pISSN 1229-1153 / eISSN 2465-9223 *J. Food Hyg. Saf.*
Vol. 33, No. 6, pp. 427~437 (2018)
https://doi.org/10.13103/JFHS.2018.33.6.427

Journal of Food Hygiene and Safety
Available online at http://www.foodhygiene.or.kr

Evaluation of Pharmacological Activities of Ethanol Extracts Prepared from Selected Korean Medicinal Plants

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(Received August 13, 2018/Revised September 17, 2018/Accepted October 11, 2018)

ABSTRACT - In this study, 23 ethanolic extracts from 20 medicinal plants were evaluated for biological activities. Results revealed that of 23 samples, seven samples have demonstrated good antimicrobial activity. Minimum inhibitory concentrations were 0.4-2.0 mg/mL, while minimum bactericidal concentrations were mostly high 0.8-2.0 mg/mL for selected seven samples. Five samples revealed > 70 mg gallic acid equivalent (GAE)/g of total phenolic contents. Among test samples, six samples exhibited > 80% inhibition of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and only two samples exhibited > 80% inhibition of 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radicals. A total of five test samples revealed Trolox equivalent antioxidant capacity more than 1000 μm/g. The MTT assay indicated that eight test samples exhibited > 90% viability of murine macrophage cells (RAW 264.7) at 250 μg/mL and suppressed iNOS mRNA expression at transcriptional level when stimulated by lipopolysaccharide (LPS). Some medicinal plants revealed promising results, and so they have prospective for further more inclusive studies.

Key words : Antioxidant, Antimicrobial, Anti-inflammatory, Cytotoxic, Medicinal plants

Medicinal plants is a gift of nature due to their chemical diversity and affability[1,2]. Natural products obtained from these plants have gained an increased interest worldwide for enhancing healthcare[3]. These products have been utilized as complementary or conventional medicines due to the potential side effects and toxicity of synthetic drugs[4]. The applications of these natural products as medicine have already been explored throughout the history in the form of remedies, traditional medicine, potions, and oils; with a big number of natural products are still unidentified. The medicinal applications of natural products is obtained as a result of man experimenting by trial and error for hundreds of centuries through palatability trials or untimely deaths, searching for available foods for the treatment of diseases[5,6]. Moreover, natural products are not only a rich source of diverse substances with an extensive range of biological activities but also as the main source for synthesized drugs[4,7]. Biological activity describes the beneficial or adverse effects of a drug on living organisms in pharmacology. Biological properties relate to the antioxidant, anticancer, anti-aging, hypocholesterolemic, anti-anxiety, anticoagulant, anti-diabetes, antithrombotic, antifungal, anti-inflammatory, antihistaminic, antihistaminic, anti-leishmanial, immunosuppressive, cytoprotective, antibacterial, and insecticidal activities[8-16].

In the current study, various medicinal plants were collected from different geographic regions of Korea and screened for different biological activities including antimicrobial, antioxidant, anti-inflammatory, cytotoxic activities.

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Materials and Methods

Plant collection and extraction

Plants material were collected from the different region and described in Table 1. All the plant specimens were identified, and voucher specimens of all accessions were prepared and are maintained at the Department of Resource Development, Forest Resource Development Institute of Gyeongbuk, Korea. The plant materials were washed with running tap water to remove the impurities and dried in the shade for one week. The dried sample was extracted with 75% ethanol at 65°C for three times every 4 hours in the heating mantle (E105, Misung Scientific Co., Yangju, Korea). And then, the extracted sample was filtered by using

Table 1. The list of medicinal plants used in the study and their respective extraction system

Sample code	Samples	Parts used	Region	Collection/ purchased*	Extraction system	Yield
1	*Staphyleabumalda*	Leaf	Gyeonju-si	Collected	75% EtOH	36.04
2	*Staphyleabumalda*	Seed	Gyeonju-si	Collected	75% EtOH	0.52
3	*Corylusheterophylla*var. *thunbergii*Blume	Leaf	Andong-si	Collected	75% EtOH	34.19
4	*Chrysanthemum morifolium*	Flower	Jecheon-si	Purchased	75% EtOH	34.56
5	*Ziziphusjujuba*Mill.	Leaf	Andong-si	Collected	75% EtOH	28.61
6	*Bassiascoparia*	Seed	Andong-si	Collected	75% EtOH	34.38
7	*Codonopsislanceolata*Skin	Rhizodermis/ Root skin	Bonghwa-gun	Collected	75% EtOH	38.26
8	*Sedum sarmentosum*	Aerial part	Andong-si	Collected	75% EtOH	37.99
9	*Helianthus tuberosus*	Flower	Andong-si	Collected	75% EtOH	32.77
10	*Pinus rigida*	Leaf	Andong-si	Collected	75% EtOH	23.27
11	*Prunusserrulata*var. *spontanea*	Flower	Andong-si	Collected	75% EtOH	43.69
12	*Elaeagnusum bellata*	Fruit	Andong-si	Collected	75% EtOH	72.67
13	*Lespedeza cuneata*G.Don	Total plant	Sancheong-gun	Purchased	75% EtOH	13.79
14	*Torilis japonica* (Houtt.) DC.	Seed	Andong-si	Collected	75% EtOH	3.54
15	*Euphorbia maculata*L.	Aerial part	Andong-si	Collected	75% EtOH	12.80
16	*Prunustomentosa*Thunb.	Fruit	Andong-si	Collected	75% EtOH	47.23
17	*Artemisia Princeps* var. *Orientalis*Hara	Aerial part	Jecheon-si	Purchased	75% EtOH	29.67
18	*Heracleummoellendorffii*Hance	Leaf	Andong-si	Collected	75% EtOH	36.19
19	*Rosa multiflora*	Flower	Andong-si	Collected	75% EtOH	13.21
20	*Rosa multiflora*	Leaf	Andong-si	Collected	75% EtOH	31.43
21	*Puerariathunbergiana*	Flower	Andong-si	Collected	75% EtOH	34.27
22	*Puerariathunbergiana*	Leaf	Andong-si	Collected	75% EtOH	11.75
23	*Sanguisorbaofficinalis*	Root	Andong-si	Collected	70% EtOH	12.68

