,1-diphenyl-2-picryl-hydrazyl) is a stable nitrogen- centered free radicals in the organic solvent. Its ethanol solution takes on the purple colour and has strong absorbing ability at 517 nm. When there exists the scavenging reagent of free radicals, the change of its absorbency and its received electrons have the quantitative relationship, because the partnership of the eliminate reagent and the DPPH single electrons makes the reduced absorbency and fade in colour at 517 nm. That is, the smaller the absorbency, the stronger the scavenging ability of the scavenging reagent of free radicals. So we can use spectrophotometer method to make a quantitative analysis.

Determination of DPPH absorption spectrum and determination wavelength: Every DPPH molecule can generate a stable nitrogenous free radicals which has the purple colour in the solution. When it reacts with the scavenging reagent containing only one electron of free radicals, it will generate a colourless substance and make the typical colour of the solution fade in colour. Through the whole-wavelength scanning of the organic free radicals solution of DPPH at the wave range between 280-680 nm. There are two character peaks (the wavelengths are 327 and 517 nm, respectively) in the organic free radicals solution of DPPH between this wave range. The research shows that after adding a small amount of ethanol extracts of *Carya cathayensis Sarg.* epicarp, leaf and tegument in the DPPH solution, the highest values of the two character peaks lower down and the peak value at 517 nm lowers the most. Hence, the absorption peak change at 517 nm wavelength is considered to be a scavenging ability on the free radicals of the antioxidants. We also choose 517 nm as the determination wavelength to raise the determining sensitivity. This is in agreement with the report that the peak value is at 517 nm.

Relationship between the light-absorption value of solution and its concentration: Take the DPPH solution and prepare it into new solutions with different concentration 0.236, 0.118, 0.059, 0.0295 and 0.01475 mg mL^{-1} and then determine each absorbance (A). Using (A) and DPPH concentration (C) to draw a plot, we can get the linear relationship equation of A and C: $A = 12.696C + 0.0448$, $R^2 = 0.9973$. The results show that there is a good linear relationship between DPPH absorbance and their concentration.

Stability experiment of the DPPH solution: In order to know the regular changing pattern with time of the absorption value (A_{517}) after adding different sample solution in the DPPH reaction system, so that we can determine the proper concentration and reaction time of DPPH solution. In this experiment, high concentration and low concentration of DPPH reaction system were determined. The high concentration system is to add respectively 0.5 mg mL^{-1} of 20 % MEF-CSEE, 40 % MEF-CSEE, 60 % MEF-CSEE, CCEE, CCEL, CCET into the DPPH (0.236 mg mL^{-1}) solution. The changing pattern with time of A_{517} can be seen in Fig. 1. The low concentration system is to add respectively 0.03125 mg mL^{-1} of 20 % MEF-CSEE, 40 % MEF-CSEE, 60 % MEF-CSEE, CCEE, CCEL, CCET into the DPPH (0.059 mg mL^{-1}) solution. The changing pattern with time of A_{517} can be seen in Fig. 2. From Fig. 1, we can know clearly that after adding 20 % MEF-CSEE and 40 % MEF-CSEE 40 %, A_{517} at DPPH reaction system becomes stable quickly. When adding the other solutions, A_{517} lowers quickly in the first 0.5 h, changes slowly between 0.5-1.0 min and there is still the tendency of falling down at 1.0 h, thus having too long a reaction time. From Fig. 2, we know that the reaction system becomes stable at 40 min no matter what sample solutions we add. So this experiment chooses the low concentration (0.059 mg mL^{-1}) solution of DPPH and 40 min as the reaction time.

