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A randomised double-blind placebo-controlled pilot trial of a combined extract of sage, rosemary and melissa, traditional herbal medicines, on the enhancement of memory in normal healthy subjects, including influence of age

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ABSTRACT

Objective: To evaluate for the first time the effects of a combination of sage, rosemary and melissa (*Salvia officinalis* L., *Rosmarinus officinalis* L. and *Melissa officinalis* L.; SRM), traditional European medicines, on verbal recall in normal healthy subjects. To devise a suitable study design for assessing the clinical efficacy of traditional herbal medicines for memory and brain function.

Methods: Forty-four normal healthy subjects (mean age 61 ± 9.26y SD; m/f 6/38) participated in this study. A double-blind, randomised, placebo-controlled pilot study was performed with subjects randomised into an active and placebo group. The study consisted of a single 2-week term ethanol extract of SRM that was chemically-characterised using high resolution LC-UV-MS/MS analysis. Immediate and delayed word recall were used to assess memory after taking SRM or placebo (ethanol extract of *Myrrhis odorata* (L.) Scop.). In addition analysis was performed with subjects divided into younger and older subgroups (≤ 62 years mean age n = 26: SRM n = 10, Placebo n = 16; ≥ 63 years n = 19: SRM n = 13, Placebo n = 6).

Results: Overall there were no significant differences between treatment and placebo change from baseline for immediate or delayed word recall. However subgroup analysis showed significant improvements to delayed word recall in the under 63 year age group ($p < 0.0123$) with Cohen's effect size $d = 0.92$. No adverse effects were observed.

Conclusion: This pilot study indicates that an oral preparation of SRM at the selected dose and for the period of administration is more effective than a placebo in supported verbal episodic memory in healthy subjects under 63 years of age. Short- and long-term supplementation with SRM extract merits more robust investigation as an

adjunctive treatment for patients with Alzheimer's disease and in the general ageing population. The study design proved a simple cost effective trial protocol to test the efficacy of herbal medicines on verbal episodic memory, with future studies including broader cognitive assessment.

Keywords:

Salvia officinalis; *Rosmarinus officinalis*; *Melissa officinalis*; Cognition; Word recall; Dementia; Traditional herbal medicine

Abbreviations:
LC-UV-MS/MS Liquid Chromatography-UltraViolet-Mass Spectroscopy, RSM Rosemary, sage and melissa (*Rosmarinus officinalis* L., *Salvia officinalis* L., and *Melissa officinalis* L.).

Introduction

The need for therapeutic strategies to counter cognitive decline in our expanding aged populations and in dementia needs no highlighting these days (Prince et al. 2013). It is reported that in the United States alone someone develops Alzheimer's every 67 seconds (Alzheimer's Dement., 2015). Recent findings in the western world suggest the incidence may be in decline due to improved brain health and that specific anti-Alzheimer's therapy should also combine preventative lifestyle interventions that target general brain health (Canevelli et al. 2016; Scheltens et al. 2016). In addition it is suggested that clinical studies (more relevant than animal studies) are key to finding effective treatments and preventative strategies (Pistollato et al. 2015).

Since the discovery of bioactivities of sage (*Saliva officinalis* L. and *S. lavandulaefolia* Vahl (the latter a synonym for *S. officinalis* subsp. *lavandulifolia* (Vahl) Gams) relevant to dementia therapy (Perry et al. 1998) several traditionally used medicinal plants have been found to enhance cognitive functions, including memory (Perry, Howes 2011) and to have actions associated with improving brain health such as antioxidant, anti-inflammatory and neuroprotection (Panickar 2013). There is evidence, from both traditional herbal practice, as well as positive controlled clinical trials, that supports the use of natural products such as bacopa *Bacopa monnieri* (L.) Wettst. (Mathur et al. 2016), curcumin (from turmeric, *Curcuma longa* L.) (Cox et al, 2015), huperzine A from *Huperzia serrata* (Thunb.) Trevis.) (Xu et al. 2012), *Ginkgo biloba* L. (Perry, Howes, 2011), peppermint (*Mentha* x *piperita* L.) (Moss et al., 2008), saffron (*Crocus sativus* L.) (Akhondzadeh et al., 2010), sage (*Salvia officinalis* L.), rosemary (*Rosmarinus officinalis* L.) and lemon balm (*Melissa officinalis* L.) in memory enhancement and/or slowing down age associated cognitive decline.

