



Screening of *In-vitro* Radical scavenging potential of Indigenous medicinal herb *Rungia repens* using DPPH, ABTS, NO and Hydrogen peroxide radical scavenging assays

J. Nandha Kumar^{*1}, V. Manjari², R. Madhavan³, N. J. Muthu Kumar⁴

¹ P.G Scholar, Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Tambaram Sanatoruim, Chennai 600047, Tamilnadu, India.

² Lecturer , Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Tambaram Sanatoruim, Chennai 600047, Tamilnadu, India.

³ Associate professor and HOD (I/C), Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Tambaram Sanatoruim, Chennai 600047, Tamilnadu, India.

⁴ Director, National Institute of Siddha, Tambaram Sanatoruim, Chennai 600047, Tamilnadu, India.

*Corresponding Author: J. Nandha Kumar

P.G Scholar, Department of Nanju Noolum Maruthuva Neethi Noolum, National Institute of Siddha, Tambaram Sanatoruim, Chennai 600047, Tamilnadu, India.

Abstract

The usage of Siddha medicine in Tamil Nadu and several parts of Southern India has considerably increased over the past two decades and it is steadily crossing the various geographies owing to its cost effectiveness compared to conventional medicines and has fairly high acceptance rates because of its herbal origin and therefore its nontoxic nature. However the information pertains to the efficacy of several Siddha herbs are not properly documented. *Rungia repens* (RR) commonly known as Kodagasalai in tamil belongs to the family Acanthaceae, is a potential medicinal herb, its has been traditionally used for treatment for several ailments, but still now there is no proper documentary evidence available with respect to the anti-oxidant potential of this herb. Hence the present investigation aimed at evaluating the radical chelating potential of the herb RR through in-vitro DPPH, ABTS, NO and Hydrogen peroxide radical scavenging assays. Results obtained from DPPH radical assay clearly shown that the herb RR has revealed highest percentage inhibition of 60.88 % with IC50 value of 81.13 (µg/ml). Similarly with NO radical scavenging assay the drug RR reveals the maximum percentage inhibition of 34.56 % with corresponding IC50 value 156.5 (µg /ml). In ABTS and hydrogen peroxide radical scavenging activity the herb RR has revealed the maximum percentage inhibition of 63.9 % and 36.41 % their corresponding IC50 values were found to be 69.26 and 143.26 (µg /ml). From the result analysis of the present investigation it was concluded that the herb RR possess significant radical scavenging activity in all the tested in-vitro assays which may be due to the presence of bioactive phytochemicals. By considering the potential of this indigenous herb *Rungia repens*, it may be ideal for management of stress related disorder's in near future.

Keywords: Oxidative stress, Herbal remedies, *Rungia repens*, Antioxidants, DPPH, ABTS, NO, Hydrogen peroxide.

1. Introduction

The oxidative stress, which occurs in cells, in general, can be combated by antioxidants since they hold oxidation stability and therefore prevent the formation of reactive species of oxygen and nitrogen. Reactive oxygen species such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide may favor the development of diseases such as cancer, cardiovascular disorders, aging, and degenerative diseases. In contrast, the consumption of natural antioxidants, such as polyphenol-rich foods, fresh fruits, and vegetables, can counteract the oxidative degradation of free radicals [1,2].

Antioxidants are important in the prevention of human diseases. Compounds with antioxidants activity may function as free radical scavengers, complexers of pro-oxidants metals, reducing agents, and quenchers of single-oxygen formation or reactive oxygen species, thereby protecting the body from degenerative diseases such as cancer. The reactive oxygen species (ROS) are harmful byproducts generated during normal cellular metabolism or from toxic insult. They lead to a state of oxidative stress that contributes to the pathogenesis of a number of human diseases by damaging lipids, proteins and DNA [3]. This has inspired much interest in antioxidant activity of phytochemicals.

