



# OPEN Baseline data collections of lipopolysaccharide content in 414 herbal extracts and its role in innate immune activation

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Some herbal extracts contain relatively high amounts of lipopolysaccharide (LPS). Because orally administered LPS activates innate immunity without inducing inflammation, it plays a role as an active ingredient in herbal extracts. However, the LPS content in herbal extracts remains extensively unevaluated. This study aimed to create a database of LPS content in herbal extracts; therefore, the LPS content of 414 herbal extracts was measured and the macrophage activation potential was evaluated. The LPS content of these hot water extracts was determined using the kinetic-turbidimetric method. The LPS concentration ranged from a few ng/g to hundreds of µg/g (Standard *Escherichia coli* LPS equivalent). Twelve samples had a high-LPS-content of > 100 µg/g, including seven samples from roots and three samples from leaves of the herbal extracts. These samples showed high phagocytosis and NO production capacity, and further investigation using polymyxin B, an LPS inhibitor, significantly inhibited macrophage activation. This study suggests that some herbal extracts contain sufficient LPS concentration to activate innate immunity. Therefore, a new approach to evaluate the efficacy of herbal extracts based on their LPS content was proposed. A database listing the LPS content of different herbal extracts is essential for this approach.

**Keywords** Lipopolysaccharide, Herbal extracts, Macrophage activation, Database

Lipopolysaccharide (LPS) is a lipid and polysaccharide molecule found in the outer membrane of gram-negative bacteria<sup>1,2</sup>. LPS has long been considered an endotoxin owing to its wide use as a potent inflammation inducer because it binds to Toll-like receptor (TLR4)<sup>3–6</sup> of immune cells and activates nuclear factor-kappa beta (NFκB)<sup>7–9</sup> to cause inflammatory cytokines, including interleukin-1 beta (IL-1β)<sup>10–12</sup>, interleukin-6 (IL-6)<sup>13</sup>, and tumor necrosis factor alpha (TNFα)<sup>14,15</sup>, inducing severe fever, diarrhea, and shock when intravenously injected<sup>16–20</sup>. Furthermore, although oral administration of LPS does not induce inflammation in healthy subjects, it has been observed that disrupted barrier system and bacterial translation may occur in diseases with persistent inflammatory lesions in the intestinal tract and periodontal tissues. Experimental models in which persistent bacterial and LPS invasion in vivo induces systemic inflammation suggest the involvement of LPS in chronic inflammatory diseases, including lifestyle-related diseases<sup>21</sup>.

However, gram-negative bacteria with LPS are found in large amounts in the human intestinal tract<sup>22</sup>, skin<sup>23,24</sup>, and other organs in contact with the outside world without causing any inflammatory effects under healthy conditions<sup>25</sup>. The decreased number of these gram-negative bacteria in the intestinal tract resulting from the use of antibiotics causes a decrease in the amount of antimicrobial peptides<sup>26,27</sup>, making individuals more susceptible to infections<sup>28,29</sup>. Thus, LPS in the intestinal tract and skin has been suggested to play a beneficial role in maintaining health. Furthermore, the lack of exposure to LPS is associated with susceptibility to allergic and infectious diseases<sup>30,31</sup>. This shows that LPS have unknowingly been taken orally and transdermally to maintain our health.

In a previous study, it was revealed that LPS is present in many plants, including herbal extracts<sup>32</sup>. It also known that several LPSs are present in rice and wheat, which are staple foods, and that their ingestion confers

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functional properties. Additionally, *Pantoea agglomerans* was isolated as the dominant LPS symbiont in wheat<sup>33</sup>. Oral consumption of *Pantoea agglomerans* LPS (LPSp) enhanced phagocytosis of abdominal macrophages in mice, but this effect was not observed in TLR4-deficient mice<sup>34</sup>. This indicates that orally administered LPS promotes foreign body removal via innate immunity using TLR4. Furthermore, in disease prevention and treatment experiments, oral LPSp administration was found to enhance the effect of anticancer drugs<sup>35</sup>, promote the treatment of lung metastases<sup>36</sup>, inhibit itching in atopic dermatitis<sup>25</sup>, prevent atherosclerosis in apolipoprotein-E (ApoE)-deficient mice<sup>37</sup>, prevent dementia in brain diabetes-induced mice<sup>38</sup> etc. Additionally, a recent study reported that orally administered LPS suppressed diabetic symptoms by increasing the expression of insulin signaling-related factors, especially adiponectin, in adipose tissue in type 2 diabetes mellitus, a disease supposedly LPS-induced<sup>39</sup>. Furthermore, LPSp has been confirmed to be highly safe in rats, with no adverse effects after oral administration at 2 g/kg body weight (BW) or higher<sup>40</sup>.