Clinical and mechanistic studies support the cognitive enhancing effects of individual extracts of three European Lamiaceae medicinal plants for memory: sage (Perry et al. 2003; Scholey et al. 2008; Tildesley et al. 2003), rosemary (Pengelly et al. 2012) and lemon balm; the latter also known by the vernacular name, melissa (Kennedy et al. 2003; Ozarowski, 2016) (SRM). These studies support their traditional use for cognitive function, however to date no study has examined clinical effects on memory of the specific SRM combination. This, combined with the observed efficacy of SRM on outcome when prescribed by medical herbalists in patients experiencing memory loss, prompted the interest for a controlled pilot study of SRM. The findings of this study

establish guidance for future similar pilot studies and outline for the first time the noteworthy effects of combined ethanol extracts of SRM.

Subjects and methods

The study was conducted at Dilston Physic Garden Centre and BodyWorks Therapy Centre (Northumberland, U.K.). The protocol was approved by the Ethics Committee of Dilston Physic Garden and all subjects gave their informed consent.

Subjects

Subjects were screened and selected on the basis of key inclusion criteria of: age ≥40y and healthy with no reported current or previous clinical diagnosis of cognitive impairment or dementia. Exclusion criteria were: use of warfarin, pregnancy and breastfeeding. 45 subjects met all criteria and participated (mean age 61 ± 9.26y SD; m/f 6/38). All subjects were required to attend a local clinic (BodyWorks Therapy Centre) on two occasions, 2 weeks apart. Subjects and administrators were blind to who was receiving the active (SRM) or placebo treatment.

Study design

A randomised, double-blind, placebo-controlled study design was performed. Subjects were assigned 5ml SRM ethanol extract or 5ml placebo, diluted in warm water, 2x per day for 2 weeks.

Memory test

A traditional pencil and paper immediate and delayed word recall test to assess verbal working and episodic memory (Moss et al 2008), performed at the pre-dose (baseline assessment) and at the end of the 14 day treatment period.

Word recall

A total of 15 words were displayed to the subject one at a time using laminated sheets (with different lists at each visit). Immediately after seeing the words the subjects were given 1 min to write down as many words as they could recall seeing (Immediate Word Recall – Verbal Working Memory). Subjects were then given a 10 min break in which they were given reading materials and asked to remain in the assessment room. After this controlled period of time subjects were again given 1 min to write down as many of the words as they could recall seeing (Delayed Word Recall – Verbal Episodic Memory.

Statistical analyses

All analyses were conducted using the SAS statistical package Version 9.4 (SAS Institute Inc., Cary, NC, USA). All values are shown as mean ± SE. Change from baseline scores were calculated and submitted to analysis of variance using the MIXED procedure. Cohen's *d* effect sizes were calculated to identify the magnitude of any differences between dosing condition. Statistical significance was defined as a two-sided *p* value less than 0.05.

Extracts

The SRM extract consisted of extracts of 3 medicinal plant species from the Lamiaceae, each having a long standing safety record from traditional use and commercial food or

beverage production. Fresh authenticated plant material of *Salvia officinalis* L. (authentication code 140613-4/2), *Rosmarinus officinalis* L. (authentication code 140618-1/1) and *Melissa officinalis* L. (authentication code 130918-2/2), collected and individually extracted 0.5 g/ml in 45% EtOH for 3 weeks in the dark at room temperature, were grown (on site) and supplied by Rutland Biodynamic, Town Park Farm, Brooke, Rutland LE15 8DG United Kingdom. Plant names were checked with the World Checklist of Selected Plant Families (http://apps.kew.org/wcsp/home.do) and all plants were grown to organic and biodynamic standards. The extracts were combined in equal proportions (equivalent to daily dose 5g original plant material) and separated into 50 ml bottles.

The placebo was 50% fresh sweet cicely [*Myrrhis odorata* (L.) Scop.; Apiaceae] extract (1 g/ml 45% EtOH extracted at room temperature for 3 weeks), not known to affect brain function, to provide a non-cognitive control and to mask the SRM. 1% Lyles Black Treacle (Waitrose Ltd, UK.), used as a colorant to mask SRM, was added to both SRM and placebo. Cicely extract was added as 10% of the total volume to both SRM and placebo preparations.

