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ANTI-MYCOBACTERIAL SCREENING OF BRITISH COLUMBIAN MEDICINAL PLANTS

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ABSTRACT

One hundred methanol plant extracts were screened for antibiotic activity against Mycobacterium tuberculosis *and* Mycobacterium avium. *Nineteen extracts exhibited some activity against* M. tuberculosis *and 16 extracts showed some activity against* M. avium. *Thirteen of these 19 active extracts were traditionally used by First Nations peoples to treat tuberculosis. Extracts made from* Heracleum maximum *(Umbelliferae) roots,* Moneses uniflora *(Ericaceae), aerial parts, and* Oplopanax horridus *(Araliaceae) inner bark completely inhibited the growth of both organisms at a concentration equivalent to 20 mg dried plant material/disc. Extracts of* Alnus rubra *(Betulaceae) bark and catkins,* Empetrum nigrum *(Empetraceae) branches,* Glehnia littoralis *(Umbelliferae) roots, and* Lomatium dissectum *(Umbelliferae) roots completely inhibited the growth of both* M. tuberculosis *and* M. avium *at a concentration equivalent to 100 mg dried plant material/disc.*

Keywords: Anti-mycobacterial activity, Ethnopharmacology (British Columbia), *Mycobacterium tuberculosis*, *M. avium*, MAC, Pharmacological activity (antibiotic), Traditional medicines (British Columbia), Tuberculosis drugs.

List of the binomials: Alnus rubra Bong. (Betulaceae), *Empetrum nigrum* L. (Empetraceae), *Glehnia littoralis* F. Schmidt (Umbelliferae), *Heracleum maximum* Bartr. (Umbelliferae), *Lomatium dissectum* (Nutt.) Math. et Const. (Umbelliferae), *Moneses uniflora* (L.) Gray (Ericaceae), and *Oplopanax horridus* Miq. (Araliaceae)

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INTRODUCTION

While tuberculosis is still the leading infectious killer of adults in the world, with approximately 3 million people dying each year, 95% of these deaths occur in the developing world (Sudre *et al.*, 1992). In the developed world, tuberculosis is commonly considered to be a disease of the past. Along with the general public, many medical practitioners have come to consider tuberculosis as a disease that no longer poses a serious public health problem. Consequently, the scientific community has been fairly slow to respond to the growing evidence that the incidence of tuberculosis in North America and Europe is increasing. Some epidemiologists have warned that the emergence of AIDS and multiple-drug resistant tuberculosis has the potential to precipitate the most disastrous public health crises since the bubonic plague (Stanford *et al.*, 1991). In addition to *Mycobacterium tuberculosis*, two other species, *M. avium* and *M. intracellulare*, commonly cause human disease, especially in immunocompromised hosts. Infections with

these species are often very hard to treat, requiring multi-drug therapy for prolonged periods (Inderleid *et al.*, 1993). These two species (commonly called the *M. avium* complex or MAC) have emerged as important pathogens of man because of the increased incidence associated with AIDS and their natural resistance to the common anti-mycobacterial drugs.

It is clear that public health measures alone cannot contain the threat of infection due to *M. tuberculosis* and MAC. Potent new anti-mycobacterial drugs are desperately needed not only for AIDS patients but also for the health care workers and members of the general public who are affected by these bacterial infections.

In Canada, the incidence of tuberculosis is significantly higher (10 times) among the aboriginal population than in the general public (Young and Casson, 1988). Many people have assumed that this was due to the fact that tuberculosis was newly introduced into the native population by European settlers. However, there is strong archaeological evidence that tuberculosis was present in Pre-Columbian America (Bulkstra and Cook, 1981; Pfeiffer, 1984; Clark *et al.*, 1987) and, therefore, it must be assumed that the North American aboriginal peoples have an equally long history of seeking out a cure for this disease. Although there are hundreds of references in the ethnobotanical literature (see Moerman, 1986) on the tuberculosis treatments that First Nations peoples had developed, few of these plant medicines have ever been assayed for anti-mycobacterial activity. Given the pressing need for new anti-mycobacterial drugs, it was deemed worthwhile to examine the potential of these traditional remedies as modern therapeutics.