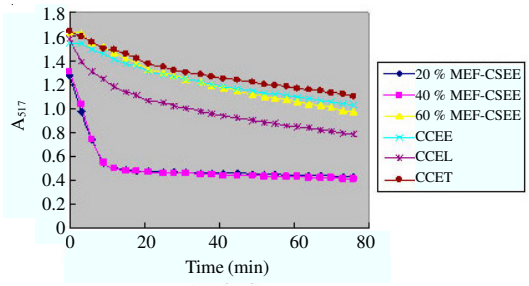

Fig. 1. High-concentration absorption spectra curve of DPPH after adding the sample solution

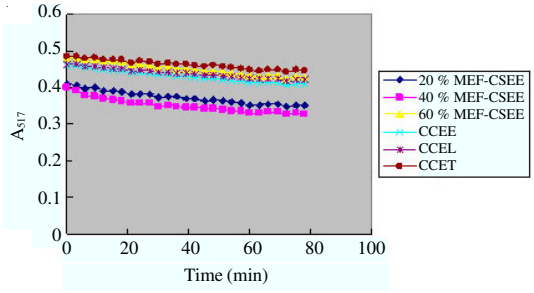

Fig. 2. Low-concentration absorption spectra curve of DPPH after adding the sample solution

RESULTS AND DISCUSSION

Content comparison between TF and TP of the extracts in different part of *Carya cathayensis Sarg.*: Many researches have shown that the flavonoids have obvious medical effect of anticancer, antioxidation[15], antiinflammation[16] and reducing cholesterol in blood and liver. The phenolic compounds are secondary metabolites widely existing in natural plants, which have many kinds of biological activity and play an important role in preventing cardiovascular and cerebrovascular diseases and in resisting aging process, scavenging free radicals, *etc*. In this experiment, we take the methods of $NaNO_2$-$Al(NO_3)_3$ and Folin-Ciocalteu respectively to determine the contents of TF and TP in different part extracts of the *Carya cathayensis Sarg.* produced in Lin'an county, Zhejiang Province and its experiment results are shown in Table-1.

TABLE-1
DETERMINATION RESULTS OF TF AND TP IN SAMPLE OF EXTRACTION

Sample of extraction	Content of TF (mg g^{-1})	Content of TP (mg g^{-1})
20 % MEF-CSEE	0.96	7.25
40 % MEF-CSEE	0.82	8.34
60 % MEF-CSEE	0.87	6.44
CCEE	0.78	5.83
CCEL	0.66	4.23
CCET	0.58	4.07

From Table-1, it is observed that there is a clear difference between the TF and TP in different samples. The TP content of CCEE and its different enrichment fraction are much higher than those in extracts of leaf and tegument. The TF contents of 20 % MEF-CSEE is higher than that in the other part. In the different enrichment part of ethanol extracts in *Carya cathayensis Sarg.* epicarp, TP content in the 40 % methanol enrichment part is relatively high and TP content in all the extracts are obviously higher than those of TF.

Scavenging effects on DPPH free radicals produced by extracts of *Carya cathayensis Sarg.* different part and the calculation of its IC_{50} value: The value of IC_{50} is the concentration of semi-restraining rate. It is the widely-used index to evaluate the free radicals scavenging effect and if the value is small, it shows that when reaching the scavenging rate of 50 %, we can use smaller concentration of free radicals scavenger to remove the free radicals and thus a better scavenging effect of free radicals.

Determination method: Use 96-hole micro-plate quantification method, MQ of the Universal Micro-plate Spectrophotometer. Add the sample solutions with different concentration and 50 µL of DPPH experimenting solution in the 96-hole micro-plate. After adding the samples, shake the solution for 30 s and kept at 37 ºC for 45 min, determined its absorbance value (Ap) under the wavelength of 517 nm. At the same time, determine blank absorbance (Ac) of sample without adding DPPH sample and determine its absorbance value (A_{max}) when adding DPPH but not contain samples (using 50 mL 95 % methanol instead sample). And then using the concentration of all the samples as the abscissa and the acquired scavenging rates (Y) as the vertical axis to make up a standard curve, we can get the regression equation. All the regression equations can be seen in Table-2. According to the regression equation, we can calculate IC_{50} value. The scavenging rate of different enrichment fraction of epicarp on DPPH and IC_{50} value can be seen in Table-3. The scavenging rate of extracts of *Carya cathayensis Sarg.* different fractions on DPPH and IC_{50} value can be seen in Table-4. The scavenging rate = 1-(Ap-Ac)/A_{max} × 100 %.

