REPORT

EVALUATION OF ANTIGLYCOSIDASE AND ANTICHOLINESTERASE ACTIVITIES OF *BOEHMERIA NIVEA*

SANDESH SANCHETI[1§], SHRUTI SANCHETI[1§] AND SUNG-YUM SEO[1,2]*

[1]*Department of Biology, Kongju National University, Kongju 314-701, Korea*
[2]*Korean Collection of Herbal Extracts, Inc., Kongju 314-701, Korea*

ABSTRACT

In this era, major community worldwide is suffering from diabetes type II, cancer and neurodegenerative disorders. To overcome these diseases, in the screening of Korean medicinal plants, we studied the whole plant of *Boehmeria nivea* (*B. nivea*). The methanolic leaf, stem and root extracts of *B. nivea* and their respective n-hexane, methylene chloride (CH_2Cl_2), ethyl acetate (EtOAc), n-butanol (BuOH) and aqueous fractions were investigated for their total phenolic content (TPC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzyme inhibition activities. Profound TPC and DPPH free radical scavenging activities were observed in the EtOAc and BuOH fractions of root, where the BuOH fraction showed high-pitched α-glucosidase inhibition and the EtOAc layer showed the maximum β-glucosidase inhibition. Furthermore, the leaf extract demonstrated the highest β-galactosidase inhibitory activity, but no α-galactosidase inhibition was seen in any of the plant parts. Notable BChE and moderate AChE inhibitory activity was found in whole plant. It can be suggested that whole plant of *B. nivea* provides a strong biochemical rationale as one of the good choices for the treatment of diabetes type II, cancer and neurodegenerative diseases (AD, etc).

Keywords: *Boehmeria nivea*, glycosidase, cholinesterase, total phenolic content, DPPH.

INTRODUCTION

Boehmeria nivea (*B. nivea*) belonging to the family Urticaceae, (common name: Ramie, China grass) is widely distributed over large areas of subtropical and tropical Asia. The leaves and roots of this plant are edible. From the leaves, dessert cakes are prepared in Korea, where roots are taken in peeled and boiled form (having sweet taste) (Reid, 1977). *B. nivea* has also been used traditionally as a diuretic, antipyretic, hepatoprotective, antioxidant and anti-inflammatory agent (Lin *et al.*, 1998). Since then, keeping in view its uses as a folk medicine, this plant, although deserved, has not been extensively subjected to the phytochemical and pharmacological investigations. Taking this into consideration, in the present study, for the first time, we investigated glycosidase and cholinesterase inhibition properties of various plant parts of *B. nivea,* as the enzyme inhibitors are of potential value in many areas of disease control and treatment.

Oxidative stress is involved in the pathogenesis of various chronic diseases, such as, diabetes, cardiovascular disease and cancer. Antioxidants protect against free radicals and they are therefore, essential in obtaining and preserving good health. Much attention has been given to polyphenols with strong antioxidant activities, as they are effective in preventing these diseases. So, the DPPH free radical scavenging activity and total phenolic content for all the fractions was carried out (Zheng and Wang, 2001).

Glycosidase inhibitors play a key role for treating diabetes type-II, viral or bacterial infections, lysosomal storage disorders, and cancer, as glycosidases are involved in digestion, biosynthesis of glycoproteins and lysosomal catabolism of glucoconjugates. As the current allopathic medicines for treatment of these diseases have numerous adverse/side effects, recently, alternative or traditional medical resources are being explored around the globe (Mehta *et al.*, 1998; Kuntz *et al.*, 2008; Bhat *et al.*, 2008).

Cholinesterase inhibitors are the only approved drugs for treating patients with mild to severe Alzheimer's disease (AD), a disorder associated with progressive degeneration of memory and cognitive function. The cholinergic hypothesis postulates that, memory impairment in patient with Alzheimer's disease results from a deficit of cholinergic function in the brain. Acetylcholinesterase inhibitors can restore the level of acetylcholine by inhibiting AChE. A potent source of AChE inhibitors is

§ These authors contributed equally to this work.

**Corresponding author*: Dr Sung-Yum Seo, Department of Biology, Kongju National University, Kongju, Korea Tel: 82-41-850-8503. Fax: 82-41-854-8503, E-mail: dnalove@kongju.ac.kr

certainly provided by the abundance of plants in nature (Lopez *et al.*, 2002; Rhee *et al.*, 2001; Mukherjee *et al.*, 2007). So, there is an interest in finding new cholinesterase inhibitors from natural source, as only few of such drugs are available for clinical use.