METHODS

Plant Collection

A sampling of the British Columbian ethnobotanical literature (Turner, 1975, 1978; Turner *et al.*, 1980, 1990) was surveyed to compile a representative list of those plants used medicinally by the native peoples of this province. The list was used in the field as a selection guide for the plant species and type of material to be collected. The collecting was carried out from May–July 1991, in five areas of the province: the Wyndel region in the Kootenay mountains, the Princeton-Penticton region in the interior, the Queen Charlotte Islands, the Fraser River canyon, and the Lower Mainland. From the several hundred plant species on the ethnobotanical list, 100 specimens were collected. In order to ensure accurate botanical identifications, only plants which were in flower were collected, introducing a seasonal bias into the selection. A voucher specimen was made for each collection and these vouchers have been filed in the University of British Columbia Herbarium.

Extract Preparation

The plant material was air-dried and ground in a Wiley grinder with a 2 mm diameter mesh. Twenty g of the ground material was extracted with (3 × 100 ml) methanol, over 24 h. The crude methanolic extract was filtered through cheesecloth and cotton wool, and then through a Buchner funnel with a No. 4 paper filter. The filtrate was evaporated to dryness and reconstituted with 10 ml of methanol.

Microorganisms

M. tuberculosis (strain Erdman, Trudeau Mycobacterial Collection [TMC] # 107; American Type Culture Collection [ATCC] # 35801) and *M. avium* (TMC # 724; ATCC # 25291) were grown, stored and assessed for viability as previously described (Stokes *et al.*, 1993).

Assay Protocol

A standard drug sensitivity testing method for mycobacteria was employed (Sommers and Good, 1985). Ten µl of plant extract (representing 20 mg of dried plant material) was applied to a 0.25 inch diameter blank paper disc (Bectin Dickinson, Cockeysville, MD) and allowed to air dry. Discs were placed in quadrant plates (Becton Dickinson) and 5 ml of molten (56°C) Middlebrook 7H10 agar + oleic acid, dextrose complex (Becton Dickinson) was plated onto each quadrant. After setting, plates were incubated overnight at 4°C to allow for diffusion of the compounds. Control discs were loaded with 10 µl methanol or 10 µl of 10 mg/ml isoniazid (one of the first choice drugs for treatment of *M. tuberculosis*). To each quadrant, 100 µl of bacterial suspension was added which contained approximately 1.5×10^6 *M. tuberculosis* or 2×10^3 *M. avium*. Preliminary studies had shown that these concentrations allowed for the identification of active extracts. Plates were incubated for up to 3 weeks in sealed bags at 37°C after which bacterial growth was assessed. To confirm the activity of those extracts which showed only slight inhibition, the assay was repeated using 50 µl/disc (the equivalent of 100 mg of dried plant material).

An arbitrary scale was used to score the anti-mycobacterial activity of each extract (Sommers and Good, 1985). Extracts scored as "−" had no discernible

effect on the bacterial growth. Extracts scored as "+" caused a small zone of clearing or a zone of inhibition with a few resistant colonies within, though colonies were too numerous to count. Extracts scored as "++" greatly inhibited the growth of the mycobacteria, to the extent that less than 50 colonies were present. Extracts scored as "+++" completely inhibited all growth. Each quadrant plate had three test quadrants with plant extracts and one control quadrant with methanol alone. Growth in the test quadrants was compared to that in the control quadrant which was always unaffected by the methanol (scored as "−").

RESULTS

The botanical names of the plants screened are listed alphabetically by genus in Table 1. The assay results for the active extracts are given in Table 2. Nineteen of the 100 methanolic plant extracts screened exhibited some antibiotic activity against *M. tuberculosis* and 16 of the extracts were active against *M. avium*. Thirteen of these 19 active extracts were traditionally used to treat tuberculosis and another four were used in the treatment of coughs.

As expected, isoniazid (positive control) at 10 µg/quadrant completely inhibited growth of *M. tuberculosis* and had no activity against *M. avium*. Methanol alone did not inhibit the growth of *M. tuberculosis* or *M. avium*.