From the above-mentioned studies, LPS from ingested food is likely to activate and regulate innate immunity. Furthermore, considering its presence in herbal extracts, there is a possibility that the consumption of herbal extracts may activate the body innate immunity regulation. Herbal extracts are defined as naturally occurring unrefined substances from any part or parts of plants, animals, and other organisms with one or more active ingredients intended to alleviate, treat, or prevent diseases<sup>41</sup>. The above-mentioned wheat is a herbal extract listed in the “The Japanese standards for nonpharmacopoeial herbal extracts 2022” and is called Shobaku<sup>42</sup>. The overall health benefits of consuming herbal extracts are generally thought to be due to the low molecular weight of the active ingredient. However, a sufficient amount of LPS in the herbal extracts can activate the innate immune system; therefore, LPS should also be considered an active ingredient of herbal extracts. As the innate immune system-activating effect of orally administered LPS is coming to light<sup>34</sup>, LPS in herbal extracts as a component of the effects of Chinese herbal medicine deserves attention. Thus, a database of the LPS content in herbal extracts and food ingredients is required to make this concept common knowledge.

In 1992, our group screened approximately 60 plant samples, including herbal extracts, for their LPS content and found that some plants had a high LPS content of over 100 µg/g<sup>32</sup>. However, since then, little effort has been made to measure the LPS content in herbal extracts. Montenegro et al. was the first to report on LPS's ability to activate macrophages, an innate immunity mechanism, in Kampo medicine<sup>43</sup>. In this study, they showed that the macrophage-activating component of Juzen-taiho-to, an immune-boosting Kampo medicine formulated from 10 herbal extracts, is correlated with the amount of LPS, which is obtained from symbiotic bacteria existing in one of its ingredients. Their study showed that LPS is a functional component that activates and controls macrophages (innate immunity) in Juzen-taiho-to; hence, LPS can be regarded as an active component of the innate immune system of numerous herbal extracts because most herbal extracts have symbiotic bacteria that supply LPS. Therefore, if information on the LPS content found in herbal extracts can be obtained, the knowledge that oral intake of LPS does not induce inflammation can be enforced, and a new perspective on the concept of LPS as an effective component of herbal extracts can be provided. However, data evaluating herbal extracts from the LPS viewpoint are currently extremely limited, as described above.

Thus, to provide a comprehensive list of the LPS content of herbal extracts and other food ingredients, the LPS content of 414 herbal extracts were measured and compared. Additionally, the macrophage activation potential of herbal extracts with particularly high-LPS-content was compared and measured to investigate the connection between LPS content and macrophage activity.

## Results

### Measurement of the LPS content of herbal extracts

By measuring Limulus activity, the amount of LPS in the herbal extracts was examined. The LPS concentrations of 414 samples of herbal extracts obtained from vascular plants, fungi, and others ranging from below the detection limit to several 100 µg/g are shown in Table 1. Figure 1 shows the distributions of the LPS concentrations within each species. Herbal extracts from vascular plants were further divided according to their parts. For this analysis, the groups were classified according to the crude drug classification method. The results showed that herbal extract ingredients with high LPS contents were mostly found in the vascular plant group. Comparisons between vascular plant parts indicated that roots (107 samples) had significantly higher LPS levels than fruits (69 samples) and seeds (22 samples), and leaves (68 samples) had significantly higher LPS levels than fruits (69 samples). The average LPS concentration in all samples was  $17.4 \pm 69.3$  µg/g. There are 12 samples containing high LPS concentration > 100 µg/g, 80 samples containing concentrations of 10–100 µg/g, and 162 samples containing concentrations of 1–10 ng/g. The 12 samples with significantly high LPS contents, which are listed in Table 2, were selected to further test the macrophage-activating effect of LPS. The measured LPS content indicated that herbal extracts contain LPS and that the amount of LPS in each plant's part varies depending on the parts from which they are derived.

### Measurement of the macrophage activation potential of the herbal extracts

Twelve herbal extract samples with LPS levels of  $\geq 100$  µg/g were tested for macrophage activation potential. Macrophage activation potential was assessed by measuring phagocytosis and nitric oxide (NO) production by stimulating RAW 264.7 cells with the herbal extracts. Stimulation using purified LPSp served as a positive control. Phagocytic activity was increased in all samples compared with that in the non-stimulated control group (Fig. 2). The phagocytosis ability of RAW 264.7 cells was increased when stimulated with Oat (*Avena sativa* L.), Sacred lotus (*Nelumbo nucifera* Gaertn.), Aralia rhizome (*Aralia cordata* Thunb.), Fortune's drynaria rhizome (*Drynaria roosii* Nakaike), Couch grass (*Elytrigia repens* (L.) Gould), Angelica dahurica root (*Angelica dahurica*), Common duckmeat (*Spirodela polyrrhiza* (L.) Schleid.), Corn silk (*Zea mays* L.), and Bupleurum root (*Bupleurum falcatum* L.) compared with the positive control LPSp. The phagocytosis ability of RAW 264.7 cells with Ginger