Taking all of the above facts into consideration, we examined the α-glucosidase, β-glucosidase, α-galactosidase, β-galactosidase, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzyme inhibition activities of the leaves, stems and roots of *B. nivea*.

MATERIALS AND METHODS

Plant material

The leaves, stems and roots of *B. nivea* were obtained from "Korean Collection of Herbal Extracts" a Biotech company in Korea. A collection of voucher specimen is available with the company (Korea Collection of Herbal Extracts, 2000).

Extraction

The dried leaves, stems and roots (2 Kg, dry weight for each plant part) were chopped into small pieces and kept for extensive decoction in 100% methanol for 8 days at room temperature. The extract was then concentrated using rotary vacuum evaporator at 20-30°C to obtain the dried crude extract for leaves (70 g), stems (61 g), and roots (52 g).

Fractionation

The crude methanolic extract of leaves, stems and roots (70 g, 61 g and 52 g, respectively) were suspended in distilled water individually (1 l) and partitioned with n-hexane, methylene chloride, ethyl acetate and n-butanol to yield the n-hexane (22 g, 8 g and 11 g, respectively), methylene chloride (3 g, 11 g and 4 g, respectively), ethyl acetate (8 g, 14 g and 7 g, respectively), n-butanol (17 g, 10 g and 18 g, respectively) and aqueous (16 g, 8 g and 22 g, respectively) fractions, respectively. The enzyme inhibition activity assays were performed using 1 mg/ml concentrations for the crude extracts and all of their fractions.

Reagents

α-Glucosidase (from *Saccharomyces cerevisiae* type I), β-glucosidase (from almonds), α-galactosidase (from green coffee beans), β-galactosidase (from *Escherichia coli*), 4-nitrophenyl α-D-glucopyranoside, 4-nitrophenyl β-D-glucopyranoside, 4-nitrophenyl α-D-galactopyranoside, 2-nitrophenyl β-D-galactopyranoside, AChE (type VI-S from Electric Eel), BChE (from horse serum), acetylthiocholine iodide (ATCI), butyrylthiocholine iodide (BTCI), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid and Folin-Ciocalteu reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). Other commercially available reagents and solvents were used as received.

Determination of Total phenolic content

TPC of the extracts was determined using Folin-Ciocalteu assay as described by Zhang *el al.* with minor modifications (Zhang *et al.*, 2006). In this assay, 10 µl of each sample solution (concentration: 1 mg/ml) and100 µl Folin-Ciocalteu reagent were mixed well and was allowed to stand for 5 min followed by addition of 80 µl of 7.5% sodium carbonate solution and mixed well. The covered plate was kept in the dark at room temperature for 30 min. and then, the absorbance was measured at 750 nm with a spectrophotometric microplate reader. TPC was expressed as gallic acid equivalent (GAE) in mg per gram dry extract.

DPPH· Free radical scavenging activity

The free radical scavenging activity of the extracts was assessed by the previously described method of Blois with minor modifications (Blois, 1958). In this method, 50 µl of methanol solution of DPPH (0.5 mM) was mixed with 10 µl of test sample (concentration: 1 mg/ml) and 50 µl of 0.1 M tris HCl buffer (pH 7.0), and the change in absorbance was measured at 517 nm 30 min later. The readings were compared with the control, which contained 10 µl of methanol instead of test samples. All experiments were carried out in triplicates.

Glycosidase inhibition assays

The enzyme inhibition activities for α-glucosidase, β-glucosidase, α-galactosidase and β-galactosidase were evaluated according to the method previously reported by Shibano *et al.* with minor modifications (Shibano *et al.*, 1997). In this, the reaction mixture consisted 50 µl of buffer, 25 µl of substrate, 10 µl of test sample (concentration: 1 mg/ml) and 25 µl of enzyme solution. This reaction mixture was then incubated at 37°C for 30 min. Then, the reaction was terminated by the addition of 100 µl of 0.2 M sodium carbonate solution. The enzymatic hydrolysis of substrate was monitored by the amount of p-nitrophenol or o-nitrophenol released in the reaction mixtures at 410 nm using microplate reader. The buffers, enzymes and substrates used in α-glucosidase, β-glucosidase, α-galactosidase and β-galactosidase enzyme inhibitions assays are detailed in table 1.

AChE and BChE inhibition assays

The enzyme inhibition activities for AChE and BChE were evaluated according to the method previously reported by Ellman *et al.* with minor modifications (Ellman *et al.*, 1961). In this method, 20 µl AChE solution (0.03 U/ml), 10 µl test sample and 180 µl buffer (pH 8) were mixed and incubated at 4°C for 30 min. Then, in the reaction mixture, 20 µl DTNB (0.3 mM) and 20 µl ATCI (1.8 mM) were added and incubated at 37°C for 20 min,

followed by the measurement of absorbance at 412 nm and the percentage inhibition was calculated. Assessment of BChE inhibition was performed as described above except that the final enzyme concentration was 0.1 U/ml and ATCI was replaced by BTCI (0.5 mM). Each sample was assayed in triplicate.

