International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 11-10-2021, Accepted: 26-10-2021, Published: 12-11-2021
Volume 6, Issue 6, 2021, Page No. 280-284

Determination of total phenolic and flavonoid contents and antioxidant activity of the stem of *Achyranthes aspera* L. from garhwal Himalaya

Seema Devi[1], Asha[2]
[1] Research Scholar, Department of Botany and Microbiology, HNB Garhwal University, Srinagar, Garhwal, Uttarakhand, India
[2] Assistant Professor, Department of Botany and Microbiology, HNB Garhwal University, Srinagar, Garhwal, Uttarakhand, India

Abstract
Achyranthes aspera L. is an important medicinal plant that grows throughout the tropical and warmer regions of the world. This plant is widely used as anti-microbial, cancer chemo-preventive, nephroprotective, diuretic hepatoprotective, analgesic, anti-inflammatory, hypolipidemic, etc. This work aimed to evaluate phytochemical content, quantification of total phenolic and flavonoid content, and antioxidant activity in the stem of *Achyranthes aspera* L. in chloroform, methanol, and distilled water extract. The total phenolic content was estimated spectrophotometrically using the Folin Ciocalteu method and the total flavonoid content was measured by aluminum chloride colorimetric assay. Antioxidant activity was evaluated by a 2, 2-diphenyl-1picrylhydrazyl (DPPH) assay. The maximum total phenolic and flavonoid content was found in methanolic extract of the stem - 13.34 mg GAE/g and 49.86mg RE/g respectively. The IC_{50} value for chloroform, methanol and distilled water were 588.69µg/mL, 26.11µg/mL, and 283.30µg/mL respectively. The highest DPPH radical scavenging activity was observed in methanol extract. This examination ropes a good answer to the utilization of *Achyranthes aspera* L. from Garhwal Himalaya as a natural antioxidant and in herbal medicine.

Keywords: *Achyranthes aspera* L., stem extract, total phenolic content, total flavonoid content, antioxidant activity

Introduction

Plants are the basis of life on earth and are central to people's livelihoods [1]**.** Plants have been the foundation of many traditional medicine systems all over the world since very ancient times and have been providing new treatments to mankind [2]. The Garhwal region of Uttarakhand is highly enriched with medicinal plants. Such plants are highly potential with their traditional value and medicinal value due to the presence of bioactive compounds, primary & secondary metabolites [3]**.** In recent times, there is an increasing interest in the role of free radical-mediated damage in the etiology of human sicknesses. In the status of normal metabolism, the levels of oxidants and antioxidants in humans are kept up in balance, which is important for sustaining optimal physiological conditions [4, 5]. Overuse of oxidants in some situations can cause an imbalance, causing oxidative damage to large biomolecules such as lipids, DNA, and proteins [6]. It is accepted that the intake of antioxidant substances strengthens defenses against free radicals. Due to the toxicity of synthetic antioxidants, their use has been limited [7]. Therefore, it is of great significance and necessity that research focuses on discovering potential natural, effective antioxidants to replace synthetic ones. *Achyranthes aspera* L. is a perennial herb belonging to the family Amaranthaceae. It grows throughout the tropical and warmer regions of the world [8]. It grows as an herb in wasteland [9]. *Achyranthes aspera* is a stiff erect herb reaching up to a height of 2 m having branched stems bearing leaves of different sizes and shapes. Flowers are arranged in long spikes in the form of inflorescence [10]. *Achyranthes aspera* L. is known for its different medicinal properties and is widely used for the treatment of various diseases in humans. It is used for the cure of fever, dysentery, asthma, hypertension, and diabetes [11]. The hot water extract of this plant has been used as an antiarthritic to reduce arthritic pain in Chinese traditional medicine [8]. In Ayurvedic medicine, *A. aspera* has been used as a diuretic in the treatment of dropsy. Unani doctors used the stem, leaves, and fruits of *A. aspera* for renal dropsy, pneumonia, cough, kidney stone, skin eruptions, snake bite, gonorrhea, and dysentery [12]. It was used to cure abdominal tumors and injured skin in Bangladesh. In the Philippines, *A. aspera* was used to treat odontalgia [13]. It was used medicinally by Maasai people to cure malaria in Kenya [14]. In Jammu, ash left after burning spike with honey is used for curing cough and fever [15]. The leaf juice is applied on boils in Uttarakhand [16]. The current study was attempted for the screening of metabolites, to determine the total phenolic and flavonoid content, and the antioxidant activity of Stem of *Achyranthes aspera* L. from Garhwal region of Uttarakhand.

