A review of medicinal plants that modulate nitric oxide activity

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Abstract

Modulation of nitric oxide (NO) may offer novel approaches in the treatment of a variety of diseases, such as Alzheimer's, cardiovascular disease, and diabetes. A strategy in the modulation of NO expression may be through the use of herbal medicines. We surveyed medicinal plant research that utilized multi-component extracts similar to what is used in clinical phytotherapy or in commerce, for demonstrated effects on NO activity. SciFinder Scholar, Pubmed, Web of Science, and BIOSIS were searched to identify human, animal, *in vivo*, *ex vivo* or *in vitro* research on botanical medicines, in whole or standardized form, that act on nitric oxide activity. iNOS was the most frequently investigated enzyme system and this system was up-regulated by many plant extracts, including, *Chicorium intybus, Cocos nucifera, Echinacea purpurea, Euonymus alatus, Ixeris dentate, Oldenlandia diffusa, Rhinacanthus nasutus, and Sida cordifolia. Many plant extracts down-regulated iNOS, including Centella asiatica, Dichroa Febrifuga, Echinacea purpurea, Evolvulus alsinoides, Fagonia cretica, Ginkgo biloba, Mollugo verticillata, Lactuca indica, Lithospermum erythrorhizon, Pueraria thunbergiana, and Taraxacum officinale*. The eNOS system was stimulated by *Eucommia ulmoides, Sida cordifolia,* and *Thymus pulegioides while Fagonia cretica, Rubia cordifolia* and *Tinospora cordifolia* down-regulated nNOS. Given the activity demonstrated by many of these herbal medicines, the increasing awareness of the effects of nitric oxide on a wide variety of disease processes and the growing incidence of these conditions in the population, further study of medicinal plants on nitric oxide signaling may lead to novel therapies and further insight into human physiology.

Introduction

Well before Furchgott and Zawadzki[1] proved the existence of a labile endothelium-derived relaxing factor (EDRF), nitric oxide (NO) had been found to be a potent vasodilator and to play a role in platelet aggregation. The eventual identification of NO as endothelial-derived relaxation factor led to the Nobel Prize for Ignarro, Furchgott and Murad. This was also the beginning of the recognition of NO as a key to not only vascular dynamics, but as a messenger molecule in a variety of tissues and organs.[2]

NO is a crucial intra- and extra-cellular communicator, modulating ion channels and messaging dynamics.[3] NO plays a pivotal role in physiological adaptation through its effects on vascular tone, proper neurotransmission, and proper immunological defense.[4] Currently, NO is reported to act as a central modulator of functions ranging from neurotransmission, gene response and second-signal messaging.[5]

As a neurotransmitter, NO plays a role in a number of processes including memory formation, sleep, neuropathologies and aging.[6,7] With regard to sleep, NO in the pontine tegmuntum enables the rapid eye movement stage.[6] NO also demonstrates a neuroprotective role for both glial and neuronal cells.[8] In the periphery, the network of non-adrenergic and non-cholinergic nerves operates through a nitric oxide-dependent mechanism regulating gastrointestinal, respiratory, and genitourinary tract functions. Additionally, erectile drugs act through neuronal generated NO.[9]

As an immunogenic messenger, substantial quantities of NO produced for host defense significantly influence the processes of immune system coordination, response, and inflammation.[10] NO is generated by activated macrophages aiding in the response to infections, directly exerting antimicrobial activity, and affecting both innate and acquired immune function through the modulation of cytokine expression and leukocyte apoptosis.[10]

The substrate for the production of NO is L-arginine, a nitrogen rich amino acid shuttled through the L-arginine-nitric oxide pathway to meet the tissue specific enzyme, nitric oxide synthase (NOS). NOS releases picomoles of nitric oxide in response to receptor stimulation.[11] NOS is unique, it is both a hemeprotein and flavoprotein. NOS includes three isoforms: neuronal NOS, named NOS1 or nNOS, inducible NOS, known as NOS2 or iNOS, and an endothelial NOS, known as NOS3 or eNOS.[12] Two of the three identified isoforms of NOS (eNOS and iNOS) are found in endothelial cells.[13] iNOS, which is integral to the inflammatory process and capable of high output of NO during inflammation, is inducible while nNOS and eNOS are constitutive.[14] The three isoforms all require cofactors and prosthetic groups for activity, including FAD, FMN, heme, calmodulin, tetrahydrobiopterin and possibly thiols.[12,15-17]

Evidence suggests that a variety of diseases are related to defects in the action and regulation of NO and NOS.[7] In pathological conditions, the isoforms of NOS are observed to be regulated differently.[18] High output of NO is injurious to a variety of tissues.[14] For example, a possible mode of action for Alzheimer's disease may involve the inappropriate expression of NO by the apoE4 genotype.[19,20] In addition, the inflammatory processes so commonly associated with obesity are associated with higher levels of NO.[21,22] In diabetes, eNOS and nNOS are down-regulated, while iNOS is up-regulated.[18] In aging, neuropathologies, and parasitemia, induction of iNOS, are observed.[6] Deficiencies in the production of NO inhibit adhesion molecules and cytokine expression and diminish leukocyte transmigration.[23] Insufficient NO release in the gastric associated lymphoid tissue, a key immunological site accounting for over 60% of all lymphoid tissue in the body, has been shown to be problematic for mucosal blood flow, gastrointestinal motility, mucus formation and bacteriostasis.[24] Thus, the NO pathways are involved in many pathological processes.