| No | Species | English name                | Scientific name                                                                            | Part                    | Limulus activity (µg/g) |
|----|---------|-----------------------------|--------------------------------------------------------------------------------------------|-------------------------|-------------------------|
| 1  | Plant   | Achyranthes root            | <i>Achyranthes bidentata</i> Blume                                                         | Root                    | 0.391                   |
| 2  | Plant   | Aconite root                | <i>Aconitum carmichaelii</i> Debeaux                                                       | Root (Tuberous root)    | 2.705                   |
| 3  | Plant   | Actinidia gall              | <i>Actinidia polygama</i> (Siebold & Zucc.) Planch. ex Maxim                               | Fruit (Gall)            | 3.891                   |
| 4  | Plant   | Adenophora root             | <i>Adenophora triphylla</i> (Thunb.) A.DC                                                  | Root                    | 5.984                   |
| 5  | Plant   | Agarwood                    | <i>Aquilaria malaccensis</i> Lam                                                           | Stem (Xylem)            | 16.284                  |
| 6  | Plant   | Agrimony                    | <i>Agrimonia eupatoria</i> L.                                                              | Stem and Leaf           | 3.282                   |
| 7  | Plant   | Ajuga herb                  | <i>Ajuga decumbens</i> Thunb                                                               | Whole plant             | 10.642                  |
| 8  | Plant   | Akebia fruit                | <i>Akebia quinata</i> (Thunb. ex Houtt.) Decne                                             | Fruit                   | 12.147                  |
| 9  | Plant   | Akebia stem                 | <i>Akebia quinata</i> (Thunb. ex Houtt.) Decne                                             | Stem                    | 1.962                   |
| 10 | Plant   | Alfalfa                     | <i>Medicago sativa</i> L.                                                                  | Stem and Leaf           | 4.089                   |
| 11 | Plant   | Alisma tuber                | <i>Alisma plantago-aquatica</i> subsp. <i>orientale</i> (Sam.) Sam                         | Root (Rhizome)          | 0.409                   |
| 12 | Plant   | Allium chinense bulb        | <i>Allium chinense</i> G.Don                                                               | Root (Bulb)             | 6.554                   |
| 13 | Plant   | Allspice                    | <i>Pimenta dioica</i> (L.) Merr                                                            | Fruit                   | 3.543                   |
| 14 | Plant   | Amomum seed                 | <i>Wurfbainia villosa</i> var. <i>xanthioides</i> (Wall. ex Baker) Škorničk. & A.D.Poulsen | Seed                    | 6.392                   |
| 15 | Plant   | Amomum tsao-ko fruit        | <i>Lanxangia tsao-ko</i> (Crevost & Lemarié) M.F.Newman & Škorničk                         | Fruit (Mature fruit)    | 0.018                   |
| 16 | Plant   | Anemarrhena rhizome         | <i>Anemarrhena asphodeloides</i> Bunge                                                     | Root (Rhizome)          | 38.905                  |
| 17 | Plant   | Angelica                    | <i>Angelica archangelica</i> L.                                                            | Root                    | 29.527                  |
| 18 | Plant   | Angelica dahurica root      | <i>Angelica dahurica</i> (Hoffm.) Benth. & Hook.f. ex Franch. & Sav                        | Root                    | 266.554                 |
| 19 | Plant   | Anise                       | <i>Pimpinella anisum</i> L.                                                                | Fruit                   | 0.100                   |
| 20 | Plant   | Apple                       | <i>Malus domestica</i> (Suckow) Borkh                                                      | Fruit                   | 8.276                   |
| 21 | Plant   | Apricot kernel              | <i>Prunus armeniaca</i> L.                                                                 | Seed                    | 0.009                   |
| 22 | Plant   | Aralia rhizome              | <i>Aralia cordata</i> Thunb                                                                | Root (Rhizome)          | 503.986                 |
| 23 | Plant   | Aralia root                 | <i>Aralia cordata</i> Thunb                                                                | Root                    | 31.014                  |
| 24 | Plant   | Aralia elata root bark      | <i>Aralia elata</i> (Miq.) Seem                                                            | Root (Root bark)        | 3.891                   |
| 25 | Plant   | Areca                       | <i>Areca catechu</i> L.                                                                    | Seed                    | 0.808                   |
| 26 | Plant   | Arisaema tuber              | <i>Arisaema heterophyllum</i> Blume                                                        | Root (Tuber)            | 1.488                   |
| 27 | Plant   | Arnica flower               | <i>Arnica montana</i> L.                                                                   | Flower                  | 29.527                  |
| 28 | Plant   | Artemisia leaf              | <i>Artemisia princeps</i> Pamp                                                             | Leaf                    | 121.750                 |
| 29 | Plant   | Artichoke                   | <i>Cynara cardunculus</i> L.                                                               | Stem and Leaf           | 4.642                   |
| 30 | Plant   | Ash bark                    | <i>Fraxinus chinensis</i> subsp. <i>rhynchophylla</i> (Hance) A.E.Murray                   | Stem (Bark)             | 1.251                   |
| 31 | Plant   | Ashitaba                    | <i>Angelica keiskei</i> (Miq.) Koidz                                                       | Leaf                    | 0.220                   |
| 32 | Plant   | Asparagus                   | <i>Asparagus officinalis</i> L                                                             | Stem                    | 0.066                   |
| 33 | Plant   | Asparagus root              | <i>Asparagus cochinchinensis</i> (Lour.) Merr                                              | Root                    | 2.389                   |
| 34 | Plant   | Aster root                  | <i>Aster tataricus</i> L.f.                                                                | Root (Root and Rhizome) | 13.635                  |
| 35 | Plant   | Astragalus root             | <i>Astragalus mongholicus</i> Bunge                                                        | Root                    | 1.256                   |
| 36 | Plant   | Asunaro                     | <i>Thujopsis dolabrata</i> (L.f.) Siebold & Zucc                                           | Branch and Leaf         | 1.445                   |
| 37 | Plant   | Atractylodes lancea rhizome | <i>Atractylodes lancea</i> (Thunb.) DC                                                     | Root (Rhizome)          | 5.609                   |
| 38 | Plant   | Bamboo culm                 | <i>Bambusa textilis</i> McClure                                                            | Stem (Culm)             | 1.151                   |
| 39 | Plant   | Banaba                      | <i>Lagerstroemia speciosa</i> (L.) Pers                                                    | Leaf                    | 0.363                   |
| 40 | Plant   | Barbed skullcup herb        | <i>Scutellaria barbata</i> D.Don                                                           | Whole plant             | 2.372                   |
| 41 | Plant   | Barberry                    | <i>Berberis vulgaris</i> L.                                                                | Fruit                   | 0.011                   |
| 42 | Plant   | Bay leaf, Laurel            | <i>Laurus nobilis</i> L.                                                                   | Leaf                    | 1.636                   |
| 43 | Plant   | Bearberry leaf              | <i>Arctostaphylos uva-ursi</i> (L.) Spreng                                                 | Leaf                    | 0.735                   |
| 44 | Plant   | Beautiful sweetgum fruit    | <i>Liquidambar formosana</i> Hance                                                         | Fruit                   | 0.778                   |
| 45 | Plant   | Beet                        | <i>Beta vulgaris</i> L.                                                                    | Root                    | 0.124                   |
| 46 | Plant   | Belvedere fruit             | <i>Bassia scoparia</i> (L.) A.J.Scott                                                      | Fruit                   | 7.322                   |
| 47 | Plant   | Bilberry                    | <i>Vaccinium myrtillus</i> L.                                                              | Leaf                    | 0.397                   |
| 48 | Plant   | Birch, Abedul, Betula       | <i>Betula pendula</i> Roth                                                                 | Leaf                    | 0.132                   |
| 49 | Plant   | Bitter bottle gourd         | <i>Cucurbita pepo</i> L.                                                                   | Fruit                   | 0.151                   |
| 50 | Plant   | Bitter melon                | <i>Momordica charantia</i> L.                                                              | Fruit                   | 118.514                 |
| 51 | Plant   | Bitter orange peel          | <i>Citrus × aurantium</i> L.                                                               | Fruit (Peel)            | 0.050                   |
| 52 | Plant   | Black tea                   | <i>Camellia sinensis</i> (L.) Kuntze                                                       | Leaf                    | 1.075                   |
| 53 | Plant   | Blackthorn                  | <i>Prunus spinosa</i> L.                                                                   | Fruit                   | 0.175                   |

