Activity and Constituents of Sage

Relevant to the Potential Treatment of Symptoms of Alzheimer’s Disease

by Peter J. Houghton, BPharm, PhD
The traditional herb sage, as commonly used in Western Europe and European cultures in North America, consists of several species of Salvia (family Lamiaceae), a fairly large genus containing hundreds of species used in traditional medicine in the parts of the world where they are endemic. Notable examples include Chinese salvia (S. miltiorrhiza Bunge), the roots and rhizomes of which are extensively used as an ingredient of Chinese medicines (known as dan shen); and divining sage (S. divinorum Epling & Játiva) from Mexico, which has a reputation as a hallucinogen. The species most often encountered in Western European medicine and cookery are common sage (S. officinalis L.) and Spanish sage (S. lavandulifolia Vahl), but several other species are encountered in commerce. It is difficult to determine from old literature sources which species is being referred to and both are used extensively for flavoring meat, especially pork and poultry (stuffing is made with common sage).

In current herbal medicine, sage is used to treat indigestion and inflammation of the throat, and to reduce sweating, including that associated with hot flashes during perimenopause. In the Commission E monographs it is recommended to be taken internally for dyspepsia, and excessive perspiration and used externally for inflammation of the membranes of the mouth and the nose. It has a reputation in Central Europe of being useful in suppressing lactation in nursing mothers.

Although some of these modern reference sources vaguely discuss its effects on the central nervous system, research into older literature reveals that sage has some activities that may be considered today to be relevant to the treatment of Alzheimer's disease (AD), particularly its traditional reputation as being good for the memory. Thus, in his late 16th century English herbal text, the English herbalist John Gerard (1545–1607) wrote about sage: "It is singularly good for the head and brain and quickeneth the nerves and memory." About 50 years later, English physician Nicholas Culpeper (1616–1654) wrote, "It also heals the memory, warming and quickening the senses." Hill (1714–1775) in 1756 poignantly encapsulated the tragic effects of a condition which appears to be AD and general aging by stating, "Sage will retard that rapid process of decay that treads upon our heels so fast in latter years of life, will preserve faculty and memory more valuable to the rational mind than life itself."

Alzheimer's disease is characterized by loss of short-term memory and is a very common disease in populations with a long life expectancy. Its etiology is still not very clear but it appears to be associated with damage caused by reactive oxygen species (ROS), pro-oxidant compounds that act on the brain cells, associated with inflammation. Characteristic features of AD observed in post-mortem examination are low levels of the neurotransmitter acetylcholine (ACh), a tangle of microscopic fibers in the brain together with plaques of a protein named beta-amyloid. A lower incidence of AD has been noted in patients taking non-steroidal anti-inflammatory drugs over a long period of time, and also in people regularly taking high doses of foods, such as tea, containing high levels of antioxidants. At one time there was thought to be a correlation with estrogen replacement therapy with low incidence, but this has since been discredited. The ancient reports, together with current usage that indicates sage might possess anti-inflammatory properties, suggested that sage might be of possible use in AD. Preliminary investigations were conducted at the Medical Research Council Neurochemistry Laboratories at Newcastle General Hospital in Northeast England into the bioactivity of sage that might be relevant to its reputation as an aid to failing memory. These initial studies formed the basis for a more detailed investigation in King's College London into other relevant activities and the chemical constituents responsible for such activity.

In Vitro Tests Carried Out on Sage Extracts and Oils

The in vitro tests were designed to monitor the effects of sage extracts on most of the factors thought at the time to be associated with AD or with a reduction in its incidence.

Cholinesterase Inhibition — Inhibition of the enzyme acetylcholinesterase (AChE) is the basis of most drugs used clinically for symptomatic relief of the early stages of AD. Inhibition of AChE (i.e., reduction of the enzyme responsible for breaking down ACh) results in elevated levels of ACh in the brain, which is associated with improvement of cognitive function including memory. Preliminary tests using the assay for choline esterase inhibition were conducted on both the 96% ethanolic extract and the steam-distilled oil of three samples of S. officinalis and three samples of oil of S. lavandulifolia. It was found that all 6 samples produced inhibition of the enzyme at very low concentrations (Table 1). The activity was considered of quite interest since all previous choline esterase inhibitors were amines, the naturally occurring types in plants being known as alkaloids, and these compounds were not known to exist in the Salvia species tested. S. lavandulifolia oil was selected for further investigation rather than S. officinalis oil since the latter contains a relatively high percentage (35–50%) of the neurotoxic thujone, which is present in only very small amounts in S. lavandulifolia. The identification of the active components is discussed below but, as part of the investigation, the dose of the S. lavandulifolia oil that decreased the enzyme activity by 50% (known in in vitro studies as the IC50 value) was determined and found to be 0.03 μL/mL (microliters per milliliter).

