**Topclass Journal of Herbal Medicine Vol. 3(2) pp. 10-13, 26 Feb., 2014**
**Available online at http://www.topclassglobaljournals.org**
**ISSN 2315-8840 ©2014 Topclass Global Journals**

**Submitted** 3/1/14 **Accepted** 23/1/14

*Full Length Research Article*

# Antioxidant activity, total phenolic contents of Mongolian herbal medicine NARU-3

**Tuguldur A[1,2], Khongorzul TS[2], Narangerel B[3], Dagvatseren B[1], Saranchimeg B[1]**

[1]Traditional Medical Science Technology and Production Corporation of Mongolia
[2]Mongolian State University of Education
[3]Veterinary Institute of Mongolia

**********

**Abstract**

**The objectives of this study were to determine total phenolic content (TPC) and antioxidant activity of Mongolia herbal medicine Naru-3. The herb was studied using the Folin–Ciocalteu method and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity assay respectively. Total phenolic content is 6% of gallic acid equivalent (GAE). In the DPPH Radical Scavenging Assay, the methanol extract showed maximum inhibition of 78% at the maximum tested dose with $IC_{50}$ value of 32.1 µg/ml. With standard substance, the gallic acid was used and its free radical of suppressing action was $IC_{50}$= 1,67 mg/ml.**

**Keywords:** DPPH, gallic acid, Traditional Mongolian medicine, Naru-3

**********

## INTRODUCTION

Many persons today take herbal medicines or herbal products for the enhancement of their health in different national health-care settings (WHO 2004). NARU-3 is a traditional Mongolian medicine used traditionally for several decades in Mongolia for the treatment of rheumatoid arthritis. It consists of fine powders of *Terminalia chebula*, *Piper longum* and *Aconitum kuznezofii (Dagvatseren et al., 2003)*. These plants are rich in phenolic compounds and alkaloids (Ligaa *et al.,* 2005).

Phenolic compounds or polyphenols constitute one of the most numerous and widely-distributed groups of substances in the plant kingdom, with more than 8,000 phenolic structures currently known. Polyphenols are products of the secondary metabolism of plants. Polyphenols exhibit a wide range of biological effects as a consequence of their antioxidant properties (Duan *et al*., 2006). Antioxidants are compounds that neutralize chemically active products of metabolism such as free radicals which damage the body (Urquiaga and Leighton 2000).

**Corresponding Author's Email**: atogoldor@yahoo.com **Tel**: 976-11-99091077 **Fax**: 976-11-342551

Natural antioxidants occur in all parts of plants. Plants may contain many different antioxidant components such as phenolic compounds, nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids) and some other endogenous metabolites which are rich in antioxidant activities (Maestri *et al*., 2006).

In this research work the main objective is to study the antioxidant activity of then traditional Mongolian medicine NARU-3.

## MATERIALS AND METHODS

### Drug material

NARU-3 herbal medicine was prepared in the traditional medical factory of Traditional Medical Science Technology and Production Corporation of Mongolia. NARU-3 herbal medicine contains medicinal herbs such as the powders of *Terminalia chebula* 220 mg, *Piper longum* 620 mg and *Aconitum kuznezofii 156* mg.

### Chemicals and Reagents

DPPH (2,2 diphenyl-1-picryl hydrazil radical), Folin–Ciocalteu phenol reagent and gallic acid (3,4.5-trihydroxybenzoic) were obtained from Fluka Chemie (Buchs, Switzerland). Methanol and Ethanol were from Riedel-de Haen (Sigma-Aldrich, Germany). Sodium carbonate was from PRS (Panreace Quimica, EU).

### Determination of total phenolics

The content of total phenolic compounds in formulations was determined by Folin-Ciocalteu reagent (Singleton and Rossi 1965). Exactly 1.0 g of the drug sample was extracted with 100 ml distilled water in a conical flask at 100°C (in a water bath) for 30 mins.

Water solution of the extract in the concentration of 0.2 mg/ml was used in the analysis. 0.5 ml of water solution were added to 2.5 ml of 1:10 diluted Folin–Ciocalteu reagent. After 5 mins, 2 ml of sodium carbonate (7%) was added. After 2 h of incubation at room temperature, the absorbance at 760 nm was measured.

Gallic acid (0.04-0.36mg/ml) was used for the standard calibration curve. The content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GAE/g of extract). Percentage total phenolic scavenging effect was calculated from the following formula:

$$X = \frac{c \; x \; V \; x \; V2}{m \; x \; V1} \; x \; 100$$

### Antioxidant Activity

### Preparation of extract

100 mg of the drug sample was extracted with 100 ml of methanol (99.8%) in a conical flask at room temperature for 24 h. Then the extract was filtered using a Whatman Filter paper no 1.

### DPPH Radical Scavenging Assay

Free radical scavenging effect was determined using the free radical generator DPPH (2,2-diphenyl-1-picrylhydrazyl) (Masafumi *et al.*, 2001). 0.006% of DPPH was prepared in methanol1.5 ml of the DPPH solution was mixed with 1.5 ml of various concentration (6.25, 12.5, 25 and 50 µg/ml) of extract and standard solution separately.

These solution mixtures were kept in dark for 30 mins and absorbance was measured in 517 nm using spectrophotometer. Methanol (1.5 ml) with 1.5 ml DPPH solution was used as control. Control sample was prepared containing the same volume without any extract and gallic acid was used as standard in 1-100 µg/ml solution. Methanol was used as blank. Percentage DPPH scavenging effect was calculated using the following formula:

$$\%\ scavenging\ \text{activity} = \frac{Abs\ control - Abs\ sample}{Abs\ control} * 100$$

## STATISTICAL ANALYSIS

All analyses were carried out in triplicates. The results of scavenger activity and total phenolic contents were performed from the averages of all samples reading Mean ± SD used Excel 2007.