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| No  | Species | English name                  | Scientific name                                                                               | Part                | Limulus activity (µg/g) |
|-----|---------|-------------------------------|-----------------------------------------------------------------------------------------------|---------------------|-------------------------|
| 54  | Plant   | Bladder wrack                 | <i>Fucus evanescens</i> C.Agardh                                                              | Whole plant         | 4.945                   |
| 55  | Plant   | Boldo, Boldus                 | <i>Peumus boldus</i> Molina                                                                   | Leaf                | 0.156                   |
| 56  | Plant   | Boston ivy, Japanese ivy      | <i>Parthenocissus tricuspidata</i> (Siebold & Zucc.) Planch                                   | Leaf                | 96.453                  |
| 57  | Plant   | Brown rice                    | <i>Oryza sativa</i> L.                                                                        | Seed                | 3.446                   |
| 58  | Plant   | Bupleurum root                | <i>Bupleurum falcatum</i> L.                                                                  | Root                | 148.514                 |
| 59  | Plant   | Burdock                       | <i>Arctium lappa</i> L.                                                                       | Root                | 4.295                   |
| 60  | Plant   | Burdock fruit                 | <i>Arctium lappa</i> L.                                                                       | Fruit               | 8.577                   |
| 61  | Plant   | Cabbage                       | <i>Brassica oleracea</i> L.                                                                   | Leaf                | 0.257                   |
| 62  | Plant   | Calendula, Marigold           | <i>Calendula officinalis</i> L.                                                               | Flower              | 21.622                  |
| 63  | Plant   | Calumba                       | <i>Jateorhiza palmata</i> (Lam.) Miers                                                        | Root                | 4.549                   |
| 64  | Plant   | Caraway                       | <i>Carum carvi</i> L.                                                                         | Fruit               | 2.004                   |
| 65  | Plant   | Cardamon                      | <i>Elettaria cardamomum</i> (L.) Maton                                                        | Fruit               | 9.203                   |
| 66  | Plant   | Carob, St. john's bread       | <i>Ceratonia siliqua</i> L.                                                                   | Fruit (Bean pod)    | 0.009                   |
| 67  | Plant   | Cassia seed                   | <i>Senna obtusifolia</i> (L.) H.S.Irwin & Barneby                                             | Seed                | 0.020                   |
| 68  | Plant   | Cassis, Black currant         | <i>Ribes nigrum</i> L.                                                                        | Fruit               | 0.033                   |
| 69  | Plant   | Cassis, Black currant         | <i>Ribes nigrum</i> L.                                                                        | Leaf                | 26.649                  |
| 70  | Plant   | Catalpa fruit                 | <i>Catalpa ovata</i> G.Don                                                                    | Fruit               | 2.199                   |
| 71  | Plant   | Catnip, Catmint               | <i>Nepeta cataria</i> L.                                                                      | Stem and Leaf       | 13.009                  |
| 72  | Plant   | Cat's whisker, Java tea       | <i>Orthosiphon aristatus</i> (Blume) Miq                                                      | Leaf                | 11.472                  |
| 73  | Plant   | Cauliflower                   | <i>Brassica oleracea</i> L.                                                                   | Stem                | 0.128                   |
| 74  | Plant   | Celandine                     | <i>Chelidonium majus</i> L.                                                                   | Whole plant         | 32.824                  |
| 75  | Plant   | Celery                        | <i>Apium graveolens</i> L.                                                                    | Root                | 0.037                   |
| 76  | Plant   | Celery seed                   | <i>Apium graveolens</i> L.                                                                    | Seed                | 4.377                   |
| 77  | Plant   | Chaenomeles fruit             | <i>Pseudocydonia sinensis</i> (Dum.Cours.) C.K.Schneid                                        | Fruit               | 3.088                   |
| 78  | Plant   | Chamaecrista herb             | <i>Chamaecrista nomame</i> (Makino) H.Ohashi                                                  | Whole plant         | 0.833                   |
| 79  | Plant   | Chaste tree                   | <i>Vitex agnus-castus</i> L.                                                                  | Fruit               | 4.124                   |