STATISTICAL ANALYSES

All assays were performed at least three times with triplicate samples. The inhibition rates for all the assays were calculated as a percentage of control (buffer containing MeOH) without inhibitor. All results are expressed as mean ± S. D.

RESULTS AND DISCUSSION

Total phenolic content (TPC) and DPPH· Free radical scavenging activity

As shown in table 2, *B. nivea* roots displayed the highest TPC (84 mg GAE/g) as compared to the TPC of leaves and stems (29 and 48 mg GAE/g, respectively). In roots, after fractionation, significantly increased TPC was observed in the n-butanol and ethyl acetate fractions (190 and 180 mg GAE/g), where in all other fractions of leaves, stems and roots, the TPC was in the range of 1 to 80 mg GAE/g of the extracts (table 2).

Phenolic compounds are known to have antioxidant activity. This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Tepe *et al.*, 2006; Zheng and Wang, 2001).

Therefore, the antioxidant activity of different plant parts of *B. nivea* was measured using the DPPH· free radical scavenging assay. The crude extract of root showed the highest activity (66%) as compared to other plant parts (table 3). After fractionation, the n-butanol and ethyl acetate fractions showed outstanding activities (76% and 72%, respectively), which illustrates the highest positive relationship between total phenols and antioxidant activity in *B. nivea*.

As there is an increasing evidence that free radicals and reactive oxygen species can induce oxidative damage in biomolecules, such as, lipids, proteins, and nucleic acids, leading to human diseases such as, atherosclerosis, cancer, diabetes, and neurodegenerative disorders, herbal drugs containing free radical scavengers like, phenolics are known for treating these disease (Beauchamp and Fridovich, 1971; Ravishankara *et al.*, 2002).

Further, to investigate the potential of the *B. nivea* extracts to treat the abovementioned diseases, we performed various *in-vitro* assays.

Glycosidase inhibition assays

Glycosidase inhibitors are prospective therapeutic agents for some degenerative diseases, including diabetes, viral attachment and cancer (Mehta *et al.*, 1998; Kuntz *et al.*, 2008; Bhat *et al.*, 2008). In literature, it has been reported that, many herbal extracts are being used as antidiabetic agents, but, no such study was done previously for *B. nivea* plant extracts. Therefore, for the first time, the *B. nivea* crude extracts (in 100% methanol) and its n-hexane, methylene chloride, ethyl acetate, n-butanol and aqueous fractions were investigated for α-glucosidase, β-

Table 1: Details of the buffers, enzymes and substrates for Glycosidase inhibition assays

Assay types → / Reagents used ↓	*α-Glucosidase Assay*	*β-Glucosidase Assay*	*α-Galactosidase Assay*	*β-Galactosidase Assay*
Buffer	0.1 M phosphate buffer (pH 7.0)	0.1 M phosphate buffer (pH 7.0)	0.5 M phosphate buffer (pH 6.5)	0.1 M phosphate buffer (pH 7.3)
Enzyme	α-glucosidase (0.1 U/mL)	β-glucosidase (2.5 U/mL)	α-galactosidase (0.1 U/mL)	β-galactosidase (0.44 U/mL)
Substrate	4-nitrophenyl α-D-glucopyranoside (0.5 mM)	4-nitrophenyl β-D-glucopyranoside (0.5 mM)	4-nitrophenyl α-D-galactopyranoside (0.5 mM)	2-nitrophenyl β-D-galactopyranoside (0.5 mM)

Table 2: TPC of various plant parts of *B. nivea* and their respective fractions

Fraction	TPC in B. nivea plant parts (mg GAE/g)		
	Leaf	Stem	Root
Crude extract	29	48	84
n-Hexane	17	2	22
Methylene chloride	43	17	60
Ethyl acetate	24	80	180
n-Butanol	37	18	190
Water	3	1	22

Table 3: Percentage inhibitions shown by different parts of *B. nivea* and their respective fractions in different assay systems