LC-UV-MS/MS Analysis

The extracts were diluted 1:4 in 70% aqueous ethanol prior to analysis using a Thermo Scientific system consisting of an 'Accela' U-HPLC unit with a photodiode array detector and an 'LTQ Orbitrap XL' MS with an electrospray source (Thermo Scientific, Waltham, MA, USA). Chromatography was performed on 5 µl sample injections onto a

150mm x 3mm, 3µm Luna C-18 column (Phenomenex, Torrance, CA, USA) using the following 400 µl/min mobile phase gradient; H_2O:CH_3OH:CH_3CN + 1% HCOOH: 90:0:10 (0 min), 90:0:10 (5 min), 0:90:10 (60 min), 0:90:10 (65 min), 90:0:10 (67 min), 90:0:10 (70 min) followed by return to start conditions and equilibration in start conditions for 5 min before the next injection. The ESI source was operated with polarity switching and the MS recorded high resolution (30 k resolution) MS1 spectra (*m/z* 125–2000) in positive mode and low resolution MS1 spectra (*m/z* 125– 2000) in negative mode and data dependent MS2 and MS3 spectra in both modes using the linear ion trap. Detected compounds were assigned by comparison of accurate mass data (based on ppm), and by available MS/MS data, with reference to the published compound assignment system (Schyanski *et al*., 2014) and with supportive UV spectra. Diterpenoids were also assigned by comparison with a reference standard of rosmanol (≥ 99% purity) (LGC Ltd, UK).

Results

Of the 45 subjects recruited 44 completed the pre and post dose test sessions at baseline and the 14 day follow up (Male = 6; Female = 38). There were no side effects observed in any subject. The treatment groups matched with 22 in each group (Table 1).

Memory test

The number of words correctly recalled, minus the number of words recalled in error (intrusions) were calculated for both immediate and delayed word recall at each visit. Changes from baseline comparisons between the SRM group to the placebo group were

analysed and whilst the treatment group did perform marginally less well at baseline, this difference between the groups did not approach statistical significance in either.

On further analysis there were no changes over time in either placebo or SRM or in differences between the two which approached statistical reliability, although numerically the SRM group did tend to improve more in delayed word recall. Analysis was conducted to establish any noteworthy effects for future studies. No benefits were found when analysing by gender, baseline scores or when excluding those who had missed >3 doses.

Although not noted during randomisation, the placebo group was on average 4.5 years younger than the SRM group (SRM mean age 63.05y ± 8.7SD; placebo mean age 58.59y ± 9.3SD) and this approached statistical significance ($p = 0.11$). The population was divided into two subgroups Younger and Older (Table 1) and the subgroups matched by age range (Younger 43-62 years: placebo 43-62y and SRM 44-61y; Older 63-80y: placebo 66-78y, SRM 63-80y) and disperse the gender of this population within the subgroups (female 86% overall populations: Younger M = 2, F = 24; Older M = 4, F = 14).

The subgroups were analysed separately using baseline performance as the covariate. This analysis showed a main effect in delayed word recall for the younger subgroup taking SRM compared to placebo ($p = 0.0347$) (Table 2, Fig. 1). This improvement in the treatment group over baseline represents 2.6 words ($p = 0.0123$) which is a 56% improvement over baseline, with a Cohen's effect size of $d = 0.92$. Treatment

compliance was similar in the 2 overall groups, but slightly less in the older group (average declared missed dose of 2.2) compared to the younger group (average declared missed dose of 1.5).

Phytochemical analysis

The LC-MS chromatograms for each SRM extract and the SRM combination are shown in Fig. 2. Phytochemical analysis revealed the compounds detected in the three Lamiaceae species are in agreement with published literature for these species (British Pharmacopeia, 2014; CCD, 2016; Fecka and Turek, 2007; Heitz et al., 2000; Kontogianni et al., 2013). The main compound classes detected in the three extracts and the SRM combination using LC-MS were flavones and their glycosides (**1**, **2**, **3**, **5**, **6**, **7**, **8**, **9**, **10**, **12**), diterpenoids (**11**, **13**, **14**, **15**, **16**) and rosmarinic acid (**4**) (Fig. 2). The flavones and their glycosides, and rosmarinic acid, were assigned from their observed $[M+H]^+$ ions, with supportive UV spectra and MS/MS interpretation. Compounds assigned as diterpenoids were only detected in the *S. officinalis* and *R. officinalis* extracts; therefore their detection in the SRM combination was due to these species and not *M. officinalis*, in which diterpenoids were not detected.

