Antimicrobial and Antiradical Activity of Nepalese Medicinal Plants

Lok Ranjan Bhatt, Jin A Lim, Chi Hwan Lim[1], Seung Hwa Baek*

Department of Herbal Resources, Professional Graduate School of Oriental Medicine, Wonkwang University,
1 : Department of Bioenvironmental Chemistry, College of Agricultural & Life Sciences, Chungnam National University

In this study fourteen ethanol extracts from Nepalese medicinal plants were screened for their *in vitro* antimicrobial and antiradical activity and their total phenolic content was evaluated. The antiradicalactivity was evaluated by free radical scavenging assay, using 2,2-diphenyl-1-picryl hydrazyl radical (DPPH). Plant extracts showed a wide range of radical scavenging activity, with IC_{50} value ranging in between 5.38 ㎍/ mL - 429.61 ㎍/mL. Strong radical scavenging activity was shown by flower extract of *Woodfordia fruticosa* (5.38 ㎍/mL) and stem bark extract of *Azadirachta indica* (5.58 ㎍/mL)that also contained high phenolic content. Most of the sample showed activity below the concentration of 100 ㎍/mL. For antimicrobial activity three test microorganisms namely *Staphylococcus aureus*, *Streptococcus epidermidis*, and *Candida albicans* were used. The minimum inhibitory concentration (MIC) of the plant extracts was determined. Most of the plant extracts were effective against bacterial strains only at higher concentration (800 – 1,600 ㎍/mL) but none of these were effective against *Candida albicans* below 1,600 ㎍/mL.

Key words : Antimicrobial activity, radical scavenging activity, phenolic content, Nepalese plants

Introduction

Higher plants produce hundreds to thousands of diverse chemical compounds with different biological activities[1]. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, much recent attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine[2,3]. Plant based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials needs to occur. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials[4].

Antioxidants are of great importance in terms of preventing oxidative stress that may cause several degenerative diseases. The harmful action of the free radicals can, however, is blocked by antioxidant substances, which scavenge the free radicals and detoxify the organism. Current research into free radicals has confirmed that foods rich in antioxidants play an essential role in the prevention of cardiovascular diseases and cancers[5,6] and neurodegenerative diseases, including Parkinson's and Alzheimer's diseases[7]. Medicinal plants are reported to contain a wide variety of free radical scavenging compounds, such as phenolics, vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity. Phenolic compounds have received considerable attention because of their antioxidant activities. The complex phenolic antioxidants in the medicinal plants may play a significant role in the prevention and treatment of many of these diseases.

Herbal medicine represents one of the most important fields of traditional medicine in rural Nepal. Traditional medical treatment, supported mainly by the use of medicinal plants, represents the main alternative method, which has its basis on indigenous knowledge. So far, only a few species have been screened for antimicrobial[8,9] and antioxidant assay[10-12]. Medicinal plants with folklore reputation should be studied in order to promote their proper use and to determine their potential as sources for new drugs and food preservatives. Hence, in this study, we assessed fourteen ethnolic extracts from Nepalese plants that are in traditional medical practice as anti-inflammatory and anti-infectious remediesfor their antimicrobial and antiradical activities. We also estimated the total phenolic contents of these plants.

* To whom correspondence should be addressed at : Seung Hwa Baek, Department of Herbal Resources, Professional Graduate School of Oriental Medicine, Wonkwang University, Iksan, 570-749, Korea
· E-mail : shbaek@wonkwang.ac.kr, · Tel : 063-850-6225
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Materials and Methods

1. Selection and collection of plant material

For the present study, thirteen plant species that are used in the treatment of infectious and inflammatory diseases were collected from different parts of Nepal. The plants were collected and identified by one of us (L.R. Bhatt) and authenticated by Dr L.R. Sharma and Mr. P.P. Kurmi, National Herbarium, Department of Plant resources, Nepal. The freshly picked parts of the plants were air-dried at room temperature for 2 weeks, with no direct sunlight and then subjected to extraction.

Table 1. Name of plants, their family and vernacular name with their traditional uses

Name of plant	Family	Vernacular name	Traditional use
Acorus calamus L.	Araceae	Bojho	Diarhhoea and dysentery, intermittent fevers, cough, cold, respiratory problems[13].
Artimisia dubia Wall.	Compositae	Titepati	Headache, Raw leaves or leaf paste used in cut/wounds
Asparagus racemosus Willd.	Liliaceae	Jhijaraena	to increase lactation in women and cattle, Diarhoea/dysentery, fever, gonorrhoea[14].
Azadirachta indica A.	Miliaceae	Neem	Malaria, insecticidal, externally applied on wounds, sores, blisters and skin diseases[14].
Calotropis gigentea L.	Asclepiadaceae	Ank	Applied for sprains, burn, dysentery and skin diseases, malarial fever
Nyctanthes arbortristis L.	Oleaceae	Parijat	Fever and rheumatism, intestinal worm[14].
Ocimum sanctum L.	Labiatae	Tulasi	Leaf decoction mixed with honey and used in flue, cough and cold, infusion of leaves stomachic, in gastric disorders of children and in hepatic affections[14].
Oxalis corniculata L.	Oxalidaceae	Chariamilo	To treat boils and pimples, cut, wounds, diarrhoea and dysentery
Swertia species	Gentianaceae	Tite, chirayato	Fever, anthelmintic, diarrhoea, cholera[13,14].
Woodfordia fruiticosa Kurz.	Lythraceae	Dhayero	Stomach troubles, diarrhea[14].