*Medicinal plants were purchased from local market

a bottle top filter (0.45 μm, corning) with a vacuum pump and concentrated by rotary vacuum evaporator (Heidolph, Laborota 4000-efficient, Schwabach Germany) at 60°C. The concentrated extract solutions were kept in shady and ventilated place in the lab to ensure the evaporation of the remaining solvent. The extracts were then collected and kept at 4°C for further use.

Antimicrobial activity

Disc diffusion assay of the test samples was performed according to the procedure previously described[17]. Mueller-Hinton Agar (MHA; Oxoid Ltd, Basingstoke, UK) was used of susceptibility testing. A stock solution of the test sample of 100 mg/mL was prepared in sterile distilled water. Blank antimicrobial discs (Thermo Scientific™ Oxoid™) received a respective concentration of tested samples. *Staphylococcus aureus*KCCM11335, *Pseudomonas aeruginosa*KCCM 11266, *Staphylococcus epidermidis*KCTC 1917 was cultured in tryptic soy broth (TSB; Difco, Sparks, MD, USA) for 18 h and at 37°C while *Propionibacterium acnes* KCTC 3314 was culture in Reinforced Clostridium Medium (RCM; Difco, Sparks, MD, USA) media anaerobically in an anaeroPack (Mitsubishi Gas Chemical Co., Inc., Tokyo, Japan) for 24 h at 37°C. The bacteria cultures were then washed twice with Buffered Peptone Water (BPW; Difco, Sparks, MD, USA) and resuspended the bacteria in BPW. The turbidity of the bacteria cultures was adjusted to an optical density of 0.08~0.1 at 600 nm of wavelength. The inoculums (1~1.5 × 10^8 CFU/mL) were spread on the tryptic soy agar (TSA; Difco, Sparks, MD, USA) using a sterile spreader and antimicrobial disc was applied to the plate. After 18 h of incubation at 37°C, the zone of inhibition was recorded. Gentamycin (10 μg/disc) was used as positive control.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values were deter-

mined only for selected test samples that higher zone of inhibition[18]. Briefly, dilutions were performed by addition of culture broth to reach concentrations ranging from 2 to 0.2 mg/mL; 100 μL of each dilution were distributed in 96-well plates, as well as a sterility control and growth control (containing culture broth plus dimethylsulfoxide, without antimicrobial substance). Each test and growth control well was inoculated with 50 μL of a bacterial suspension (10^8 CFU/mL or 10^5 CFU/well). The 96 well (SPL, Life Science, Pochun, South Korea) titre plates were incubated at 37°C for 24 h. MBC was determined by sub-culturing the test dilutions on to a fresh solid medium and incubated further for 18-24 h. The highest dilution that yielded no bacterial growth on solid medium was taken as MBC. For this assay gentamycin (range 0.2-128 μg/mL) was used as positive control.

Determination of total phenolic content (TPC)

The TPC of all the test samples were determined by Folin-Ciocalteu's (Sigma Chemical Co. (St. Louis, MO) colorimetric method[19]. A total of 1 mg/mL of the sample was mixed with 1 mL of 2% of sodium carbonate solution and 1 mL of 10% of Folin-Ciocalteu's phenol reagent. After 1 h, the absorbance was measured at 750 nm using microplate spectrophotometer (Spectramax i3, molecular device, Sunnyvale, CA, USA). For standard curve determination, gallic acid (GA; Sigma Chemical Co. St. Louis, MO) was used. The measurement was compared to a calibration curve of gallic acid and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample (mg GAE/g).