The extracts of *Heracleum maximum* Bartr. (Umbelliferae) roots, *Moneses uniflora* (L.) Gray (Ericaceae) aerial parts and *Oplopanax horridus* Miq. (Araliaceae) inner bark completely inhibited the growth of both *M. tuberculosis* and *M. avium* at a concentration equivalent to 20 mg of dried plant material (10 µl extract/disc). The extracts of *Alnus rubra* Bong. (Betulaceae) bark and catkins, *Empetrum nigrum* L. (Empetraceae) branches, *Glehnia littoralis* F. Schmidt (Umbelliferae) roots and *Lomatium dissectum* (Nutt.) Math. et Const. (Umbellif-

Table 1. Botanical names of species screened[a].

Achillea millefolium L. ssp. *lanulosa* (Nutt.) Piper var. *lanulosa* (Compositae) P-10
Alnus rubra Bong. (Betulaceae) Q-1 Bark, Q-2 Catkins
Ambrosia chamissonis (Less.) Greene var. *chamissonis* (Compositae) Q-29
Amelanchier alnifolia Nutt. var. *humptulipensis* (Jones) Hitchc. (Rosaceae) P-6, P-15
Antennaria microphylla Rydb. (Compositae) P-21
Arctostaphylos uva ursi (L.) Spreng. (Ericaceae) P-42a Branches, P-42b Roots
Argentina egedii (Wormsk.) Rydb. (Rosaceae) Q-20
Arnica cordifolia Hook. (Compositae) P-31
Arnica sororia Greene (Compositae) P-7
Artemisia ludoviciana Nutt. ssp. *latiloba* Nutt. (Compositae) W-5
Artemisia michauxiana Bess. (Compositae) P-29
Artemisia tridentata Nutt. ssp. *tridentata* (Compositae) W-19
Aruncus dioicus (Walt.) Fern. var. *vulgaris* (Maxim) Hara (Rosaceae) F-1
Asarum caudatum Lindl. (Aristolochiaceae) W-12

Balsamorhiza sagittata (Pursh) Nutt. (Compositae) W-18 Aerial, P-2 Roots
Betula papyrifera Marsh (Betulaceae) P-38

Capsella bursa-pastoris Medic. (Cruciferae) W-8
Cardamine angulata Hook. (Cruciferae) Q-16
Ceanothus velutinus Dougl. (Rhamnaceae) P-39
Chaenactis douglassi (Hook.) H. et A. (Compositae) P-3
Chrysothamnus nauseosus (Pall.) Britt. var. *albicaulis* (Nutt.) Rydb. (Compositae) P-25
Clematis ligusticifolia Nutt. in T. et G. (Ranunculaceae) P-14
Conocephalum conicum (L.) Dum. (Conocephalaceae) [Hepaticae] Q-28
Cornus canadensis L. (Cornaceae) Q-12
Cornus sericea L. ssp. *sericea* (Cornaceae) W-6
Crataegus douglasii Lindl. (Rosaceae) W-14

Delphinium nutallianum Pritz. var. *nutallianum* (Ranunculaceae) P-33
Disporum trachycarpum (Wats.) Benth. et Hook. (Liliaceae) W-11

Empetrum nigrum L. (Empetraceae) Q-17
Epilobium minutum Lindl. (Onagraceae) P-1
Equisetum arvense L. (Equisetaceae) W-3
Equisetum hyemale L. (Equisetaceae) P-37
Erigeron filifolia Nutt. (Compositae) P-9
Eriogonum heracleoides Nutt. (Polygonaceae) P-11 Aerial, P-17 Roots

Table continues

(Table 1. *cont.*)

Fauria crista-galli (Menzies ex Hook.) Makino (Menyanthaceae) Q-19
Fragaria chiloensis (L.) Duchesne (Rosaceae) Q-7
Fragaria vesca L. var. *bracteata* (Heller) Davis (Rosaceae) W-1

Gaillardia aristata Pursh (Compositae) P-8
Ganoderma applanatum L. (Polyporaceae) Q-10
Geum macrophyllum Willd. var. *macrophyllum* (Rosaceae) Q-23
Glehnia littoralis F. Schmidt ssp. *leiocarpa* (Mathias) Hult. (Umbelliferae) Q-13

Heracleum maximum Bartr. (Umbelliferae) P-32a Aerial, P-32b Roots
Heuchera cylindrica Dougl. var. *cylindrica* (Saxifraceae) W-4
Holodiscus discolor (Pursh) Maxim (Rosaceae) F-3
Hylocomium splendens (Hedw.) B.S.G. (Hylocomiaceae) [Bryidae] Q-9
Hypericum perforatum L. (Hypericaceae) P-30