2. Preparation of crude extracts

Each 10 gm of the air- dried and powdered plant materials was extracted using Soxhlet extractor with 95% ethanol (100 mL). The obtained extracts were evaporated by using vacuum evaporator under 40℃ to give the crude dried extract. The yields on dried weight of the extracts ranged from 5.41% for the extract of *Asparagus racemosus* (aerial parts) to 22.9% for the leaf extracts of *Calotropis gigentea.*

3. Microorganisms and media

The test organisms used in this study were as followed: *Staphylococcus aureus* (KCTC 1927), *Staphylococcus epidermidis* (KCTC 1917) and *Candida albicans* (KCTC 7965). *S. aureus* and *S. epidermidis* were grown in Nutrient broth and *C. albicans* in Sabouraud Dextrose broth.

4. Antimicrobial assay

Minimum inhibitory concentration (MIC) of the crude ethanolic extracts of plants against bacterial strains and *Candida albicans* were determined following[15] with some modifications. The organisms to be tested were grown in appropriate media at 37℃. The inocula of microorganisms were prepared from 16r broth cultures. The 96 well plates were prepared by dispensing 98 µL of media and 2 µL of inoculums. 100 µL of extract of each plant was prepared and each selected plant extract was subjected to a serial dilution. The plant extracts dissolved in dimethylsulfoxide (DMSO) were first diluted to the highest concentration (1,600 µg/mL to be tested, and then serial two-fold dilutions were made in a concentration range from 25 µg/mL to 1,600 µg/mL. The final concentration of DMSO in the assay did not exceed 2.5%. The last well containing 198 µL of nutrient broth without compound and 2 µLof the inoculum on each strip was used as negative control. The final volume of each well was maintained 200 µL. After completing the treatment, the plates were covered and incubated at 37℃ for 24 hours. The minimum inhibitory concentration (MIC) was the lowest concentration able to inhibit any visible growth of microorganism.

5. DPPH assay

DPPH radical scavenging assay of the samples was carried out after following with slight modifications. Briefly, five hundred microlitres of various concentrations of the extracts in methanol was added to Five hundred microlitres a 0.12 mM methanol solution of DPPH. After vortexing, the mixture was incubated for 30 minutes at room temperature and the absorbance was read against a blank at 517 nm. Extract concentration providing 50% inhibition (IC_{50}) was calculated using the graph by plotting inhibition percentage against extract concentration. All tests were carried out in triplicate.

6. Total phenolic content

The total phenolic content was determined following the Folin-Ciocalteu method[16]. The reaction mixture containing 200 μL of sample, 750 μL of the Folin-Ciocalteu reagent was mixed thoroughly. After one minute, 2 mL of 7.5% sodium carbonate solution was added. The final mixture was diluted to 7mL with deionized water. After 2 hr incubation in dark at room temperature the absorbance was measured at 765 nm against the blank (solution contained all the reaction reagents except the sample). Gallic acid (0-500 mg/L) was used for calibration of a standard curve. Total phenolic content was determined as

Gallic acid equivalents (GAE) and values were expressed as mg of acid/g of plant material (in GAE).

7. Statistical analysis

The data are results of triplicate experiments. Microsoft Excel was used to compute means, standard deviation and regression. The significance of difference was calculated by Student's t-test and values <0.05 were considered to be significant.

Results and Discussion

The results showed that many of the extracts showed activity against tested bacterial strains between 800-1,600 μg/mL concentration and little activity with *C. albicans* below 1,600 μg/mL. Ethanolic extracts of *Artimisia dubia*, *Azadirachta indica* and *Woodfordia fruticosa* showed activity at 800 μg/ml and rest of extracts showed activity at higher concentrations(Table 1).

Table 2. Antimicrobial activities of medicinal plants

Name of plant	Part tested	MIC (μg/mL)		
		S. aureus	*S. epiderdimis*	*C. albicans*
Acorus calamus	Whole plant	1,600	1,600	>1,600
Artimisia dubia	Leaf	800	800	>1,600
Asparagus racemosus	Twigs	>1,600	>1,600	>1,600
Asparagus racemosus	Tuber	1,600	1,600	>1,600
Azadirachta indica	Leaf	800	1,600	>1,600
Azadirachta indica	Stem bark	800	800	>1,600
Calotropis gigentea	Leaf	>1,600	1,600	>1,600
Nyctanthes arbortristis	Twigs	1,600	1,600	>1,600
Oxalis corniculata	Leaf	1,600	800	>1,600
Swertia chirata	Stem	>1,600	>1,600	>1,600
Woodfordia fruiticosa	Leaf	800	800	>1,600
Woodfordia fruticosa	Flower	1,600	1,600	>1,600
Ocimum sactum	Whole plant	>1,600	>1,600	>1,600

Previous study[17] reported that methanolic extracts of various species of *Artimisia* showed activity against gram-positive bacteria at higher concentration (10 mg/mL) but less active against *C. albicans*. It is reported that *Azadirachta indica* stem bark extracts inhibit 50% growth at 250 μg/mL and 90 % at 1,000 μg/mL against *S. aureus*[18]. Similarly, NIM-76, a spermicidal fraction from *A. indica* oil, showed stronger anti-microbial activity[19] than the whole neem oil against *S. aureus* (1,000 μg/mL) and *C. albicans* (250 μg/mL). However, acetone extract of flowers of same plant was found inactive against *S. aureus*[20].