Even though they are structurally similar, NOS isoforms differ in their dependence on Ca^{++}.[25] The constitutive isoforms in the brain and vasculature are regulated by calcium and calmodulin while the inducible iNOS, found in

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Key words: nitric oxide synthase, traditional medicine, traditional Chinese medicine, Ayurveda, herbs, immunomodulators, immune, medicinal plants.

Contributions: JB, LM reviewed accepted papers, wrote sections of the manuscript and entered data into the charts; KS organized the study, performed the searches, reviewed accepted papers, wrote sections of the manuscript, entered data into the charts and checked the validity of the data.

Conflict of interests: the authors declare had no potential conflict of interests.

Received for publication: 16 October 2011.
Revision received: 9 February 2012.
Accepted for publication: 15 February 2012.

Alternative Medicine Studies 2012; 2:e6
doi:10.4081/ams.2012.e6

macrophages, is calcium independent.[7] Thus, transcriptional and post-transcriptional regulation of these various isoforms is distinct rendering therapeutic intervention specific to iNOS, eNOS or nNOS.[18,26,27] This heterogeneity provides the potential for specific therapeutic targets in the modulation of NO expression; a goal of pharmaceutical research due to the panoply of diseases with which NO is associated. For example, corticoids inhibit the induction of iNOS while proinflammatory cytokines may induce iNOS.[7]

Previous studies have shown dietary variables to have an impact on NO expression.[28-34] It is sensible, therefore, to consider the effect of plants in both a dietary and a medicinal scenario. Medicinal plants have been shown to influence a number of fundamental cellular processes and more research is being carried out into the effects of medicinal plants on NO. For example, Wang and Mazza[35] demonstrated that the anti-inflammatory effects of specific anthocyanin and proanthocyanin rich plants may be due in part to the inhibition of NO production. A review of the primary literature suggests that many of the effects of herbal remedies involve up- or down-regulation of such messengers as NO, cytokines and adhesion molecules.[36-39] This review of the primary literature demonstrates the effects of plant-based medicines on the expression of NO. Historically, the therapeutic use of medicinal plants included an array of diverse constituents or groups of constituents due to the phytochemical matrix of plant-based medicines.[40] As Islam and Carter suggest,[41] research into isolated constituents to reveal the modes of activity of herbal medicines loses sight of the principles of phytotherapy. Moreover, clinicians using medicinal plants in their practice generally use phytotherapeutic remedies that contain multiple constituents. Therefore, in order to maintain relevance to modern phytotherapy, this review was limited to herbal medicines that are available in the market place or preparations that represent multi-component phytochemical mixtures representative of a plant-based medicine approach.

Materials and Methods

Search strategy

The primary literature was searched in Pubmed, Web of Science, BIOSIS, and SciFinder Scholar. Titles were screened for all hits to the terms herbs and nitric oxide, medicinal plants and nitric oxide, Chinese medicine and nitric oxide, and Ayurveda and nitric oxide. The search only included English language papers.

Criteria for inclusion

The following inclusion criteria were adopted: i) Investigations on whole herbs or plant parts (*e.g.* seed, leaf, root, stem, flower or entire plant) or standardized extracts. Research on isolated constituents or herbal formulations were rejected. Fungi, although technically not plants, were included as they are commonly used in phytotherapy. ii) Model types were considered. *In vitro, ex vivo, in vivo,* animal and human models were accepted. iii) Two out of three of the following criteria were required to be transparent in the investigation: method of preparation of the botanical medicine, concentration of the plant preparation, or dose/exposure time. iv) Only studies demonstrating activity with regard to nitric oxide that showed a statistically significant result ($P<0.05$) were included.