The World Health Organization estimated that over 80% of the people in developing countries rely on traditional remedies such as herbs for their daily needs and about 855 traditional medicines include crude plant extracts. This means that about 3.5 to 4 billion of the global population rely on plants resources for drugs. However, the traditional usages of just some of these medicinal plants have been investigated in vitro and clinical trial studies [4,5].

The use of medicinal herbs and herbal medicines is an age-old tradition, and the recent progress in modern therapeutics has stimulated the use of natural products worldwide for diverse ailments and diseases [6]. In traditional medicine, folk people used medicinal plants in diverse manners to treat diseases [7]. Traditional medicine is popular in all regions of the world, and its use is rapidly expanding even in developed countries [8].

Rungia repens known as Kodagasalai in tamil belongs to the family Acanthaceae, is a potential medicinal herb spreading throughout India [9,10]. The herb is

dried and pulverized and traditionally used for treatment of cough and fever; it is also credited with vermifugal and diuretic properties [11]. Fresh, bruised leaves are mixed with castor oil and applied to scalp to cure *Tinea capitis*, a scaly fungoid infection, usually occurring amongst children [12-15]. Investigation on the flavonoid pigments in ivory-white and pale yellow flowers showed the presence of luteolin and chrysoeriol (3'-o-methyluteolin) and their glucosides [16]. It was reported for the presence of isosalipurposide, luteolin and delphinidin-3,5-diglucoside [17].

2. Materials and Methods

2.1. DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay

The antioxidant activity of test drug sample RR was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay. Sample RR was mixed with 95% methanol to prepare the stock solution in required concentration. From the stock solution 1ml, 2ml, 4ml, 6ml 8ml and 10ml of this solution were taken in five test tubes and by serial dilution with same solvent were made the final volume of each test tube up to 10 ml whose concentration was then 10 µg/ml, 20 µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml respectively. Ascorbic acid were used as standard was prepared in same concentration as that of the test drug by using methanol as solvent. Final reaction mixture containing 1 ml of 0.3 mM DPPH methanol solution was added to 2.5 ml of sample solution of different concentrations and allowed to react at room temperature. Absorbance in the presence of test sample RR at different concentration of (10 µg, 20 µg, 40 µg, 60 µg, 80 µg and 100µg/ml) was noted after 15 min incubation period at 37°C. Absorbance was read out at 517 nm using double-beam U.V Spectrophotometer by using methanol as blank [18].

$$\% \text{ scavenging} = \left[\frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \right] \times 100$$

The effective concentration of test sample RR required to scavenge DPPH radical by 50% (IC₅₀ value) was obtained by linear regression analysis of dose-response curve plotting between %inhibition and concentrations

2.2. Nitric Oxide Radical Scavenging Assay

The concentrations of test sample RR are made into serial dilution from 10–100 µg/mL and the standard

gallic acid. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylenediaminedihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 mL of the different concentrations of the test drug (10–100 µg/mL) and incubated at 25°C for 180 mins. The test drug RR was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the test drug but with an equal volume of buffer were prepared in a similar manner as was done for the test samples [19]. The absorbance was measured at 546 nm using a Spectra Max Plus UV-Vis microplate reader (Molecular Devices, GA, USA). Gallic acid was used as the positive control. The percentage inhibition of the test drug RR and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the test drug RR and gallic acid were calculated using the following formula:

percentage nitrite radical scavenging activity:

$$\text{nitric oxide scavenged (\%)} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100,$$

where A_{control} = absorbance of control sample and A_{test} = absorbance in the presence of the samples extracts or standards.