| 80  | Plant   | Cherry bark                   | <i>Prunus jamasakura</i> (Makino) Siebold ex Koidz                                            | Stem (Bark)         | 1.300                   |
| 81  | Plant   | China berry                   | <i>Melia azedarach</i> L./ <i>Melia azedarach</i> var. <i>subtripinnata</i> Miq               | Leaf                | 0.331                   |
| 82  | Plant   | Chinese blackberry, sweet tea | <i>Rubus chingii</i> var. <i>suavissimus</i> (S.K.Lee) L.T.Lu                                 | Leaf                | 0.304                   |
| 83  | Plant   | Chinese honeylocust spine     | <i>Gleditsia sinensis</i> Lam.                                                                | Stem (Hook)         | 84.297                  |
| 84  | Plant   | Chinese prickly ash           | <i>Zanthoxylum simulans</i> Hance                                                             | Fruit (Peel)        | 13.399                  |
| 85  | Plant   | Chinese pulsatilla root       | <i>Pulsatilla chinensis</i> (Bunge) Regel                                                     | Root                | 58.676                  |
| 86  | Plant   | Chokeberry                    | <i>Aronia melanocarpa</i> (Michx.) Elliott                                                    | Fruit               | 0.106                   |
| 87  | Plant   | Chrysanthemum flower          | <i>Chrysanthemum indicum</i> L.                                                               | Flower (Capitula)   | 13.399                  |
| 88  | Plant   | Chundan, Kathala hibutu tea   | <i>Salacia reticulata</i> Wight                                                               | Root (Root bark)    | 0.678                   |
| 89  | Plant   | Cimicifuga rhizome            | <i>Actaea dahurica</i> (Turcz. ex Fisch. & C.A.Mey.) Franch                                   | Root (Rhizome)      | 13.349                  |
| 90  | Plant   | Cimicifuga rhizome            | <i>Actaea simplex</i> (DC.) Wormsk. ex Prantl                                                 | Root (Rhizome)      | 4.822                   |
| 91  | Plant   | Cinnamon                      | <i>Neolitsea cassia</i> (L.) Kosterm                                                          | Stem (Bark)         | 0.047                   |
| 92  | Plant   | Cinnamon bark (Crude drug)    | <i>Neolitsea cassia</i> (L.) Kosterm                                                          | Stem (Bark)         | 12.386                  |
| 93  | Plant   | Citrus peel                   | <i>Citrus × aurantium</i> L./ <i>Citrus reticulata</i> Blanco                                 | Fruit (Peel)        | 0.106                   |
| 94  | Plant   | Citrus unshiu peel            | <i>Citrus × aurantium</i> f. <i>deliciosa</i> (Ten.) M.Hiroe/ <i>Citrus reticulata</i> Blanco | Fruit (Peel)        | 0.133                   |
| 95  | Plant   | Clematis root                 | <i>Clematis terniflora</i> var. <i>mandshurica</i> (Rupr.) Ohwi                               | Root                | 2.953                   |
| 96  | Plant   | Clove (Crude drug)            | <i>Syzygium aromaticum</i> (L.) Merr. & L.M.Perry                                             | Flower (Flower bud) | 0.216                   |
| 97  | Plant   | Clove                         | <i>Syzygium aromaticum</i> (L.) Merr. & L.M.Perry                                             | Flower (Flower bud) | 0.075                   |
| 98  | Plant   | Club moss                     | <i>Lycopodium clavatum</i> L.                                                                 | Whole plant         | 2.434                   |
| 99  | Plant   | Cnidium monnieri fruit        | <i>Cnidium monnieri</i> (L.) Cusson                                                           | Fruit               | 36.730                  |
| 100 | Plant   | Cnidium rhizome               | <i>Ligusticum officinale</i> (Makino) Kitag                                                   | Root (Rhizome)      | 5.264                   |
| 101 | Plant   | Codonopsis root               | <i>Codonopsis pilosula</i> (Franch.) Nannf                                                    | Root                | 0.322                   |
| 102 | Plant   | Cola                          | <i>Cola nitida</i> (Vent.) Schott & Endl                                                      | Seed                | 0.121                   |
| 103 | Plant   | Coltsfoot                     | <i>Tussilago farfara</i> L.                                                                   | Leaf                | 2.162                   |
| 104 | Plant   | Coltsfoot flower              | <i>Tussilago farfara</i> L.                                                                   | Flower (Flower bud) | 1.916                   |
| 105 | Plant   | Comfrey, Boneset              | <i>Symphytum officinale</i> L.                                                                | Root                | 29.459                  |
| 106 | Plant   | Comfrey, Boneset              | <i>Symphytum officinale</i> L.                                                                | Whole plant         | 21.986                  |
| 107 | Plant   | Common Curculigo rhizome      | <i>Curculigo orchoides</i> Gaertn                                                             | Root (Rhizome)      | 0.778                   |
| 108 | Plant   | Common ducksmeat herb         | <i>Spirodela polyrhiza</i> (L.) Schleid                                                       | Whole plant         | 366.554                 |