Results

Out of 451 papers reviewed, only 44 papers met the established selection criteria. Data collected as a result of searches are shown in Tables 1-6.[42-84] The majority of the research used *in vitro* models, but *in vivo* animal models were also utilized. Data in Tables 1-6 list the *Genus species* of the plants researched, followed by the plant parts used, methods of preparation, concentration/dose, duration of exposure, model utilized, nitric oxide pathway affected and references. Tables 1 and 2 show *ex vivo* results. Tables 3-6 list the *in vitro* results utilizing cell culture categorized by solvents used for the medicinal plant extractions (aqueous, ethanolic, and other extractions). The tables are divided into columns denoting the medicinal plant species, type of preparation/extraction (aqueous, ethanolic, powder etc.), concentration/dosage used, duration of exposure, the cell type, inducing agent and model (*in vivo, ex vivo* or *in vitro*). If one or more of these variables differed in an investigator's series of experiments (*e.g.* plant part, cell model or dose) a separate listing for each experimental condition is listed.

The most common model employed for assaying NO activity was based on the Greiss assay that uses sulfanilamide and N-1-napthylethylenediamine to monitor the output of nitrite, a stable by product of NO production. The terms *increase* or *decrease* were used for these models. Investigations measuring enzyme concentration of NOS isoforms utilized Western blots (showing direct upregulation of NOS protein) or PCR was utilized to show the direct upregulation of NOS genes. The terms *induction* or *inhibition* were used for these models. The term *stimulation* was used to denote measures of increased NOS activity.

A large volume of research did not meet the inclusion criteria. Much of the rejected research was based on isolated constituents and offered little relevance for clinical phytotherapy.[85-87] Research of semi-purified compounds like curcumin (a mixture of curcuminoids) or bromelain (a mixture of proteolytic enzymes) or isolates such as indole-3-carbinol were included only if they were readily available in commerce and/or frequently used by phytotherapists. One particular paper reporting a series of investigations that screened 83 plant extracts was included in this review.[75] However, due to the high dosage utilized in the screening process (80 μg/mL), only the six plant extracts that demonstrated dose dependent responses at the lowest dose that achieved over 40% inhibition of NO expression were included from this series of investigations.[75]

The majority of the available studies located on NO and medicinal plants generated data based on *in vitro* work. Of these, only 15 studies had concentrations of extracts that were at or below 10 μg/mL. These included *Crataegus pinnatifida, Ixeris dentate, Chasalia chartacea, Hedyotis verticillata, Lasianthus oblongus, Leea indica, Litsea cubeba, Spermacoce articularis, Thymus pulegioides, Brassica* spp. (as the isolate indole-3-carbinol), *Cocos nucifera, Echinacea angustifolia* (combined with *E. purpurea*), *Echinacea purpurea, Kalopanax pictus* and *Phellinus linteus*. With rare exceptions, most *in vitro* models utilized macrophages and thus studied iNOS activity.

The significant findings of this literature review are that there are a multitude of medicinal plants that modulate the NO/NOS system. These effects are highlighted in Tables 1-6. Some of the most frequently used medicinal plants appear to interface with the NOS system. For example, *Centella asiatica* and *Echinacea purpurea*, both common medicinal plants, show activity on immune related NO release. However, *C. asiatica* was found to be an inhibitor of iNOS, while *E. purpurea* demonstrated increases in NO release in *ex vivo* models. In an *in vitro* murine macrophage model *E. purpurea* was found to both increase and decrease iNOS activity as assessed by NO concentrations. *Ginkgo biloba* has also demonstrated decreases in iNOS activity and, as a result, in NO release in human macrophages. Two plants from the Ayurvedic system, *Rubia cordifolia* and *Tinospora cordifolia*, have both been found to inhibit iNOS activity and NO release in *ex vivo* models. Multiple other plants have also been found to be active in the reviewed studies as shown in Tables 1-6.

Discussion

The limitations in this collection of data are

due to the use of *in vitro* and animal models in the bulk of the reviewed investigations. Replicating physiologically relevant models of human physiology are difficult with *in vitro* methods. Cells that survive, divide and then are utilized by *in vitro* experimental models are often poorly representative of cellular conditions *in vivo*, especially with regard to gene expression and enzyme levels.[88] In addition, *in vitro* models fail to account for the digestive and metabolic processing of the multiple compounds present within a plant extract. *In vitro* models are also often poor representations of physiologically relevant serum concentrations. Moreover, the increased oxygen exposure in cell-culture conditions leads to more reactive oxygen species (ROS) generation and may impair cellular antioxidant defences.[41] In considering *in vitro* research involving NOS and NO that are dependent on redox reactions, increased ROS generation due to cell culture conditions may have a dramatic impact on the results of these assays.

Animal models also may misrepresent human physiology. Artificially generated pathology, confounding variables, differences in anatomy and biochemical pathways all call into question the validity of data gathered from animal models. Accordingly, caution must be used when extrapolating data from *in vitro* and *in vivo* animal models. While *in vitro* and animal research models provide less than strong evidence, traditional use of medicinal plants has been shown to offer leads to biochemical and physiological effects of traditional remedies. For example, of 6,350 proven antimicrobial plant species, around 63% have ethnomedical documentation as antimicrobials.[89] Additionally, 75% of new drugs made from nat-

Table 1. Medicinal plants demonstrating nitric oxide activity in *ex vivo* models.

