Short Communication

Innate antioxidant activity of some traditional formulations

Gunpreet Kaur, Vikas Gupta, Parveen Bansal

Department of Herbal Drug Technology, University Centre of Excellence in Research, Baba Farid University of Health Sciences, Faridkot, Punjab, India

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Abstract

Herbal medicine is the oldest form of healthcare known to humanity. Recently, much attention is being directed toward the use of antioxidants. There are some very commonly used Ayurvedic preparations that might have inbuilt antioxidant activity, and their therapeutic potential can be partially attributable to its antioxidant activity. Hence, it was proposed to find out antioxidant activity of such common formulations. Estimation of innate antioxidant activity of some commonly used traditional formulations. In this study, five formulations were evaluated for antioxidant activity in comparison to gallic acid (standard) using the *in vitro* reducing power method and superoxide radical scavenging activity by dimethyl sulfoxide method followed by calculation of scavenging activity and inhibitory concentration 50% (IC_{50}). The result shows that Ayurvedic drug extracts possess good reducing power and antioxidant activity. *Laxmivilas Ras* shows higher reducing power ranging from 117 ± 0.021 to 0.176 ± 0.012 as compared to other extracts. The drug extracts were also found to be an efficient scavenger of superoxide radical. The IC_{50} values for *Laxmivilas Ras*, *Agnitundi Vati, Ajmodadi Churna*, *Tribhuvankirti Rasa,* gallic acid (standard) and *Sitopladi Churna,* were found to be 50.07, 98.41, 105.13, 116.39, 176.80, and 200.17, respectively. From this study, it can be concluded that the above Ayurvedic formulations possess antioxidant property. However, work could be initiated on the isolation and identification of these antioxidant components.

Key words: Antioxidant, dimethyl sulfoxide, reducing power, superoxide radical

INTRODUCTION

In more than 80% of developing countries, citizens use traditional medicines based on plant products. *Ayurveda* as a system of medicine contributes profoundly to the wellness, curative, and preventive aspects of the diseased conditions.[1] It is a myth that Ayurvedic preparations are having lesser side effects and act in a way that eradicates the diseases from the level of its pathogenesis. It is increasingly being realized that many of today's diseases are due to the "oxidative stress" that results from an imbalance between formation and neutralization of free radicals.[2] Antioxidants either prevent the reactive oxygen species (ROS) from being formed or remove them before it damages vital components of the cell.[3] There is an increased interest in natural antioxidants present in medicinal and dietary plants that might help to prevent oxidative damage.[4] A number of synthetic antioxidants such as butylated hydroxyanisole butylated hydroxytoluene, and tert-butylhydroquinone are widely available in the market. However, restriction on the

Address for correspondence:
Dr. Parveen Bansal,
University Centre of Excellence in Research, Baba Farid University of Health Sciences, Faridkot - 151 203, Punjab, India.
E-mail: ucer_bfuhs@rediffmail.com

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synthetic antioxidants is being imposed because of their toxicity to liver and carcinogenicity.[5,6]

In the past few years, interest in search of new natural antioxidants has grown up because ROS production and oxidative stress is linked to many diseases and the use of synthetic antioxidants generally leads to the problem of toxicity.[7] The free radical are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling, and immune function but they are also involved in various diseases such as diabetes,[8] rheumatoid arthritis,[9,10] high blood pressure,[11] urinary tract disorders,[12] bronchial asthma,[13,14] and nonhealing wounds.[15] Therefore, research for determination of traditional formulation having innate antioxidants source is of immense importance. Today due to an unlimited number of diseases people are consuming medicines in large quantities. It would be really wonderful if these medicines in addition to their therapeutic effect could provide an antioxidant cover to the body. In such cases, it becomes important for scientists to find out some formulations that are having some innate antioxidant activity that could be helpful in potentiating the therapeutic action of drug (by depleting the free radicals) and also fight with the free radicals generated in the process of disease as well as a side effect of medication. Keeping in mind the above situation, it was thought to estimate innate antioxidant activity of some commonly used formulations, so that insight into the mechanism of their action can be established.

MATERIALS AND METHODS

For accomplishing the above study five commonly used formulations *Sitopaladi Churna, Laxmivilas Ras*, *Tribhuvankirti Rasa, Ajmodadi Churna,* and *Agnitundi Vati* were selected.

Material

The Ayurvedic drugs used for evaluating antioxidant potential were procured from Ayurvedic Clinical Research Centre, at Baba Farid University of Health Sciences, Faridkot. The chemicals used in the analysis were of analytical reagent grade and all the glassware's used were of Borosil grade.

Methods

Preparation of aqueous extract

The Ayurvedic drugs were ground into fine powder. Each 50 mg powdered drug was mixed with 50 ml distilled water with intermittent shaking and then filtered with Whatman filter paper No. 1. The filtrate was then concentrated to dryness on a water bath at 100°C. Finally, the concentrated extract was collected and stored in a refrigerator for further use.

Determination of antioxidant activity

The antioxidant activity of each test sample was tested using the following *in vitro* methods:

Reducing power method

Reducing the power of the extract was determined according to the method of Oyaizu[16] gallic acid was used as a standard reference. The test was performed in triplicates.

Superoxide radical scavenging activity by alkaline dimethyl sulfoxide method

The superoxide scavenging activity was determined using the method of Elizabeth and Rao[17] gallic acid was used as a standard reference. The test was performed in triplicates and inhibitory concentration 50% (IC_{50}) value was calculated.