Materials and Methods

Chemicals and reagents

Glucose, Gallic acid, Rutin, Aluminium chloride, Sodium nitrite, Sodium hydroxide, Folin's Ciocalteau reagent, Sodium carbonate, DPPH, Ascorbic acid and, other reagents were laboratory grade purchased from Hi-Media. The plant extracts were prepared by using laboratory-grade solvent methanol, chloroform, and aqueous extract.

Sample collection

The *Achyranthes aspera* L. stem was collected from the Chauras campus of Hemvati Nandan Bahuguna Garhwal University, Srinagar Garhwal, Uttarakhand in November 2018. After collection, the stem was cleaned with running tap water to eliminate dust particles, dried in the open air

under shade and, powdered with the help of a mixer grinder. The powdered dried stem was stored in air-tight polybags at 4°c.

Extract preparation

Different extracts were prepared by sequential solvents extraction from non-polar to polar (chloroform, methanol and, distilled water). 50g of the powdered stem was extracted with chloroform using the Soxhlet apparatus followed by methanol and distilled water. The plant extract was filtered through Whatman no.1 filter paper. These extracts were concentrated and dried using a vacuum rotary evaporator at 40 ℃ [17].

Determination of extraction yield

The percentage of extraction yield was calculated as follows:

$$Extraction\ yield = \frac{We}{Ws} X100$$ [18]

where, We = Weight of the extract after evaporation solvent and freeze-drying, Ws = dry weight of the sample

Qualitative phytochemical screening

Preliminary phytochemical screening for detection of metabolites such as carbohydrates, proteins, Amino-acids, alkaloids, phenols, flavonoids, terpenes, saponins, tannins, gums and mucilages, steroids, glycosides, volatile oils, was carried out on chloroform, methanol, and distilled water extracts according to the standard methods [17, 19–26].

Quantitative estimation

Estimation of total phenolic content

The total phenolic content of the extract was performed by the Folin-Ciocalteu method described by Makkar [27] with slight modifications. 100µL of each extract and 0.5mL of 1N Folin-Ciocalteu reagent were mixed and allowed to stand for 5 min. at room temperature. After 5 minutes, 2.5mL of 5 % sodium carbonate solution was added. The mixture was kept in dark for 40 min. at room temp. After incubating all the samples, their absorbance was measured at 725 nm against blank by UV-Vis spectrophotometer. The calibration curve for gallic acid (0-100µg/mL) was established to calculate total phenolic content. The estimation of the phenolic content was carried out in triplicates. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram dry weight of extract (mg GAE/g DW). The equation of the standard curve was y=0.0197x+0.0289, R^2= 0.9976

Estimation of total flavonoid content

The total flavonoid content of each extract was determined using the aluminum chloride method described by Zhishen *et al*. [28] with slight modifications. 100 µL of each extract was mixed with 150 µL of 5 % sodium nitrite and kept at room temperature for five minutes. After that 150 µL of 10 %, aluminum chloride solution was added and allowed to stand for 6 minutes. After 6 minutes 2 mL of 4 % sodium hydroxide was added and made the final volume up to 5mL using distilled water and incubated at room temperature for 15 minutes. After incubating all the samples, their absorbance was measured against the reagent blank at 510 nm using a Systronics double beam UV-Vis spectrophotometer (Model AU-2701). The calibration curve for Rutin (0-200µg/mL) was established to calculate total flavonoid content. The estimation of the flavonoid content was performed in triplicates. The concentration of TFC was expressed as mg rutin equivalents per gram dry weight of each extract (mg RE/g DW). The equation of the calibration curve was y=0.0021x-0.0019, R^2=0.9957

