ISSN 2211-3835
CN 10-1171/R

APSB

Acta Pharmaceutica Sinica B

Xuejun Wang, Zhaofang Bai, Xiaohe Xiao, et al.
T cell–associated immunoregulation and antiviral effect of oxymatrine in hydrodynamic injection HBV mouse model

2017
Vol.7 No.3

Chinese Pharmaceutical Association
Institute of Materia Medica,
Chinese Academy of Medical Sciences

http://www.elsevier.com/locate/apsb
http://www.yxxb.com.cn

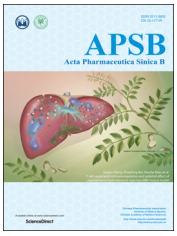
Available online at www.sciencedirect.com

ScienceDirect

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



Graphical Abstracts/Acta Pharmaceutica Sinica B, 7 (2017) iii–vii

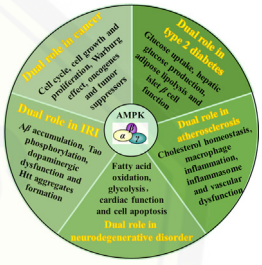
Reviews

Acta Pharmaceutica Sinica B, 7 (2017) 249

Dissecting the role of AMP-activated protein kinase in human diseases

Jin Li[a], Liping Zhong[b], Fengzhong Wang[a], Haibo Zhu[c,d,e]

[a]*Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences (CAAS), Beijing 100193, China*
[b]*Life Science College of Tarim University, Xinjiang 843300, China*
[c]*State Key Laboratory for Bioactive Substances and Functions of Natural Medicines, Beijing 100050, China*
[d]*Beijing Key Laboratory of New Drug Mechanisms and Pharmacological Evaluation Study, Beijing 100050, China*
[e]*Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China*



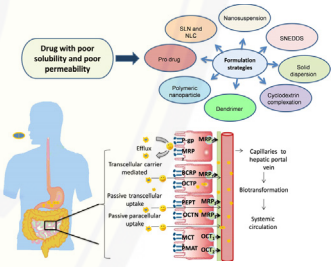
AMP-activated protein kinase (AMPK) has been identified as an attractive therapeutic target for cancer, type 2 diabetes, atherosclerosis, myocardial ischemia/reperfusion injury and neurodegenerative disease. This review summarizes and updates the paradoxical role of AMPK implicated in these human diseases and put forward the challenge encountered.

Acta Pharmaceutica Sinica B, 7 (2017) 260

Understanding *peroral* absorption: regulatory aspects and contemporary approaches to tackling solubility and permeability hurdles

Prachi B. Shekhawat, Varsha B. Pokharkar

Department of Pharmaceutics, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Pune 411038, India



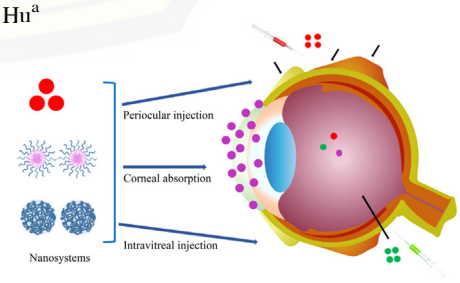
Bioavailability of a drug depends on physicochemical properties of the drug and physiological factors and dosage-related factors. Drug absorption occurs from the gut wall by passive diffusion, carrier-mediated uptake and paracellular transport. Several formulation strategies can be used to combat poor bioavailability.

Acta Pharmaceutica Sinica B, 7 (2017) 281

Nanotechnology-based strategies for treatment of ocular disease

Yuhua Weng[a,b], Juan Liu[b,c], Shubin Jin[b,c], Weisheng Guo[b,c], Xingjie Liang[b,c], Zhongbo Hu[a]

[a]*College of Materials Science and Opto-Electronic Technology, University of Chinese Academy of Sciences, Beijing 100049, China*
[b]*Laboratory of Controllable Nanopharmaceuticals, Chinese Academy of Sciences (CAS) Center for Excellence in Nanoscience; and CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology of China, Beijing 100190, China*
[c]*University of Chinese Academy of Sciences. Beijing 100049, China*



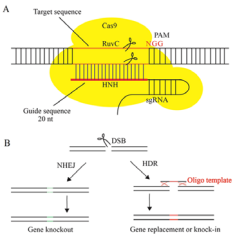
In this review, we highlights recent advances in development of nanotechnology-based systems, which could deliver both ocular drugs and gene to the eye *via* cornal absorption, periocular injection, and intravitreal injection, for ocular disease therapy and diagnosis. Both of nanosystems application and challenge in ophthalmology have been discussed and prospected.

Acta Pharmaceutica Sinica B, 7 (2017) 292

Application of CRISPR/Cas9 in plant biology

Xuan Liu, Surui Wu, Jiao Xu, Chun Sui, Jianhe Wei

Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences, Peking Union Medical College, Beijing 100193, China



The CRISPR/Cas (clustered regularly interspaced short palindromic repeats/CRISPR-associated proteins) system has received extensive attention owing to its easy manipulation, high efficiency, and wide application in gene mutation and transcriptional regulation in mammals and plants as a revolutionary technology. This review introduces the mechanism of the Type II CRISPR/Cas called CRISPR/Cas9, updates its recent advances in various applications in plants, and discusses its future prospects to provide an argument for its use in the study of medicinal plants.