Ipomopsis aggregata (Pursh) Grant var. *aggregata* (Polemoniaceae) P-13a Aerial, P-13b Roots

Juniperus communis L. (Cupressaceae) Q-25

Kalmia microphylla (Hook.) Heller ssp. *occidentalis* (Small) Taylor et MacBryde (Ericaceae) Q-5

Larix occidentalis Nutt. (Pinaceae) W-15
Ledum groenlandicum Oeder (Ericaceae) Q-4
Lobaria oregana (Tuck) Mull. (Lobariaceae) Q-11
Lomatium dissectum (Nutt.) Math. et Const. var. *multifidum* (Nutt.) Math. et Const. (Umbelliferae) W-10
Lonicera ciliosa (Pursh) DC. (Caprifoliaceae) F-2
Lonicera involucrata (Rich.) Banks (Caprifoliaceae) F-5
Lupinus sericeus Pursh var. *sericeus* (Leguminosae) P-12
Lycopodium clavatum L. (Lycopodiaceae) Q-6
Lysichiton americanum Hulten et St. John (Araceae) Q-26

Mahonia aquifolium (Pursh) Nutt. (Berberidaceae) W-2
Maianthemum racemosum (L.) Link (Liliaceae) W-17
Maianthemum stellatum (L.) Link (Liliaceae) W-13
Moneses uniflora (L.) A. Gray (Ericaceae) Q-8
Monotropa uniflora L. (Ericaceae) P-19

Nuphar lutea (L.) Sm. ssp. *polysepala* (Engelm.) E.O. Beal (Nymphaceae) Q-3b Roots, Q-3c Rhizomes

Oplopanax horridus (Smith) Miq. (Araliaceae) Q-14
Opuntia fragilis (Nutt.) Haw. (Cactaceae) F-4
Osmorhiza purpurea (Coult. et Rose) Suksd. (Umbelliferae) Q-24

Penstemon fruticosus (Pursh) Greene (Scrophulariaceae) P-4
Philadelphus lewisii Pursh (Hydrangeaceae) P-22
Pinus contorta Dougl. var. *contorta* (Pinaceae) Q-18
Pinus ponderosa Dougl. (Pinaceae) W-20
Plantago major L. (Plantaginaceae) Q-22
Polypodium glycyrrhiza DC. Eaton (Polypodiaceae) Q-27
Polystichum munitum (Kaulf.) Presl (Polypodiaceae) Q-15
Populus tremuloides Michx. (Salicaceae) P-34
Potentilla arguta Pursh (Rosaceae) W-7
Prunus virginiana L. (Rosaceae) W-9, P-40

Rhus glabra L. (Anacardiaceae) P-23
Ribes sanguineum Pursh (Grossulariaceae) P-18
Rosa nutkana Presl var. *nutkana* (Rosaceae) P-5
Rubus parviflorus Nutt. (Rosaceae) P-28

Salix bebbiana Sarg. (Salicaceae) P-36
Sambucus caerulea Raf. (Caprifoliaceae) P-16
Sambucus racemosa L. ssp. *pubens* (Michx.) House var. *arborescens* (Caprifoliaceae) Q-21
Sedum lanceolatum Torr. (Crassulaceae) P-20
Shepherdia canadensis (L.) Nutt. (Elaeagnaceae) W-16
Spiraea betulifolia Pall. (Rosaceae) P-41
Spiraea pyramidata Greene (Rosaceae) P-35
Symphoricarpos albus (L.) Blake var. *laevigatus* Fern. (Caprifoliaceae) P-26

Urtica dioica L. ssp. *gracilis* (Ait.) Seland. var. *lyallii* (Wats.) Hitchc. (Urticaceae) P-27

Verbascum thapsus L. (Scrophulariaceae) P-24

[a] Key to collection site: F = Fraser River Canyon; P = Princeton-Penticton region; Q = Queen Charlotte Islands; W = Wyndel region. Collectors for all species were A.R. McCutcheon and S.M. Ellis. Voucher specimens filed in the University of British Columbia Herbarium.

Table 2. Anti-mycobacterial screening results.