Antioxidant Activity — Antioxidant effects were shown to be present in the ethanolic extract of S. lavandulifolia, but it was not possible to test the oil in the system used. The inhibition given by a 5 mg/mL extract was 75 ± 9% of that given by the standard antioxidant 10 mM propyl gallate which can be considered weak, though significant (P < 0.05). Both water-soluble and chloroform-soluble fractions of the extract gave similar antioxidant activity to the propyl gallate, thus indicating that a mixture of substances are present which might prevent brain cells from damage by ROS.

Illustration of Salvia officinalis, from: Medical botany: containing systematic and general descriptions, with plates, of all the medicinal plants, indigenous and exotic, comprehended in the catalogues of the materia medica, as published by the Royal Colleges of Physicians of London and Edinburgh: accompanied with a circumstantial detail of their medicinal effects, and of the diseases in which they have been most successfully employed. Volume 1 of 4, by William Woodville. © 1995-2004 Missouri Botanical Garden http://ridgeway.db.mobot.org/mobot/rarebooks/.
Effect of Extract on Eicosanoid Synthesis — Eicosanoid synthesis is part of the inflammatory response and a reduced level of these compounds is indicative of reduction in inflammation. Eicosanoids include thromboxanes and leukotrienes. The *S. lavandulifolia* ethanol extract at 50 microg/mL showed only weak inhibition of eicosanoid synthesis, giving only about 10% inhibition of thromboxane B₂ (TXB₂) synthesis but more inhibition (60%) of leukotriene B₄ (LTB₄) synthesis. When the ethanol extract was fractionated, the chloroform-soluble portion produced a greater inhibition than the water-soluble fraction.

Binding to Estrogen Receptor — Estrogen-receptor binding studies were carried out at a time when AD was formerly thought to be prevented by estrogen replacement therapy. Estrogenic activity was observed in the ethanolic extract and the essential oil of *S. lavandulifolia*. A dose-dependent activity was observed with the ethanol extract over the range 1 to 5 mg/mL and appeared to be concentrated in the water-soluble fraction. No dose-response activity was obtained by the essential oil and the possible explanation offered was the volatility of components of the oil that may have evaporated and spread their activity to surrounding cells across the glass plate.

Identification of Compounds Responsible for Activity Detected

Cholinesterase Inhibition — The oil of *S. lavandulifolia* was fractionated by droplet counter-current chromatography and the chemical composition of the fractions analyzed by thin-layer and gas chromatography (TLC and GC). The cholinesterase inhibitory activity of each fraction was also measured and, from the active fractions, several cyclic monoterpenes were identified as the most active compounds. These are shown in Table 2, together with their inhibitory activities. The extent of inhibition was found to be directly related to the amount of compound used in the test and could be removed by ceasing to add the extract to the system. Attempts at relating the chemical structure of the compounds to their strength of enzyme inhibition were unsuccessful. It can be seen that, of the active components, 1,8-cineole is likely to contribute most to the activity of the oil since it is present in the greatest concentration. Its ability to inhibit cholinesterase has also been reported by other research. It should be noted that 1,8-cineole is a relatively common compound in essential oils and is found in several other plant species. The cholinesterase inhibitory properties of these monoterpenes was only recently reported, although it should be noted that they are considerably less active, by a factor of at least 10³, than the alkaloid inhibitors such as physostigmine derived from the calabar-bean (*Physostigma venenosum* Balf., *Fabaceae*). However, it was noted that the inhibitory activities of the major terpene constituents did not readily account for the inhibitory effect of the total essential oil. It was calculated that 50% enzyme inhibition for the oil would occur at approximately 160 mg/L if the values of the constituent terpenes individually were taken into account — approximately 5,000 times the concentration of essential oil (0.03 mg/L) providing 50% inhibition. This suggested either that there was a high degree of synergy in the combined action of the terpenes, or, less likely, that there was present in the oil an as-yet unidentified minor constituent of high potency. The argument in herbalism for the use of extracts, rather than isolated compounds, is based to a considerable extent on the concept of synergy (i.e., the activity of an extract is much greater than might be expected from the activities of its isolated components). The data for sage oil appears to support this occurring as regards its anticholinesterase effects.

Antioxidant Activity — No compounds were isolated from the fractions showing activity, but some of the individual components of the volatile oil were tested for antioxidant effect since these would be expected to be present in the chloroform-soluble fraction. Antioxidant effects were noted with 1,8-cineole, alpha-pinene and beta-pinene, but a pro-oxidant effect was produced by camphor, a relatively major component of the oil. Given that the monoterpenoids with antioxidant activity in this study are present in the essential oil at a slightly higher relative percentage (collectively over 30%) than camphor (27% of essential oil), it is likely that the pro-oxidant activity of camphor is eclipsed by the antioxidant compounds so that the total oil would have an overall antioxidant effect.

