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Original Article

The evaluation of nitric oxide radical-scavenging property of Scutellaria Barbata

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ABSTRACT
Scutellaria barbata also known as barbed skullcap is a species of flowering plant in the mint family, Lamiaceae. It is an herb used in traditional Chinese medicine for the treatment of multiple ailments, especially cancer. It is found to possess anti-viral, anti-inflammatory, anti-oxidation and immunity enhancement properties. Aim is to study the Nitric oxide assay free radical scavenging property of the ethanolic extract of Scutellaria barbata. A dried sample of Scutellaria barbata was extracted using 85% ethanol. Various concentrations of the extract were mixed with Sodium nitroprusside (SNP) in Phosphate Buffer Saline (PBS). Then Griess reagent was added and the absorbance studied using a spectrophotometer at 546nm. Quercetin solution was used as the standard. The herb exhibited maximal activity of 72.18% at the concentration of 1000 µg/ml. The IC50 value of Quercetin & Herb was found to be 10.24 µg/ml & 104.18 µg/ml respectively. The ethanolic extract of Scutellaria barbata demonstrated a concentration-dependent free radical scavenging property which can be postulated as the mechanism of action in its anti-cancer, anti-inflammatory and anti-oxidation properties.

Keywords: Scutellaria barbata, Free radicals, nitric oxide assay, anti-cancer

INTRODUCTION
Herbs have been used as medicine for centuries before modern medicine came into being. Nature has bestowed us with millions of plant species and ancient medicinal systems have studied and utilized the properties of different parts of plants and applied them to cure various diseases since time immemorial. There has been an explosion of research into the chemical constituents, properties and mechanism of action of these herbal formulations. And the knowledge thus gained can be applied to further the frontiers of modern medicine. The Scutellaria barbata and Hedyotis diffusa (SH) herb pair is extensively used in Traditional Chinese Medicine for efficacy enhancement in cancer treatment in China and Asian countries.[1]. It is found to possess anti-viral, anti-inflammatory, anti-oxidation and immunity

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enhancement properties [2] Animal models also suggest antitumor activity with S. barbata across a range of cancers [3,4]

Scutellaria barbata is a perennial herb that is used along with other botanicals in traditional Chinese medicine to treat bacterial infections, hepatitis, and tumors. A number of constituents including terpenoids, anthraquinones, flavonoids are thought to be responsible for these activities.[5] S. barbata possesses a range of antitumor activities via multiple intracellular targets [6], with major anticancer constituents identified as scutellarin, apigenin5-O-β-glucopyranoside, apigenin, p-coumaric acid, luteolin and 4'-hydroxywogonin [7] Preclinical studies of S. barbata have focused mostly on colon, lung, and liver cancer models.These are the postulated mechanisms of its anti-cancer effect. Further study is still under way to elucidate its various actions. The following study was undertaken to determine the free radical scavenging activity of this herb.

MATERIALS AND METHODS

1. Griess reagent (1% sulphonilamide, 0.1% N 1-naphthylethylenediamine, 2% orthophosphoric acid),
2. Sodium nitroprusside (SNP),
3. Phosphate buffer saline (PBS) and
4. Quercetin were purchased from RR Herbs Pharma,Chennai, India.
5. A dried sample of Scutellaria barbata was purchased locally.

Preparation of the extracts: Reflux method was used to extract 500g of Scutellaria barbata with 5000ml of 85% ethanol and then filtered. A rotary evaporator was used to evaporate the ethanolic extract. This was then brought to a relative density of 1.05 by concentrating it, and a spray dryer was used to produce the dried powder of the ethanolic extract by spraying desiccation. The required concentrations of the extract were then prepared by dissolving the powder in saline (0.6g/mL) [8]

Principle: Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates Nitric oxide which interacts with oxygen to produce Nitrite ions, which is measured at 546nm by spectrophotometer in the presence of Griess reagent [9].

Procedure: 1 ml of SNP (5mM) in PBS was taken in 7 different test tubes and 7 different concentrations (10, 50,100, 200, 400, 800 & 1000µg/ml) of ethanolic extracts of the herb were added to the test-tubes. The test tubes were then incubated at 29°C for 3 hrs. Similar concentrations of quercetin were prepared and incubated in a similar manner which was taken as reference anti-oxidant in the study. For control, a test tube filled with distilled water was taken and conducted in an identical manner. After 3 hours, one ml of Griess reagent was used to dilute the incubated samples. The absorbance that is formed as a result of diazotization of nitrite with sulphanilamide and consecutive coupling with naphthylethylenediamine dichloride was analyzed on Spectrophotometer at 546 nm [10].