Continued

| No        | Species | English name                      | Scientific name                                                                  | Part                    | Limulus activity (µg/g) |
|-----------|---------|-----------------------------------|----------------------------------------------------------------------------------|-------------------------|-------------------------|
| 109       | Plant   | Common knotgrass herb             | <i>Polygonum aviculare</i> L.                                                    | Whole plant             | 14.243                  |
| 110       | Plant   | Common mullein, Great mullein     | <i>Verbascum thapsus</i> L.                                                      | Stem and Leaf           | 2.649                   |
| 111       | Plant   | Common reed                       | <i>Phragmites australis</i> (Cav.) Trin. ex Steud                                | Stem                    | 6.554                   |
| 112       | Plant   | Coptis rhizome                    | <i>Coptis japonica</i> (Thunb.) Makino                                           | Root (Rhizome)          | 0.289                   |
| 113       | Plant   | Coriander                         | <i>Coriandrum sativum</i> L.                                                     | Fruit                   | 20.986                  |
| 114       | Plant   | Corn silk                         | <i>Zea mays</i> L.                                                               | Flower (Flower's style) | 180.068                 |
| 115       | Plant   | Cornflower                        | <i>Centaurea cyanus</i> L.                                                       | Flower                  | 0.155                   |
| 116       | Plant   | Cornus fruit                      | <i>Cornus officinalis</i> Siebold & Zucc                                         | Fruit                   | 0.043                   |
| 117       | Plant   | Corydalis tuber                   | <i>Corydalis yanhusuo</i> (Y.H.Chou & Chun C.Hsu)<br>W.T.Wang ex Z.Y.Su & C.Y.Wu | Root (Tuber)            | 8.796                   |
| 118       | Plant   | Couch grass, Quack grass          | <i>Elymus repens</i> (L.) Gould                                                  | Root (Rhizome)          | 392.635                 |
| 119       | Plant   | Cowherb                           | <i>Gypsophila vaccaria</i> (L.) Sm                                               | Seed                    | 0.354                   |
| 120       | Plant   | Crataegus fruit                   | <i>Crataegus cuneata</i> Siebold & Zucc                                          | Fruit                   | 0.244                   |
| 121       | Plant   | Cumin                             | <i>Cuminum cyminum</i> L.                                                        | Fruit                   | 80.054                  |
| 122       | Plant   | Curcuma rhizome                   | <i>Curcuma zedoaria</i> (Christm.) Roscoe                                        | Root (Rhizome)          | 65.608                  |
| 123       | Plant   | Cyperus rhizome                   | <i>Cyperus rotundus</i> L.                                                       | Root (Rhizome)          | 2.801                   |
| 124       | Plant   | Daisy fleabane                    | <i>Erigeron annuus</i> (L.) Pers                                                 | Whole plant             | 0.389                   |
| 125       | Plant   | Damiana                           | <i>Turnera diffusa</i> Willd. ex Schult                                          | Leaf                    | 1.962                   |
| 126       | Plant   | Dandelion                         | <i>Taraxacum</i> Weber                                                           | Root                    | 4.945                   |
| 127       | Plant   | Dayflower                         | <i>Commelina communis</i> L.                                                     | Whole plant             | 6.058                   |
| 128       | Plant   | Devil's claw                      | <i>Harpagophytum procumbens</i> (Burch.) DC. ex Meisn                            | Root (Tuber)            | 0.188                   |
| 129       | Plant   | Dioscorea rhizome                 | <i>Dioscorea japonica</i> Thunb.                                                 | Root (Rhizome)          | 0.010                   |
| 130       | Plant   | Dipsacus root                     | <i>Dipsacus asper</i> Wall. ex DC                                                | Root                    | 0.170                   |
| 131       | Plant   | Echinacea                         | <i>Echinacea purpurea</i> (L.) Moench                                            | Stem and Leaf           | 10.635                  |
| 132       | Plant   | Elder                             | <i>Sambucus nigra</i> L.                                                         | Flower                  | 9.359                   |
| 133       | Plant   | English hawthorn                  | <i>Crataegus laevigata</i> (Poir.) DC                                            | Leaf                    | 36.730                  |
| 134       | Plant   | Ephedra herb                      | <i>Ephedra sinica</i> Stapf                                                      | Stem                    | 0.723                   |