The antioxidant potential of the plants was determined by using very stable free radicals 1,1-diphenyl-2-picrylhydrazyl (DPPH). Plant extracts showed high to moderate radical scavenging activity. The IC_{50} value varied from 5.38 μg/mL to 429.61 μg/mL(Table 2).

Table 3. Antiradicalactivity and total phenolic content of plants

Plant	Part tested	Yield (%)	IC_{50} (μg/mL)	TPC(mg GA/g)
Acorus calamus	Whole plant	10.50	212.69 ± 8.0*	21.10±3.11
Artimisia dubia	Leaf	20.16	21.42 ± 2.05**	108.43 ±7.21
Asparagus racemosus	Leaf	5.41	62.26 ± 1.22**	91.2±1.03
Asparagus racemosus	Tuber	10.35	76.95± 3.41 **	77.55±7.10
Azadirachta indica	Stem bark	17.21	5.58 ± 0.26**	409.18±15.5
Azadirachta indica	Leaf	16.13	13.39 ± 1.86*	246.85 ±10.64
Calotropis gigentea	Leaf	22.9	278.49 ± 7.36**	14.82±1.60
Nyctanthus arbortrtis	Aerial parts	17.03	26.26 ± 1.29**	143.7±10.65
Oxalis corniculata	Leaf	17.17	429.61 ± 10.11**	17.25±1.14
Swertia chirata	Stem	19.36	29.39 ± 1.89**	85.12±4.71
Swertia chirata	Leaf	20.63	82.42 ± 2.11**	155.33±4.08
Woodfordia fruticosa	Leaf	18.80	12.05 ± 0.67**	328.10±5.19
Woodfordia fruticosa	Flower	28.22	5.38 ± 0.15**	565.18±3.09

The values represent the mean ± standard deviations for triplicate experiments, significantly different from the control values; *$p < 0.05$, **$p < 0.01$ (Student's t-test)

Among the tested samples, strong radical scavenging activity was shown by the flower extract of *Woodfordia fruticosa* and stem bark extract of *Azadirachta indica*, followed by their leaf extracts(Table 2). Leaf aqueous and stem bark ethanol extracts of *Siamese neem* (*Azadirachta indica* A. Juss var. *siamensis* Valeton) has been reported to exhibit high antioxidant activities[21] and, bark aqueous extract of *Azadirachta indica* has potent antisecretory and antiulcer activity[22]. The bark extract is rich in phenols, unsaturated sterols, triterpenes and saponine[23] and number of phenolic diterpenoids, limonoids, c-secomeliacins, c-secolimonoids, polysachharides. Flowers of *Woodfordia fruticosa* are reported to have immunomodulatory activity[24] and Tannins isolated from the plant inhibited DNA topoisomerase II[25] and shown antitumor activity[26]. The strong scavenging capacity of these plant extracts might be due to the combined effect of tannins and other phenolic compounds that are reported to have ideal structural chemistry for free radical scavenging.[27] Besides, leaf extract of *Artimisia dubia* and *Nyctanthus arbortrtis*, stem extract of *Swertia chirata* and extract from Ocimum sanctum efficiently scavenged the DPPH free radical. Medium activity was exhibited by *Asparagus racemosus* extracts and leaf extract of *Swertia chirata* where as Acorus calamus, *Calotropis gigente* and *Oxalis corniculata* were less active(Table 2).

The total phenolic content of extracts was reported as gallic acid equivalent concentration (mg/g). The results showed thatthe extract with higher phenolic content exhibited stronger radical scavenging activity(Table 2). Extracts of *Azadirachta indica, Woodfordia fruticosa, Artimisia dubia, Nyctanthus arbortrtis* contained higher phenolic content and stronger radical scavenging activity. Plant-derived phenolic compounds are well known to exhibit antioxidant activity through a variety of mechanisms, including free

radical-scavenging, lipid peroxidation and chelating of metal ions[28]. The results of the present study in part indicatedthe contribution of phenolics towards antioxidant capacity, besides other factors.

In conclusion, of the 14 plant extracts tested, many appeared to have antimicrobial activity and high radical scavenging activity. Extracts of *Azadirachta indica* and *Woodfordia fruticosa* showed better antimicrobial activity and were the strongest radical scavengers that contained high phenolic content among the plants screened. They are promising plants for more detailed investigation of their antioxidant properties and application possibilities. Further investigations should be done on their *in vivo* antioxidant activity, and the different antioxidant mechanisms.

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