The present study evaluated the free radical scavenging and anticholinesterase activity of the methanolic extracts of *Acorus calamus* (ACME) and *Nardostachys jatamansi* (NJME) rhizomes in vitro. In addition, total phenolics (TP) were also estimated. NJME contained significantly higher (*p* < 0.05) phenolics (37 µg GAE/mg) than ACME (23 µg GAE/mg). Consequently, NJME exhibited significantly higher (*p* < 0.05) radical scavenging activity than ACME and BHT, a synthetic antioxidant. Further, the IC\(_{50}\) values were 704, 237 & 335 µg/ml for ACME, NJME and BHT respectively. In case of anticholinesterase activity also NJME exhibited significantly higher (*p* < 0.05) activity with lower IC\(_{50}\) value than ACME. However, the anticholinesterase activity of both ACME and NJME were significantly lower (*p* > 0.05) than neostigmine, a standard drug wherein, neostigmine exhibited significantly lower (*p* > 0.05) IC\(_{50}\) value than ACME and NJME. Furthermore, a significant correlation between the total phenolic content, antioxidant and anticholinesterase activities of both the extracts indicating that total phenolics might be responsible for the observed antioxidant and anticholinesterase activities. This is the first report in this direction.

**Keywords:** Acetylcholinesterase, antioxidant, *Acorus calamus*, *jatamansi*, phenolics.

**INTRODUCTION**

Ayurveda, an alternative system of medicine in India, uses a number of plants for the treatment of a variety of diseases. The Medhya rasayana are a group of medicines in Ayurveda known to act on the nervous system. These drugs mainly contain extracts from plants such as *Acorus calamus*, *jatamansi* and *Bacopa monnieri*. These medhya rasayana have been claimed to improve mental ability. The acetylcholinesterase (AChE) is a biologically important enzyme that hydrolyzes acetylcholine (ACh), a neurotransmitter considered to play role in the pathology of Alzheimer’s disease. One of the most important approaches for treatment of this disease involves the enhancement of acetylcholine level in brain using AChE inhibitors. Several studies have reported anti-cholinesterase activity of the plant extracts and drugs. Certain reports have claimed that, a few herbal extracts can act on the central nervous system, thereby enhancing the faculties of learning and memory. A recent study has shown that *B. monnieri* improves memory in humans. *Clitoria ternatea* and *jatamansi* have also been reported to be excellent memory enhancers. The roots and rhizomes of *Acorus calamus* LIND (AC), commonly known as sweet flag, sweet grass and sweet cane (Araceae), have been used in the Indian and Chinese systems of the medicine for hundreds of years for their beneficial role in improving learning performance, and for their anti-aging effect. The roots and the rhizomes of *Nardostachys jatamansi* DC, (Valerianaceae) mentioned in Ayurveda, have been used to treat epilepsy, hysteria, syncope and mental weakness. The same plant has also been used as herbal combinations with other herbs to evaluate depressant activity. Recent studies show that, the extracts of the rhizomes of *Nardostachys jatamansi* also contain hepatoprotective compounds and it is also reported that, studies have been conducted to elucidate the chemical structure of the active ingredients of this plant.

Pharmacological effects of the medicinal plants are related to their free-radical scavenging properties which include inhibition of lipid peroxidation, maintaining integrity and permeability of cell walls and the protection of neurons against oxidative stress. Free radical-induced lipid peroxidation has been associated with many neurodegenerative diseases. Hence, the present study was planned to explore the antioxidative activity and anticholinesterase activity of two indigenous medicinal plants namely, *Acorus calamus* and *Nardostachys jatamansi*.

**MATERIALS AND METHODS**

**Chemicals**

1,1-diphenyl-2-picrylhydrazyl (DPPH), 5,5-Dithio (bis) nitro benzoic acid (DTNB), acetylthiocholine iodide were purchased from Sigma Aldrich, India. All the reagents and chemicals used in the study were of analytical grade.

**Plant material**

The rhizomes of *Acorus calamus* and *Nardostachys jatamansi*...
were purchased from a local herbalist in Mysore. The samples were further dried (50°C), powdered, passed through 60 mesh sieve and stored in an air tight container at 4°C till further use.