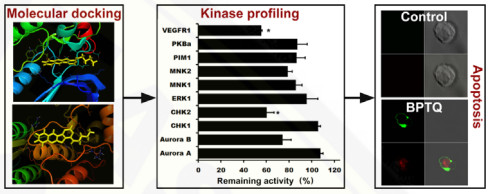
Original Articles

Acta Pharmaceutica Sinica B, 7 (2017) 303

Inhibition of protein kinases by anticancer DNA intercalator, 4-butylaminopyrimido[4′,5′:4,5]thieno(2,3-*b*)quinoline

Heggodu G. Rohit Kumar[a], Chethan S. Kumar[b], Hulihalli N. Kiran Kumar[a], Gopal M. Advi Rao[a]

[a]*Department of Biochemistry, Davangere University, Shivagangotri, Tholahunase, Davangere 577002, India*
[b]*SS Institute of Medical Sciences and Research Centre, Jnanashankara, Davangere 577005, India*



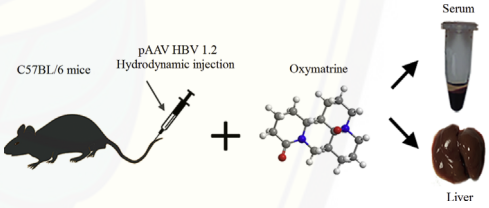
In this study, the ability of pyrimido[4′,5′:4,5]thieno(2,3-*b*)quinoline derivatives to interact with different protein kinases was analyzed by molecular docking. 4-Butylaminopyrimido[4′,5′:4,5]thieno(2,3-*b*)quinoline (BPTQ) showed a higher order of interaction compared to other derivatives, and effectively inhibited the VEGFR1 and CHK2. BPTQ also induced apoptosis in cancer cells.

Acta Pharmaceutica Sinica B, 7 (2017) 311

T cell–associated immunoregulation and antiviral effect of oxymatrine in hydrodynamic injection HBV mouse model

Xiuxiu Sang[a,b], Ruilin Wang[c], Yanzhong Han[a,b], Cong'en Zhang[b], Honghui Shen[b], Zhirui Yang[b], Yin Xiong[e], Huimin Liu[a,b], Shijing Liu[c] Ruisheng Li[f], Ruichuang Yang[f], Jiabo Wang[b], Xuejun Wang[d], Zhaofang Bai[b], Xiaohe Xiao[c]

[a]*Chengde Medical College, Chengde 067000, China*
[b]*China Military Institute of Chinese Medicine, 302 Military Hospital, Beijing 100039, China*
[c]*Integrative Medical Center, 302 Military Hospital, Beijing 100039, China*
[d]*Department of Biotechnology, Beijing Institute of Radiation Medicine, Beijing 100850, China*
[e]*Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming 650500, China*
[f]*Research Center for Clinical & Translational Medicine, 302 Military Hospital, Beijing 100039, China*



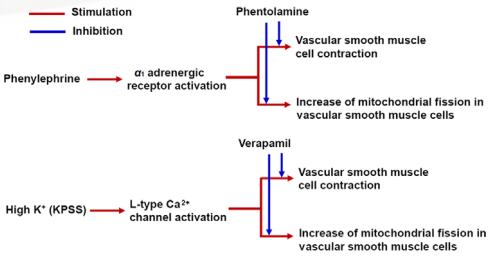
In the present study, the antiviral effect of oxymatrine (OMT) was investigated in an immunocompetent mouse model. Our findings demonstrate the beneficial effects of OMT on immunologic enhancement and controlling hepatitis B virus (HBV) antigens, offering a good antiviral therapeutic candidate to HBV infection.

Acta Pharmaceutica Sinica B, 7 (2017) 319

Arterial relaxation is coupled to inhibition of mitochondrial fission in arterial smooth muscle cells: comparison of vasorelaxant effects of verapamil and phentolamine

Jing Jin[a,b], Xin Shen[a,b], Yu Tai[a,b], Shanliang Li[a,b], Mingyu Liu[a,b], Changlin Zhen[a,b], Xiuchen Xuan[a,b], Xiyue Zhang[a,b], Nan Hu[a,b], Xinzi Zhang[a,b], Deli Dong[a,b]

[a]*Department of Pharmacology (the State-Province Key Laboratories of Biomedicine-Pharmaceutics of China, Key Laboratory of Cardiovascular Research, Ministry of Education), College of Pharmacy, Harbin Medical University, Harbin 150086, China*
[b]*Translational Medicine Research and Cooperation Center of Northern China, Heilongjiang Academy of Medical Sciences, Harbin Medical University, Harbin 150086, China*



The effects of verapamil and phentolamine in PE- and KPSS-induced vasoconstriction models demonstrated that arterial relaxation was coupled to inhibition of mitochondrial fission in arterial smooth muscle cells.

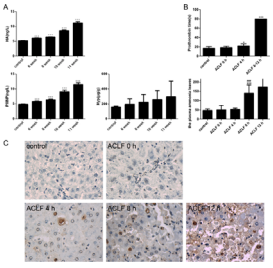
Acta Pharmaceutica Sinica B, 7 (2017) 326

Establishment of a new acute-on-chronic liver failure model

Fangfang Li[a], Luyang Miao[b], Hua Sun[a], Yuyang Zhang[b], Xiuqi Bao[a], Dan Zhang[a]

[a]*State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China*
[b]*Shenyang Pharmaceutical University, Shenyang 110016, China*

A rat acute-on-chronic liver failure (ACLF) model induced by porcine serum and D-galactosamine/lipopolysaccharide was established and underlying mechanisms of ACLF development were explored.



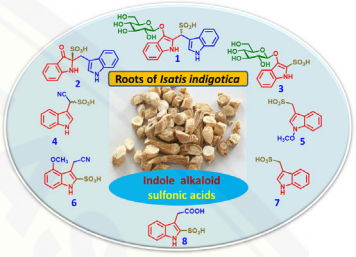
Acta Pharmaceutica Sinica B, 7 (2017) 334

Indole alkaloid sulfonic acids from an aqueous extract of *Isatis indigotica* roots and their antiviral activity

Lingjie Meng, Qinglan Guo, Yufeng Liu, Minghua Chen, Yuhuan Li, Jiandong Jiang, Jiangong Shi

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

Six new indole alkaloid sulfonic acids (**1**–**6**), together with two analogues (**7** and **8**) that were previously reported as synthetic products, were isolated from an aqueous extract of the *Isatis indigotica* root. Their structures were determined by spectroscopic data analysis, combined with enzyme hydrolysis and comparison of experimental circular dichroism and calculated electronic circular dichroism spectra. In the preliminary assay, compounds **2** and **4** showed antiviral activity against Coxsackie virus B3 and influenza virus A/Hanfang/359/95 (H3N2), respectively.



