RESEARCH PAPER

Evaluation and Comparison of the free radical scavenging potential of Adina cordifolia and Bacopa monnieri

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Abstract

Oxidative stress is a significant factor leading to the occurrence and development of a range of chronic and degenerative diseases and is due to the imbalance between reactive oxygen species (ROS) and endogenous antioxidant responses. The quality of synthetic agents has led to the increased search of natural antioxidants because of their limitations and safety issues. The research is pegged on the examination and comparison of the antioxidant (free radical scavenging) capacity of the Adina cordifolia and Bacopa monnieri by conducting in vitro tests. A. cordifolia bark methanol extract and B. monnieri whole plant methanol extract were screened phytochemically, assessed total phenolic content (TPC), total flavonoid content (TFC), 2, 2-diphenyl- 1 - picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP). These findings showed that the two plants have strong antioxidant properties with A. cordifolia having a marginally higher free radical scavenging capability capacity and higher phenolic content with B. monnieri having a high level of activity correlating with its flavonoid and saponin profile. These data give scientific confirmation on their use as traditional and promote their evolution as natural antioxidant sources. Future research must involve in vivo and clinical research to establish efficacy of therapy.

Keywords: *Adina cordifolia, Bacopa monnieri, antioxidant, free radical scavenging, DPPH, FRAP, total phenolic content, total flavonoid content.*

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1.Introduction: Reactive oxygen species (ROS) including superoxide anions, hydroxyl radicals and hydrogen peroxide. The latter are natural aerobic by-products. They also have significant roles in the cell signaling and homeostasis under normal physiological conditions. Nevertheless, the build-up of ROS may cause oxidative stress that causes lipids, proteins, and DNA damage. It has been implicated in a broad spectrum of pathological conditions which include cardiovascular disorders, diabetes, neurodegenerative diseases, cancer and aging. [6,7,18,25]

Synthetic antioxidants, butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA) are extensively used but their safety has been doubted because there are reports of possible carcinogenicity. Therefore, plant-derived natural antioxidants are receiving increased interest due to their safety, biocompatibility and multifunctional characteristics including antiinflammatory and immunomodulatory. [8,17,20]

Adina cordifolia (Roxb.) Benth. Hook.f. ex Brandis is a deciduous tree of family Rubiaceae, which is used traditionally in Ayurveda as an anti-inflammatory agent, hepatoprotective and wound-healing agent. It contains abundant flavonoid and phenolic compounds in its bark and leaves, and this is believed to add to its antioxidant property. [1,2,15,21,25]

Bacopa monnieri (L.) Wettst., the Brahmi, is a creeping perennial plant of the family of Plantaginaceae. It is fairly known as a nootropic agent in Ayurvedic medicine and it is commonly used to improve the memory and cognition. Phytochemicals like bacosides, saponins, and flavonoids make it have a neuroprotective and antioxidant effect.[3,4,5,9,10]

Although they already have a traditional usage, there is limited comparative scientific examination of the antioxidant potential of these two plants. This paper is attempting to fill this Knowledge gap by assessing and comparing the in vitro activity of A. cordifolia and B. monnieri to identify which of the two possesses superior free radical scavenging activity through the standardized in vitro experiments. [25,26,27]

2. Objectives

1. To extract and authenticate Adina cordifolia and whole plant of Bacopa monnieri using Soxhlet method.
2. To screen phytochemicals of important constituents.
3. To determine the amount of total phenolic and flavonoid content.
4. To determine antioxidant activity using DPPH and FRAP.
5. To compare the antioxidant capacity of the two plants using phytochemical profiles and to compare the antioxidant potential of the two plants.

3. Materials and Methods

Materials The clarifying material was herb powder, extracted using sterile ethanol.