DPPH radical scavenging activity assay

The free radical scavenging activity of test samples was measured by DPPH (Sigma) according to the procedure[20]. Briefly, 0.1 mM solution of DPPH was prepared in methanol. Then, 50 μL of this solution was added to 150 μl of extracts of plants. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. For positive control ascorbic acid (100 μg/mL) was used. Then the absorbance was measured at 517 nm in microplate spectrophotometer (Spectramaxi 3, molecular device, Sunnyvale, CA, USA). The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH scavenging effect calculated using the following equation:

$$\textit{Scavenging effect}\ (\%) = \frac{\textit{Control absorbance} - \textit{sample absorbance}}{\textit{control absorbance}} x\,100$$

$ABTS^{\bullet+}$ radical scavenging activity

The $ABTS^{\bullet+}$ (Sigma) cation scavenging activity was performed as per Saeed, Khan[21] guidelines. Briefly, $ABTS^{\bullet+}$ solution (7 mM) was mixed with potassium persulfate (2.45 mM) solution and kept for 12hin the dark to yield a dark colored solution containing $ABTS^{\bullet+}$ radical cations. Before starting the assay, the $ABTS^{\bullet+}$ solution was diluted with 50% methanol to attain an initial absorbance of about 0.70 at 750 nm, with temperature control set at 25°C. Free radical scavenging activity was assessed by mixing 300 μL of the test sample with 3.0 mL of $ABTS^{\bullet+}$ working standard. The decrease in absorbance was measured exactly one minute after mixing the solution, then up to 6 min. The percentage inhibition was calculated according to the formula:

$$\textit{Scavenging effect}\ (\%) = \frac{\textit{Control absorbance} - \textit{sample absorbance}}{\textit{control absorbance}} x\,100$$

Trolox equivalent antioxidant capacity (TEAC)

The standard TEAC assay was carried out with minor modifications for determination of the TEAC value[22]. Briefly, $ABTS^{\bullet+}$ solution (7 mM) was mixed with potassium persulfate (2.45 mM) solution and kept for 12hin the dark to yield a dark colored solution containing $ABTS^{\bullet+}$ radical cations. The $ABTS^{\bullet+}$ solution was diluted with 50% methanol to attain an initial absorbance of about 0.70 at 750 nm. Trolox (TCI, Tokyo, Japan) stock solution was prepared in ethanol. Ten microliters of test samples were added to 0.990 mL $ABTS^{\bullet+}$ solution, and the absorbance at 734 nm was measured in time. This was compared to a blank where 10 μL of the solvent was added to 990 μL of the $ABTS^{\bullet+}$ solution. The reduction in absorbance 6 min after the addition of the antioxidant was determined. Results were expressed as μmolTrolox equivalent per gram sample of dried plant (μmol equivalent Trolox/g), based on the Trolox calibration curve.

Cell viability assay

The murine macrophage cell line RAW 264.7 was purchased from the Korea Cell Line Bank (Seoul, Korea). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (v/v) (Invitrogen-Gibco), 5% penicillin (100 U/mL), and streptomycin (100 μg/mL; Gibco, Grand Island, NY, USA) at 37°C in a humidified atmosphere of 5% CO_2. Cells were treated with increasing doses of test samples for 24 h. Cell survival was determined by adding 500 μg/mL of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT; Sigma Chemical Co., St. Louis, MO) to each well and incubated for another 4 h at 37°C. After removal of medium, cells were lysed with dimethyl sulfoxide. The absorbance of the colored solution was

measured at 450 nm with a microplate reader (Spectramax i3, molecular device, Sunnyvale, CA, USA).

Anti-inflammatory assay

RAW 264.7 cells were pretreated with 400-100 μg/mL of test samples for 24 h then stimulated with 1 μg/mL of LPS (from *E. coli* O127: B8; Sigma, Israel). After 24 h, NO levels in the culture media were determined using NO detection kit (iNtRON, Korea). A total of 0.2 mL of media was transferred to 96 well plates, and absorbance was measured at 540 nm using SpectraMax® i3 plate reader (Molecular Devices, Sunnyvale, CA, USA).

iNOS mRNA expression

Inducible nitric oxide synthase (iNOS) mRNA expressions of the selected test samples were performed. Macrophage cell line RAW 264.7 cells were incubated with test samples (400 μg/mL) for 24 h then induced by LPS (1 μg/mL) for 4 h. Total RNA from LPS-induced RAW 264.7 cells was prepared using RNeasy mini kits (QIAGEN, Valencia, CA, USA) according to manufacturer's protocol and stored at −20°C before use. Total RNA was reverse transcribed with Super Script First-Strand Synthesis Systems kits (Invitrogen, Carlsbad, CA, USA) to obtain cDNA. All PCR analyses using a real-time polymerase chain reaction were subsequently carried out in a 20 μL volume containing 10 μL of SYBR Green, 10 pmol of 5' and 3' primers, and cDNA. PCR primers used in this study are the following: iNOS: 5'-AGTGGTGTTCTTTGCTTC-3' (forward) and 5'-GCTTGC-CTTATACTGGTC-3' (reverse).