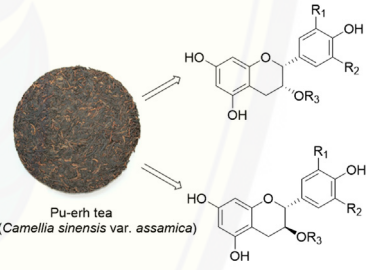
Acta Pharmaceutica Sinica B, 7 (2017) 342

Flavanols from the *Camellia sinensis* var. *assamica* and their hypoglycemic and hypolipidemic activities

Xin Wang, Quan Liu, Hongbo Zhu, Hongqing Wang, Jie Kang, Zhufang Shen, Ruoyun Chen

State Key Laboratory of Bioactive Substance and Function of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

One new flavanol, named (–)-epicatechin-3-*O*-(*Z*)-coumarate (**1**), and 16 known analogs (**2**–**17**) were isolated from the aqueous extract of the pu-erh tea. Compound **15** showed moderate inhibitory effect on sucrase with an IC_{50} value of 32.5 μmol/L and significant inhibitory effect on maltase *in vitro* with an IC_{50} value of 1.3 μmol/L; compounds **8**, **10**, **11** and **15** displayed moderate activity against a lipase with IC_{50} values of 16.0, 13.6, 19.8, and 13.3 μmol/L, respectively.



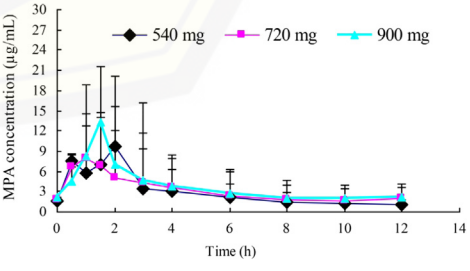
Acta Pharmaceutica Sinica B, 7 (2017) 347

Nonlinear relationship between enteric-coated mycophenolate sodium dose and mycophenolic acid exposure in Han kidney transplantation recipients

Jun Zhang[a], Mengmeng Jia[a], Lihua Zuo[a], Na Li[a], Yonggang Luo[b], Zhi Sun[a], Xiaojian Zhang[a], Zhenfeng Zhu[a]

[a]*Department of Pharmacy, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China*
[b]*Department of Integrated Intensive Care Unit, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China*

This research investigated the pharmacokinetics (PK) of enteric-coated mycophenolate sodium (EC-MPS) by quantification of the active metabolite of mycophenolic acid (MPA) after multiple escalating oral doses in Han kidney transplant recipients. Nonlinear PK properties were discovered at doses ranging from 540 to 900 mg after multiple-dose administration. This may have important contributions to clinical practice.



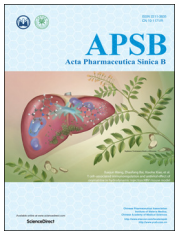
Short Communications

Acta Pharmaceutica Sinica B 2017;7(3):395–400

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



SHORT COMMUNICATION

Scanometry as microplate reader for high throughput method based on DPPH dry reagent for antioxidant assay

Mochammad Amrun Hidayat, Aulia Fitri, Bambang Kuswandi*

Chemo and Biosensor Group, Faculty of Pharmacy, University of Jember, Jember 68121, Indonesia

Received 10 November 2016; revised 2 January 2017; accepted 27 January 2017

KEY WORDS

Scanometry;
High throughput;
DPPH;
Optical sensor;
Antioxidant

Abstract The stable chromogenic radical 1,1′-diphenyl-2-picrylhydrazyl (DPPH) solution was immobilized on the microwell plate as dry reagent to construct a simple antioxidant sensor. Then, a regular flatbed scanner was used as microplate reader to obtain analytical parameters for antioxidant assay using one-shot optical sensors as scanometry technique. Variables affecting the acquisition of the images were optimized and the analytical parameters are obtained from an area of the sensing zone inside microwell using the average luminosity of the sensing zone captured as the mean of red, green, and blue (RGB) value using ImageJ® program. By using this RGB value as sensor response, it is possible to determine antioxidant capacity in the range 1–25 ppm as gallic acid equivalent (GAE) with the response time of 9 min. The reproducibility of sensor was good (RSD < 1%) with recovery at 93%–96%. The antioxidant sensor was applied to the plant extracts, such as sappan wood and Turmeric Rhizome. The results are good when compared to the same procedure using a UV/Vis spectrophotometer.

*Corresponding author. Tel./fax: +62 331324736.
E-mail address: b_kuswandi.farmasi@unej.ac.id (Bambang Kuswandi).

Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

http://dx.doi.org/10.1016/j.apsb.2017.02.001

1. Introduction

Antioxidant capacity is a broadly used term as a parameter to characterize different substances and food samples with the ability of scavenging or neutralizing free radicals. This capacity is associated to the presence of compounds capable of protecting a biological system against harmful oxidation[1]. There are several synthetic radicals, such as 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 1,1′-diphenyl-2-picrylhydrazyl (DPPH), and *N*,*N*-dimethyl-*p*-phenylendiamine (DMPD), employed for determining antioxidant capacity of various samples[2–5]. The DPPH method is one of the most frequently used to assess the ability of compounds as free radical scavengers or hydrogen donors, and to evaluate the antioxidant capacity of food samples. The method was introduced by Blois[5], and improved by some authors for measuring antioxidant activity of numerous substances and determining antioxidant capacity of various food and plant samples[3,6,7]. In brief, this method is based on the reduction of the chromogenic DPPH radical by an antioxidant, such as in a plant extract, which causes the radical to change color, and this change can be monitored and quantified using spectrophotometer at 515–520 nm[8]. The radical DPPH is stable and does not have to be generated for hours before the analysis, as in other radical scavenging assays[9].