2.3. ABTS Assay

This assay carried out for the purpose of evaluating the anti-oxidant potential of test drug RR against 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS radicals. The ABTS radical cation method was modified to evaluate the free radical-scavenging effect of one hundred pure chemical compounds. The ABTS reagent was prepared by mixing 5 mL of 7 mM ABTS with 88 µL of 140 mM potassium persulfate. The mixture was then kept in the dark at room temperature for 16 h to allow free radical generation and was then diluted with water (1:44, v/v). To determine the scavenging activity, 100 µL ABTS reagent was mixed with 100 µL of test sample (10-100 µg/ml) and was incubated at room temperature for 6 min. After incubation, the absorbance was measured 734 nm [20]. 100% methanol was used as a control. Gallic acid with same concentrations of test drug RR was measured following the same procedures described above and was used as positive controls. The antioxidant activity of the test sample RR was calculated using the following equation: The ABTS scavenging effect was measured using the following formula:

Radical scavenging (%)

$$= \left[\frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}} \right] \times 100.$$

2.4. Hydrogen Peroxide Radical Scavenging Assay

A hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 mL) of the test sample RR (different concentration ranging from 10-100 µg/ml) were transferred into the test tubes and their volumes were made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4). After adding 0.6 mL hydrogen peroxide solution, tubes were vortexed and the absorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against a blank. BHA was used as the positive control [21]. The percentage inhibition of the test drug RR and standard was calculated and recorded. The percentage radical scavenging activity of the test drug RR and BHA were calculated using the following formula:

Radical scavenging (%)

$$= \left[\frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}} \right] \times 100$$

3. Results

3.1. Result Analysis of DPPH radical scavenging activity of RR

The results of DPPH radical scavenging activity of the sample RR reveals significant DPPH radical scavenging activity dose dependently. Sample RR were screened for DPPH radical scavenging activity and the percentage inhibition ranges from 8.71 to 60.88 % when compared with standard ascorbic acid with percentage inhibition ranges from 38.63 to 95.92 %. The IC₅₀ value of the trial drug was found to be 81.13 (µg /ml) when compared with standard ascorbic acid with (IC₅₀ value 16.42 µg/ml). The values of DPPH free radical scavenging activity of the RR was tabulated in table 1 and 2.

Table 1: Percentage inhibition of test drug RR on DPPH radical scavenging assay

Concentration (µg/ml)	% Inhibition of RR	% Inhibition of Ascorbic Acid
10 µg/ml	8.718 ± 1.282	38.63 ± 3.357
20 µg/ml	18.6 ± 7.213	59.61 ± 3.647
40 µg/ml	28.5 ± 8.827	67.57 ± 6.914
60 µg/ml	40.42 ± 5.041	75.19 ± 5.329
80 µg/ml	47.94 ± 4.176	81.76 ± 2.077
100 µg/ml	60.88 ± 10.15	95.92 ± 0.2162

Data are given as Mean ± SD (n=3)

Table 2: IC50 Values for DPPH radical scavenging Assay by RR and standard.

Test Drug / Standard	IC50 Value DPPH Assay ± SD (µg /ml)
Ascorbic acid	16.42 ± 3.063
RR	81.13 ± 13.82

Data are given as Mean ± SD (n=3)

3.2. Result Analysis of Nitric oxide radical scavenging activity of RR

NO radical scavenging activity of the sample RR revealed that the percentage inhibition of the test drug ranges from 4.53 to 34.56 % when compared with standard gallic acid with percentage inhibition ranges

from 29.85 to 89.28 % .The corresponding IC50 value of the trial drug was found to be 156.5 (µg /ml) when compared with standard gallic acid with (IC₅₀ value 37.44µg/ml). The values of NO radical scavenging activity of the RR was tabulated in table 3 and 4.

Table 3: Percentage inhibition of test drug RR on Nitric Oxide radical scavenging assay

Concentration (µg/ml)	% Inhibition of RR	% Inhibition of Gallic Acid
10 µg/ml	4.53 ± 2.862	29.85 ± 0.5689
20 µg/ml	10.08 ± 3.721	44.3 ± 1.599
40 µg/ml	17.25 ± 5.992	53.25 ± 0.9429
60 µg/ml	20.84 ± 6.38	58.75 ± 0.9429
80 µg/ml	27.43 ± 6.989	76.49 ± 1.683
100 µg/ml	34.56 ± 9.823	89.28 ± 0.279

Data are given as Mean ± SD (n=3)

Table 4: IC50 Values for Nitric Oxide radical scavenging assay by RR and standard.