Genus species	Plant part	Preparation used	Dose	Exposure time	Cell type	Induced?	System affected	Direction of effect	Ref.
Centella asiatica	Herba and radix	Freeze-dried aqueous extract	0.10 g/kg	24 h	Murine gastric tissue homogenate	None	iNOS	Inhibition	[42]
Centella asiatica	Herba and radix	Freeze-dried aqueous extract	0.10 g/kg	24 h	Murine gastric tissue homogenate	None	NO	Decrease	[42]
Dichroa febrifuga	Radix	Aqueous decoction	100 mg/kg Not specified	20 h	Murine serum	LPS	iNOS	Inhibition	[43]
Echinacea purpurea	Herba and radix	Extract enriched in *cichoric acid, ♯polysaccharides **alkylamides	*120 g/kg/d ♯3000 g/kg/d **12 g/kg/d	4 days	Murine alveolar macrophage	LPS	NO	Increase	[44]
Emblica officinalis	Fructus	Aqueous extract	25 mg/kg	10-20 d	Murine peritoneal macrophage	TG	NO	Inhibition	[45]
Eucommia ulmoides	Folia	Aqueous extract	400 g/mL		Murine thoracic artery	L-NAME	eNOS	Stimulation	[46]
Eucommia ulmoides	Cortex	Aqueous extract	200 g/mL		Murine thoracic artery	L-NAME	eNOS	Stimulation	[46]
Eucommia ulmoides	Folia and cortex	Aqueous extract	200 g/mL		Canine thoracic artery	L-NAME	eNOS	Stimulation	[46]
Evolvulus alsinoides	Herba	Aqueous	25 mg/kg	10 d	Murine peritoneal Macrophage	TG	NO	Decreased	[45]

Decrease/Increase, levels; Induction/Inhibition, enzyme models (NOS); Stimulation, increased enzyme activity; TG, thioglycolate; L-NAME, L-N(G)-nitroarginine methyl ester hydrochloride; LPS, lipopolysaccharide.

Table 2. Medicinal plants demonstrating nitric oxide activity in *ex vivo* models.

Fagonia cretica	Radix	Not specified	2 mg/mL	2 h	Murine hippocampus	Oxygen-glucose deprivation	NO nNOS	Decrease inhibition	[47]
Mollugo verticillata	Herba	Ethanol, lyophilized	500 mg/g	7 d	Murine macrophage	BCG Ag	NO	Inhibition	[48]
Perilla frutescens	Folia	Aqueous extract	50 mg/kg/d	34 wks	Murine Serum	None	NO	Increased	[49]
Rubia cordifolia	Radix	Not specified	2 mg/mL	2 h	Murine hippocampus	Oxygen-glucose deprivation	NO nNOS	Decrease Inhibition	[45]
Sida cordifolia	Folium	Aqueous fraction	20 mg/kg, iv	One-time dose	Murine aorta/ vena cava	None	eNOS	Stimulation	[50]
Spirodela polyrhixa	Not specified	Aqueous extract	1 mg/mL	1 h	Murine peritoneal macrophage	LPS	NO	Inhibition	[51]
Spirodela polyrhixa	Not specified	Aqueous extract	1 mg/mL	1 h	Murine peritoneal macrophage	LPS	NO	Inhibition	[51]
Tinospora cordifolia	Radix	Not specified	2 mg/mL	2 h	Murine hippocampus	Oxygen-glucose deprivation	NO nNOS	Decrease Inhibition	[47]

BCG Ag, Bacille Calmette Guérin Antigen from Mycobacterium bovis; LPS, lipopolysaccharide.

ural products were *discovered* by following leads from traditional use of medicinal plants.[90] Thus, the reviewed data, which demonstrates the effects of herbal extracts on NO activity and NOS expression, may offer meaningful insight into the physiological activity and potential therapeutic indications when put into the context of traditional use. Furthermore, given the broad spectrum activity via stimulation of cell-to-cell communication by NO and, in turn, the broad spectrum activity of phytomedicines which can be viewed as complex chemical remedies, it is likely that some of the organ and tissue effects of these remedies are due, at least in part, to modulation of NO expression.