## RESULTS AND DISCUSSION

### Phenolic Content of the Extracts

Table 1 shows the total phenol contents that were measured by Folin-Ciocalteu reagent in terms of gallic acid equivalent (GAE). The calibration curve showed linearity for gallic acid in the range of 25 - 400 µg·ml–1, with a correlation coefficient (R2) of 0.999 (Figure 1). From the chemical result of NARU-3 medicine, it was determined that the phenolic content in ethanolic extract reached 8.2 %.

### Antioxidant Activity of the Extracts

Methanol extract of NARU-3 showed a significant DPPH

**Table 1.** Polyphenol contents of NARU-3

| | Water extract of NARU-3 | 70% ethanolic extract of NARU-3 |
|---|---|---|
| Total phenolic content (%) | 6.0 | 8.2 |

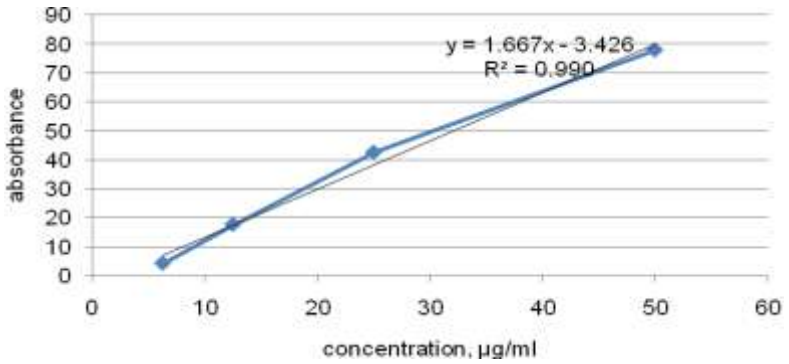


**Figure 1.** Calibration curve of gallic acid for total phenolics

**Table 2.** DPPH radical scavenging activity of Gallic acid

| Effective concentration (µg/ml) | % inhibition of DPPH radical scavenging activity | $IC_{50}$ value, µg/ml |
|---|---|---|
| 0.3125 | 27.48 | 1.67 |
| 0.625 | 36.57 | |
| 1.25 | 42.61 | |
| 2.5 | 62.77 | |

**Table 3.** DPPH radical scavenging activity of NARU-3

| Effective concentration (µg/ml) | % inhibition of DPPH radical scavenging activity | $IC_{50}$ value, µg/ml |
|---|---|---|
| 6.25 | 4.26 | 32.1 |
| 12.5 | 17.7 | |
| 25 | 42.6 | |
| 50 | 78 | |

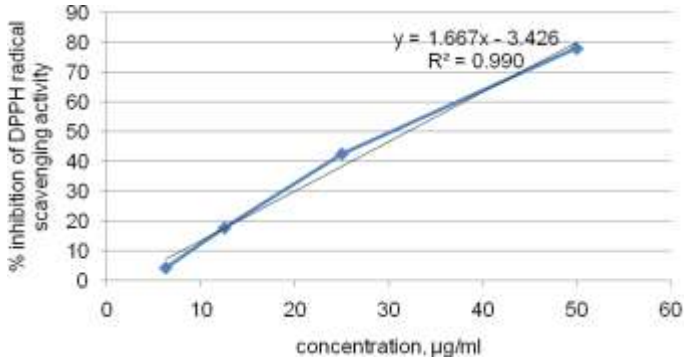


**Figure 2.** DPPH free radical scavenging activity of NARU-3

radical scavenging activity of free radicals. The $IC_{50}$ values of standard and samples were found to be 1.67 µg/ml and 32.1 µg/ml where $R^2$ was found to be 0.9988 and 0.9904 (Table 2 and 3; Figure 2 and 3).

## CONCLUSION

It can be concluded that methanolic extract of Naru-3 and its ingredients have good antioxidant property and could be attributed to the presence of flavonoids, alkaloids, tannins and phenolic compounds. The results of this study show that methanolic extract of Naru-3 and its constituents can be of use as an easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry.

### REFERENCES

Dagvatseren B, Khishigjargal L, Narantsetseg D (2003). Reference book of traditional medicine and herbal materials. Ulaanbaatar. Admon. P. 146

Duan XJ, Zhang WW, Li XM, Wang BG (2006). Evaluation of antioxidant property of extract and fractions obtained from a red alga, Polysiphonia urceolata. Food Chem. 95:37-43

Ligaa U, Davaasuren B, Ninjil N (2005). Medicinal Plants of Mongolia Used in Western and Eastern Medicine. Ulaanbaatar: JCK Printing. P. 220

Maestri DM, Nepote V, Lamarque AL, Zygadlo JA (2006). Natural products as antioxidants. Phytochemistry: Advances in Research. P. 105-135

Masafumi O, Junei K, Toshihiro N, Ono M (2001). DPPH Radical Scavenging Activity of Flavonoids Obtained from Some Medicinal Plants. Biol. Pharm. Bull. 24(10):1202-1205

Singleton VL, Rossi JA 1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am. J. Enol. Viticult. 16:144-158

Urquiaga I, Leighton F (2000). Plant Polyphenol Antioxidants and Oxidative Stress Biol. Res. 33(2):55-64.

Waterman PG, Mole S (1994). Analysis of Phenolic Plant Metabolites. Blackwell Cientific Publications, Oxford, UK. P. 85-87

WHO (2004). Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems. P. 82