A compound with *m/z* 347.1845 eluting at the retention time (Rt) 36.5 min (**11**) was assigned as $C_{20}H_{26}O_5$ from the observed $[M+H]^+$ ion and was designated as rosmanol, a diterpenoid known to occur in both *S. officinalis* and *R. officinalis* (Miura et al., 2001; Borrás Linares et al., 2011). At Rt 39.8 min (*m/z* 377.1581) a compound (**13**) was designated the molecular formula $C_{20}H_{24}O_7$ from the observed $[M+H]^+$ ion, which was proposed to be an abietane diterpenoid (or isomer) previously reported to occur in *S. gilliesii* Benth. [synonym for *S. cuspidata* subsp. *gilliesii* (Benth.) J.R.I.Wood] and in *S.*

palaestina Benth. [6,12,19-trihydroxy-11,14-diketo-8,12-abietadien-20,7-olide and salvipalestinoic acid, respectively] (Nieto et al., 2000; CCD, 2016).

A compound (**14**) eluting at 45.6 min with *m/z* 405.1896 was assigned with the molecular formula $C_{22}H_{28}O_7$, determined from the $[M+H]^+$ ion, which is the molecular formula of dihydroxy-clerodadiene-diolide, and is known to occur in *Salvia* species (CCD, 2016). With supportive MS/MS interpretation, the molecular formula $C_{22}H_{30}O_5$ was designated to the compound (**15**) eluting at Rt 48.1 min with *m/z* 375.2151, and is that of the ethyl ether derivative of rosmanol (or isomer). Indeed, 7-ethoxyrosmanol, epiisorosmanol ethyl ether (Borrás Linares et al., 2011) and epirosmanol ethyl ether (Zhang et al., 2012) are proposed to occur in *R. officinalis*; whilst the 9-ethyl ether derivative of rosmanol is reported in *S. officinalis* (Djarmati et al., 1991). The compound (**16**) eluting at 54.6 min, with *m/z* 715.4154, was assigned as a methyl ether derivative of carnosic acid from the observed $[2M + Na]^+$ ion, with the molecular formula calculated as $C_{21}H_{30}O_4$. The 12-methyl ether derivative of carnosic acid has previously been reported to occur in *R. officinalis* (CCD, 2016) and has also been known as methyl carnosate (Zhang et al., 2012).

Discussion

This pilot study using a combined extract of sage, rosemary and melissa in healthy subjects for the first time, shows encouraging indications of value in supporting memory in brain health and disorders of memory impairment. Results support findings of previous cognitive benefits of the individual extracts (Kennedy et al. 2003; Pengelly et al. 2012; Scholey et al. 2008).

The mechanisms behind the delayed word recall effects are likely to be due to anticholinesterase actions of these species and due to the interactions of their constituents such as Lamiaceae monoterpenoids (1,8-cineole, α-pinene, borneol and camphor) and other constituents such as rosmarinic acid, and/or their metabolites (Williams et al., 2011). The anticholinesterase activity of a rosemary extract was concluded to explain its memory-enhancing effect in a scopolamine-induced animal model of dementia (Habtemariam, 2016) and reduced levels of AChE were found in rat brain following oral administration of sage essential oil, suggesting monoterpenoids present in orally taken extracts of sage, rosemary and melissa can reach the brain (Perry et al., 2003).