Antioxidant activity

The antioxidant activity of the stem of different extracts was determined using DPPH- free radical scavenging assay described by Abi Beaulah and Truong *et al*. [29, 18] with slight modifications. To 1 mL extracts in various concentrations (100-600 µg/mL) of stem, 1 mL DPPH solution (methanolic 0.1 mM DPPH) was added. The mixture was shaken vigorously, allowed to stand at room temperature for 40 min and absorbance was measured at 517 nm by UV-Vis Spectrophotometer. All experiments were performed in triplicate. Ascorbic acid in the concentration range 100-600 µg/mL was used as standard. The percent DPPH scavenging effect was calculated using the following equation: DPPH scavenging effect (%) = [(A0-A)/A0] x 100 Where A0-Absorbance of Negative control. A1-Absorbance of Standard or Sample. The value of IC_{50} (inhibitory concentration), was calculated by plotting the percentage of residual DPPH against the sample concentration.

Results and Discussion

Effect of different solvents on extraction yield

Among the selected solvents the highest extraction yield was observed in distilled water (15.51%), followed by methanol (6.62%) and chloroform (0.96%) signifying that the extraction yield favors the highly polar solvents [18] (Table 1).

Qualitative screening

Achyranthes aspera L. stem revealed the presence of various primary and secondary metabolites such as carbohydrates, proteins, amino acids, phenols, flavonoids, saponins, glycosides, tannins, terpenes. Among these carbohydrate, protein, phenols, flavonoid, and glycoside were present in all three extracts. Saponin and tannin were present in methanol, and distilled water extract. The amino acid was detected only in methanol extract. Terpenes were present only in chloroform extract (Table 2). Here, methanol was observed to be the best solvent to extract phenolic and flavonoid compounds from *Achyranthes aspera* L. stem. The presence of tannin and phenolics like flavonoids in the plant extracts may act as primary antioxidants [30]. Several studies reported that polyphenols and flavonoids have a strong antioxidant activity and they are considered as anticancer, anti-inflammatory, antiviral, and antibacterial agents due to their antioxidant and free radical scavenging properties [31].

Total phenolic and flavonoid content

The total amount of phenol and flavonoid content was calculated from gallic acid (Fig 1) and rutin standard curves (Fig 2). The distribution of phenolic compounds in stem extract demonstrated that methanol extract contained the highest amount with a value of 13.34 mg of GAE/g dry weight and the lowest phenolic content was observed in chloroform extract with a value of 4.01 mg of GAE/g (Table 3). The total flavonoid content of stem extract contained the

highest amount in methanol extract with a value of 49.86 mg of RE/g and the lowest flavonoid content was observed in chloroform extract with a value of 38.49 mg of RE/g (Table 4). Furthermore, the methanolic extract of the stem showed higher flavonoid content than phenol. In earlier studies, it was reported that methanol extract of stem showed slightly high phenolic content than aqueous extract [11]. Methanol extract showed higher flavonoid and phenol content as compared to other solvents [18, 32]. Moreover, the solubility of the phenolic compound is governed by the type of extraction and solvent used, the degree of polymerization of phenolics, and their interactions [31].

Antioxidant activity

The antioxidant activity of different extracts was illustrated in (Fig. 3). The samples were prepared at different concentrations (100-600 µg/mL). Ascorbic acid was used as a standard with IC_{50} value was calculated to be 8.52µg/mL. The IC_{50} value for chloroform, methanol and distilled water was 588.69 µg/mL, 26.11µg/mL, and 283.30 µg/mL respectively (Table 5).

Among these, methanol extract was the most potent with a low IC_{50} value of 26.11µg/mL. It is in consonance with earlier workers who have reported a higher antioxidant activity with a low IC_{50} value [33-35]. The maximum scavenging percentage of methanol extract is in tune with the works of Priya *et al*. and Khuma [11, 18, 32]. The IC_{50} value of all the extracts was higher than ascorbic acid.

Conclusion

Our result concluded that the methanolic extract of the stem of *Achyranthes aspera* L. from Garhwal Himalaya possesses almost all important types of phytochemical constituents and antioxidant potential thus validating the ethnopharmacological claims. In plant parts, flavonoids could be a significant source of antioxidant molecules. Phenolics in plants are effective free radical scavengers and antioxidants. This plant has diversified pharmacological potential and was used since ancient times. This plant is locally available in abundance so it can be utilized for the pharmacological purpose which will help the financial upliftment of the local people. Further studies need to be carried out to explore the maximum pharmacological potential of the whole plant from Garhwal Himalaya.