Plant parts	Fractions	Percentage (%) inhibition activities in different assay systems						
		DPPH	α-Glucosidase	β-Glucosidase	α-Galactosidase	β-Galactosidase	AChE	BChE
Concentration per reaction mixture		10 µg/ 110 µl	10 µg/ 210 µl	10 µg/210 µl	10 µg/210 µl	10 µg/210 µl	10 µg/250 µl	10 µg/250 µl
Leaf	Crude extract	24 ± 0.8	14 ± 0.2	ND	ND	73 ± 2.1	20 ± 0.9	48 ± 1.2
	n-Hexane	3 ± 0.2	32 ± 0.6	ND	ND	85 ±1.4	50 ± 1.4	50 ± 2.1
	Methylene chloride	8 ± 0.5	62 ± 0.8	ND	ND	95 ± 2.7	45 ± 2.1	48 ± 3.1
	Ethyl acetate	4 ± 0.1	ND	ND	2 ± 0.1	32 ± 2.6	12 ± 1.2	47 ± 1.1
	n-Butanol	50 ± 0.5	ND	ND	ND	2 ± 0.1	25 ± 3.1	43 ± 0.2
	Water	1 ± 0.1	ND	ND	ND	ND	ND	29 ± 0.5
Stem	Crude extract	37 ± 0.8	41 ± 0.6	73 ± 1.4	ND	44 ± 2.3	5 ± 0.8	46 ± 0.9
	n-Hexane	7 ± 0.2	ND	ND	2 ± 0.3	ND	10 ± 0.5	29 ± 1.1
	Methylene chloride	49 ± 0.3	66 ± 1.3	ND	ND	20 ± 1.1	17 ± 2.1	74 ± 2.1
	Ethyl acetate	58 ± 1.2	71 ± 1.9	84 ± 2.1	2 ± 0.2	77 ±2.2	28 ±3.1	52 ± 1.4
	n-Butanol	44 ± 0.1	19 ± 2.1	23 ± 1.3	ND	ND	12 ± 1.1	36 ± 2.1
	Water	4 ± 0.3	ND	12 ± 0.2	ND	ND	15 ± 0.6	50 ± 3.1
Root	Crude extract	66 ± 0.9	90 ± 3.2	86 ± 0.9	ND	ND	19 ± 0.7	47 ± 1.6
	n-Hexane	12 ± 0.3	67 ± 2.7	ND	ND	ND	11 ± 0.2	66 ± 0.8
	Methylene chloride	52 ± 1.4	51 ± 1.8	52 ± 0.8	ND	1 ± 0.3	8 ± 1.1	46 ± 1.3
	Ethyl acetate	72 ± 1.8	75 ± 2.4	93 ± 2.1	1 ± 0.1	7 ± 0.4	26 ± 2.1	40 ± 1.9
	n-Butanol	76 ± 1.5	94 ± 3.6	ND	ND	2 ± 0.2	ND	34 ± 0.3
	Water	29 ± 0.9	63 ± 2.9	51 ± 1.7	ND	ND	ND	27 ± 1.3

ND: Not detected

glucosidase, α-galactosidase and β-galactosidase inhibition activities.

In comparison to the leaf and stem extracts, the fractions of root extract of *B. nivea* demonstrated remarkable α-glucosidase and β-glucosidase inhibitory activities ranging from 51 to 94% and 0 to 93%, respectively, and are detailed in table 3. The highest inhibition was reported in n-butanol and ethyl acetate fractions with 94% and 93% α-glucosidase and β-glucosidase inhibitions, correspondingly.

The β-galactosidase inhibition was profound in leaf extract within the range of 0 to 95%, where methylene chloride showed 95% inhibition. In stem extract, the noticeable inhibition was observed in ethyl acetate fraction (77%), where no inhibition was found in root extract for β-galactosidase (table 3). In all extracts of *B. nivea*, α-galactosidase activity was found to be almost nil.

In summary, *B. nivea* is the effective bioresource for the α-glucosidase, β-glucosidase and β-galactosidase inhibitory activities.

AChE and BChE inhibition assays

Since AD, one of the most common cause of death worldwide, has become threaten to public health, new treatment strategies based on medicinal plants have been focused. The ability of *B. nivea* extract has been evaluated using AChE and BChE assays. The results obtained in this study propose that, the stem and root extracts of *B. nivea* are a strong BChE inhibitor, in which the methylene chloride fraction of stem and the n-hexane fraction of root showed 74% and 66% inhibition respectively, and also moderate AChE inhibition was observed in n-hexane fraction of leaf extract (50%). The AChE and BChE inhibitions in all of the fractions of the various parts of *B. nivea* ranged from 0 to 50% and 27 to 74 % (table 3). This indicates that the *B. nivea* extracts may prove a potential candidate for the treatment of AD and other neurodegenerative diseases like Parkinson disease, senile dementia, ataxia and myasthenia gravis.