Results and Discussion

Antimicrobial activity

There is an alarming increase in resistance of bacterial strain towards some antibiotics demands that progressive

Table 2. Zone of inhibition against various microorganisms (2 mg/disc)

Samples	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Pseudomonas aeruginosa*	*Propionibacterium acnes*
1	8.5 ± 0.1ab	8.25 ± 0.05a	10.45 ± 0.15	7.53 ± 0.24a
2	--	--	8.35 ± 0.04ab	8.37 ± 0.04b
3	15.3 ± 0.05	18.25 ± 0.05e	19.3 ± 0.01d	14.57 ± 0.05d
4	8.9 ± 0.25bc	--	8.65 ± 0.05b	7.67 ± 0.33bc
5	9.3 ± 0.15d	8.1 ± 0.12a	9.35 ± 0.04c	10.60 ± 0.28
6	10.4 ± 0.05	12.5 ± 0.24c	--	8.33 ± 0.38bc
7	9.31 ± 0.15d	8.42 ± 0.04a	--	8.43 ± 0.24b
8	--	9.7 ± 0.1b	--	7.43 ± 0.09a
9	21.1 ± 0.3f	15.5 ± 0.1d	19.35 ± 0.15d	15.73 ± 0.05d
10	9.1 ± 0.15cd	--	14.35 ± 0.12	13.17 ± 0.24
11	22.1 ± 0.2	18.85 ± 0.75e	11.45 ± 0.14	14.43 ± 0.24c
12	--	7.55 ± 0.52a	--	--
13	17.6 ± 0.1	16.25 ± 0.04d	--	11.47 ± 0.05
14	9.6 ± 0.15d	7.45 ± 0.045	--	--
15	13.1 ± 0.3	15.55 ± 0.25d	8.7 ± 0.4bc	--
16	--	8.25 ± 0.05a	8.4 ± 0.3a	--
17	--	8.25 ± 0.15a	8.95 ± 0.25bc	14.87 ± 0.03c
18	20.3 ± 0.2f	17.85 ± 0.23e	17.05 ± 0.55	--
19	8.0 ± 0.15a	7.9 ± 0.3a	8.35 ± 0.05ab	--
20	--	--	7.6 ± 0.3a	--
21	--	7.9 ± 0.7a	--	8.77 ± 0.19b
22	11.8 ± 0.1e	10.35 ± 0.05b	--	7.60 ± 0a
23	11.01 ± .01e	13.22 ± 0.31	15.36 ± 0.15	15.90 ± 0.04d
PC	23.23 ± 0.14	20.87 ± 0.24	21.87 ± 0.6	14.32 ± 0.37c

The column represents means of three replications ± standard deviation. Within a column, two consecutive values followed by different letters are significantly different ($p < 0.05$). Two values in same column sharing same letters are not significantly different ($p < 0.05$). PC: positive control (gentamycin 10 μg/disc).

Table 3. MIC and MBC values of test samples against microorganisms (mg/mL)

Samples	*Staphylococcus aureus*		*Staphylococcus epidermidis*		*Pseudomonas aeruginosa*		*Propionibacterium acnes*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
3	0.6	1.0	0.4	0.8	1.0	1.4	1.0	1.6
9	0.6	1.2	0.6	1.2	0.6	1.0	1.0	1.4
11	0.6	1.2	0.6	1.0	0.6	1.0	1.0	1.6
13	1.0	1.6	0.6	1.2	1.0	1.4	1.2	1.8
15	1.2	1.6	1.6	> 2.0	2.0	> 2.0	2.0	> 2.0
18	0.6	1.0	0.8	1.2	0.6	1.0	1.0	1.6
24	1.6	> 2.0	1.0	1.4	0.4	1.0	0.6	1.2
PC (μg/mL)	2.0	4.0	8.0	32.0	16.0	32.0	> 128	> 128

Selected test samples were screened for MIC and MBC. The test concentration used in the study was ranged from 0.2-2.0 mg/mL for both MIC and MBC. PC: positive control (gentamycin ranges from 0.2 to 128 μg/mL)