In conventional spectrophotometric method, a large volume (1.0–5.0 mL) of freshly prepared DPPH solution in a cuvette is required[10–15]. In order to reduce large amount of DPPH solution, a microwell plate can be used in the assay, as it was done by Lee et al.[16]. Lately, it was known that no significant different parameters (repeatability, reproducibility, percentage recovery) were observed between microwell and cuvette-based method within intra-laboratory validation[9]. The DPPH microwell-based method is continuously used then by some authors as high throughput screening for antioxidant capacity[17–20]. To make the assay simpler and faster as high throughput screening, the microwell was used as solid support for DPPH in dry reagent format as described elsewhere[19]. By adding methanol or ethanol into the wells, the system can be used for high throughput antioxidant screening of various samples (banana, green tea, pink guava, and honey dew), and it was shown that the results were in good agreement with that of conventional DPPH-microwell platform. However, this method is not suitable for field analysis since specialized, cost-expensive instrument such as microplate reader (spectrophotometer) is required to conduct the assay. To overcome this limitation, flatbed scanner can be used as a microplate reader to obtain digital color image which can be further analyzed quantitatively.

Recently, using scanner as scanometric technique gained its popularity due to its application in various chemical and biochemical assays. The scanometric technique which relies on either a light scattering instrument or flatbed scanner coupled with various probes or sensors can be used for the detection of bacteria, dopamine, magnesium ions, lead ions, thrombin, and mercury ions[21–26]. Being subclass of colorimetry, scanometry uses a gray scale as opposed to the various color space. The gray intensity, typically a result of silver enhancement is the measured signal in scanometry[27]. Scanometry was used to characterize optical feature of various dyes, such as disperse orange 3, methyl orange, fluorescein, eosin Y, rhodamine B, trypan blue, prussian blue, malachite green, methylene blue, chlorophyll b, and DPPH, in a microwell plate[28]. As the color intensity in red, green, and blue (RGB) value was mathematically converted to RGB-resolved absorbance, it was shown that flatbed scanner was comparable with spectrophotometer.

Here, we propose a scanometric technique for conducting DPPH assay. In this work, DPPH solution was immobilized on a 96 microwell as dry reagent to construct antioxidant sensor. Then, a regular flatbed scanner was used as microplate reader to evaluate antioxidant capacity of several plant extracts. When it was compared with other DPPH-based sensors[29,30], the proposed sensor is simpler, since in other optical sensors the DPPH solution has to be immobilized in polymer (e.g., PVA, PVC) in a long time chemical synthetic reaction to construct the sensor. Moreover, the sensors have to be transferred into a cuvette prior to antioxidant assay in UV/Vis spectrophotometer which made the afore mentioned methods[29,30] need longer procedure than the proposed method. As regular flatbed scanner was employed for obtaining sensor response, the developed method is extremely cheaper than microplate reader (ELISA reader) or UV/Vis spectrophotometer. In the microplate reader, the absorbance of DPPH after antioxidant addition was measured, while in the proposed scanometric technique, the color intensity (mean RGB) of DPPH after antioxidant addition has been measured for determining antioxidant capacity. Hence, it is obvious that, using scanometric technique, no need the samples to be transparence, as in our previous work[19]. In this paper, even non-transparence or opaque sample can be used, since the different analytical response and different instrument were used as a reader for antioxidant sensor response. In addition, we also used less reagent concentration (125 μg/L) compared to the previous one (150 μg/mL)[19]. Thus, it make reagent used more efficient, as it is used in one shot measurement. Furthermore, the developed method can be suitable for field analysis and/or in remote area, where medicinal plant extracts can be screened for their antioxidant capacity on site.

2. Materials and methods

2.1. Chemicals

Gallic acid (GA) and DPPH were obtained from Sigma–Aldrich (USA). Methanol was purchased from Merck (Germany). All chemicals were of analytical reagent grade.

2.2. Herbal samples

Herbal samples used in this work, *i.e.*, Sappan wood (*Caesalpinia sappan* L.), and Turmeric Rhizome (*Curcuma domestica* Val.), were purchased from local market of Jember, East Java. All herbal samples were authenticated and deposited at Pharmacognosy Laboratory, Faculty of Pharmacy, University of Jember, Indonesia. Herbal samples were air dried and powdered until their particle size freely passed through sieve 100 mesh.

2.3. Sensor fabrication

Sensor fabrication was done as in our previous work[19] with slight modification. A solution of DPPH in methanol at various concentrations (50, 100, 125, and 150 ppm) were transferred (200 μL) into 96 microwell plate as matrix sensor. The solvent

was then evaporated under mild condition at room temperature to construct antioxidant sensor based on DPPH. Afterward, the antioxidant sensor was ready to be used. For long term used and avoid photodecomposition of DPPH, the antioxidant sensor was sealed with aluminum foil.

2.4. Extraction of herbal samples

All herbal powder were extracted using method reported by Ningsih et al.[31] with slight modification. The dried herbal powder (10 g) was extracted with methanol (300 mL) for an hour at 30 °C using an ultrasonicator bath (Elmasonic S180H, Germany). The extracts were then filtered through Whatman filter paper No. 1 (USA) by using vacuum funnel. Afterward, the filtrates were separated and stored in well-capped tubes prior to antioxidant assay at room temperature.

2.5. Optimization study

In order to find the optimum DPPH concentration that give best calibration curve in term of linear correlation and the slope, GA was used as standard solution, since it is classified as intermediate based on its antioxidant kinetic, which is more suitable for the optimization study than that of antioxidants with rapid kinetic (ascorbic acid) or slow kinetic (ferulic acid)[7]. GA solution was added in different concentration (1, 5, 10, 15, 20, and 25 ppm) to each well 150 μL of methanol and 50 μL of GA solution were added. After 9 min, the color change of each well was quantified using the measurement procedure, where the calibration curve can be constructed.