In the past decade, NO has been recognized as a primary messenger molecule of paramount functional significance with broad spectrum activity. The activity of NO is not dependent primarily on NOS isoforms but rather on NO concentrations, leukocyte priming, and cellular context.[23] NO has been found to be a pleiotropic mediator of inflammation.[91] In addition, dependent on concentration, NO is involved in protection against or induction of oxidative stress in many tissues. With this dual role in mind, Cooke[92] suggests a *yin/yang* effect of NO in physiological function. Overproduction of NO is of primary importance and is proinflammatory, inducing cellular damage via oxidative stress, characterizing atherosclerosis, as well as insulin resistance. Conversely, a reduction in production of NO may be involved in initiation and progression of atherosclerosis.[23,92-94] The endothelial isoform of NOS, eNOS, is believed to play a role in limiting the acute phase of inflammation by inhibiting leukocyte activation and platelet aggregation, and by inducing vasodilatation.[95]

Achike and Kwan[95] also recognize pleiotropic activity of NO as *yin/yang* suggesting that an imbalance in vascular function relates to the *yin* (vascular relaxation) and yang (vascular contraction) balance of the system. They suggest that hypertension is due to an excessive yang nature, while hypotension is considered excessive *yin* nature. Ou *et al*.[96] suggest that herbs that show strong antioxidant activity were *yin* in nature after comparing the results of herbs known in traditional Chinese medicine as *yin* or *yang* that were evaluated by the oxygen radical absorbance capacity (ORAC) assay. Intriguingly, of the herbs suggested to be *yin* in nature, many are, in the proper clinical context, used to treat cardiac conditions, including hypertension which is commonly considered a yang condition. These include *Astragalus membranaceous, Crataegus pinnatifida, Emblica officinalis, Eucommia ulmoides, Evolvulus alsinoides, Nigella sativa* and *Oldenlandia diffusa*.

Cooke's[92] suggestion of NO having an *amphoteric* effect relates to the activity of some of the reviewed medicinal plants that are believed to amphoterically regulate physiology. For example, extracts from *Echinacea* spp., once thought to be simple immune stimulants, have shown both immune enhancing and immune dampening effects. *Echinacea* extracts have demonstrated immune stimulatory activity in mice,[97] cattle,[98] and humans.[99] The alkylamides of *Echinacea* spp. have also shown increases in the proinflammatory tumor necrosis factor alpha in macrophages (TNF-α).[100] Conversely, recent studies demonstrate downregulation of the inflammatory cytokines by the alkylamides, including IL-1,[101] IL-2,[102] IL-12,[101] TNF-α,[101,103] as well as by an *Echinacea* extract (IL-1β, IL-8, TNF-α) consumed by adult humans.[104] With regard to the

Table 3. Aqueous extracts of medicinal plants demonstrating nitric oxide activity, *in vitro* models.

Genus species	Plant part	Preparation used	Active concentration	Exposure time	Cell type	Induced?	System affected	Direction of effect	Ref.
Astragalus membranaceous	Radix	Aqueous extract	20 µg/mL 2000 µg/mL	24 h	Murine macrophage	LPS+	NO iNOS	Decrease inhibition	[52]
Centella asiatica	Herba and radix	Aqueous extract	125 µg/mL	24 h	Murine macrophage	None LPS +	NO NO	Increase Increase	[53]
Crataegus pinnatifida	Fructus	Aqueous extract	10 µg/mL	Not specified	Murine macrophage	SNP	NO	Decrease	[54]
Dichroa febrifuga	Radix	Aqueous extract	83 µg/mL 250 µg/mL 150 µg/mL 250 µg/mL	22 h	Murine peritoneal macrophage	LPS LPS + IFN-γ	NO iNOS NO iNOS	Decrease inhibition Decrease inhibition	[43]
Euonymus alatus	Cortex and cork	Aqueous extract	100 µg/mL 1000 µg/mL	6 h 12 h	Mouse peritoneal macrophage	IFN-γ iNOS	NO	Increase induced	[55]
Ixeris dentata	Not specified	Fresh plant mash	1.0 µg/mL 100 µg/mL	6 h 12 h	Mouse peritoneal	rIFN-γ	NO	Increase	[56]
Kalopanax pictus	Ramulus cortex	H_2O extraction KPR-1 K-1	34.15 ug/mL 43.24 ug/mL	10 min	Murine macrophage	LPS	NO	Inhibition	[57]
Lactuca indica	Fresh plant	Hot water extract	50 µg/mL	6 h	Murine macrophage	LPS	iNOS	Inhibition	[58]
Lithospermum erythrorhizon	Radix	Aqueous 1:1	100 µg/mL 1000 µg/mL	48 h	Murine macrophage	rIFN-γ+ LPS+	NO iNOS	Decreased inhibition	[59]
Nigella sativa	Semen	Boiled aqueous extract 1:3	50 µL	2 h	Murine peritoneal macrophage	LPS	NO	Inhibition	[60]
Oldenlandia diffusa	Folia	Decoction	1000 µg/mL	6 h 12 h	Mouse peritoneal macrophage	rIFN-γ+	NO iNOS	Increased induction	[61]
Pueraria thunbergiana	Flos	Aqueous extraction	55.8 ug/mL	10 min	Murine macrophage	LPS	NO	Decrease	[62]
Rhus verniciflua	Lignum	Aqueous extraction	22 ug/mL	10 min	Murine macrophage	LPS	NO	Inhibition	[62]