efforts to be made to search novel antibacterial agents against bacterial strains which are resistant to or less sensitive to the available antibiotics[23]. A variety of medicinal plant products have been investigated for antimicrobial activity and have shown good antimicrobial activity[24-26]. A high number of these agents appear to have structures and modes of actions that are distinct from those of the antibiotic currently in use[27]. In the current study, we have screened all the test samples for their potential antimicrobial activity against *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *P. acnes* using disc diffusion method. The zones of inhibitions (mm) produced by test samples at 2 mg/mL are depicted in Table 2. Highest zone of inhibition was recorded for *P. serrulata* (22.1 ± 0.2 mm) followed by *H. tuberosus* (21.1 ± 0.3 mm), *H. moellendorffii* (20.3 ± 0.2 mm) and *L. cuneata* (17.6 ± 0.1 mm) against *S. aureus* bacterium. There were no significant different ($p < 0.05$) was observed for *H. tuberosus* and *H. moellendorffii*. For *S. epidermidis* spp. The highest zone of inhibition was recorded for *P. serrulata* (18.85 ± 0.75 mm), *C. heterophylla* (18.25 ± 0.05 mm) and *H. moellendorffii* (17.85 ± 0.23 mm) and showed no significant difference from each other ($p < 0.05$), which were followed by *L. cuneata* (16.25 ± 0.04 mm), *E. maculata* (15.55 ± 0.25 mm) and *H. tuberosus* (15.5 ± 0.1 mm) and showed no significant difference ($p < 0.05$). Previous studies showed that Kaempferol isolated from *P. serrulata* and caffeoylquinic acid from *H. tuberosus* has shown to have antimicrobial activity[28-30]. As evident from the literature, the essential oils from genus *Heracleum* has shown good antibacterial and anti-fungal activity[31,32]. For *P. aeruginosa* spp. The highest zone of inhibition was recorded for *C. heterophylla* (19.3 ± 0.01 mm), *H. tuberosus* (19.35 ± 0.15 mm) and showed no significant difference ($p < 0.05$) with each other while significantly higher as compared to other samples ($p < 0.05$), which were followed by *H. moellendorffii* (17.05 ± 0.55 mm) and *S. officinalis* (15.36 ± 0.15 mm). The bacterium *P. acnes* has shown resistance as compared to other microbes against tested samples and highest zone of inhibition was recorded for *S. officinalis* (15.9 ± 0.04 mm) and *H. tuberosus* (15.73 ± 0.05 mm) which were significantly different as compared to other tested samples ($p < 0.05$). The current results are in agreement with previous observation in antimicrobial activity of the plants[33,34]. Table 3 shows the MIC and MBC values of selected test samples against *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *P. acnes.* The MIC and MBC values of the test samples were found to be in the range of 0.4-2.0 mg/mL and 0.8->2.0 mg/mL, respectively. From this study, we can conclude that the plants *S. officinalis*, and *C. heterophylla, H. tuberosus, H. moellendorffii* possessed the highest antimicrobial activity.

Total phenolic contents

The TPC of all the test samples is depicted in Fig. 1. TPC was calculated from the calibration curve ($R^2 = 0.997$) and ranged from 7.63 ± 0.23-86.23 ± 0.08 mg GAE/g of the sample. *C. heterophylla* (86.23 ± 0.08 mg GAE/g) has the highest TPC followed by *P. rigida* (76.97 ± 0.21 mg GAE/g) and *R. multiflora* leaf (70.81 ± 0.13 mg GAE/g). The highest TPC of *C. heterophylla* may be due to the various tannins present in the plant extract[34,35]. Methanolic extract of *P. rigida* has higher TPC (119.9 mg/g dry weight) as compared to the current study[36]. The difference in the TPC may be attributed to the extraction system used in the current study[37]. However, the phenolic compounds consisting of several phenol groups are important plant metabolites. Some of them are very reactive in neutralizing free radicals by donating a hydrogen atom or an electron, chelating metal ions in aqueous solutions[38]. Moreover, the phenolic compounds derived from medicinal plants possess a number of biological properties such as antimutagenic, antitumor and

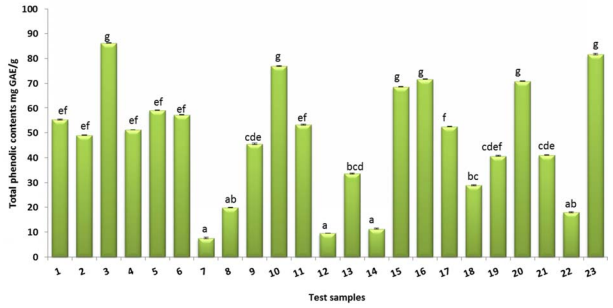

Fig. 1. The total phenolic contents of the test samples depicted as mg GAE/g. Value bar represents the mean ± standard deviation. Letters (a-g) represent significant difference among test samples. Bar sharing the same letter is not significantly different ($p < 0.05$).

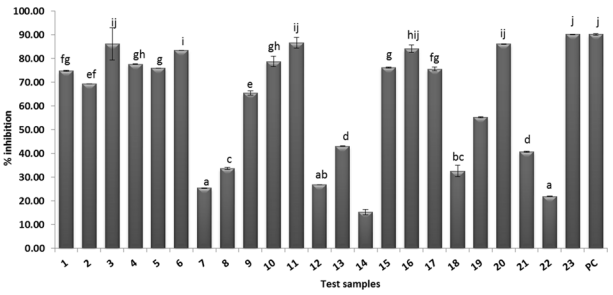

Fig. 2. Percent inhibition of DPPH free radical by test samples. Value bar represents the mean ± standard deviation. Letters (a-g) represent significant difference among test samples. Bar sharing the same letter is not significantly different ($p < 0.05$). PC: positive control (ascorbic acid was used at 100 μg/mL).

antibacterial properties, and these properties might be associated with their antioxidant activity[39,40]. In the present study it is found that the aqueous ethanolic extract of some test samples contains a substantial amount of phenolics.