2.6. Measurement procedure

The color change of sensor was captured using flatbed scanner (Canoscan, LIDE 110, Japan) in which color photo mode with resolution at 300 dpi was set for image scanning. The color intensity was then analyzed with ImageJ® program for Windows®. The color intensity of sensors (ΔRGB) was obtained by subtracting the intensity value of mean RGB of the application of sample from the intensity value of mean RGB without the sample. All of the experiments were carried out in triplicate measurements.

3. Results and discussion

3.1. The antioxidant sensor

The fabrication of antioxidant sensor based on DPPH was performed by surface coating on the bottom of the 96 microwell plate with the 200 μL DPPH solution (125 ppm) as it can be seen in our previous work[19]. The process was very simple, by just evaporate the DPPH solvent in 40 min, then leaving DPPH dry reagent as immobilized reagent on the bottom of each well. After this immobilization process, the antioxidant sensor was ready to be used as antioxidant sensor.

3.2. Sensing scheme

In this sensing scheme, DPPH as radicals is used as the basis for optical detection in the assessment of antioxidant activity[5–7]. This is due to the fact that DPPH has been widely used for the determination of antioxidant activity of phytochemicals, such as flavonoids and polyphenols[18,19]. In this assay, the purple of chromogen radical ($DPPH^{\bullet}$) is reduced by antioxidant (*e.g.*, GA) to the corresponding pale yellow hydrazine (DPPH-H). Reduction of the chromogenic purple radical ($DPPH^{\bullet}$) by hydrogen-donating antioxidant is monitored by capturing its color change to the pale yellow of the antioxidant sensor. The sensing mechanism is shown in Fig. 1, where AH is donor molecule, and $A^{\bullet}$ is free radical produced.

This DPPH as chromogenic radical reagent was chosen, since it is widely used in well-established procedures for the antioxidants assay, together with sufficient chemical stability in a dry reagent format, avoiding problems associated with leaching[19], as it will dissolve again when sample introduced. In addition, there is no need for regeneration of radicals *in situ*, which makes the antioxidant sensor simpler and more practical.

3.3. Optimization of DPPH concentration

The antioxidant sensor works as the color change from purple to yellow by introducing antioxidant such as GA; therefore, the optimum color change of the sensor would be depend on the DPPH concentration. In order to optimize the DPPH concentration in term of its calibration curve, various concentration of DPPH have been immobilized as antioxidant sensor and tested toward the increased concentration of GA (1–25 ppm). After scanometric measurements, the calibration curves of GA *vs*. color intensity (ΔRGB) were made for each DPPH concentration (50, 100, 125 and 150 ppm) tested as given in Table 1. The antioxidant sensor with 125 ppm DPPH was selected as optimum DPPH concentration in term of its linear correlation and slope. The slope and coefficient correlations (r) of the 125 ppm DPPH have been found to

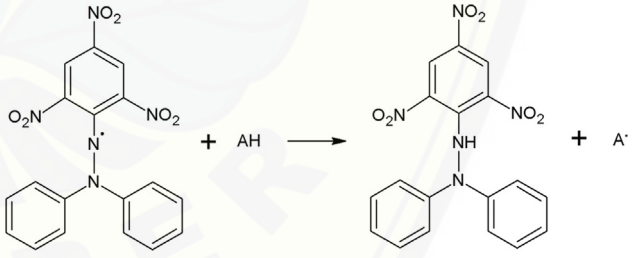


Figure 1 The reaction mechanism of DPPH radical with antioxidant (AH).

Table 1 The calibration curves of gallic acid (1, 5, 10, 15, 20, and 25 ppm) towards various DPPH concentrations for the antioxidant sensor ($n=3$).

DPPH conc. (ppm)	r^2	Equation of calibration curve	Slope
50	0.9873	$y=3.2809x+0.4224$	3.2809
100	0.9911	$y=3.3118x-1.1945$	3.3118
125	0.9922	$y=3.4257x-3.3301$	3.4257
150	0.9912	$y=3.2353x-1.3858$	3.2353

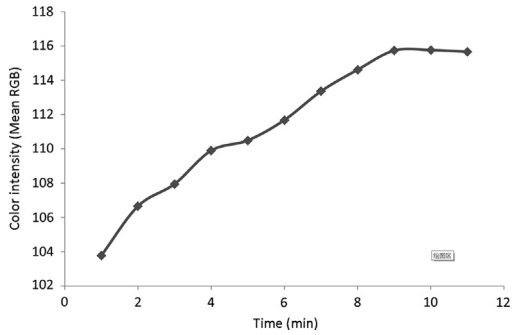


Figure 2 Response time of the antioxidant sensor towards gallic acid (15 ppm).

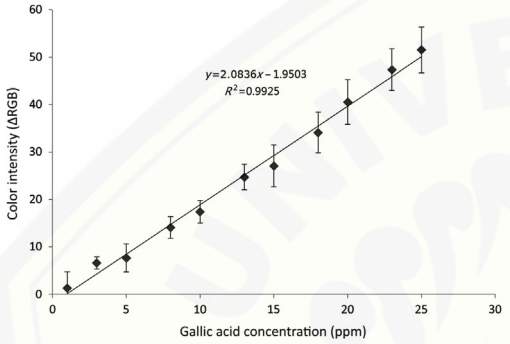


Figure 3 Sensor response towards gallic acid (1–28 ppm), $n=3$.

be 3.426 and 0.996, respectively, which is higher compared to other DPPH concentrations (50, 100 and 150 ppm) as it can be seen in Table 1. Therefore, the antioxidant sensor with DPPH concentration of 125 ppm was used for further measurements.

3.4. Response time

The response time of antioxidant sensor was investigated using 15 ppm GA solution. The response of sensor was recorded at every 2 min until stable color intensity value was obtained. The response time of sensor was observed at 9 min. After this period, the sensor gave stable response as shown in Fig. 2. Therefore, this response times was used for further measurements.