KPR-1, aqueous extraction of Kalopanax pictus; K-1, aqueous extraction of Kalopanax pictus; LPS, lipopolysaccharide.

effect of Echinacea extracts on NO, Goel *et al.*[44] and Rininger *et al.*[81] report an increase in the production of NO from macrophages. In contrast, Stevenson *et al*. revealed an inhibitory effect on NO production from macrophages. Both Rininger and Stevenson's *in vitro* models were in the range of physiologically relevant serum concentrations and used lipopolysaccharide as a macrophage primer. Nonetheless, they show opposing activity. This may be due to one extract undergoing simulated digestion while the other represented only the lipophilic fraction of *E. purpurea radix*. These studies demonstrate the folly of relying only on *in vitro* results for understanding pharmacology where the context of pathophysiological condition is difficult to replicate. This may also be an example of various constituents having opposing activities in medicinal plant extracts.

Another example of a medicinal plant that influences the NO pathways is turmeric (*Curcuma longa*) which has been used historically as both a food spice and medicine throughout India and southeast Asia. Traditional applications of the plant include treatment of inflammation, skin wounds and tumors.[105] Clinical trials have investigated the efficacy of both turmeric extract and curcumin, an isolated mixture of the yellow/orange pigments known as curcuminoids that give turmeric its characteristic color. Curcumin is widely available on the market and used in irritable bowel syndrome, colorectal cancer, pancreatitis, osteoarthritis and peptic and gastric ulcers.[106] Research has demonstrated the anti-inflammatory,[107] antioxidant,[108] and anticarcinogenic[109] activity of curcumin. These effects are suggested to involve multiple pathways and modes of activity, such as inhibition

Table 4. Alcohol extracts of medicinal plants demonstrating nitric oxide activity; *in vitro* models.

Genus species	Plant part	Preparation used	Active concentration	Exposure time	Cell type	Induced?	NOS affected	Direction of effect	Ref.
Acorus calamus	Rhizome	Ethanol Extract	100 µg/mL	24 h	Murine macrophage	LPS None	NO NO	Decrease Decrease	[63]
Bidens pilosa	Herba	Ethanol Extract	100 ug/mL	24 h	Murine macrophage	LPS	NO	Inhibition	[64]
Catalpa ovata	Caulis cortex	Methanol Extract	100 ug/mL 100 ug/mL	24 h 4 h	Murine macrophage	LPS	NO iNOS	Decrease inhibition	[65]
Centella asiatica	Herba and radix	Ethanol extract	500 µg/mL 100 µg/mL	24 h	Murine LPS macrophage	NO	iNOS	Decrease inhibition	[53]
Chasalia chartacea	Herba and radix	Methanol extract	2.5 µg/mL	24 h	Murine macrophage	IFN-γ+LPS	NO	Decrease	[66]
Cupania vernalis	Folium	Ethanol extract	50 µg/mL	48 h	Murine Macrophage	LPS + IFN-γ	iNOS	Inhibition	[67]
Ganoderma lucidum	Fruiting body	Ethanol extract	100 ug/mL	24 h	Murine Macrophage	LPS + IFN-γ	NO	Decrease	[68]
Hedyotis verticillata	Herba and radix	Methanol extract	2.5 µg/mL	24 h	Murine macrophage	IFN-γ + LPS	NO	Decrease	[66]
Lasianthus oblongus	Herba and radix	Methanol extract	2.5 µg/mL	24 h	Murine macrophage	IFN-γ + LPS	NO	Decrease	[66]
Leea indica	Herba and radix	Methanol extract	2.5 µg/mL	24 h	Murine macrophage	IFN-γ + LPS	NO	Decrease	[66]
Litsea cubeba	Cortex	Methanol extract	10 ug/mL	24 h	Murine macrophage	LPS	iNOS	Inhibition	[69]
Mollugo verticillata	Herba	Ethanol extract	25 µg/mL	48 h	Murine macrophage	None BCG Ag	NO	Increases decreases	[48]
Propolis	-	Warm ethanol extract	30 µg/mL	24 h	Murine macrophage	LPS	NO iNOS	Decrease inhibition	[70]
Rhinacanthus nasutus	Caulis and folia	Ethanol extract	250 µg/mL	24 h	Murine macrophage	LPS	NO	Increase	[53]
Serjania lethalis	Ramulus lignum	Ethanol extract	50 µg/mL	48 h	Murine Macrophage	LPS + IFN-γ	iNOS	Inhibition	[67]
Spermacoce articularis	Herba and radix	Methanol extract	2.5 µg/mL	24 h	Murine macrophage	IFN-γ + LPS	NO	Decrease	[66]
Taraxacum officinale	Flos	Ethanol extraction	130 ug/mL	24 h	Murine macrophage	LPS	NO	Inhibition	[71]
Taxus yunnanensis	Lignum	H_2O/methanol extract	40.3 ug/mL	24 h	Murine macrophage	LPS	iNOS	Inhibition	[72]
Taxus yunnanensis	Lignum	Methanol extract	34.2 ug/mL	24 h	Murine macrophage	LPS	iNOS	Inhibition	[72]
Thymus pulegioides	Folium	Hot ethanol extract	1 uM GAE	16 h	Porcine aortic endothelial cells	A23187	eNOS	Stimulation	[73]
Ulmus davidiana	Ramulus cortex	Hot methanol extract	100 ug/mL	48 h	Murine macrophage	LPS + IFN-γ	NO	Decrease	[74]