DPPH-free radical scavenging assay

The DPPH-free radical scavenging potential of the test samples at 100 μg/mL were investigated and depicted in Fig. 2. All the test samples showed low to good scavenging potential. The highest % inhibition was recorded for *S. officinalis* with 90.21 ± 0.22% inhibition, followed by *C. heterophylla* (88.19 ± 6.73), *P. serrulata* (86.67 ± 2.22), *R. multiflora* leaf (86.1 ± 0.12), *P. tomentosa* (84.11 ± 1.57) and *B. scoparia* (83.45 ± 0.07). Results showed that *S. officinalis*, *C. heterophylla*, *P. serrulata*, *P. tomentosa*, and *R. multiflora* leaf extracts exhibited highest DPPH radical scavenging. The free radical scavenging activity of the test samples may contribute to the various antioxidant compounds in the plants. Plants, the primary sources of natural antioxidants that trap free radicals, have sourced antioxidants, such as vitamin E, vitamin C, phenolic acids, carotenes, phytate, and

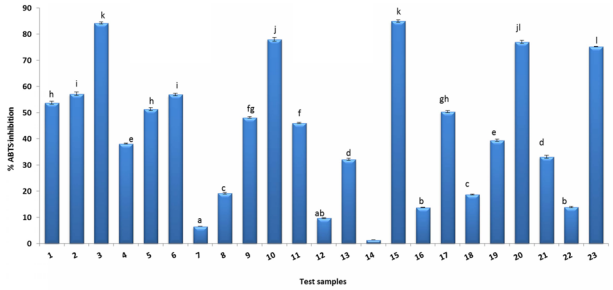

Fig. 3. Percent inhibition of $ABTS^{\bullet+}$ radicals by test samples. Value bar represents the mean ± standard deviation. Letters (a-g) represent significant difference among test samples. Bar sharing the same letter is not significantly different ($p < 0.05$).

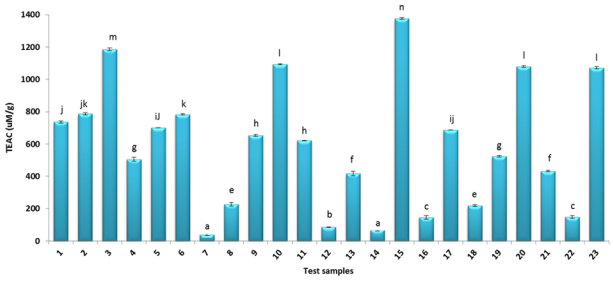

Fig. 4. TEAC values for test samples depicted as μM/g. Value bar represents the mean ± standard deviation. Letters (a-g) represent significant difference among test samples. Bar sharing the same letter is not significantly different ($p < 0.05$).

phytoestrogens[41]. The antioxidant activity of the test samples may be attributed to the extent of phenolics present in extract being responsible for its marked antioxidant activity as assayed through various *in vitro* models. Numerous reports have shown a very close relationship between TPC and antioxidative activity of the fruits, plants and vegetable[42-46].

$ABTS^{\bullet+}$ radical scavenging activity

$ABTS^{\bullet+}$ radical scavenging activity of all the tested samples was evaluated at 100 μg/mL and presented in Fig. 3. The current results showed that the $ABTS^{\bullet+}$ radical scavenging ability of samples could be ranked as *E. maculate* > *C. heterophylla* > *P. rigida* > *R. multiflora* leaf > *S. officinalis*. *E. maculata* and *C. heterophylla* exhibited prominent $ABTS^{\bullet+}$ radical scavenging activities. $ABTS^{\bullet+}$ assay depends on the antioxidant compound ability to scavenge $ABTS^{\bullet+}$ radical. By this assay, we can measure the antioxidant capacity of lipophilic and hydrophilic compounds in the same sample. $ABTS^{\bullet+}$, a protonated radical, has specific absorbance maxima at 734 nm that decreases with the scavenging of the proton radicals[47]. The $ABTS^{\bullet+}$ percent inhibition was reduced for *C. heterophylla*, *B. scoparia*, *R.*

multiflora leaf and *S. officinalis* as compared to DPPH. It was reported that there are a number of factors like stereoselectivity of the radicals or the solubility of the extract in different testing systems have been documented to affect the capacity of the plant extracts to react and quench different radicals[48]. Wang et al.[49] found that the test compounds exhibited strong $ABTS^{\bullet+}$ activity did not show DPPH free radical scavenging activity. In addition, $ABTS^{\bullet+}$ decolorization depends non-linearly on the concentration of certain antioxidants, such as quercetin[50,51].

Trolox equivalent antioxidant capacity

The TEAC of the test samples was determined and depicted in Fig. 4. Results showed that the highest TEAC was recorded for *E. maculata* with 1377.05 μm Trolox/g, followed by *C. heterophylla* with 1186.13 μm Trolox/g, *P. rigida* with 1094.29 μm Trolox/g, *R. multiflora* leaf with 1080.07 μm Trolox/g and *S. officinalis* with 1071.32 Trolox/g of the test samples. The TEAC values of a test sample are the ratio between the Trolox concentration and that of the test samples when both have the same antioxidant activity. Since, TEAC depends on the test sample and time of reaction, as some compounds may not react as quick as Trolox, exhibiting low TEAC values for early measuring times, and may not be more effective than Trolox if $ABTS^{\bullet+}$ amount scavenged is considered[51].