It can be seen in Fig. 2 that the developed sensor has response time at 9 min which is faster than that of other DPPH-based optical sensor[30], while the similar GA concentration was applied (±1 mmol/L). Moreover, our sensor takes a short operating time in the term of time of analysis, while DPPH absorbance was typically read at 30 min after addition of tested samples in other microwell-based methods[18,19]. Similar time of analysis (10 min) was also reported with observed in other microwell-based method[20]; however, the method was suffered from co-solvent and/or buffer addition. Hence, it can be drawn that our developed method is simpler and faster than afore mentioned methods.

3.5. Antioxidant determination

GA was used as standard for antioxidant assay in this work. The calibration curve was constructed by plotting concentration of GA *vs*. sensor response as ΔRGB value as depicted in Fig. 3. It can be seen that the linear range of the sensor response is in the range of 1–25 ppm with the coefficient correlation (r) of 0.996. The detection limit (LOD) of the antioxidant sensor, which is defined as the concentration of sample yielding a signal equal to the blank signal plus three times of its standard deviation[32], was calculated to be 0.762 ppm. The reproducibility of the sensor response was tested toward 10 ppm of GA solution as given in Table 2, the precision of sensor can be observed. It can be seen that RSD value was lower than 2%, indicating the developed method has good precision[32].

Table 2 The antioxidant sensor response towards 10 ppm of gallic acid ($n=6$).

Sample	RGB	ΔRGB
Blank	43.62	–
1	71.724	28.104
2	70.814	27.194
3	71.237	27.617
4	71.603	27.983
5	71.712	28.092
6	71.301	27.681
Mean		27.779
RSD (%)		1.271

3.6. Recovery

The percentage recovery values for the accuracy of the sensor on the determination of antioxidant activity are performed by adding 30%, 45% and 60% to initial 12, 13, and 15 ppm of GA in sample simulation. The mean of percentage recovery (%) was calculated to be 91%–96% as it can be seen in Table 3. This results were in good agreement with theoretical recovery values (%) for unit concentration of 10 ppm (80%–110%)[33]. Therefore, the developed method indicated a good accuracy for antioxidant measurements.

3.7. Sensor stability

In this work, various storage conditions were applied for stability test of the developed sensor. The sensor was stored separately in a well-capped cabinet at room (30 °C) and chiller (4 °C) temperature. Then, the sensor response towards GA (1–25 ppm) was

Table 3 Recovery study of gallic acid (%) in simulated sample using the antioxidant sensor ($n=3$).

Sample	Found concentration (ppm)	Recovery (%)
Initial (10 ppm)	10.135±0.008	101.350±0.084
30% addition	2.546±0.075	83.747±2.397
45% addition	4.417±0.063	96.840±1.462
60% addition	6.115±0.365	100.551±5.924

observed in every week, until 10% decrease of initial response was obtained. After two weeks, sensor response was observed to decrease more than 10% when sensor was stored at room temperature as it can be seen in Table 4. Hence, it can be noted that stability of sensor was only maintained during one week storage at room temperature. This finding seemed to be found in other DPPH-based optical sensor, in which the absorbance of DPPH-polymer film was completely loss within one week storage at room temperature[30].

Table 4 The decrease of sensor response towards gallic acid (1–25 ppm) after it was stored in room temperature (30 °C).

Gallic acid	Storage time (week)				
conc. (ppm)	1	2	3	4	5
1	9.16	12.93	15.45	17.22	19.14
5	2.19	11.02	13.34	13.50	14.59
13	7.34	12.68	13.89	15.83	44.21
25	3.46	12.53	11.85	12.02	15.02

In order to obtain the stable sensor response, the sensor was also stored in chiller temperature right after fabrication step as it was described earlier. Table 5 shows that sensor response towards GA (1–25 ppm) was found to be stable during six-week storage at chiller temperature. After six week, the sensor response was decreased more than 10% (data not shown), suggesting that stability of sensor was only preserved during six week storage at chiller temperature.

Table 5 The decrease of sensor response towards gallic acid (1–25 ppm) after it was stored in chiller temperature (4 °C).

Gallic acid	Storage time (week)					
conc. (ppm)	1	2	3	4	5	6
1	0.81	1.40	2.33	3.21	7.45	7.67
5	3.12	3.67	3.68	3.92	4.87	5.29
13	4.80	6.07	6.40	7.26	7.43	8.22
25	1.50	2.61	2.83	4.69	7.12	7.49

3.8. *Application on plant extracts*

In order to demonstrate the practical used of scannometry, various plant extract, such as sappan wood and Turmeric Rhizome, were carried out. Here, Sappan Lignum and Turmeric Rhizome were used with some purposes. Firstly, they were used to show that the color of herbal extracts did not affect the measurement of antioxidant capacity (the color of sappan extract is red, while the color of turmeric is yellow), and that the sensing mechanism relied on redox activity only. Secondly, the sample is taken based on the part of plant organ used for medication, *e.g.*, sappan is represented woods, while turmeric is represented rhizome. Thus, the proposed antioxidant sensor can be used for determination of antioxidant activity in any part of plant organ (leaf, root, flower, etc.) extracts or other plants extracts.

In this work, antioxidant capacity of plant extracts were compared with that of GA, as this approach was also reported in some literatures[34–37]. Using calibration curve of GA (5–25 ppm) made by the scanometric technique, the antioxidant capacity of extract was calculated, as it was expressed as gallic acid equivalent in ppm (ppm GAE). As comparison, UV/Vis spectrophorometry was used for the assay of antioxidant capacity of sample plant extracts with absorbance reading at 515 nm. Then, the results based on the scanometry were compared with the assay using UV/Vis spectrophotometer. The results showed that the proposed method was in good agreement with the spectrophotometric method, as it can be seen in Table 6, indicating the feasibility of the proposed scanometry for the determination of antioxidant capacity of the plant extracts.