Test Drug / Standard	IC50 Value NO Assay ± SD (µg /ml)
RR	156.5 ± 42.04
Gallic acid	37.44 ± 1.484

Data are given as Mean ± SD (n=3)

3.3. Result Analysis of ABTS radical scavenging activity of RR

The results of ABTS radical scavenging activity of the sample RR shown that the test drug has significant

ABTS radical scavenging activity dose dependently. Trial drug RR were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 13.62 to 63.9 % when compared with standard gallic acid with percentage

inhibition ranges from 27.65 to 94.18 % .The corresponding IC₅₀ value of the trial drug was found to be 69.26 (µg /ml) when compared with

standard gallic acid with (IC₅₀ value 28.66µg/ml). The values of ABTS radical scavenging activity of the RR was tabulated in table 5 and 6.

Table 5: Percentage inhibition of test drug RR on ABTS radical scavenging assay

Concentration (µg/ml)	% Inhibition of RR	% Inhibition of Gallic Acid
10 µg/ml	13.62 ± 2.295	27.65 ± 1.628
20 µg/ml	26.05 ± 2.465	49.88 ± 1.928
40 µg/ml	33.99 ± 2.29	61.48 ± 1.125
60 µg/ml	47.06 ± 2.089	75.19 ± 1.055
80 µg/ml	57.63 ± 1.673	86.05 ± 1.555
100 µg/ml	63.9 ± 0.5398	94.18 ± 0.38

Data are given as Mean ± SD (n=3)

Table 6: IC₅₀ Values for ABTS radical scavenging assay by RR and standard.

Test Drug / Standard	IC ₅₀ Value ABTS Assay ± SD (µg /ml)
RR	69.26 ± 0.7764
Gallic acid	28.66 ± 1.558

Data are given as Mean ± SD (n=3)

3.4. Result Analysis of hydrogen peroxide radical scavenging activity of RR

Trial drug RR were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 5.44 to 36.41% when compared with standard BHA with percentage inhibition ranges

from 29.23 to 79.91 % .The corresponding IC₅₀ value of the trial drug was found to be 143.26 (µg /ml) when compared with standard BHA with (IC₅₀ value 41.12µg/ml). The values of hydrogen peroxide radical scavenging activity of the RR was tabulated in table 7 and 8.

Table 7: Percentage inhibition of test drug RR on Hydrogen peroxide radical scavenging assay

Concentration (µg/ml)	% Inhibition of RR	% Inhibition of BHA
10 µg/ml	5.448 ± 2.094	29.23 ± 3.035
20 µg/ml	11.73 ± 1.047	41.26 ± 1.715
40 µg/ml	17.66 ± 2.18	51.19 ± 3.455
60 µg/ml	22.2 ± 2.094	61.48 ± 3.396
80 µg/ml	29.53 ± 2.77	70.99 ± 2.748
100 µg/ml	36.41 ± 2.621	79.91 ± 1.574

Data are given as Mean ± SD (n=3)

Table 8: IC₅₀ Values for Hydrogen peroxide radical scavenging assay by RR and standard.

Test Drug / Standard	IC ₅₀ Value Hydrogen peroxide radical scavenging Assay ± SD (µg /ml)
RR	143.2 ± 11.87
BHA	41.12 ± 2.604

Data are given as Mean ± SD (n=3)

4. Discussion

Reactive oxygen species (ROS) react with free radicals to become radicals themselves. Oxygen, although essential to life, is the source of the potentially damaging free radicals. Antioxidants counteract these cellular by-products, called free radicals, and bind them before they can cause damage. In fact, free radicals are believed to play a role in more than 60 different health conditions, including the aging process, cancer, and atherosclerosis [22]. Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents, and pesticides. Therefore, ROS can cause lipid peroxidation in foods leading to their deterioration. In addition, these ROS can easily initiate the peroxidation of membrane lipids leading to the accumulation of lipid peroxidation [23,24]. As a result of this, much attention has been focused on the use of antioxidants, especially natural antioxidants to inhibit lipid peroxidation and to protect from damage due to free radicals. A great number of aromatic and other medicinal plants contain chemical compounds that exhibit antioxidant properties.

Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer or coronary heart disease. The hypothesis that antioxidant supplements might promote health has not been confirmed experimentally. Trials including β -carotene, vitamin A, and vitamin E singly or in different combinations have indicated that supplementation has no effect on mortality or might increase it [25]. Randomized clinical trials of taking antioxidants including β -carotene, vitamin E, vitamin C, and selenium have shown no effect on cancer risk or have increased cancer risk [26]. Supplementation with selenium or vitamin E does not reduce the risk of cardiovascular disease [27].

The electron donation ability of natural products can be measured by 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) purple-coloured solution bleaching. The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test [28]. The results of DPPH radical scavenging activity of the sample RR reveals

significant DPPH radical scavenging activity dose dependently. Sample RR were screened for DPPH radical scavenging activity and the percentage inhibition ranges from 8.71 to 60.88 % when compared with standard ascorbic acid with percentage inhibition ranges from 38.63 to 95.92 %. The IC₅₀ value of the trial drug was found to be 81.13 ($\mu\text{g}/\text{ml}$) when compared with standard ascorbic acid with (IC₅₀ value 16.42 $\mu\text{g}/\text{ml}$).

Nitric oxide is a lipophilic molecule. At physiological pH it reacts with oxygen and produce nitrite ion. Nitric oxide is very essential for controlling of vasodilation; signal transmission, inflammatory response etc [29]. Scavengers of nitric oxide compete with oxygen and inhibit the production of nitric oxide [30]. NO radical scavenging activity of the sample RR revealed that the percentage inhibition of the test drug ranges from 4.53 to 34.56 % when compared with standard gallic acid with percentage inhibition ranges from 29.85 to 89.28 %. The corresponding IC₅₀ value of the trial drug was found to be 156.5 ($\mu\text{g}/\text{ml}$) when compared with standard gallic acid with (IC₅₀ value 37.44 $\mu\text{g}/\text{ml}$).

Hydrogen is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals ($\cdot\text{OH}$) that can initiate lipid peroxidation and cause DNA damage [31]. In the present study test drug RR were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 5.44 to 36.41% when compared with standard BHA with percentage inhibition ranges from 29.23 to 79.91 %. The corresponding IC₅₀ value of the trial drug was found to be 143.26 ($\mu\text{g}/\text{ml}$) when compared with standard BHA with (IC₅₀ value 41.12 $\mu\text{g}/\text{ml}$).

The ABTS radical cation is generated by the oxidation of ABTS with potassium persulfate, its reduction in the presence of hydrogen-donating antioxidants is measured spectrophotometrically [32]. The results of ABTS radical scavenging activity of the sample RR shown that the test drug has significant ABTS radical scavenging activity dose dependently. Trial drug RR were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 13.62 to 63.9 % when compared with standard gallic acid with percentage inhibition ranges from 27.65 to 94.18 %. The corresponding IC₅₀ value of the trial drug was found to be 69.26 ($\mu\text{g}/\text{ml}$) when compared with standard gallic acid with (IC₅₀ value 28.66 $\mu\text{g}/\text{ml}$).

5. Conclusion

The single herbal formulation of Kodagasalai choornam having antioxidant property as mentioned in Siddha literature³³. Hence the study have been proved by Screening of *In-vitro* Radical scavenging potential of Siddha medicinal herb *Rungia repens* using DPPH, ABTS, NO and Hydrogen peroxide radical scavenging assays.

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