Cell viability assay

Medicinal plants have an indefinite capacity for the synthesis of bioactive compounds that are effective and have less adverse effects compared to synthetic drugs[52]. A major concern about bioactive compounds from plants is that some of these compounds are toxic to our normal system; therefore safety is critical in the development of novel drugs[53]. In this study, the toxic effect of test samples was investigated against RAW 264.7 macrophage cells as shown in Table 4. Percentage cell viability was calculated by measuring the absorbance of pink color formazan formed from the reduction of MTT solution by the presence of mitochondrial dehydrogenase in viable cells. All test samples at the highest concentration (1000 μg/mL) to cell line and this concentration, *P. serrulata*, *E. umbellata*, *L. cuneata* and *P. tomentosa* has achieved > 80% cell viability. However, at the lowest concentration (500 μg/mL), *H. tuberosus*, *P. serrulata*, *E. umbellata*, *L. cuneata*, *P. tomentosa*, *R. multiflora* leaf, *P. thunbergiana* flower and *P. thunbergiana* leaf extracts have achieved > 90% viability.

Table 4. Effects of test samples on cell viability of RAW 264.7 macrophage cells

Samples	% survival/MTT (μg/mL)		
	250	500	1000
1	79.49 ± 8.59	68.43 ± 7.23	59.14 ± 9.43
2	82.18 ± 7.27	70.72 ± 5.92	32.50 ± 3.21
3	31.95 ± 1.27	25.59 ± 1.01	14.89 ± 0.29
4	**83.05 ± 6.01**	**81.71 ± 7.43**	**79.96 ± 8.83**
5	89.22 ± 2.01	56.48 ± 9.29	21.92 ± 4.52
6	94.44 ± 3.80	50.92 ± 12.12	17.21 ± 0.53
7	69.63 ± 5.32	53.56 ± 4.90	21.42 ± 4.37
8	**98.28 ± 3.92**	**78.81 ± 9.84**	**26.44 ± 2.22**
9	**96.52 ± 8.44**	**92.70 ± 5.06**	**42.58 ± 7.41**
10	18.79 ± 1.02	14.57 ± 0.43	13.47 ± 0.82
11	**98.04 ± 4.43**	**96.01 ± 5.38**	**89.21 ± 5.01**
12	**99.35 ± 0.90**	**98.52 ± 2.03**	**87.85 ± 2.17**
13	**95.47 ± 2.92**	**94.16 ± 2.46**	**92.86 ± 5.67**
14	12.21 ± 0.28	13.32 ± 0.34	14.22 ± 0.43
15	27.11 ± 0.61	22.21 ± 1.96	17.99 ± 2.29
16	**94.06 ± 8.22**	**93.15 ± 4.17**	**81.98 ± 4.41**
17	75.72 ± 2.03	18.85 ± 1.57	24.62 ± 0.90
18	7.21 ± 3.01	6.17 ± 0.53	7.16 ± 0.72
19	66.14 ± 5.55	18.15 ± 2.69	13.92 ± 0.35
20	**97.47 ± 8.63**	**95.78 ± 5.01**	**64.34 ± 6.45**
21	**99.72 ± 0.54**	**90.60 ± 1.47**	**54.53 ± 7.42**
22	**101.11 ± 0.96**	**93.96 ± 4.32**	**52.34 ± 1.60**
24	41.95 ± 6.54	34.70 ± 1.97	28.470.36

Cells were treated with test samples at (250, 500 and 1000 μg/mL) for 24 h then percentage of cell viability was measured by MTT assay. On the basis of toxicity level, samples in bold were selected for further studies.

Anti-inflammatory assay

Inflammation is part of complex biological response of the body to harmful stimuli, such as pathogens, damaged cells, or irritants. Macrophages in the tissues play a critical role in the initiation and propagation of inflammatory responses by releasing proinflammatory mediators, i.e., NO, prostaglandin $(PG)E_2$ and cytokines to stimulate inflammatory responses[54-56]. Therefore, the extent of proinflammatory mediators and cytokines may exhibit the severity of inflammation and give information to study the effect of pharmacological agents on the inflammatory processes. In our study, as shown in Table 5, the release of nitrite was inhibited by test samples in a dose-dependent manner in LPS-induced RAW 264.7 macrophage cells. LPS induced control cell produced 39.01 ± 2.39 μg/mL. The highest activity was observed for *R. multiflora* leaf (1.26 μg/mL), followed by *P. tomentosa* (4.01 μg/mL) and *P. serrulata* (4.66 μg/mL) at the highest concentration of 400 μg/mL and are in agreement with previous results[57-60].