Preparation of extracts

Powdered rhizomes (40 g) were extracted with methanol (500 ml) in a mechanical shaker for 24 hrs at room temperature. The extract was filtered and evaporated to dryness under reduced pressure in a rotary evaporator (Lab systems, India) to yield methanolic extracts of *Acorus calamus* (ACME) *Nardostachys jatamansi* (NJME). Both the extracts were subjected to free radical scavenging assay (RSA) and AChE inhibition assay in vitro.

Estimation of total Phenolics

The total phenolics (TP) content was estimated according to Folin Ciocalteu micro method19. Extract solution (20 µl) was mixed with distilled water (1.58 ml) and Folin Ciocalteu reagent (100 µl) followed by the addition of Na$_2$CO$_3$ (20 %), after 1 min and before 8 min. Subsequently, the mixture was incubated at 40°C for 30 min and the absorbance was measured at 760 nm in a spectrophotometer (Systronics 117). Gallic acid was used as standard for calibration curve and total phenolics content was expressed as Gallic acid equivalents (GAE).

DPPH Radical scavenging assay

The hydrogen atom or electron donation ability of various extracts and some pure compounds (BHT) was measured from the bleaching of purple colored methanol solution of DPPH. This spectrophotometric assay uses stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a reagent20. The radical, DPPH was reduced to the corresponding colorless hydrazine upon its reaction with hydrogen donors (Okusa et al, 2007). Various concentrations of the extracts in 3 ml methanol were added to 1 ml of a 0.1 mM solution of DPPH. After 30 min incubation period at room temperature the absorbance was read against a blank (methanol) at 517 nm using a semi auto analyzer. The percent Inhibition of DPPH was calculated using the following formula:

\[
\%\text{ inhibition} = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100
\]

*Abs control* is the absorbance of the control reaction (containing all reagents except the test compound), and *Abs sample* is the absorbance of the test compound. Neostigmine bromide was used as positive control and all tests were carried out in triplicate.

In vitro acetylcholinesterase inhibition assay

AChE inhibition activities of selected extracts were measured by slightly modifying the spectrophotometric method developed by Ellman21. Acetylthiocholine iodide was used as substrate and 5,5'-Dithiobis [2-nitrobenzoic acid] was used for the measurement of cholinesterase activity and rat brain homogenate was used as source of acetylcholinesterase enzyme.

Preparation of the enzyme

Male rat of Wistar strain weighing 150 g was sacrificed by cervical dislocation, the brain was immediately excised and homogenized with 0.1 mM sodium phosphate buffer (pH 7.0) in cold condition. The homogenate was stored at -80°C till use.

Assay procedure

Various concentrations of the extracts in 2.6 ml of 0.1 mM sodium phosphate buffer (pH 8.0) were added to 100 µl of DTNB (0.75 mM) and 5 µl brain homogenate (Crude enzyme) and incubated for 5 min at 25°C. The reaction was then initiated by the addition of 20 µl of acetylthiocholine. The hydrolysis of acetylthiocholine was monitored by the formation of yellow 2-nitro-5-sulfidobenzenecarboxylate anion as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine for 10 min, at a wavelength of 412 nm.

The percentage Inhibition of cholinesterase activity was calculated using the following formula:

\[
\%\text{ inhibition} = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100
\]

*Abs control* is the absorbance of the control reaction (containing all reagents except the test compound), and *Abs sample* is the absorbance of the test compound. Neostigmine bromide was used as positive control and all tests were carried out in triplicate.

Statistical analysis

Data was analyzed by ANOVA followed by Tukey’s multiple comparisons test for significant differences and the correlations between antioxidant activity and anticholinesterase activity were calculated by Pearson correlation using SPSS 14.0 software. Maximum inhibition and the IC$_{50}$ values were calculated by Boltzmann’s dose response analysis using Origin 6.1 software.

RESULTS AND DISCUSSION

The data on total phenolics content is presented in Table 1. NJME contained significantly higher amounts of phenolic compounds (36.7 µg GAE/mg extract) than ACME (23.4 µg GAE/mg extract), which is comparably higher than the phenolic content reported in common herbal extracts22. Ultraviolet light, ionizing radiation, chemical reactions and metabolic processes can induce the production of reactive oxygen species in the cells. These reactive oxygen species cause lipid peroxidation, protein peroxidation, DNA damage and cellular degeneration in the cells and can induce numerous diseases including cari9.
Phenolics have been known to possess a capacity to scavenge free radicals. They are commonly found in both edible and non-edible plants and have multiple biological effects, including antioxidant activity.