The activity of the water-soluble fraction, which was also active, was not investigated but it is likely to contain flavonoids and other phenolic compounds that might well be antioxidant.

Effect on Eicosanoid Synthesis — The *S. lavandulifolia* extracts showed only weak inhibition of eicosanoid synthesis. Since the chloroform-soluble fraction showed greater activity, some of the oil constituents likely to be present were tested in

### Table 1. Acetylcholine esterase inhibition of samples of different sage oils at 0.03 mcg/mL

<table>
<thead>
<tr>
<th>Sample</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salvia officinalis</em> A</td>
<td>34.1</td>
</tr>
<tr>
<td><em>S. officinalis</em> B</td>
<td>39.9</td>
</tr>
<tr>
<td><em>S. officinalis</em> C</td>
<td>46.9</td>
</tr>
<tr>
<td><em>S. lavandulifolia</em> A</td>
<td>31.2</td>
</tr>
<tr>
<td><em>S. lavandulifolia</em> B</td>
<td>45.8</td>
</tr>
<tr>
<td><em>S. lavandulifolia</em> C</td>
<td>44.6</td>
</tr>
</tbody>
</table>
the system. Alpha-pinene, comprising 5% of the essential oil, was the only constituent present which gave significant activity (52% inhibition at 200nM) and showed weak selectivity for the leukotriene LTB₄. LTB₄ is produced via the enzyme 5-lipoxygenase, the gene of which is upregulated during neurodegeneration and, although the role of this inflammatory mediator in AD is not entirely apparent, selective inhibition over cyclo-oxygenase may be relevant therapeutically since this would reduce the activity of the upregulated 5-lipoxygenase, which is associated with this. The three other monoterpenoids (collectively 44% of essential oil) tested did not show anti-inflammatory action in this analysis.

**Binding to Estrogen Receptor** — Since the oil gave indication of binding to the estrogen receptors, some of the major components were also tested. Of the 5 monoterpenoid constituents of *S. lavandulifolia* essential oil screened, only geraniol (0.1-2 mM; <1% of essential oil) exhibited estrogenic activity (*P < 0.001*), which was weak in comparison to 17-beta-estradiol (1,000 nM). The highest concentrations of all the monoterpenoids interfered with cell growth. Thus, it seems unlikely that the constituents tested contribute very much to the effect of the oil. It should be noted that compounds that produce estrogenic activity in the *in vitro* test system used may not produce activity in an *in vivo* environment where compounds can be metabolized and the metabolites can have more, less, no, or different effects. That is, one must be very careful to impute results from such *in vitro* tests for estrogenic activity to human physiology and thus, their clinical significance is unclear.

**Table 2. Acetylcholine esterase inhibitory activity of major constituents in sage oil**

<table>
<thead>
<tr>
<th>Constituent</th>
<th>% in oil (gas chromatography)</th>
<th>% inhibition 4.7 mM</th>
<th>IC₅₀ mM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>(-)-Bornyl acetate</td>
<td>14.8</td>
<td>48</td>
<td>~4.7</td>
</tr>
<tr>
<td>Camphor</td>
<td>27.3</td>
<td>27</td>
<td>&gt;4.7</td>
</tr>
<tr>
<td>1,8-Cineole</td>
<td>17.2</td>
<td>–</td>
<td>0.67</td>
</tr>
<tr>
<td>Beta-pinene</td>
<td>12.6</td>
<td>68</td>
<td>–</td>
</tr>
<tr>
<td>Alpha-pinene</td>
<td>5.0</td>
<td>–</td>
<td>0.63</td>
</tr>
<tr>
<td>Total oil</td>
<td>100</td>
<td>–</td>
<td>0.05 mcg/ml</td>
</tr>
</tbody>
</table>

* Decreased enzyme activity by 50%.
Recent research has shown that estrogenic compounds actually increase the incidence of AD rather than reduce it, so, with hindsight, these findings are of little consequence.

**Conclusions regarding activities noted** — It appears that the cholinesterase inhibition shown by the oil of sage is most likely due to the cyclic monoterpenes 1,8-cineole and alpha-pinene with some contribution from camphor. There seems to be some evidence that synergistic effects occur. The identity of the active compounds responsible for the other effects noted are less clear but the antioxidant effect is due, at least in part, to 1,8-cineole, alpha-pinene, and beta-pinene. These compounds are present in several other essential oils, but little work has been done on the antioxidant activity of these.

**In Vivo and Clinical Studies**

Most of the activities of sage so far reported in the scientific literature are concerned with *in vitro* studies, but it is important that work is carried out to show that these activities are retained *in vivo*.