Other mechanisms of action of constituents present in the extracts may also be responsible such as cholinergic receptor activation (Kennedy et al. 2003) and, though more likely evident with longer term treatment, anti-inflammatory, antioxidative (such as pinenes, geraniol and apigenin or metabolites), anti-amyloid, oestrogenic and neuroprotective actions of terpenoids and phenolics or their metabolites present in these species may play a role (Howes and Houghton, 2012; Huang et al, 2008; Oliveira 2015; Porres-Martínez et al. 2015; Soodi et al, 2017). Rosemary and sage terpenoids are associated with a range of antioxidant, anti-inflammatory and anti-amyloid mechanisms that have been considered to contribute to the cognitive enhancing effects of extracts (Habtemariam, 2016). Rosmanol, a diterpenoid detected in the SRM combination, has been suggested as a potent anti-inflammatory agent (Lai et al., 2009). However, whilst rosemary diterpenoids (including carnosic acid methyl ether, detected in the SRM) have been associated with potent antioxidant effects, it has been suggested that their antioxidant potency is reduced during the digestion process and digestive products of

the diterpenoids may mediate antioxidant effects following their oral ingestion (Soler-Rivas et al., 2010). Regarding bioavailability, one study associated rosemary flavonoids with passive diffusion in Caco-2 cells to predict their intestinal absorption, with cirsimaritin (detected in SRM) having amongst the highest permeation potency; although rosemary diterpenoids including rosmanol (detected in SRM) demonstrated even higher permeability (Pérez-Sánchez et al., 2017). Thus, the flavonoid, monoterpenoid, and diterpenoid constituents in SRM may be adequately bioavailable to contribute to the observed effects of SRM on cognitive functions in the present study. In general, it has been concluded that flavonoids are poorly absorbed following oral administration and are extensively metabolised in the intestine and liver (Lotito and Frei, 2006). Furthermore, studies using an *in vitro* model of the blood-brain barrier (BBB) indicate some flavonoids and their glucuronide metabolites may be able to cross the BBB; and *in vivo*, flavonoid aglycones were detected in rat brain tissue following oral administration of flavonoid glycosides (Jäger and Saaby, 2011). Other studies associate diterpenoids from *Salvia* species with clogP values indicative of their ability to cross the blood-brain barrier (Ren et al., 2004). The results from our study associate SRM with improved verbal episodic memory in healthy subjects under 63 years of age, compared to placebo, thus indicate that constituents of SRM, or their metabolites, are adequately bioavailable and cross the BBB. This concurs with previous studies where oral administration of members of the Lamiaceae is associated with improved cognitive functions in controlled trials (Perry and Howes, 2011). We therefore emphasise the need for continued investigations on the pharmacokinetics of plant species and their constituents to further elucidate their therapeutic potential for use in cognitive disorders.

It is notable that rosmarinic acid modulates a range of pathways implicated in Alzheimer's pathology, including amyloid accumulation, DNA fragmentation, capase-3 activation and tau protein hyperphosphorylation (Williams et al., 2011), therefore its detection in all three Lamaiceae species in the SRM extract is of particular relevance when investigating plant species to favourably impact against cognitive decline. Subchronic oral administration of rosmarinic acid to rats (2 mg/mg for 2 or 3 weeks) enhanced cognitive performance (Park et al., 2010), thus indicating the potential relevance of this phenolic acid (or its metabolites) for cognitive functions. However, more studies on the bioavailability and pharmacokinetics of rosmarinic acid (and other phenolic/ terpenoid constituents present) in humans, and the ability of rosmarinic acid, and others such as salvianolic acid, metabolites to cross the blood-brain barrier, are needed to more fully elucidate its clinical relevance. Recent studies reveal that rosmarinic acid has poor oral bioavailability [< 2% *in vivo*] (Wang et al., 2017), but in humans it is rapidly absorbed following oral administration prior to methylation and conjugation by glucuronidation and/or sulfation, with one of the major metabolites reported as trimethoxycinnamic acid monoglucuronide; whilst it is important to consider that pharmacokinetics studies in animals report different metabolites to those observed in humans (Amoah et al., 2016). Other studies provide conflicting results on whether rosmarinic acid and/or its metabolites can cross the BBB, with rosmarinic acid not detected in the brain of rats following intragastric administration of an extract prepared from another Lamiaceae species (*Plectranthus barbatus* Andrews); but detection of rosmarinic acid was observed in rat brain following intraperitoneal administration of the same preparation (Falé et al., 2011).