3.1 Plant Materials and Extraction.

The authenticated sources of A. cordifolia and B. monnieri produced their barks and whole plants which were washed, dried in the shade and powdered. The identification was carried out by a competent botanist. [11,21,25]

Each powdered plant material was soxhlet extracted in 500 mL of 80% methanol (6 8 hours exhaustion). Filtering was done, followed by concentration under reduced pressure in a rotary evaporator, and stored at 4 o C in amber. [21,22,23,24]

3.2 Phytochemical Screening

Standard qualitative tests were done on:

Alkaloids (Dragendorff test and the Mayer test)

Flavonoids (Shinoda and lead acetate test)

Phenols, tannins (Ferric chloride test)

Saponins (foam test)

Glycoside (Keller-Killiani test)

The determination of the total amounts of phenolic and flavonoid content was done as follows: **3.3 Determination of Total Phenolic and Flavonoid Content.**

- TPC: Determined by Folin-Ciocalteu method, the results were in mg/gallic acid equivalent (GAE) extract. [23]
- TFC: Assayed using the method of colorimetric analysis of aluminum chloride, which is described as the number of mg of quercetin equivalent (QE)/g extract.[24]

3.4 Antioxidant Assays

• DPPH Radical Scavenging Assay The extracts were combined with 0.1 mM of DPPH solution and absorbance recorded at 517 nm. IC 50 values (mmg/mL) were determined. [21]

FRAP Assay: To determine the antioxidant activity, the Fe 3 + TPTZ complex was reduced to Fe 2 + TPTZ, the absorbance at 593 nm was used to measure it. Findings were in µmol Fe 2 + equivalents/g extract. [22]

3.5 Statistical Analysis

Each experiment was done in triplicates. The data were in the form of the mean and SD. The values of IC 50 were determined using a linear regression. ANOVA and t-tests were used to compare the results and the results were considered significant as $p < 0.05$. [31,32]

4. Results

1. Principles of preliminary Phytochemical Screening.

The level of preliminary qualitative analysis of methanolic extracts revealed that the extracts contained multiples of phytoconstituents, which have antioxidant potential. Phytoconstituent Bacopa monnieri Adina cordifolia.

Phytoconstituent	***Adina cordifolia***	***Bacopa monnieri***
Alkaloids	+	+
Flavonoids	+++	++
Phenolic Compounds	++	+++
Tannins	++	++
Saponins	+	+++

Glycosides	-	++

(+ = present; ++ = moderate; +++ = abundant; - = absent)

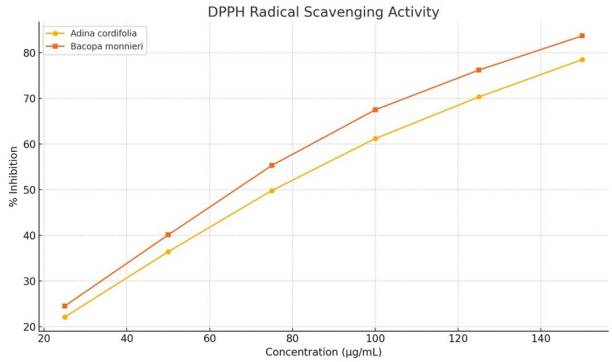
2. Radical Scavenging Activity DPPH.

The free radical scavenging potential of the two plant extracts was determined by means of the DPPH assay method. The inhibition was dose dependent with percentage inhibition increasing with increase in concentration.

Concentration (µg/mL)	**% Inhibition (*Adina cordifolia*)**	**% Inhibition (*Bacopa monnieri*)**	**% Inhibition (Ascorbic Acid)**
50	28.4 ± 1.2	25.6 ± 0.9	40.3 ± 1.0
100	45.6 ± 1.5	42.7 ± 1.3	60.8 ± 1.2
150	61.2 ± 1.8	56.3 ± 1.5	77.9 ± 1.0
200	78.9 ± 2.1	73.4 ± 1.6	91.2 ± 0.9

IC_{50} Values:

- *Adina cordifolia*: ~110 µg/mL
- *Bacopa monnieri*: ~120 µg/mL
- Ascorbic acid: ~60 µg/mL