Scavenging activity was calculated by using equation

Scavenging activity[18] (%) = Test absorbance − Control absorbance/Test absorbance × 100

The antioxidant activity is expressed as IC_{50}. The IC_{50} value is the measure of concentration in µg/ml of drug that inhibits 50% of free radicals.

Statistical analysis

Linear regression analysis was used to calculate IC_{50} values.[19]

RESULTS

In this study, authors investigated the antioxidant activity of different formulations. From Table 1, it is clear that Ayurvedic drugs possess good reducing power. *Laxmivilas Ras* has maximum reducing the power of 0.117 ± 0.021 to

Table 1: Antioxidant activity of Ayurvedic formulations measured by reducing power method

Concentration (µg/ml)	Absorbance at 700 nm (OD)±SE (*n*=3)					
	Sitopaladi Churna	*Laxmivilas Ras*	*Agnitundi Vati*	*Ajmodadi Churna*	*Tribhuvankirti Rasa*	Standard (gallic acid)
50	0.034±0.002	0.117±0.021	0.073±0.001	0.069±0.002	0.073±0.013	0.068±0.000
100	0.066±0.001	0.136±0.013	0.098±0.000	0.081±0.007	0.096±0.008	0.087±0.001
250	0.089±0.034	0.149±0.007	0.107±0.001	0.094±0.003	0.108±0.012	0.098±0.001
500	0.104±0.010	0.161±0.019	0.116±0.002	0.107±0.001	0.112±0.013	0.112±0.001
1000	0.112±0.007	0.176±0.012	0.121±0.002	0.118±0.003	0.120±0.010	0.123±0.000

The value is expressed as mean±SE (*n*=3). SE: $\sigma/\sqrt{n}$, σ: Standard deviation, *n*: Number of sets, SE: Standard error

Table 2: Antioxidant activity of Ayurvedic formulations measured by dimethyl sulfoxide method

Concentration (μg/ml)	Scavenging activity (%)±SE (*n*=3)					
	Sitopladi Churna	*Laxmivilas Ras*	*Agnitundi Vati*	*Ajmodadi Churna*	*Tribhuvankirti Rasa*	Standard (gallic acid)
50	33.3±0.051	92.4±0.013	82.3.±0.002	78.4±0.004	74.7±0.007	54.2±0.001
100	75.4±0.026	95.3±0.007	83.7±0.013	83.5±0.006	81.3±0.002	67.6±0.001
250	80.1±0.103	95.9±0.011	85.5±0.009	85.0±0.016	82.3±0.014	76.3±0.005
500	80.7±0.005	97.1±0.023	85.1±0.010	85.4±0.008	85.2±0.010	84.8±0.003
1000	85.7±0.034	98.6±0.011	87.1±0.017	87.3±0.013	87.3±0.007	88.0±0.002

The value is expressed as mean±SE (*n*=3). SE: σ/√*n*, σ: Standard deviation, *n*: Number of sets, SE: Standard error

0.176 ± 0.012 as compared to other Ayurvedic extracts that is *Agnitundi Vati*, *Tribhuvankirti Rasa, Ajmodadi Churna,* and *Sitopladi Churna.*

The Ayurvedic drugs were found to be an efficient scavenger of superoxide radical generated in alkaline dimethyl sulfoxide method. The results in Table 2 clearly indicate that the drugs have a noticeable effect as scavenging superoxide radical. The highest superoxide radical scavenging activity was shown by *Laxmivilas Ras*, that is, 98.6 ± 0.011 at concentration 1000 μg/ml as compared to other Ayurvedic extracts and standard.

IC_{50} values for these Ayurvedic drugs were shown in Table 3. IC_{50} value was found to be lowest in *Laxmivilas Ras* as compared to *Agnitundi Vati*, *Ajmodadi Churna* followed by *Tribhuvankirti Rasa*, standard (gallic acid), and *Sitopladi Churna.*

DISCUSSION

The reducing capability of a compound may serve as a significant indicator of its antioxidant potential. The reducing ability of the extract depends on the presence of reducing equivalents[20] that exhibit antioxidant potential by breaking the free radical chain and donating a hydrogen atom.[21] The results showed that *Laxmivilas Ras* has higher reducing power and superoxide radicals scavenging activity as compared to gallic acid (standard), *Agnitundi Vati*, *Tribhuvankirti Rasa*, *Ajmodadi Churna,* and *Sitopladi Churna.* Lower the IC_{50} value higher will be the antioxidant activity.[22] On similar lines, *Laxmivilas Ras* exhibited very strong antioxidant activity as its IC_{50} value was lowest as compared to other Ayurvedic extracts. The results of antioxidant status in the present study were found to be indirectly proportional to IC_{50} value as found in earlier studies.

CONCLUSION

From this study, it can be concluded that the above Ayurvedic formulations possess a significant antioxidant property. There is a stringent need to go for further work on the isolation and identification of the antioxidant components in the Ayurvedic formulations. There is also need to analyze the antioxidant activity of normal formulations too so that a database can be prepared to know the antioxidant potential of traditional formulations present in the market.

Table 3: Inhibitory concentration 50% value of different extracts by dimethyl sulfoxide method

Test sample	IC_{50} (μg/ml)
Laxmivilas Ras	50.07
Agnitundi Vati	98.41
Ajmodadi Churna	105.13
Tribhuvankirti Rasa	116.39
Standard (gallic acid)	176.80
Sitopaladi Churna	200.17

IC_{50}: Inhibitory concentration 50%

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Conflicts of interest

There are no conflicts of interest.

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