Percentage of Inhibition of NO scavenging activity is given by the formula:

Nitric Oxide scavenged (%) =

$$\frac{A(Control) - A(Test)}{A(Control)} \times 100$$

Where, A(control)= Absorbance of control and A(test)= Absorbance of test sample.

STATISTICAL METHODS:

The IC50 values were obtained by Probit analysis using Graphpad Prism 6 software.

RESULTS

The Nitric oxide scavenging activity of the ethanolic extract of Scutellaria barbata and Quercetin solution which was used as the standard at varying concentrations is represented in Table 1. In the study we found the IC50 value of Quercetin & herb to be 10.24 µg/ml & 104.18 µg/ml respectively. The coefficient of determination, r2 for Scutellaria barbata was 0.9345, whereas r2 for Quercetin was 0.8069. This result is statistically significant (>0.8).

DISCUSSION

Oxygen, although indispensable to human life, can also create havoc in the human body by creating free radicals. These free radicals are, in turn, highly reactive species with an extra free electron which can react with various biomolecules like DNA, proteins, glycoproteins and lipids and damage them, causing premature ageing, multiple sclerosis, atherosclerosis, cancer, Alzheimer's disease, diabetes, etc [10]. NO has been associated with a variety of physiological processes in the human body since it was identified as a novel signal molecule. It transmits signals from vascular endothelial cells to vascular smooth muscle cells and causes vascular dilation. It also plays an important role in vital physiological functions in respiratory, immune, neuromuscular and other systems [11]

Thus, the ability to scavenge excess nitric oxide by Scutellaria barbata can be tapped to prevent and treat cancers, delay ageing and prevent numerous diseases where oxidative stress is the problem

CONCLUSION

From the study, we found that the ethanolic extract of the Scutellaria barbata plant has significant concentration-dependent Nitric oxide free radical-scavenging property. This property can be implicated in the mechanism of action in the anti-inflammatory, anti-cancer uses of this herb. This finding may open up new avenues for further research on this herb in the future.

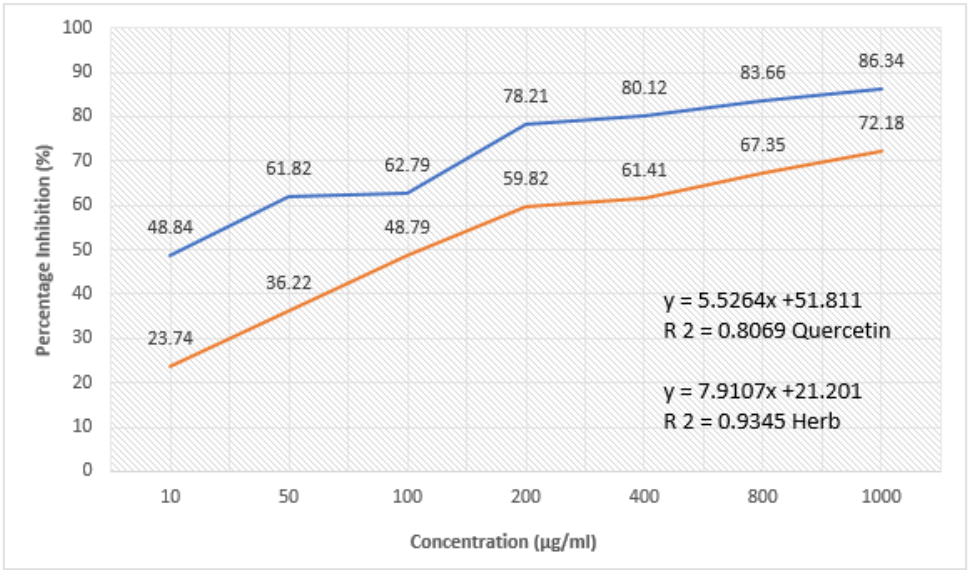
Table 1. Table showing percentage inhibition of Nitric oxide free radical by Scutellaria barbata and Quercetin at varying concentrations

Concentration (µg/ml)	Percentage Inhibition (%)	
	Standard (Quercetin)	Scutellaria barbata
10	48.84±0.72	23.74±0.18
50	61.82±0.84	36.22±0.91
100	62.79±0.84	48.79±1.79
200	78.21±1.16	59.82±1.52
400	80.12±1.06	61.41±0.68
800	83.66±1.98	67.35±0.49
1000*	86.34±1.02*	72.18±0.98*

Note: The maximum free radical scavenging activity of the herb Scutellaria barbata was 72.18±0.98% and it was seen at a concentration of 1000 µg/mL.

Dried sample of Scutellaria

Graph 1: Graph showing the percentage of inhibition of Quercetin versus Scutellaria barbata



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