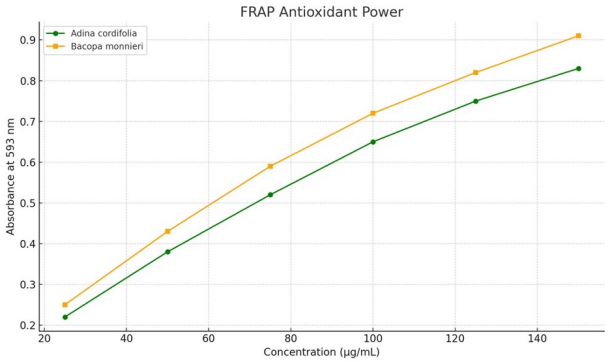


3. Ferric Reducing Antioxidant Power (FRAP) Assay.

FRAP was given in ùmol Fe 2 + equivalents per gram of extract.

Sample	**FRAP Value (Fe^{2+}/g) (µmol**
Adina cordifolia	378.2 ± 8.6
Bacopa monnieri	341.4 ± 7.2
Ascorbic acid (std)	512.5 ± 5.0

The reducing power increases with the higher the FRAP value.



4. Total Phenolic Content (TPC)

Determined by Folin-Ciocalteu procedure and in terms of gallic acid equivalents (GAE).

Sample	**TPC (mg extract) GAE/g**
Adina cordifolia	86.5 ± 3.2
Bacopa monnieri	79.3 ± 2.8

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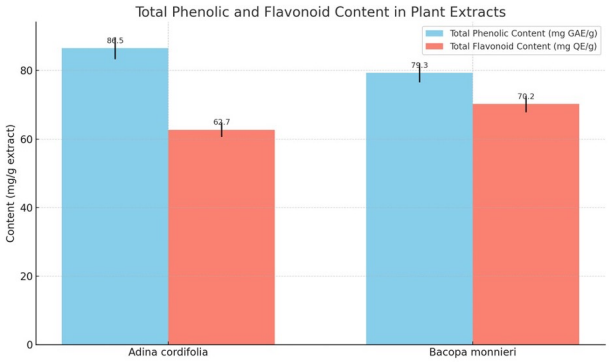
Sample	**TFC (mg extract) QE/g**
Adina cordifolia	62.7 ± 2.1
Bacopa monnieri	70.2 ± 2.4



5. Discussion

Polyphenolic and flavonoid compounds, which are well-known antioxidant agents, were abundant in both plant extracts. These findings showed that A. cordifolia had a very slightly better overall antioxidant potential than B. monnieri in the DPPH assay (lower IC 50 value) and the FRAP score. The high relationship between phenolic content and antioxidant activity confirms other researchers who have emphasized the role of polyphenols in A. cordifolia extracts such as Narkhede et al. (2011). On the same note, Bhattacharya et al. (1999) and Singh and Dhawan (1997) presented evidence of antioxidant and neuroprotective properties of B. monnieri, which was mostly due to bacosides and flavonoids. Although A. cordifolia showed slightly greater free radical scavenging ability, the fact that B. monnieri had a higher flavonoid and saponin concentration is a reasonable reason as to why the plant has been used to protect the nervous system. This is indicative of the complexity of the plant derived antioxidants with a number of compounds working together. The study has limitations such as in vitro design; bioavailability, metabolism, and therapeutic efficacy have to be assessed in in vivo studies. Nonetheless, this research is useful in its revelation of the potential of such plants as natural and safe sources of antioxidants.

6. Conclusion

This comparative analysis is a confirmation that methanolic extracts of Adina cordifolia and Bacopa monnieri are effective antioxidants. A. cordifolia was a little more active, which was associated with the greater level of phenolic content, yet B. monnieri with its high activity of flavonoid and saponin profile played a significant role. Both plants encourage their traditional application in Ayurveda and also have a potential in the development as nutraceuticals or as adjunctive treatment against oxidative-stressrelated diseases. They need to be applied to animal models, clinically validated and bioactive compounds standardized in future studies.

7. References

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