TABLE-2
REGRESSION EQUATION OF THE SCAVENGING EFFECTS ON DPPH FREE RADICALS IN ALL DIFFERENT EXTRACTS

Sample of extraction	Regression equation	R^2
CCEE	Y = 0.7548X + 0.1559	0.9729
CCEL	Y = 0.5882X + 0.1847	0.9538
CCET	Y = 0.4454X + 0.1551	0.9889
20 % MEF-CSEE	Y = 1.2462X + 0.1601	0.9703
40 % MEF-CSEE	Y = 1.2813X + 0.3223	0. 9858
60 % MEF-CSEE	Y = 0.7633X + 0.1585	0.9538

Influence of the sample with different concentration on scavenging rate of DPPH free radicals: Take the sample of the *Carya cathayensis Sarg.* epicarp ethanol extracts with different concentration and add them into DPPH solutions, respectively. Then determine the influence of different concentration extracts of *Carya cathayensis Sarg.* epicarp on scavenging rate of DPPH free radicals (Fig. 3).

TABLE-3
SCAVENGING RATE OF DIFFERENT ENRICHMENT FRACTION OF EPICARP ON DPPH AND IC_{50} VALUE

Concentration of sample (mg mL^{-1})	Rate of scavenging (%)		
	20 % MEF-CSEE	40 % MEF-CSEE	60 % MEF-CSEE
0.5	0.8036	0.9228	0.5446
0.25	0.4345	0.6822	0.3510
0.125	0.2802	0.6106	0.2243
0.0625	0.2994	0.3441	0.2160
0.03125	0.1901	0.2933	0.1960
IC_{50} (mg mL^{-1})	0.2727	0.1387	0.4474

TABLE-4
SCAVENGING RATE OF EXTRACTS OF *Carya Cathayensis Sarg.* DIFFERENT FRACTION ON DPPH AND IC_{50} VALUE

Concentration of sample (mg mL^{-1})	Rate of scavenging (%)		
	CCEE	CCEL	CCET
0.5	0.5167	0.4752	0.3754
0.25	0.3783	0.3273	0.2760
0.125	0.2636	0.2780	0.1993
0.0625	0.1767	0.2467	0.1875
0.03125	0.1754	0.1660	0.1698
IC_{50} (mg mL^{-1})	0.4559	0.5360	0.7751

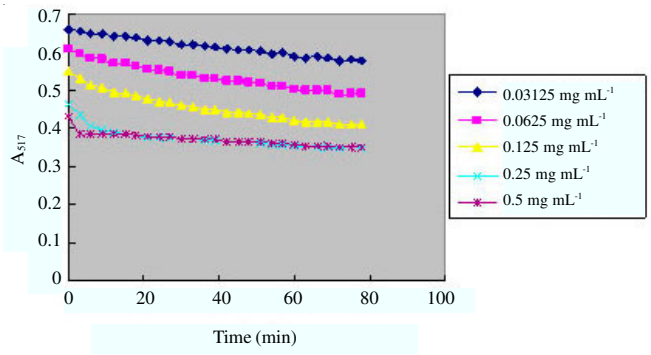

Fig. 3. Influence of the samples with different concentration on scavenging rate of DPPH free radicals

From Fig. 3, it is observed that when the sample are at the low concentration (0.03125 mg mL^{-1}), the reaction speed of scavenging free radicals is relatively slow. After 40 min of reaction, it still doesn't become stable and that the absorbency value still has the tendency of falling down. But when the sample are at higher concentration (0.5-0.25 mg mL^{-1}), the reaction speed of scavenging free radicals is faster, after 20-30 min of reaction, its absorbency value almost becomes stable. The result shows that the concentration of the sample has a strong influence on the scavenging speed on DPPH free radicals. When the concentration of sample is low, the reaction time for scavenging the free radicals is long, thus there is a slow changing speed. When the sample reach a certain concentration, the reaction time for scavenging the free radicals is short, thus there is a faster changing speed and its absorbency value can quickly become stable.