Acknowledgments

The authors are grateful to the Department of Botany and Microbiology, H. N. B., Garhwal University, Srinagar Garhwal, Uttarakhand for support.

Conflicts of Interest

The authors declare no conflicts of interest.

Table 1: Determination of extractive value of *Achyranthes aspera* L. stem

S. No	Quantity (g)	Solvent used	Amount of extraction after evaporation	% of extract
1.	50	Chloroform	0.488g	0.96
2.	50	Methanol	3.311g	6.62
3.	50	Distilled water	7.753g	15.51

Table 2: Result of qualitative phytochemical screening of *Achyranthes aspera* L. stem

S. No.	Phytochemicals	Tests	Chloroform	Methanol	Distilled water
1.	Carbohydrates	Benedict’s test	+	+	+
		Molish's test	+	+	+
		Fehling’s test	-	+	+
2.	Proteins	Biuret test	-	-	-
		Xanthoprotenic test	+	-	+
3.	Amino-acids	Ninhydrin test	-	+	-
4.	Alkaloids	Wagner's	-	-	-
		Mayer's	-	-	-
		Dragendroff's test	-	-	-
5.	Phenols	Lead acetate test	+	+	+
6.	Tannins	Ferric chloride test	-	-	+
		Gelatin test	-	-	-
7.	Flavonoids	Lead acetate test	+	+	+
8.	Saponins	Honeycomb	-	+	+
		Foam test	-	+	+
9.	Steroids	Salkowski’s test	-	-	-
10.	Terpenes		+	-	-
11.	Glycosides	Keller-Killiani test	-	-	-
		Glycoside test	-	-	-
12.	Gums and mucilage		-	-	-
13.	Fixed oil and fats		-	-	-
14.	Volatile oils		-	-	-

+ = presence, - = absence

Table 3: Total phenolic content of different extracts of stem of *Achyranthes aspera* L.

S. No.	Extract 100µg/mL	Total phenolic content (mg GAE /g DW)
1.	Chloroform	4.01 ± 0.1
2.	Methanol	13.34 ± 0.5
3.	Distilled water	5.84 ± 0.2

All values are the mean ± SD (n=3)

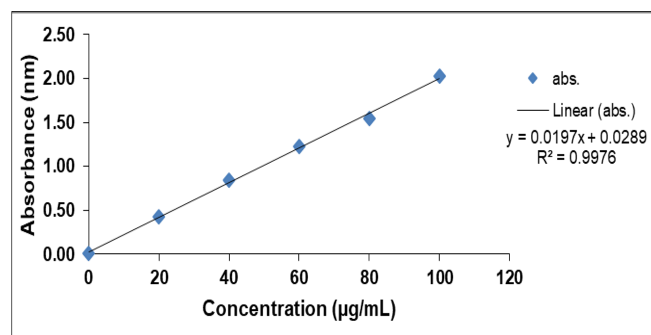

Fig 1: Standard calibration curve of Gallic acid for the determination of total phenolic content

Table 4: Total Flavonoid Content of different extracts of stem of *Achyranthes aspera* L.

S. No.	Extract 500µg/mL	Total Flavonoid content (mg RE/g DW)
1.	Chloroform	38.49 ± 0.8
2.	Methanol	49.86 ± 1.0
3.	Distilled water	41.37 ± 0.5

All values are the mean ± SD (n=3)

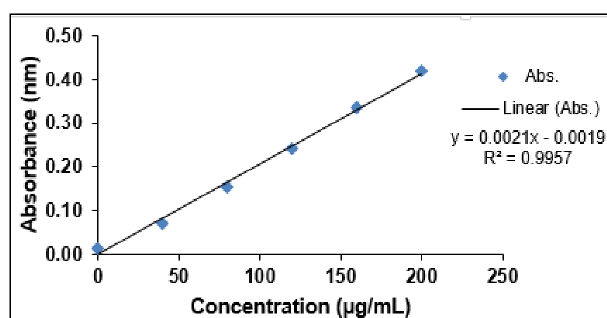

Fig 2: Standard calibration curve of Rutin for the determination of total flavonoid content

Table 5: The 50% inhibitory concentration (IC_{50}) values of DPPH scavenging activity of different *Achyranthes aspera* L. stem extract.

