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Development of a neuroprotective potential algorithm for medicinal plants

Weixi Liu [a, 1], Hang Ma [b, 1], Nicholas A. DaSilva [b], Kenneth N. Rose [b], Shelby L. Johnson [b], Lu Zhang [b], Chunpeng Wan [b], Joel A. Dain [a, **], Navindra P. Seeram [b, c, *]

[a] Department of Chemistry, University of Rhode Island, Kingston, RI 02881, USA
[b] Bioactive Botanical Research Laboratory, Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston, RI 02881, USA
[c] George & Anne Ryan Institute for Neuroscience, University of Rhode Island, Kingston, RI 02881, USA

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ABSTRACT

Medicinal plants are promising candidates for Alzheimer's disease (AD) research but there is lack of systematic algorithms and procedures to guide their selection and evaluation. Herein, we developed a Neuroprotective Potential Algorithm (NPA) by evaluating twenty-three standardized and chemically characterized Ayurvedic medicinal plant extracts in a panel of bioassays targeting oxidative stress, carbonyl stress, protein glycation, amyloid beta (Aβ) fibrillation, acetylcholinesterase (AChE) inhibition, and neuroinflammation. The twenty-three herbal extracts were initially evaluated for: 1) total polyphenol content (Folin-Ciocalteu assay), 2) free radical scavenging capacity (DPPH assay), 3) ferric reducing antioxidant power (FRAP assay), 4) reactive carbonyl species scavenging capacity (methylglyoxal trapping assay), 5) anti-glycative effects (BSA-fructose, and BSA-methylglyoxal assays) and, 6) anti-Aβ fibrillation effects (thioflavin-T assay). Based on assigned index scores from the initial screening, twelve extracts with a cumulative NPA score ≥40 were selected for further evaluation for their: 1) inhibitory effects on AChE activity, 2) in vitro anti-inflammatory effects on murine BV-2 microglial cells (Griess assay measuring levels of lipopolysaccharide-induced nitric oxide species), and 3) in vivo neuroprotective effects on *Caenorhabditis elegans* post induction of $A\beta_{1-42}$ induced neurotoxicity and paralysis. Among these, four extracts had a cumulative NPA score ≥60 including *Phyllanthus emblica* (amla; Indian gooseberry), *Mucuna pruriens* (velvet bean), *Punica granatum* (pomegranate) and *Curcuma longa* (turmeric; curcumin). These extracts also showed protective effects on H_2O_2 induced cytotoxicity in differentiated cholinergic human neuronal SH-SY5Y and murine BV-2 microglial cells and reduced tau protein levels in the SH-SY5Y neuronal cells. While published animal data support the neuroprotective effects of several of these Ayurvedic medicinal plant extracts, some remain unexplored for their anti-AD potential. Therefore, the NPA may be utilized, in part, as a strategy to help guide the selection of promising medicinal plant candidates for future AD-based research using animal models.

1. Introduction

Alzheimer's disease (AD) is a common neurodegenerative disorder characterized by the progression of cognitive decline leading to severe dementia (Buckner et al., 2005). The accumulation of senile plaques and neurofibrillary tangles in cerebral cortex and hippocampus are two major pathological hallmarks of AD (Ittner and Götz, 2011). However, due to the complexity of the disease, the precise factors which trigger the development of AD remains unknown (Alzheimer's Association, 2015). Moreover, given the unclear etiology of AD, current therapeutic approaches focus mainly on symptom management but no treatment is available to alter or reverse the course of the disease (Alzheimer's Association, 2015; Citron, 2010). Although the pathogenesis of AD is still under investigation, increasing evidence suggest that AD is a multifactorial disease which develops as a result of several risk contributors instead of a single cause alone (Norton et al., 2014; Reitz and

* Corresponding author. 7 Greenhouse Road, Kingston, RI, USA.
** Corresponding author. 140 Flagg Road, Kingston, RI, USA.
E-mail addresses: jdain@chm.uri.edu (J.A. Dain), nseeram@uri.edu (N.P. Seeram).
[1] These authors contributed equally to this work.

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Abbreviations

AD	Alzheimer's disease
ROS	reactive oxygen species
AGEs	advanced glycation end-products
RAGE	receptor for advanced glycation end-products
Aβ	beta amyloid
BBB	blood brain barrier
RCS	reactive carbonyl species
APP	Aβ precursor protein
AG	aminoguanidine
FRAP	ferric reducing antioxidant power
AChE	acetylcholinesterase
CNS	central nervous system
NOS	nitric oxide species
TCM	traditional Chinese medicine
NPA	Neuroprotective Potential Algorithm
BHT	Butylated hydroxytoluene
BSA	bovine serum albumin
MGO	methylglyoxal
RESV	resveratrol
PD	1,2-phenylenediamine
DQ	2,3-dimethylquinoxaline
TFA	trifluoroacetic acid
ThT	thioflavin T
$A\beta_{1-42}$	beta amyloid 1–42
DMSO	dimethyl sulfoxide
GAEs	gallic acid equivalents
DMEM/F12	Dulbecco's modified eagle medium: nutrient mixture F-12
FBS	fetal bovine serum
CTG 2.0	CellTiter-Glo 2.0
LPS	lipopolysaccharide
NGM	nematode growth medium

Mayeux, 2014).