BCG Ag, Bacille Calmette Guérin Antigen from Mycobacterium bovis; GAE, Gallic Acid Equivalents; LPS, lipopolysaccharide.

Table 5. Other extracts of medicinal plants demonstrating nitric oxide activity; *in vitro* models.

Genus species	Plant part	Preparation used	Active concentration	Exposure time	Cell type	Induced?	NOS affected	Direction of effect	Ref.
Abies koreana	Ramulus cortex	MeOH, ethyl ether	40 ug/mL	20 h	Murine macrophage	LPS	iNOS	Inhibition	[75]
Artemisia iwayomogi	Ramulus cortex	MeOH, ethyl ether	40 ug/mL	20 h	Murine macrophage	LPS	iNOS	Inhibition	[75]
Ananus comosus	Herba	Bromelain	15 ug/mL	24 h	Murine macrophage	IFN-γ	NO	Increase	[76]
Ananus comosus	Herba	Bromelain	50 ug/mL	24 h	Murine bone marrow macrophage	None IFN-γ	NO NO	Increase Increase	[76]
Bidens pilosa	Herba	Ethyl acetate	36.2 ug/mL	24 h	Murine macrophage	LPS	NO	Inhibition	[64]
Brassica spp.	Isolate	Indole-3-carbinol	0.001 uM	24 h	Murine macrophage	LPS + IFN-γ	NO iNOS	Decrease Inhibition	[77]
Broussonetia kazinoki	Ramulus cortex ethyl ether	MeOH,	20 ug/mL	20 h	Murine macrophage	LPS	iNOS	Inhibition	[75]
Casearia Sylvestris	Ramulus cortex	Hexane	50 µg/mL	48 h	Murine macrophage	LPS IFN-γ	iNOS	Inhibition	[67]
Chicorium intybus	Radix	Inulin isolate	10 ug/mL 1000 ug/mL	24 h 24 h	Murine macrophage	LPS IFN-γ	NO iNOS	Increase Induction	[78]
Cocos nucifera	Husk fiber	Ethyl acetate	10 µg/mL	1.9 h	Murine peritoneal macrophage	Leishmania infection	NO	Increased	[79]
Curcuma zedoaria	Rhizome	Curcumin	20 uM	18 h	Murine macrophage	LPS	iNOS	Inhibition	[80]

Table 6. Other extracts of medicinal plants demonstrating nitric oxide activity; *in vitro* models.

Genus species	Plant part	Preparation used	Active concentration	Exposure time	Cell type	Induced?	NOS affected	Direction of effect	Ref.
Echinaceapurpurea	Herba and radix	Simulated digestion	5 ug/mL	24 h	Murine macrophage	LPS	NO	Increase	[81]
E.purpurea & E. angustifolia	Radix	Lipophilic extract	2.0 ug/mL	21 h	Murine macrophage	LPS	NO	Decrease	[82]
Ginkgo biloba	Folia	Standardized	100 µg/mL 100 ug/mL	4 h	Human macrophage	PMA	NO iNOS	Decrease Inhibition	[83]
Idesia polycarpa	Ramulus cortex	MeOH, ethyl ether	40 ug/mL	20 h	Murine macrophage	LPS	iNOS	Inhibition	[75]
Machilus thunbergii	Ramulus cortex	MeOH, ethyl ether	40 ug/mL	20 h	Murine macrophage	LPS	iNOS	Inhibition	[75]
Morus bombycis	Ramulus cortex	MeOH, ethyl ether	40 ug/mL	20 h	Murine macrophage	LPS	iNOS	Inhibition	[75]
Kalopanax pictus	Ramulus cortex	Chloroform, ethyl acetate	9.5 ug/mL	10 min	Murine macrophage	LPS	NO	Inhibition	[56]
Phellinus linteus	Fruiting body	Polysaccharide precipitate	10 µg/mL	48 h	Murine peritoneal macrophage	None	NO	Increase	[84]
Populus davidiana	Ramulus cortex	MeOH, ethyl ether	20 ug/mL	20 h	Murine macrophage	LPS	iNOS	Inhibition	[75]
Populus maximowiczii	Ramulus cortex	MeOH, ethyl ether	20 ug/mL	20 h	Murine macrophage	LPS	iNOS	Inhibition	[75]
Pueraria thunbergiana	Flos	Chloroform, ethyl acetate	26.1 ug/mL	10 min	Murine macrophage	LPS	NO	Inhibition	[62]
Rhus verniciflua	Lignum	Chloroform ethyl acetate	16.7 ug/mL	10 min	Murine macrophage	LPS	NO	Inhibition	[62]