Test Material	Usage[a]	*M. tuberculosis*[b]		*M. avium*[b]	
		20 mg	100 mg	20 mg	100 mg
Positive control (Isoniazid)		+++	+++	−	−
Alnus rubra (Betulaceae) Q-1 Bark	T	+	+++	−	+++
Alnus rubra (Betulaceae) Q-2 Catkins	T	+	+++	−	+++
Balsamorhiza sagittata (Compositae) P-2	T	+	++	−	−
Chaenactis douglasii (Compositae) P-3	T	+	+++	−	+
Empetrum nigrum (Empetraceae) Q-17	T	++	+++	+	+++
Fragaria vesca (Rosaceae) W-1	T	−	+	−	−
Geum macrophyllum (Rosaceae) Q-23	P	+	+++	−	−
Glehnia littoralis (Umbelliferae) Q-13	M	++	+++	++	+++
Heracleum maximum (Umbelliferae) P-32b	T	+++	+++	+++	+++
Hypericum perforatum (Hypericaceae) P-30	C	−	+	++	++
Juniperus communis (Cupressaceae) Q-25	T	+	++	−	+
Lomatium dissectum (Umbelliferae) W-10	T	++	+++	++	+++
Moneses uniflora (Ericaceae) Q-8	C	+++	+++	+++	+++
Nuphar lutea (Nymphaceae) Q-3c	T	−	+++	+	+
Oplopanax horridus (Araliaceae) Q-14	T	+++	+++	+++	+++
Pinus contorta (Pinaceae) Q-18	T	+	+++	−	+
Polystichum munitum (Polypodiaceae) Q-15	C	−	+	+	+
Populus tremuloides (Salicaceae) P-34	T	+	+++	−	+
Rosa nutkana (Rosaceae) P-5	C	+	+	−	+
Total number active		15	19	9	16

[a] Traditional usage: C = coughs; M = unspecified medicinal plant; P = physic; T = tuberculosis medicine.
[b] Key to scoring: −, no inhibition; +, zone of inhibition with a few resistant colonies within it or small zone of clearing (colonies too numerous to count); ++, large zone of clearing or greatly inhibited growth (less than 50 colonies present) ; +++, complete inhibition.

erae) roots completely inhibited the growth of both test organisms at a concentration equivalent to 100 mg dried plant material (50 µl extract/disc). Three extracts exhibited a slight inhibitory effect on *M. tuberculosis* but did not effect the growth of *M. avium.* These three extracts were made from *Balsamorhiza sagittata* (Pursh) Nutt. (Compositae) aerial parts, *Fragaria vesca* L. (Rosaceae) leaves and *Geum macrophyllum* Willd. (Rosaceae) roots.

DISCUSSION AND CONCLUSIONS

The infamy of tuberculosis is due not only to the fact that it is the greatest killer in human history, responsible for over a billion deaths in the last two centuries alone (Ryan, 1992) but also because each death was preceded by a prolonged, painful decline in health. Tuberculosis is contracted simply by inhaling the airborne bacteria, making every human, regardless of race, sexual preference or economic status, susceptible to the disease. In the majority of people that come in contact with the bacteria, the immune system is able to successfully contain the organism and disease symptoms do not develop. However, in some individuals pathogenic mycobacteria are able to survive the killing mechanisms of the immune system, enabling them to persist and multiply within the host's macrophages. Unable to eradicate the bacteria, the immune system walls off the bacteria within a granuloma to contain the infection. The bacteria remain as a latent threat within these tubercules, capable of reactivating if the immune system of the host is compromised in any way.

It has been estimated that there are approximately 1.7 billion people or roughly one third of the world's population infected with tuberculosis (Sudre *et al.*, 1992). In the normal host, the lifetime risk of developing tuberculosis following infection is 10%; 5% in the first one or two years after infection and 5% throughout the rest of the life of the individual. An estimated 2.9 million people died from tuberculosis in 1990, making this disease the largest cause of death from a single pathogen in the world (Murray, 1991).

The HIV virus destroys the white blood cells (CD4 lymphocytes) which are essential in enabling most people to fight off mycobacterial infections. Forty percent of all HIV cases are co-infected with *M. tuberculosis*, making tuberculosis the number one opportunistic infection

of AIDS patients worldwide. In addition, as a result of AIDS, patients are more susceptible to infection with the *M. avium* complex (MAC), the development of which leads to early morbidity and mortality in AIDS patients.