Future combination therapy could consider addition of other plant extracts reputed to counter cognitive decline, including bacopa (*Bacopa monnieri* (L.) Wettst.), clubmoss (*Huperzia serrata* (Thunb.) Trevis.) and ginseng (*Panax ginseng* C.A.Mey.), nigella (*Nigella sativa* L.), periwinkle (*Catharanthus roseus* L.), ashwagandha (*Withania somnifera* L.) and turmeric (*Curcuma longa* L.) amongst others (Perry and Howes, 2011). Plants that have other beneficial mechanistic effects, such as increasing blood flow and cholesterol metabolism, and benefits for behavioural symptoms such as anti-agitation (for example chamomile (*Matricaria chamomilla* L.), passion flower (*Passiflora* species)) and sleep enhancers (such as lavender (*Lavandula angustifolia* Mill.) and valerian (*Valeriana officinalis* L.)) may also provide the warranted polypharmaceutical (and even preventative) approach to multifaceted disease processes such as Alzheimer's (Akhondzadeh et al. 2010; Ji et al, 2011; Perry and Howes 2011; Russo et al. 2013).

The absence of any effect in the older population was unexpected, and may reflect the need for a higher dose and/or treatment duration in individuals whose cerebral function may be compromised by age; and also as a preventative agent to help prevent long term memory decline given the additional antioxidative and anti-inflammatory properties of such plant species. Since a major objective of this pilot controlled trial was to investigate medicinal plants to counter age associated cognitive decline, the results have indicated the value for a future trial, with a longer duration, more extensive cognitive assessments, SRM at a higher dose or using other methods of administration to assess and enhance bioavailability. A recent 20-year follow-up population-based study concluded that cognitive decline in a non-demented elderly population was lower in

subjects who took *Ginkgo biloba* compared to those who did not (Amieva et al., 2013), equally indicates a longer term trial may be relevant to examine the preventative potential of medicinal plants for dementia, given the numerous and polypharmacologic neuromechanisms behind a number of these plants and considering their relative safety of use. Furthermore, for future medicinal plant clinical studies in general, care should be taken with patient cohorts to allow for any potential contraindications (*S. officinalis* in epilepsy) and adverse effects of specific doses of sage (blood pressure), melissa and rosemary (anxiety) (Pengelly et al. 2012; Scholey et al. 2014).

For the majority of plant medicines, there are few robust clinical trials to assess their efficacy, with the major issue for many trials to date being the investigation of plant medicines that are not adequately authenticated or chemically-characterised. The type of extract and the ability of the 'active' compounds to cross the blood-brain barrier to reach the CNS must also be considered. In addition to characterising phytochemical differences (or similarities), exploring alternative methods of administration may also be key to discrepancies between laboratory and clinical data (McClure et al. 2015), although the explicit phytochemical analysis and characterisation of extracts remains primary in this objective.

Conclusion

This double-blind, placebo-controlled pilot study has left clear water for piloting other plant medicines (and their combinations) by this method, demonstrating a simple cost effective model that could be used clinically by medical herbalists, aromatherapists and in other clinical settings to test efficacy. All future pilot studies of medicinal plants will

benefit from a randomised double-blind placebo-controlled cross-over design and increased scope of cognitive function testing, that would provide more robust (cognitive) data and understanding of the therapeutic indications of SRM (and other chemically-characterised polyherbal formulations), not only for core aspects of cognitive function known to decline with age, but for other CNS disorders such as anxiety, depression, sleep and pain.

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Conflict of interest

With reference to 'A randomised double-blind placebo-controlled pilot trial of a combined extract of rosemary, sage and melissa, traditional herbal medicines, on the enhancement of memory in normal healthy subjects, including influence of age' we wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Fig. 1. Delayed Word Recall in the Younger (≤63) and Older (≥ 63 years) Subgroups mean change from baseline. [colour]