4. Conclusion

A scanometry has been used as a microplate reader for high throughput screening of antioxidant based on DPPH as dry reagent on microwell plate as also used by other[28]. However, the proposed method is the first application for antioxidant sensor, measuring antioxidant capacity of GA and plant extracts. In this work, the developed method has linear range at 1–28 ppm with LOD at 0.794 ppm, and it was found to be reproducible, and good recovery for antioxidant determination of several plant extracts. The used of scanner in this proposed method is simple, easy to

Table 6 The comparison results of antioxidant capacity (ppm GAE) of various plant extracts determined by the antioxidant sensor and the UV/Vis spectrophotometer ($n=3$, $\alpha=0.05$).

Sample extracts	Antioxidant sensor-scanometric	UV/Vis Spectrophotometer	Significance value (P)
Sappan wood (10%, *w/v*)	10.22 ± 0.07	10.55 ± 0.21	0.062
Turmeric Rhizome (0.1%, *w/v*)	10.72 ± 0.26	11.08 ± 0.09	0.082

operate, and low-cost, as well as it can be used as an alternative reader for the microwell plate.

References

1. Pyrzynska K, Pękal A. Application of free radical diphenylpicrylhydrazyl (DPPH) to estimate the antioxidant capacity of food samples. *Anal Methods* 2013;**5**:4288–95.
2. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin Sci* 1993;**84**:407–12.
3. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci Technol* 1995;**28**:25–30.
4. Fogliano V, Verde V, Randazzo G, Ritieni A. Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. *J Agric Food Chem* 1999;**47**:1035–40.
5. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature* 1958;**181**:1199–200.
6. Bondet V, Brand-Williams W, Berset C. Kinetics and mechanisms of antioxidant activity using the DPPH. Free radical method. *LWT Food Sci Technol* 1997;**30**:609–15.
7. Sánchez-Moreno C, Larrauri JA, Saura-Calixto F. A procedure to measure the antiradical efficiency of polyphenols. *J Sci Food Agric* 1998;**76**:270–6.
8. Molyneux P. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J Sci Technol* 2004;**26**:211–9.
9. Bobo-García G, Davidov-Pardo G, Arroqui C, Vírseda P, Marín-Arroyo MR, Navarro M. Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods. *J Sci Food Agric* 2015;**95**:204–9.
10. Bener M, Özyürek M, Güçlü K, Apak R. Optimization of microwave-assisted extraction of curcumin from *Curcuma longa* L. (Turmeric) and evaluation of antioxidant activity in multi-test systems. *Rec Nat Prod* 2016;**10**:542–54.
11. Dolinsky M, Agostinho C, Ribeiro D, Rocha GD, Barroso SG, Ferreira D, et al. Effect of different cooking methods on the polyphenol concentration and antioxidant capacity of selected vegetables. *J Culin Sci Technol* 2016;**14**:1–12.
12. Nisar T, Iqbal M, Raza A, Safdar M, Iftikhar F, Waheed M. Estimation of total phenolics and free radical scavenging of Turmeric (*Curcuma longa*). *Am-Eur J Agric Environ Sci* 2015;**15**:1272–7.
13. Nirmal NP, Panichayupakaranant P. Antioxidant, antibacterial, and anti-inflammatory activities of standardized brazilin-rich *Caesalpinia sappan* extract. *Pharm Biol* 2015;**53**:1339–43.
14. Pękal A, Pyrzynska K. Effect of pH and metal ions on DPPH radical scavenging activity of tea. *Int J Food Sci Nutr* 2015;**66**:58–62.
15. Seo J, Lee S, Elam ML, Johnson SA, Kang J, Arjmandi BH. Study to find the best extraction solvent for use with guava leaves (*Psidium guajava* L.) for high antioxidant efficacy. *Food Sci Nutr* 2014;**42**:174–80.
16. Lee SK, Mbwambo ZH, Chung H, Luyengi L, Gamez EJ, Mehta RG, et al. Evaluation of the antioxidant potential of natural products. *Comb Chem High Throughput Screen* 1998;**1**:35–46.
17. Cheng Z, Moore J, Yu L. High-throughput relative DPPH radical scavenging capacity assay. *J Agric Food Chem* 2006;**54**:7429–36.
18. Abderrahim F, Arribas SM, Carmen Gonzalez M, Condezo-Hoyos L. Rapid high-throughput assay to assess scavenging capacity index using DPPH. *Food Chem* 2013;**141**:788–94.
19. Musa KH, Abdullah A, Kuswandi B, Hidayat MA. A novel high throughput method based on the DPPH dry reagent array for determination of antioxidant activity. *Food Chem* 2013;**141**:4102–6.
20. Condezo-Hoyos L, Abderrahim F, Arriba SM, Carmen González M. A novel, micro, rapid and direct assay to assess total antioxidant capacity of solid foods. *Talanta* 2015;**138**:108–16.
21. Shi X, Wen J, Li Y, Zheng Y, Zhou J, Li X, et al. DNA molecular beacon-based plastic biochip: a versatile and sensitive scanometric detection platform. *ACS Appl Mater Interfaces* 2014;**6**:21788–97.
22. Ma S, Tang Y, Liu J, Wu J. Visible paper chip immunoassay for rapid determination of bacteria in water distribution system. *Talanta* 2014;**120**:135–40.
23. Abbaspour A, Khajehzadeh A, Ghaffarinejad A. A simple and cost-effective method, as an appropriate alternative for visible spectrophotometry: development of a dopamine biosensor. *Analyst* 2009;**134**:1692–8.
24. Shokrollahi A, Hemmatidoust K, Zarghampour F. Determination of magnesium by the solution scanometric method in a coloured titan yellow magnesium hydroxide complex form. *J Taibah Univ Sci* 2016;**10**:161–7.
25. Chen B, Wang Z, Hu D, Ma Q, Huang L, Xv C, et al. Scanometric nanomolar lead (II) detection using DNA-functionalized gold nanoparticles and silver stain enhancement. *Sens Actuators B Chem* 2014;**200**:310–6.
26. Vijitvarasan P, Oaew S, Surareungchai W. Paper-based scanometric assay for lead ion detection using DNAzyme. *Anal Chim Acta* 2015;**896**:152–9.
27. Wong JX, Liu FS, Yu HZ. Mobile app-based quantitative scanometric analysis. *Anal Chem* 2014;**86**:11966–71.
28. Christodouleas DC, Nemiroski A, Kumar AA, Whitesides GM. Broadly available imaging devices enable high-quality low-cost photometry. *Anal Chem* 2015;**87**:9170–8.
29. Ahn YH, Yoo JS, Kim SH. An antioxidant capacity assay using a polyvinyl alcohol-based DPPH pellet. *Bull Korean Chem Soc* 2010;**31**:2557–60.
30. Steinberg IM, Milardović S. Chromogenic radical based optical sensor membrane for screening of antioxidant activity. *Talanta* 2007;**71**:1782–7.
31. Ningsih IY, Zulaikhah S, Hidayat MA, Kuswandi B. Antioxidant activity of various Kenitu (*Chrysophyllum cainito* L.) leaves extracts from Jember, Indonesia. *Agric Agric Sci Procedia* 2016;**9**:378–85.
32. Yuwono M, Indrayanto G. Validation of chromatographic methods of analysis. *Profiles Drug Subst Excipients Relat Methodol* 2005;**32**:243–59.
33. Huber L. *Validation and qualification in analytical laboratories*. 2nd Edition. New York: Informa Healthcare; 2007.
34. Lu Y, Shipton FN, Khoo TJ, Wiart C. Antioxidant activity determination of citronellal and crude extracts of *Cymbopogon citratus* by 3 different methods. *Pharmacol Pharm* 2014;**5**:395–400.
35. Sharma S, Vig AP. Evaluation of in vitro antioxidant properties of methanol and aqueous extracts of *Parkinsonia aculeata* L. leaves. *Sci World J* 2013;**2013**:604865.
36. Zhang A, Fang Y, Wang H, Li H, Zhang Z. Free-radical scavenging properties and reducing power of grape cane extracts from 11 selected grape cultivars widely grown in China. *Molecules* 2011;**16**:10104–22.
37. Apetrei CL, Tuchilus C, Aprotosoaie AC, Oprea A, Malterud KE, Miron A. Chemical, antioxidant and antimicrobial investigations of *Pinus cembra* L. bark and needles. *Molecules* 2011;**16**:7773–88.