Ginkgo Standardized-24% flavonoid glycosides, 6% terpenoids; LPS, lipopolysaccharide; PMA, phorbol myristate acetate.

of IL-8 production and signal transmission,[109] inhibition of TNF-α,[107] inhibition of the transcription factor NFκB,[110] and the scavenging effects and induction of antioxidant enzymes.[111] Of interest, the NO pathways link turmeric to all three activities: anti-inflammatory, antioxidant and anticarcinogenic activity.

Since nitric oxide is found to be elevated when inflammation is present, the ability of turmeric to inhibit nitric oxide is a possible partial explanation for its anti-inflammatory properties. In the course of screening Asian anti-inflammatory herbs for inhibitory activity of nitric oxide synthesis, a crude extract of *Curcuma zedoaria* showed significant inhibitory activity as compared to other herbs. The crude methanolic extract of *Curcuma zedoaria* was found to inhibit the synthesis of nitric oxide by 78% *in vitro*.[80]

Nitric oxide inhibition may also be a causative factor in the anticarcinogenic activity of turmeric and curcumin. iNOS is overexpressed in colonic tumors of humans and also in murine models. In an animal study on colon tumor development, curcumin was found to inhibit colonic aberrant crypt foci formation by 45% ($P<0.001$), an indicator of its anticarcinogenic and iNOS inhibitory activity.[112]

There is evidence to suggest that the antioxidant activity of turmeric and curcumin are also linked to nitric oxide modulation. The effects of curcumin on the nitric oxide pathway in cardiac tissue and cultured cells were studied on rats with induced diabetes. One month of induced diabetes caused an upregulation of both eNOS and iNOS mRNA levels in the heart. Treatment of the diabetic rats with curcumin reduced eNOS and iNOS levels in association with reduced oxidative DNA and protein damage.[113]

Many medicinal plants, which influence the NOS system also contain reasonable concentrations of folate.[114] Serum homocysteine concentrations, which are reduced by folate, are suspected to cause the accumulation of asymmetrical dimethylarginine (ADMA). ADMA impairs NO synthesis and therefore accelerates atherosclerosis.[115] Hence the combination of NOS modulating constituents in the reviewed plants and the nutrient folate may additively or synergically act against atherosclerosis.

Besides secondary metabolites, primary metabolites of plants have also shown an effect on NO. For example, α-linolenic acid has been shown to lower the release of eicosanoids and NO from human aortic endothelial cells.[116] Arginine, another primary metabolite of plants and the substrate of NOS, yields NO as a product. Clinical trials on patients with coronary or peripheral arterial disease have demonstrated increases in walking distance, exercise capacity, and quality of life, with a decrease in symptoms when supplemented with arginine.[31,32,34] This suggests that arginine rich foods, which generally come from plant-based proteins, may have positive NO activity resulting in beneficial cardiovascular effects.[117,118] This provides yet another pathway, in addition to folate and secondary metabolites, by which medicinal plants may have an influence on vascular dynamics. NO signaling represents a fundamental process in the body that appears to be modulated by a large number of plants. While this is pharmacologically intriguing, it frames a larger question about the role of phytochemistry in mammalian physiology. Considered from an evolutionary time scale, NO activity appears to have been modulated by plant compounds for millions of years. While the study of NO modulation by phytochemicals is enticing and deserves further investigation, future research should also consider the effects on physiological processes when NO signaling is not routinely modulated due to decreased phytochemical intake.[119] This may offer further insights into chronic disease processes.

Conclusions

The reviewed data demonstrate that many medicinal plants have effects on the nitric oxide pathways. Considering the observations made by traditional healers, modern phytotherapists, and laboratory researchers regarding the reviewed plants, the beneficial activity of these medicinal plants may be partially due to effects on the nitric oxide pathways. Many of these reviewed medicinal plant species may be appropriate in nitric oxide based conditions. Further studies are needed to clarify the clinical therapeutics of these chemically complex remedies.

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