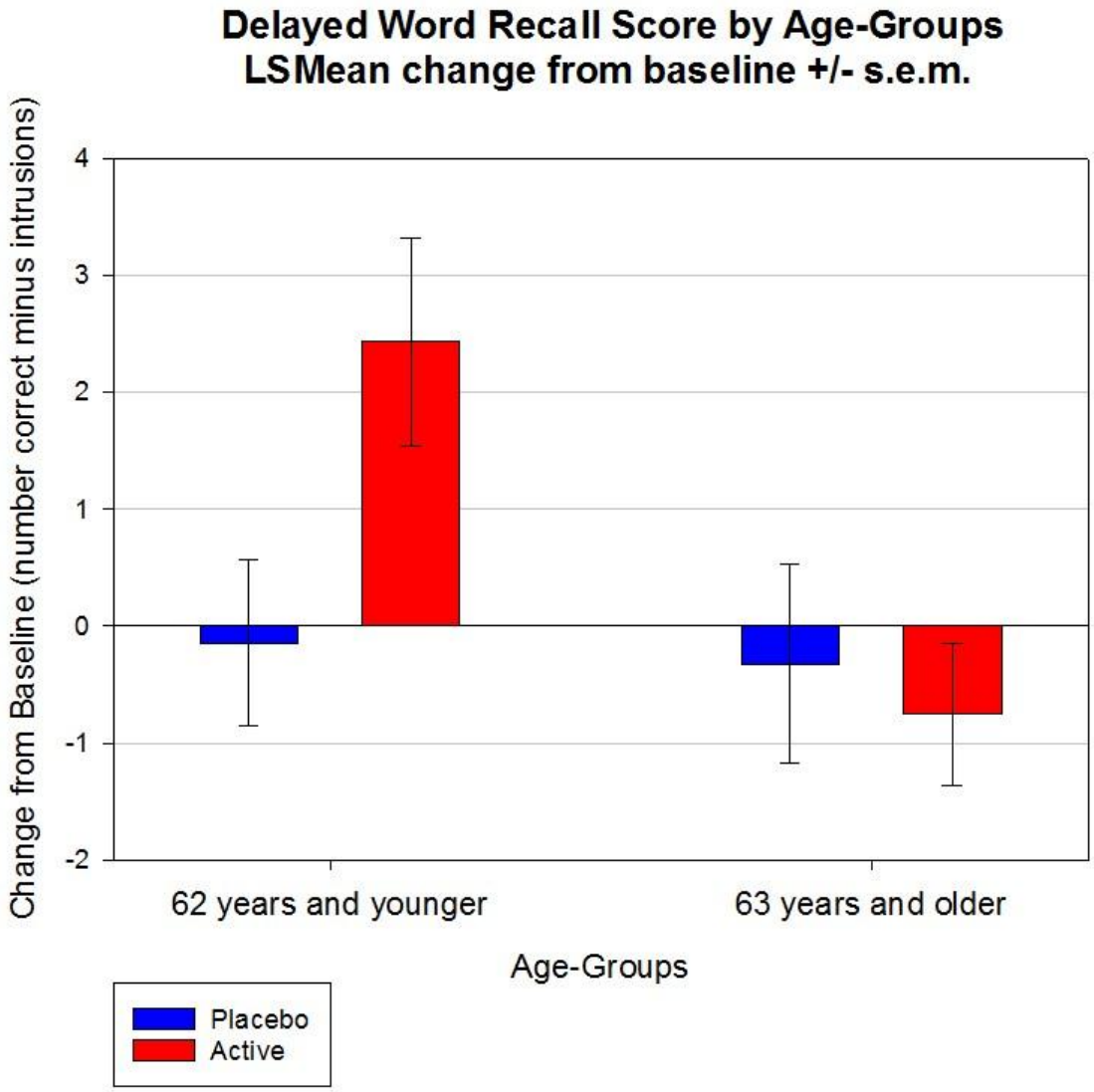

Fig. 2. LC-MS chromatograms (positive mode) of the *S. officinalis*, *R. officinalis* and *M. officinalis* extracts and the SRM extract combination. Assigned compounds: (**1**) luteolin 7,4′-di-*O*-glucuronide; (**2**) luteolin 7-*O*-glucoside; (**3**) luteolin 7-*O*-glucuronide; (**4**) rosmarinic acid; (**5**) chrysoeriol 7-*O*-glucuronide; (**6**) luteolin 3′-*O*-glucuronide; (**7**) luteolin; (**8**) 6-methoxyluteolin; (**9**) apigenin; (**10**) scutellarein 6-methyl ether; (**11**) rosmanol; (**12**) cirsimaritin; (**13**) diterpenoid: $C_{20}H_{24}O_7$; (**14**) dihydroxy-clerodadiene-diolide; (**15**) rosmanol ethyl ether; (**16**) carnosic acid methyl ether.