Acta Pharmaceutica Sinica B

Editorial Board

136 editors and editorial board members in 14 countries/regions

CN China (68) US United States of America (47) IT Italy (3) GB United Kingdom (3)
BE Belgium (2) CA Canada (2) DE Germany (2) KR South Korea (2) SE Sweden (2)
AT Austria DK Denmark FR France HU Hungary JP Japan

ISSN: 2211-3835

Editor-in-Chief

Jian-Dong Jiang
Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Materia Medica, Beijing, China

Associate Editors-in-Chief

Xin-Xin Ding
The University of Arizona, Tucson, Arizona, United States of America

Jianfeng Cai
University of South Florida, Tampa, Florida, United States of America

Shi-lin Chen
China Academy of Chinese Medical Sciences Institute of Chinese Materia Medica, Beijing, China

Wen-Xing Ding
University of Kansas Medical Center, Kansas City, Kansas, United States of America

De-An Guo
China Academy of Chinese Medical Sciences Institute of Chinese Materia Medica, Beijing, China

Rong-Guang Shao
Chinese Academy of Medical Sciences Institute of Medicinal Biotechnology and Peking Union Medical College, Beijing, China

Jian-Gong Shi
China Academy of Chinese Medical Sciences Institute of Chinese Materia Medica and Peking Union Medical College, Beijing, China

Duxin Sun
University of Michigan College of Pharmacy, Ann Arbor, Michigan, United States of America

Xiao-Liang Wang
China Academy of Chinese Medical Sciences Institute of Chinese Materia Medica and Peking Union Medical College, Beijing, China

Wen Xie
University of Pittsburgh, School of Pharmacy, Department of Pharmaceutical Sciences, Pittsburgh, Pennsylvania, United States of America

Jian-Ping Ye
Shanghai Institute of Diabetes, Shanghai, China

Qiang Zhang
Peking University School of Pharmaceutical Sciences, Beijing, China.

Bo Yang
Zhejiang University, Hangzhou, Zhejiang, China
Ling Yang
The University of Iowa Roy J and Lucille A Carver College of Medicine, , United States of America
Ming-shi Yang
University of Copenhagen Department of Pharmacy, København, Denmark
Peter York
University of Bradford School of Pharmacy and Medical Sciences, Bradford, United Kingdom
Qi-dong You
China Pharmaceutical University, Nanjing, China
Ai-ming Yu
University of California Davis School of Medicine, Sacramento, California, United States of America
Shan-ping Yu
Emory University Department of Anesthesiology, Atlanta, Georgia, United States of America
Shi-Shan Yu
China Academy of Chinese Medical Sciences Institute of Chinese Materia Medica, Beijing, China
Su Zeng
Laboratory of Pharmaceutical Analysis and Drug Metabolism,College of Pharmaceutical Sciences,Zhejiang University, Hangzhou, China
Ao Zhang
Shanghai Institute of Materia Medica Chinese Academy of Sciences, Shanghai, China
Jian Zhang
Shanghai Jiao Tong University, Shanghai, China
Ji-wen Zhang
Shanghai Institute of Materia Medica Chinese Academy of Sciences, Shanghai, China
iang-ren Zhang
Peking University School of Pharmaceutical Sciences, Beijing, China
Zhiping Zhang
Huazhong University of Science and Technology, Wuhan, Hubei, China
Zhong-yin Zhang
Purdue University, West Lafayette, Indiana, United States of America
Ai-lian Zheng
Chinese Academy of Medical Sciences & Peking Union Medical College Institute of Materia Medica, Beijing, China
Xiao-bo Zhong
University of Connecticut, Storrs, Connecticut, United States of America
Hua Zhu
The Ohio State University, Columbus, Ohio, United States of America
Yi Zhu
Baylor College of Medicine, Houston, Texas, United States of America