S. No.	Samples	IC_{50} value µg/Ml
1.	Chloroform	588.69 ± 5.7
2.	Methanol	26.11 ± 10.54
3.	Distilled water	283.30 ± 13.34
4.	Ascorbic acid	8.52 ± 5.62

All values are the mean ± SD (n=3)

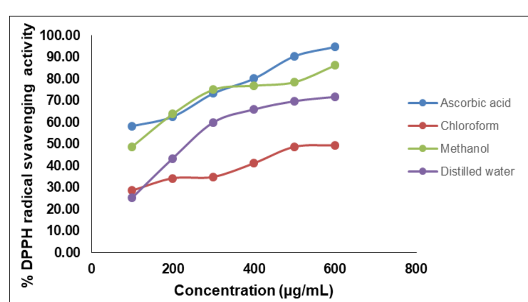

Fig 3: Result for DPPH radical Scavenging model of *Achyranthes aspera* L. stem extracts.

References

1. Sajem AL, Gosai K. Traditional use of medicinal plants by the Jaintia tribes in North Cachar Hills district of Assam, northeast India. Journal of Ethnobiology and Ethnomedicine,2006:2(1):1-7.
2. Mondal M, Hossain MS, Das N, Khalipha AB, Sarkar AP, Islam MT, Smrity SZ, Biswas S, Kundu SK. Phytochemical screening and evaluation of pharmacological activity of leaf Methanolic extract of *Colocasia affinis* Schott. Clinical Phytoscience,2019:5(1):1-11.
3. Dogra S, Vidyarthi S, Chandra S, Saklani S. *In Vitro* Antimicrobial Activity and Phytochemical Screening of *Rubus Laciocarpus* Leaves. World journal of pharmaceutical research,2018:7(11):566-573.
4. Temple NJ. Antioxidants and disease: more questions than answers. Nutrition research,2000:20(3):449-459.
5. Thompson LU. Antioxidants and hormone-mediated health benefits of whole grains. Critical Reviews in Food Science and Nutrition,1994:34(5-6):473-497.
6. Liu RH. Supplement quick fix fails to deliver. Food Technol. Int,2002:1:71-72.
7. Valentão P, Fernandes E, Carvalho F, Andrade PB, Seabra RM, Bastos ML. Antioxidative properties of cardoon (*Cynara cardunculus* L.) infusion against superoxide radical, hydroxyl radical, and hypochlorous acid. Journal of agricultural and food chemistry,2002:50(17):4989-4993.
8. Hossain MJ, Khaleda L, Chowdhury AM, Arifuzzaman M, Al-Forkan M. Phytochemical screening and evaluation of cytotoxicity and thrombolytic properties of *Achyranthes aspera* leaf extract. Journal of Pharmacy and Biological Sciences,2013:6(3):30-38.
9. Singh S, Verma SK, Singh SK. In-vitro anticancer activity of *Achyranthes aspera* root extract against different human cancer cell lines. Biolife,2017:5(1):119-122.
10. Sharma V, Chaudhary U. An overview on indigenous knowledge of *Achyranthes aspera*. Journal of Critical Reviews,2015:2(1):7-19.
11. Priya CL, Kumar G, Karthik L, Rao KV. Antioxidant activity of *Achyranthes aspera* Linn stem extracts. Pharmacologyonline,2010:2(2):228-237.
12. Dey A. *Achyranthes aspera* L: phytochemical and pharmacological aspects. International journal of pharmaceutical sciences review and research,2011:9(2):72-82.
13. Zafar R. Medicinal plants of India. CBS Publishers and Distributors, New Delhi, India,2000, 1-15.
14. Bussmann RW, Gilbreath GG, Solio J, Lutura M, Lutuluo R, Kunguru K *et al*. Plant use of the Maasai of Sekenani Valley, Maasai Mara, Kenya. Journal of ethnobiology and ethnomedicine,2006:2(1):1-7.
15. Bhatia H, Sharma YP, Manhas RK, Kumar K. Ethnomedicinal plants used by the villagers of district Udhampur, J&K, India. Journal of ethnopharmacology,2014:151(2):1005-1018.
16. Sharma J, Gairola S, Sharma YP, Gaur RD. Ethnomedicinal plants used to treat skin diseases by Tharu community of district Udham Singh Nagar, Uttarakhand, India. Journal of Ethnopharmacology,2014:158:140-206.
17. Banu KS, Cathrine L. General techniques involved in phytochemical analysis. International Journal of Advanced Research in Chemical Science,2015:2(4):25-32.
18. Truong DH, Nguyen DH, Ta NT, Bui AV, Do TH, Nguyen HC. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and *in*

vitro anti-inflammatory activities of *Severinia buxifolia*. Journal of food quality, 2019, 2-9