| 135       | Plant   | Epimedium herb                    | <i>Epimedium grandiflorum</i> var. <i>koreanum</i> (Nakai)<br>K.Suzuki           | Leaf                    | 11.359                  |
| 136       | Plant   | Erythrina bark                    | <i>Erythrina variegata</i> L.                                                    | Stem (Bark)             | 16.959                  |
| 137       | Plant   | Eucalyptus                        | <i>Eucalyptus globulus</i> Labill.                                               | Leaf                    | 0.023                   |
| 138       | Plant   | Eucommia bark                     | <i>Eucommia ulmoides</i> Oliv.                                                   | Stem (Bark)             | 0.188                   |
| 139       | Plant   | Eucommia leaf                     | <i>Eucommia ulmoides</i> Oliv.                                                   | Leaf                    | 2.791                   |
| 140       | Plant   | Euodia fruit                      | <i>Tetradium ruticarpum</i> (A.Juss.) T.G.Hartley                                | Fruit                   | 10.642                  |
| 141       | Plant   | European verbena herb             | <i>Verbena officinalis</i> L.                                                    | Stem                    | 7.001                   |
| 142       | Plant   | Eyebright                         | <i>Euphrasia officinalis</i> L.                                                  | Stem and Leaf           | 8.577                   |
| 143       | Plant   | Feather cockscomb seed            | <i>Celosia argentea</i> L.                                                       | Seed                    | 2.791                   |
| 144       | Plant   | Fennel                            | <i>Foeniculum vulgare</i> Mill.                                                  | Fruit                   | 0.045                   |
| 145       | Plant   | Fenugreek                         | <i>Trigonella foenum-graecum</i> L.                                              | Stem and Leaf           | 36.351                  |
| 146       | Plant   | Fermented black soybean           | <i>Glycine max</i> (L.) Merr.                                                    | Seed                    | 5.264                   |
| 147       | Plant   | Feverfew                          | <i>Tanacetum parthenium</i> (L.) Sch.Bip.                                        | Whole plant             | 8.714                   |
| 148       | Plant   | Figwort flower Picrorhiza rhizome | <i>Neopicrorhiza scrophulariiflora</i> (Pennell) D.Y.Hong                        | Root (Rhizome)          | 1.678                   |
| 149       | Plant   | Finger citron                     | <i>Citrus medica</i> L.                                                          | Fruit (Peel)            | 18.716                  |
| 150       | Plant   | Flatstem milkvetch seed           | <i>Phyllobium chinense</i> Fisch.                                                | Seed                    | 4.295                   |
| 151       | Plant   | Forsythia fruit                   | <i>Forsythia suspensa</i> (Thunb.) Vahl.                                         | Fruit                   | 10.530                  |
| 152       | Plant   | Fortune windmill palm petiole     | <i>Trachycarpus fortunei</i> (Hook.) H.Wendl.                                    | Leaf                    | 1.151                   |
| 153       | Plant   | Fortune's drynaria rhizome        | <i>Drynaria roosii</i> Nakaike                                                   | Root (Rhizome)          | 423.041                 |
| 154       | Plant   | Foxtail millet                    | <i>Setaria italica</i> (L.) P.Beauv.                                             | Seed                    | 0.006                   |
| 155       | Plant   | Fragrant rosewood                 | <i>Dalbergia odorifera</i> T.C.Chen                                              | Root (Heart wood)       | 1.418                   |
| 156       | Plant   | Fragrant solomonseal rhizome      | <i>Polygonatum odoratum</i> (Mill.) Druce                                        | Root (Rhizome)          | 0.894                   |
| 157       | Plant   | Frankincense                      | <i>Boswellia sacra</i> Flück.                                                    | Resin                   | 0.003                   |
| 158       | Plant   | French bean                       | <i>Phaseolus vulgaris</i> L.                                                     | Fruit (Bean pod)        | 0.936                   |
| 159       | Plant   | Garden burnet root                | <i>Sanguisorba officinalis</i> L.                                                | Root (Root and Rhizome) | 6.392                   |
| 160       | Plant   | Gardenia fruit                    | <i>Gardenia jasminoides</i> J.Ellis                                              | Fruit                   | 0.014                   |
| 161       | Plant   | Gastrodia tuber                   | <i>Gastrodia elata</i> Blume                                                     | Root (Tuber)            | 0.155                   |
| 162       | Plant   | Gentiana macrophylla root         | <i>Gentiana macrophylla</i> Pall.                                                | Root                    | 0.385                   |
| Continued |         |                                   |                                                                                  |                         |                         |