Oxidative stress and the production of reactive oxygen species (ROS) have been implicated in the pathogenesis of AD and are believed to be leading causative factors for neuronal cell dysfunction and cell death (Lin and Beal, 2006; Smith et al., 2000). It has been demonstrated that the products of protein oxidation and lipid peroxidation are elevated in AD patients (Christen, 2000). In addition, in the AD brain, the activities of antioxidant enzymes are altered, accompanied with a decline in the expression of these antioxidant enzymes (Christen, 2000; Smith et al., 2000). Given the established links between oxidative stress and AD, antioxidants, including those from natural products, are extensively studied for their neuroprotective abilities and constitute dietary intervention strategies for AD prevention and treatment (Alzheimer's Association, 2015; Choi et al., 2012; Praticò, 2008).

Apart from oxidative stress, carbonyl stress and the formation of advanced glycation end-products (AGEs) resulting from protein glycation are also believed to be vital contributors to AD (Srikanth et al., 2011; Vicente Miranda and Outeiro, 2010). Glycation is one type of post-translational modification of proteins, resulting in the formation of AGEs both intracellularly and extracellularly. Glycation and AGEs formation are associated with AD due to several reasons. First, AGEs bind to the transmembrane receptor, RAGE (receptor for AGEs), upregulate RAGE expression, and activate RAGE-mediated neuronal dysfunction and neuron damages (Srikanth et al., 2011). Second, RAGE mediates the transportation of beta amyloid (Aβ) across the blood brain barrier (BBB) (Donahue et al., 2006). Therefore, the activation of RAGE by AGE can cause Aβ accumulation in the brain. Third, during the course of glycation and AGE formation, ROS and reactive carbonyl species (RCS) are generated as by-products which, in turn, promote AGE formation and cause neurotoxicity (Ahmed et al., 2005; Münch et al., 2012; Picklo et al., 2002). Consequently, all of the factors involved in this positive feedback loop including AGEs, RCS, and ROS are considered to be promising targets for AD prevention and treatment.

Another common target for AD therapy is the Aβ peptide which consists of 40–42 amino acids and is generated from the cleavage of the Aβ precursor protein. Aβ is the major component of senile plaques and neurofibrillary tangles, two pathological hallmarks of AD (Buckner et al., 2005; Palop and Mucke, 2010). In AD patients, elevated Aβ levels were observed in both cerebrospinal fluid and blood (Mawuenyega et al., 2010). In addition, certain forms of Aβ, including fibrillated Aβ and glycated Aβ (Aβ-AGEs), have been shown to be neurotoxic (Butterfield, 2002; Li et al., 2013). Fibrillated Aβ can induce neurotoxicity by enhancing neuronal oxidative stress and neuroinflammation (Butterfield, 2002). Aβ-AGEs can induce intracellular oxidative stress and inflammation by activating RAGE and upregulating RAGE expression in neuronal cells (Li et al., 2013). Therefore, considerable research efforts have been directed to finding inhibitors which may prevent or reverse the formation of Aβ fibrils and Aβ-AGEs. For example, aminoguanidine (AG), a synthetic glycation inhibitor, can reduce glycated Aβ formation, attenuate RAGE upregulation, and restore the cognitive deficit in AD animal models (Li et al., 2013). However, AG failed in human clinical trials due to severe side effects (Thornalley, 2003) leading to the search for non-toxic alternatives including medicinal plants and their derived natural products and botanical extracts (Solanki et al., 2016; Venigalla et al., 2016).

In addition to oxidative stress, glycation, and Aβ formation, neuroinflammation is another pivotal factor implicated in the development of neurodegenerative diseases with increased inflammation observed in AD (Eikelenboom et al., 2002). In addition, inflammatory stress leads to the activation of microglia cells, the immune cells in the central nervous system, which release nitric oxide species (NOS) including nitrates and nitrites. These NOS are neurotoxic and cause massive neuronal death further exacerbating neurodegenerative diseases (Eikelenboom et al., 2002, 2006).

For centuries, traditional systems of medicines such as Ayurveda [from India, a country which has one of the lowest incidences of AD worldwide (Chandra et al., 2001; Vas et al., 2001)] and traditional Chinese medicine (TCM) (Steele et al., 2013), have used medicinal plants to treat several ailments including neurodegenerative diseases. While neurochemical/biological studies have been conducted on some these traditional medicinal plants, there is a lack of systematic procedures and algorithms to help guide the selection and evaluation of the most promising candidates for further AD-based research using animal models. This is urgently needed given the large variety of medicinal plant species (and combinations thereof) used worldwide in the traditional systems of medicines of various cultures. Furthermore, although medicinal plants are consumed as foods, herbs, spices, beverages, and botanical extracts, their underlying mechanisms of neuroprotective effects remain unclear. Therefore, given all of the aforementioned factors, herein, we utilized a panel of bioassays including total polyphenol contents, antioxidant capacities, anti-glycation effects, carbonyl