19. Geetha TS, Geetha N. Phytochemical screening, quantitative analysis of primary and secondary metabolites of *Cymbopogan citratus* (DC) Stapf. leaves from Kodaikanal hills, Tamilnadu. International Journal of pharmtech research,2014:6(2):521-529.
20. Kokate CK, Purohit AP, Ghokale SB. Text book of Pharmacognosy, 22nd edition, Nirali prakashan; Pune, India, 2002, 1-280.
21. Semwal P, Anthwal P, Kapoor T, Thapliyal A. Preliminary investigation of phytochemicals of *Saussurea obvallata* (Brahm Kamal) and *Pittosporum eriocarpum* (Agni): two endangered medicinal plant species of Uttarakhand. International Journal of Pharmacognosy,2014:1(4):266-269.
22. Talreja T, Goswami A, Sharma T. Preliminary phytochemical analysis of *Achyranthes aspera* and *Cissus quadrangularis*. Journal of Pharmacognosy and Phytochemistry,2016:5(5):362.
23. Devmurari VP. Phytochemical screening study and antibacterial evaluation of *Symplocos racemosa* Roxb. Archives of applied science research,2010:2(1):354-359.
24. Kumar V, Jat RK. Phytochemical estimation of medicinal plant *Achyranthes aspera* root. International Journal of Research in Pharmacy and Pharmaceutical Sciences,2017:3(1):190-193.
25. Obianime AW, Uche FI. The Phytochemical screening and the effects of methanolic extract of *Phyllanthus amarus* leaf on the Biochemical parameters of Male guinea pigs. Journal of Applied Sciences and Environmental Management, 2008, 12(4).
26. Sivasankari S, Banu BJ, Sadiq AM, Kanagavalli U. Phytochemical evaluation and Antioxidant potential of ethanolic leaf extract of *Achyranthes aspera*, 2017.
27. Makkar HP. Quantification of tannins in tree and shrub foliage: a laboratory manual. Springer Science & Business Media, 2003, 30.
28. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chemistry,1999:64(4):555-559.
29. Abi Beaulah G, Mohamed Sadiq A, Jaya Santhi R. Antioxidant and antibacterial activity of *Achyranthes aspera*: An *in vitro* study. Annals of Biological Research, 2011:2(5):662 – 670.
30. Sharma V, Chaudhary U, Singh R, Agarwal A. *Achyranthes aspera*: phytochemical estimation. American Journal of Pharmtech Research,2013:3(2):242-251.
31. El Atki Y, Aouam I, Taroq A, Lyoussi B, Taleb M, Abdellaoui A. Total phenolic and flavonoid contents and antioxidant activities of extracts from *Teucrium polium* growing wild in Morocco. Materials Today: Proceedings,2019:13:777-783.
32. Dhital KS. Screening of phytochemicals, estimation of total phenolic and total flavonoid content and Antioxidant activity in *Achyranthes aspera* L: International Journal of Chemical Science,2018:2(3):01-06.
33. Rama P, Vignesh A, Lakshmanan G, Murugesan K. *In vitro* antioxidant activity of *Achyranthes aspera* Linn. International Journal of Medicine and Pharmaceutical Sciences,2013:3(2):67-78.
34. Jadid N, Hidayati D, Hartanti SR, Arraniry BA, Rachman RY, Wikanta W. Antioxidant activities of different solvent extracts of *Piper retrofractum* Vahl. using DPPH assay. In AIP conference proceedings. AIP Publishing LLC,2017:1854(1):020019.
35. Rana PS, Saklani P, Chandel C. Influence of altitude on secondary metabolites and antioxidant activity of *Coleus forskohlii* root extracts. Research Journal of Medicinal Plants,2020:14(2): 43-52.