CONCLUSION

Profound TPC and DPPH free radical scavenging activities were observed in the EtOAc and BuOH fractions of root extract of *B. nivea,* where the BuOH fraction showed high-pitched α-glucosidase inhibition and the EtOAc layer showed the maximum β-glucosidase inhibition. Furthermore, the leaf extract demonstrated the highest β-galactosidase inhibitory activity. Notable BChE and moderate AChE inhibitory activity was found in whole plant. Thus, in conclusion it can be suggested that whole plant of B. nivea provide a strong biochemical rationale as one of the good choices for the treatment of diabetes type II, cancer and neurodegenerative diseases (AD, etc). Based on these *in vitro* studies, it is recommended that, further animal and clinical studies need to be investigated to prove *B. nivea* as one of the useful medicinal bioresource for the healthier world.

ACKNOWLEDGEMENT

This research was financially supported by Seo Chun Gun, Korea.

REFERENCES

Beauchamp C and Fridovich I (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal. Biochem.*, **44**: 276-287.

Bhat M, Zinjarde SS, Bhargava SY, Kumar AR and Joshi BN (2008). Antidiabetic Indian Plants: a Good Source of Potent Amylase Inhibitors. *Evid. Based Complement Alternat. Med.*, pp.1-6.

Blois MS (1958). Antioxidant determinations by the use of a stable free radical. *Nature,* **181**: 1199-1200.

Ellman GL, Courtney KD, Andres V Jr and Feather-Stone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, **7**: 88-95.

Kuntz DA, Tarling CA, Withers SG and Rose DR (2008). Structural Analysis of Golgi α-Mannosidase II Inhibitors Identified from a Focused Glycosidase Inhibitor Screen. *Biochemistry*, **47**: 10058-10068.

Lin CC, Yen MH, Lo TS and Lin JM (1998). Evaluation of the hepatoprotective and antioxidant activity of *Boehmeria nivea* var. *nivea* and *B. nivea* var. *tenacissima. J. Ethnopharmacol.*, **60**: 9-17.

Lopez S, Bastida J, Viladomat F and Conida C (2002). Acetylcholinesterase inhibitory activity of some Amaryllidaceae alkaloids and *Narcissus* extracts. *Life Sci.*, **72**: 2521-2529.

Mehta A, Zitzmann N, Rudd PM, Block TM and Dwek RA (1998). Alpha-glucosidase inhibitors as potential broad based anti-viral agents. *FEBS Lett.*, **430**: 17-22.

Mukherjee PK, Kumar V, Mal M and Houghhton J (2007). Acetylcholinesterase inhibitors from plants. *Phytomedicine*, **14**: 289-300.

Ravishankara MN, Shrivastava N, Padh H and Rajani M (2002). Evaluation of antioxidant properties of root bark of *Hemidesmus indicus* R. Br. *(Anantmul). Phytomedicine*, **9**: 153-160.

Reid BE (1977). Famine Foods of the Chiu-Huang Pen-ts'ao. Southern Materials Centre, Taipei.

Rhee IK, Meent MVD, Ingkaninan K and Verpoorte R (2001). Screening for acetylcholinesterase inhibitors from *Amaryllidaceae* using silica gel thin layer chromatography in combination with bioactivity staining. *J. Chromatogr. A*, **915**: 217-223.

Shibano M, Kitagawa S, Nakamura S, AkazawaN and Kusano G (1997). Studies on the constituents of Broussonetia species. II. Six new pyrrolidine alkaloids, broussonetine A, B, E, F and broussonetinine A and B, as inhibitors of glycosidases from Broussonetia kazinoki Sieb. *Chem. Pharm. Bull.*, **45**: 700-705.

Tepe B, Sokmen M, Akpulat HA and Sokmen A (2006). Screening of the antioxidant potentials of six Salvia species from Turkey. *Food Chem.*, **95**: 200-204.

Zhang Q, Zhang J, Shen J, Silva A, Dennis DA and Barrow CJ (2006). A simple 96-well microplate method for estimation of total polyphenols content in seaweeds. *J. Appl. Phycol.*, **18**: 445-450.

Zheng W and Wang SY (2001). Antioxidant Activity and Phenolic Compounds in Selected Herbs. *J. Agric. Food Chem.*, **49**: 5165-5170.

Vol. 23, No.2, April 2010

Vol. 23, No.2, April 2010

Vol. 23, No.2, April 2010

Vol. 23, No.2, April 2010

April 2010 Vol. 23, No.2, Pages 125-240

April 2010 Vol. 23, No.2, Pages 125-240

April 2010 Vol. 23, No.2, Pages 125-240

April 2010 Vol. 23, No.2, Pages 125-240