Table 5. Production of NO by RAW 264.7 macrophage cell treated with test samples

Samples	Production of NO (μg/mL)		
	100	200	400
4	27.37 ± 0.76^{ab}	27.54 ± 0.3^{b}	11.06 ± 2.82^{bc}
8	32.94 ± 1.23^{c}	17.44 ± 0.44^{a}	14.38 ± 3.37^{c}
9	23.59 ± 1.16^{a}	12.64 ± 3.55^{a}	9.80 ± 0.99^{bc}
11	38.94 ± 0.87^{d}	27.54 ± 3.55^{b}	4.66 ± 0.17^{ab}
12	40.23 ± 0.67^{d}	16.10 ± 3.91^{a}	30.45 ± 5.36^{d}
13	19.34 ± 0.26	12.81 ± 2.12^{a}	6.94 ± 1.04^{abc}
16	34.49 ± 0.35^{c}	16.77 ± 1.15^{a}	4.01 ± 0.12^{ab}
20	39.37 ± 1.11^{d}	28.09 ± 2.62^{b}	1.26 ± 0.5^{a}
21	42.37 ± 0.29^{d}	30.19 ± 0.61^{b}	27.17 ± 3.29^{d}
22	29.29 ± 1.20^{b}	11.92 ± 3.45^{a}	5.47 ± 0.92^{ab}

The experiment was performed in duplicate. Values are given as mean ± standard deviation. RAW cells were treated with test samples at 100, 200 and 400 μg/mL for 24 h, then following LPS treatment for 4 h and the production of nitrite was measured using a microplate reader. Within a column, two consecutive values followed by different letters are significantly different ($p < 0.05$). Two values in the same column sharing same letters are not significantly different ($p < 0.05$).

iNOS mRNA expression

To elucidate the mechanism responsible in the inhibition of NO generation by test samples in LPS-stimulated RAW 264.7 cells, we studied the effects of test samples on iNOS mRNA expression by reverse transcriptase polymerase chain reaction. iNOS was undetectable in unstimulated RAW 264.7 cells. All the results showed that LPS induced RAW 264.7 cells caused higher expression of iNOS gene as shown in Fig. 5. All the test samples were positive for iNOS gene expression. However, the level of expression is significantly lower ($p < 0.05$) as compared to LPS induced positive control. These results indicate that the reduced expression of iNOS at the transcriptional level contributed to the inhibitory effect of test samples on LPS-induced NO production. Various studies reported that NO is the critical mediator of inflammation. NO plays a vital role in numerous body functions; however, its excessive production, especially in macrophages, can lead to inflammation, cytotoxicity, and autoimmune disorders[61,62]. iNOS is one of the main enzymes producing NO from arginine in response to different inflammatory stimuli. Therefore, generation of endotoxin-induced NO can be used as a measure of the development of inflammation, and inhibition of their generation might have important therapeutic value for inhibiting inflammatory reactions and disease. This suppression may be attributed to inhibiting iNOSupregulation at the transcriptional level during RAW 264.7 cell activation by LPS.

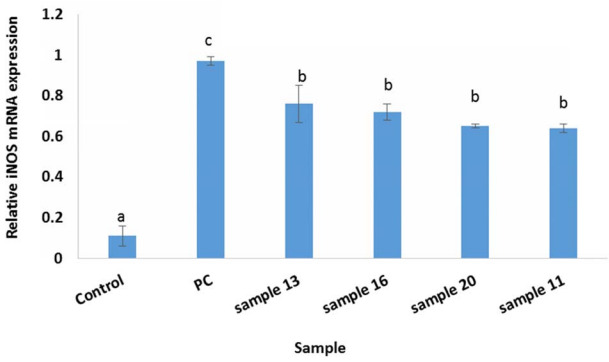

Fig. 5. Effects of test samples on iNOS expression in LPS induced RAW 264.7. LPS induced RAW 264.7 cells were treated with test sample at 400 μg/mL concentration for 24 h then activated with LPS (1 μg/mL) for 4 h. Values not sharing the same letter were significantly different ($p < 0.05$). PC: positive control (LPS induced RAW 264.7 cells).

Conclusion

The current research is the first comprehensive study that presented detailed information for antimicrobial, total phenolic, antioxidant, cytotoxic and anti-inflammatory activity of 20 medicinal plants of Korea. Out of 20 medicinal plants, 10 plants have passed the MTT screening and showed no or low cytotoxicity. Out of 10 medicinal plants, only 4 medicinal plants *R. multiflora, P. tomentosa*, *P. serrulata,* and *L. cuneata* have successfully inhibited the production of NO in LPS induced RAW 264.7 cells. These samples could be used as a dietary source of antioxidants, thus reducing risks of cancer, heart disease as well as other disorders like arthritis, etc. Completion and promising outcomes obtained from following studies would certainly strengthen its potential as a novel and cost-effective agent for the development of functional foods against various chronic infections.

Acknowledgment

This study was supported by the forestry technology research and development projects (S111515L130100) from Korea Forest Service and Forest Resources Development Institute of Gyeongsangbuk-do and partly supported by Kangwon National University in 2015. We also thankful to the central laboratory, Kangwon National University.

Disclosure statement

No potential conflict of interest was reported by the authors.

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