Another reason for the resurgence of mycobacterial infections in the developed world is thought to be the increasing incidence of multiple-drug resistant strains of *M. tuberculosis* and *M. avium*. The innate capacity of *Mycobacterium* to develop resistance to a drug was observed in trials of the first antibiotics against tuberculosis. Resistance was also observed to emerge when only two drugs are used. Therefore the standard treatment for tuberculosis became a combination of drugs, typically isoniazid, rifampin and pyrazinamide. During the 1980's, the number of reports of multiple-drug resistant (MDR) tuberculosis began to increase. More alarmingly, many of these MDR strains were resistant not only to the first line antibiotics but also many of the secondary drugs, some strains being resistant to seven of the most effective tuberculosis drugs available and as virulent as the wild type strains (Iseman and Madsen, 1985). The increasing incidence of MDR strains worldwide signifies the desperate need for new anti-mycobacterial drugs.

The search for tuberculosis therapeutics, however, is both far more difficult and dangerous than most antibiotic development programs. The extreme virulence of these airborne pathogens necessitates extraordinary containment facilities and specially trained personnel in order to safely conduct research in this area. Other genera of bacteria cannot reliably substitute for anti-mycobacterial screenings as the unusual waxy coat that makes *Mycobacterium* impervious to the killing mechanisms of white blood cells is also an impenetrable barrier to many antibiotics. In addition, some of the compounds with anti-mycobacterial activity have no effect against other bacterial species. Therefore, leads towards the discovery of new drugs and findings which may improve the efficacy of the search are both of value.

In both of these contexts, the results of this antibiotic screening of 100 methanol plant extracts against *M. tuberculosis* and *M. avium* appear promising. Nineteen extracts showed activity against *M. tuberculosis* and 16 extracts were active against *M. avium*. Of these active extracts, six were particularly outstanding in their ability to completely inhibit the growth of both organisms: *Empetrum nigrum, Glehnia littoralis, Heracleum maximum, Lomatium dissectum, Moneses uniflora* and *Oplopanax horridus*. Chemical isolation work to identify the active constituents is currently in progress.

It is noteworthy that three of the extracts with the greatest activity against the mycobacteria are members of the same plant family, the Umbelliferae: *G. littoralis*, *L. dissectum* and *H. maximum*. A pair of unstable tetronic acids were identified as the antimicrobial constituents of *L. dissectum* (Cardellina and Vanwagenen, 1985; Vanwagenen and Cardellina, 1986), but it is not yet known if these compounds are also responsible for this plant extract's anti-mycobacterial activity. The Umbelliferae family is well-known for its cytotoxic furano-coumarin constituents and these compounds may be responsible for the anti- mycobacterial activity observed in these family members.

Can the traditional usage of a plant to treat tuberculosis also be used as an effective selection criterion for anti-mycobacterial screenings? In this study, a comparison of the ethnobotanical literature and the screening results shows that 13 of the 19 active extracts (68%) were prepared from plant species which were specifically reported to have been used for the treatment of tuberculosis. These active tuberculosis remedies are indicated with a letter "T" in Table 2. There were no reports that the extracts of the six other plant species which exhibited anti-mycobacterial activity were used specifically to treat tuberculosis, however, four of these plants were reported to have been used to treat coughs (these extracts are indicated by a letter "C" in Table 2).

Of the 100 extracts screened, a total of 37 samples were prepared from plant species which were reported to have been used to treat tuberculosis or consumption (see McCutcheon, 1996 for summaries of the ethnobotanical literature). Analysed in this context, it may be concluded that roughly one-third of traditional tuberculosis remedies assayed did exhibit *in vitro* anti-mycobacterial activity. These results suggest that there may be a positive correlation between traditional usage in the treatment of tuberculosis and anti-mycobacterial activity. Although *in vitro* activity is not necessarily indicative of *in vivo* activity, these preliminary findings are very encouraging. It seems reasonable to suggest that some of these traditional tuberculosis remedies, representing the cumulative knowledge from hundreds of years of experience, may indeed have been efficacious. Research is now being conducted to determine whether similar results are obtained in a cellular assay system.

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