Normal rats were orally administered on separate occasions (4 months apart) either 20 mL or 50 mL of *S. lavandulifolia* essential oil in a standard dose of sunflower oil once per day for 5 days, with a control group given sunflower oil alone. After the 5-day period (during which the behavior, eating, and motor function of both groups of animals was observed as normal/healthy), the rats were sacrificed and three parts of the brain were assessed for AChE activity.

At the lower dose there was a trend for decreased AChE activity in the striatum (the part of the brain associated with excitatory responses) of the *S. lavandulifolia*-treated rats compared to the control group without prior inhibition of butyrylcholine esterase (BuChE). The difference was more pronounced and significant when BuChE was inhibited prior to analysis, suggesting that only AChE and not BuChE had been inhibited *in vivo* by *S. lavandulifolia*. At the higher dose there was also a trend for decreased striatal AChE activity. At both doses there was no change in the AChE activity in the cortex of the *S. lavandulifolia*-treated rats compared to the control group.

These results suggested that one or more constituents of the *S. lavandulifolia* oil, or their metabolites, following oral administration, reach the brain (crossing the gastrointestinal and blood-brain barriers) and inhibit cholinesterase in select brain areas, consistent with evidence of inhibition of the brain enzyme *in vitro*.

So far, no clinical studies using sage preparations on patients with AD have been reported, but a recent study describes the effect of sage in healthy volunteers.** In this placebo-controlled, double-blind, balanced, crossover design study, participants received 3 doses of a standardized extract of *S. lavandulifolia* in sunflower oil as well as the sunflower oil alone (34 female, 10 male; 18–37 years old, mean age 23.2 years). Doses were administered on different days, each separated by a 7-day wash-out period using a pseudo-random treatment order. Cognitive assessment and subjective mood ratings measurements took place immediately prior to treatment and 1 hour, 2.5 h, 4 h and 6 h thereafter. A number of significant effects on cognition were associated with the lowest (50 mL) dose of *Salvia*. These included improvements in both immediate and delayed word recall scores coupled with decreases in both accuracy and speed of attention. The same dose was associated with reductions in self-rated “alertness” and “calmness.” Following the highest dose, both “calmness” and “contentedness” were reduced across most time-points. These results represent the first systematic evidence that *Salvia* oil is capable of acute modulation of mood and cognition in healthy young adults.

**General Conclusions**

The recent *in vivo* studies provide some evidence that the *in vitro* activities observed for sage oil may translate into effects that may be clinically relevant to AD. The activity of major importance is the inhibition of cholinesterase but the possibility of a combination of effects, all contributing to some degree of prevention or alleviation of early symptoms, merits further research, particularly as clinical studies. It is particularly important to determine a dose which would achieve observable improvement. The fact that sage is widely used in food suggests that its use is associated with comparatively low risk.

Even if sage is never accepted as a treatment for AD — and that is a conclusion that is too early to predict — the work performed so far is an interesting example of how a traditional use has been given some credence through modern scientific studies. **Peter Haughton has taught pharmacognosy in the Department of Pharmacy, Kings College London, UK, since 1971. He was awarded a personal chair as Professor of Pharmacognosy in 1999 and heads the largest research group in the UK investigating medicinal plants. He has published more than 175 research articles on the chemistry and biological activity of medicinal plants.**
He is a member of the Board of the Society for Medicinal Plant Research and President of the Medicinal and Aromatic Plants Section of the International Pharmaceutical Federation (FIP).

References:
5. Gerard J. The Herball or Generall Historie of Plants. London (UK); 1597.

GARLIC AND ONION
Continued from page 47

The approach in these Jewish sources is preventative, seeking to teach people how to avoid illness.

Conclusion
Throughout their history, the Jewish people have consumed garlic and onions both as common foods and medicinal plants, as shown by the many references to them in Jewish sources. We have chosen to investigate the uses of garlic and onions with the aim of filling the gap between the knowledge of an ancient people living in their land and the medical arts as practiced in our times. Modern medicine, which in the Land of Israel emerged in the late nineteenth century, has ignored a vast body of knowledge gathered over several millennia and passed down through the populations that continued to dwell in the land in the years after its destruction.

Through the study of this ancient knowledge, we hope to encourage future research that will result in the cultivation and use of the medicinal plants of Israel for the benefit of all. 🌶️

Born in Jerusalem, Mina Ferne is a biologist who has always been interested in botany. She earned her BSc and MSc in botany and biochemistry at the Jerusalem University, then her PhD on plant biochemistry at the University of Strasbourg, France. From 1972–1990 she worked at Hadassah Hospital and the Ministry of Health, Jerusalem, on medical research in Biochemistry, Immunology and Microbiology, especially Streptococci. In 1983 she began studying Chinese medicine, and continues these studies in Israel and France. Since 1990 she has taught and researched medicinal plants, writing two books in Hebrew and many articles.

References

General Bibliography