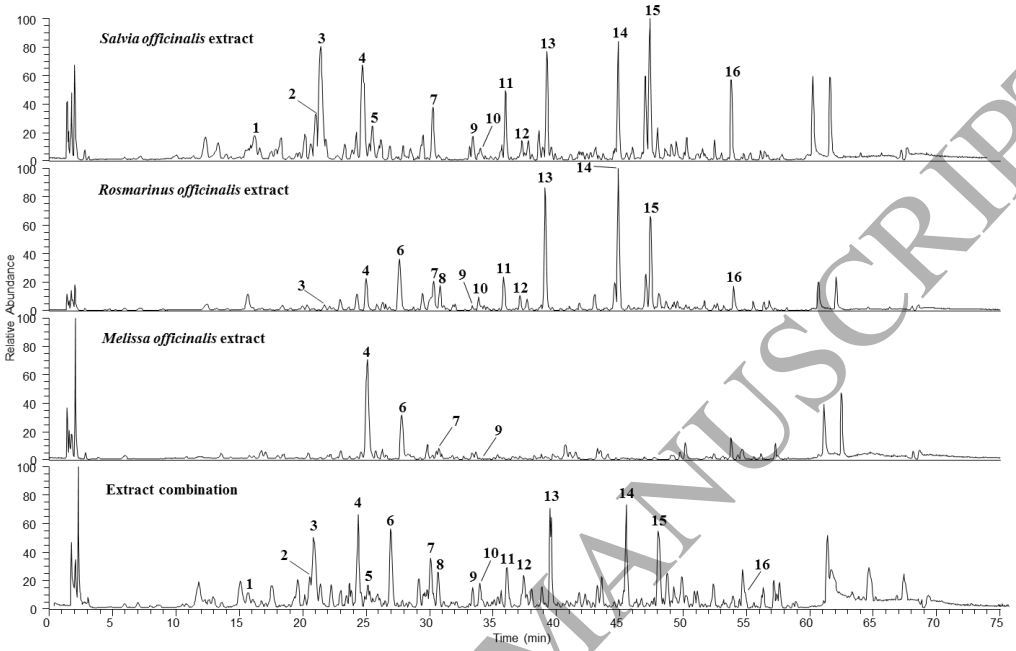

Table 1 Subject subgroup characteristics

Group	Treatment	N	Gender	Age Mean	SD	Min	Max	Group
Older	SRM	10	Female	69.50	4.9	63.00	80.00	63 to
≥ 63 years	SRM	2	Male	68.50	7.7	63.00	74.00	80
	Placebo	4	Female	70.50	5.2	66.00	78.00	66 to
	Placebo	2	Male	68.00	.	68.00	68.00	78
Younger	SRM	9	Female	55.44	6.1	44.00	61.00	44 to
(≤62years)	SRM	1	Male	56.00	.	56.00	56.00	61
	Placebo	15	Female	54.27	7.2	43.00	62.00	43 to
	Placebo	1	Male	57.00	.	57.00	57.00	62

Table 2

Immediate and delayed word recall results by subgroup and treatment

Subgroup	Treatment	N	Visit	Test	Mean number of words recalled	SE
OLDER	SRM	n = 12	Baseline	Immediate Word Recall	6	0.86
≥ 63 years			Baseline	Delayed Word Recall	4.16	0.99
			14 days	Immediate Word Recall	6	0.75
			14 days	Delayed Word Recall	3.5	0.85
	Placebo	n = 6	Baseline	Immediate Word Recall	6.16	0.74
			Baseline	Delayed Word Recall	5	0.81
			14 days	Immediate Word Recall	6.16	0.79
			14 days	Delayed Word Recall	4.5	1.11
YOUNGER	SRM	n = 10	Baseline	Immediate Word Recall	6.9	1.02
≤ 62 years			Baseline	Delayed Word Recall	4.6	0.71
			14 days	Immediate Word Recall	7.2	1.13
			14 days	Delayed Word Recall	7.2	1.29
	Placebo	n = 16	Baseline	Immediate Word Recall	7.37	0.83
			Baseline	Delayed Word Recall	5.62	0.81
			14 days	Immediate Word Recall	7.31	0.59
			14 days	Delayed Word Recall	5.37	0.75

Graphical abstract