Conclusion

The different part of *Carya cathayensis Sarg.* have the biological activity substances like the flavonoids and the phenolic. Among these, different enrichment fractions of the ethanol extracts of *Carya cathayensis Sarg.* epicarp has a

obvious effect of scavenging DPPH free radicals and the scavenging ability can increase with enhancement of the sample concentration and it has a quantity-effect relationship for the scavenging rate of DPPH free radicals. Whereas the ethanol extracts of *Carya cathayensis Sarg.* different part has some effect for the scavenging of DPPH free radicals, but with unconspicuous effect. Its scavenging ability can increase with enhancement of the solution concentration and it also has a quantity-effect relationship for the scavenging rate of DPPH free radicals.

The scavenging ability of the different enrichment fractions of ethanol extracts of *Carya cathayensis Sarg.* epicarp for the DPPH free radicals is more than that of ethanol extracts of *Carya cathayensis Sarg.* different part, which shows that *Carya cathayensis Sarg.* epicarp has a strong ability for scavenging DPPH free radicals.

Through the experiment of the antioxidant activity, we know that different parts of *Carya cathayensis Sarg.* all have the components of the flavonoids and the phenolics. Among them, TP content in the 40 % MEF-CSEE is the highest, while TF content in 20 % MEF-CSEE is the highest. The ethanol extracts in different parts of *Carya cathayensis Sarg.* all have the effect of scavenging free radicals and the 40 % MEF-CSEE has the strongest antioxidant activity. Here, the flavonoids and the phenolics play the important part in the antioxidation process. And there is a direct relationship between the contents of the flavonoids and the phenolics and the antioxidant activity. Our country possess of rich resource of *Carya cathayensis Sarg.*, but its epicarps have not been utilized, which is really a great waste of natural resource. People will gradually pay attention to the fact that the epicarps of *Carya cathayensis Sarg.* have a good ability to scavenging free radicals. It can be used as a natural resource which can have the effect of antioxidation. The experimental result provides a scientific basis for the further researches of its medical effect and its development of *Carya cathayensis Sarg.* epicarp, leaves and tegument.

ACKNOWLEDGEMENTS

The authors are grateful to the Zhejiang Provincial Key Laboratory for Modern Silvicultural Technology, Zhejiang Agriculture and Forestry University for performing Universal Microplate Spectrophotometer.

REFERENCES

1. J.Q. Huang, W. Fang, Y.L. Ding and F.J. He, *J. Zhejiang Forestry College*, **19**, 227 (2002).
2. H. Y. Yao, J. C. Tang, A. L. Zhang and Y. P. Xu, *Acta Bot. Boreal-Occident*, **23**, 1650 (2003).
3. J.H. Shi, J.H. Wang, D. Che, Y.R. Song and X. Li, *Res. Inform. Tradit. Chin. Med.*, **7**, 7 (2005).
4. R.L. Rong, Free Radical Biology [M], Higher Education Press, Beijing, p. 46 (1992).
5. Q. Kong and K.O. Lillehei, *Medical Hypotheses*, **51**, 405 (1998).
6. A.C. Kaliora, G.Z. Dedoussis and H. Schmidt, *Atherosclerosis*, **12**, 187 (2006).
7. W.A. Pryor, *Free Radic. Biol. Med.*, **28**, 141 (2000).
8. Y.M. Pan, Y. Liang and H.S. Wang, *Food Chem.*, **88**, 347 (2004).
9. X.L. Li, L.X. Huang and X.L. Xu, *Food Sci.*, **27**, 930 (2006).
10. D.Y. Zheng and X.N. An, *J. Fujian College of Forestry*, **24**, 88 (2004).
11. T.B. Cui and M.Y. Shi, *Guangxi Agric. Biosci.*, **21**, 266 (2002).
12. X.F. Yang and Y.L. Zhu, *Spectrum Lab*, **23**, 812 (2006).
13. K. Dastmalchi, D.H. Damien and M. Kosar, *Food Sci. Technol.*, **40**, 239 (2007).
14. B.L. Cotellen and P. Catteauj, *Free Radic. Biol. Med*, **20**, 35 (1996).
15. P.K. Wilmsen, D.S. Spada and M. Salvador, *Agric. Food Chem.*, **53**, 4757 (2005).
16. A.E. Rotelli, T. Guardia and A.O. Juare, *Pharmacol. Res.*, **48**, 601 (2003).