To determine the free radical scavenging activity of the extracts, DPPH assay was performed and the results are presented in Figure 1. The total phenolic content, maximum inhibition and IC50 values are shown in Table 1. The radical scavenging activity of both the extracts was dose-dependent in nature. Nardostachys jatamansi exhibited significantly higher (p = 0.05) RSA than Acorus calamus. The radical scavenging activity of Nardostachys jatamansi was comparable with that of BHT, a synthetic antioxidant. The IC50 value for Nardostachys jatamansi was 237 µg/ml which was significantly lower than BHT (335.3 µg/ml) and Acorus calamus (703.9 µg/ml). A significant correlation (r = 0.986, p = 0.01) was observed between the total phenolic content and the radical scavenging activity of both the extracts justifying their usage in traditional system of medicine for treating stress-related disorders.

AChE is found among neurofibrillary tangles and neuritic plaques and its inhibition is an effective tool for the treatment of Alzheimer’s disease and related Dementia. Tacrine, a standard drug, exerts its pharmacological effect by increasing the acetylcholine level in the mouse brain. Hence, the AChE inhibitory effects of plant extracts indicate their potential in the development of natural therapeutics for Alzheimer’s disease and related problems. In the present study, a dose-dependent inhibition of AChE was found and the results are presented in figure 2. Neostigmine inhibited AChE to an extent of 93% which was significantly higher (p = 0.05) than Nardostachys jatamansi and Acorus calamus. Nardostachys jatamansi was found to inhibit AChE to an extent of 65.7% at 1 mg/ml concentration which was significantly higher than the inhibitory activity exhibited by Acorus calamus (52.8%). The IC50 value for Nardostachys jatamansi was 562 µg/ml which was significantly lower than Acorus calamus (791 µg/ml). The IC50 value of neostigmine was 9.09 µg/ml which was significantly lower than both the extracts, probably active component may exert similar activity upon purification. A significant positive correlation (r = 0.973, p = 0.01) was observed between the total phenolic content and anticholinesterase activity of both the extracts. There was also a positive correlation between the radical scavenging activity and anticholinesterase activity of Acorus calamus (r = 0.986, p = 0.01) and

---

**Table 1. Total phenolic content, antioxidant activity and anti acetylcholinesterase activity of Acorus calamus and Nardostachys jatamansi**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Phenolics µg GAE/mg extract</th>
<th>Maximum inhibition (%)</th>
<th>DPPH Radical Scavenging Assay IC50 µg/ml</th>
<th>Maximum inhibition (%)</th>
<th>Anticholinesterase Assay IC50 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACME</td>
<td>23.4 ± 2.57a</td>
<td>68.60 ± 2.20a</td>
<td>703.9 ± 22.29a</td>
<td>52.8 ± 0.6a</td>
<td>791.35 ± 77.67c</td>
</tr>
<tr>
<td>NJME</td>
<td>36.7 ± 0.72b</td>
<td>86.68 ± 2.04a</td>
<td>237.0 ± 30.64a</td>
<td>65.7 ± 1.40b</td>
<td>562.21 ± 25.53c</td>
</tr>
<tr>
<td>BHT</td>
<td>ND</td>
<td>81.76 ± 2.30a</td>
<td>335.3 ± 40.0a</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>NSG</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>93.4 ± 1.8c</td>
<td>9.09 ± 0.06c</td>
</tr>
</tbody>
</table>

*ACME: Acorus calamus methanol extract, NJME: Nardostachys jatamansi methanol extract, BHT: Butylated hydroxytoluene, NSG: Neostigmine Bromide, ND: Not determined. **Mean values carrying different superscript letters a, b, c… in columns differ significantly (p=0.05).
Nardostachys jatamansi (r = 0.916, p = 0.01) indicating possible role of phenolics for the observed antioxidant and anticholinesterase activities.

From the results of the present study, it is concluded that Acorus calamus and Nardostachys jatamansi rhizomes possesses significant antioxidant and anticholinesterase activity and phenolic compounds are mainly responsible for these biological activities. However, there is a need to isolate and characterize these compounds for their effective utilization in the treatment of Alzheimer’s disease and other stress related disorders. Studies in this direction are currently underway in our laboratory.

REFERENCES


Source of support: Nil, Conflict of interest: None Declared