| No        | Species | English name                     | Scientific name                                                                               | Part                      | Limulus activity (µg/g) |
|-----------|---------|----------------------------------|-----------------------------------------------------------------------------------------------|---------------------------|-------------------------|
| 163       | Plant   | Geranium herb                    | <i>Geranium thunbergii</i> Siebold & Zucc. ex Lindl. & Paxton                                 | Stem and Leaf             | 10.642                  |
| 164       | Plant   | German chamomile                 | <i>Matricaria chamomilla</i> L.                                                               | Flower                    | 0.322                   |
| 165       | Plant   | Ginger                           | <i>Zingiber officinale</i> Roscoe                                                             | Root (Rhizome)            | 122.020                 |
| 166       | Plant   | Ginkgo                           | <i>Ginkgo biloba</i> L.                                                                       | Leaf                      | 6.936                   |
| 167       | Plant   | Ginseng                          | <i>Panax ginseng</i> C.A.Mey                                                                  | Root                      | 0.023                   |
| 168       | Plant   | Glechoma hederacea herb          | <i>Glechoma grandis</i> (A.Gray) Kuprian.                                                     | Stem and Leaf             | 3.470                   |
| 169       | Plant   | Glycyrrhiza                      | <i>Glycyrrhiza uralensis</i> Fisch. ex DC.                                                    | Root                      | 3.101                   |
| 170       | Plant   | Goldenrod                        | <i>Solidago virgaurea</i> subsp. <i>asiatica</i> (Nakai ex Hara) Kitam. ex Hara L.            | Leaf                      | 18.378                  |
| 171       | Plant   | Gooseberry, European gooseberry  | <i>Ribes uva-crispa</i> L.                                                                    | Fruit                     | 0.000                   |
| 172       | Plant   | Gorgon euryale seed              | <i>Euryale ferox</i> Salisb.                                                                  | Seed                      | 0.081                   |
| 173       | Plant   | Grape                            | <i>Vitis</i> L.                                                                               | Leaf                      | 1.151                   |
| 174       | Plant   | Green tea leaf                   | <i>Camellia sinensis</i> (L.) Kuntze.                                                         | Leaf                      | 0.115                   |
| 175       | Plant   | Guarana seed                     | <i>Paullinia cupana</i> Kunth.                                                                | Seed                      | 5.058                   |
| 176       | Plant   | Guava                            | <i>Psidium guajava</i> L.                                                                     | Fruit                     | 0.080                   |
| 177       | Plant   | Gymnema                          | <i>Gymnema sylvestre</i> (Retz.) R.Br. ex Sm.                                                 | Leaf                      | 0.346                   |
| 178       | Plant   | Haichow Elsholtzia herb          | <i>Elsholtzia splendens</i> var. <i>splendens</i>                                             | Whole plant               | 3.839                   |
| 179       | Plant   | Hairyveine agrimonia herb        | <i>Agrimonia pilosa</i> Ledeb.                                                                | Whole plant               | 23.892                  |
| 180       | Plant   | Heather                          | <i>Calluna vulgaris</i> (L.) Hull.                                                            | Flower (Flower bud)       | 4.124                   |
| 181       | Plant   | Hedysarum root                   | <i>Hedysarum polybotrys</i> Hand.-Mazz.                                                       | Root                      | 1.364                   |
| 182       | Plant   | Henna                            | <i>Lawsonia inermis</i> L.                                                                    | Leaf                      | 1.104                   |
| 183       | Plant   | Heterophylly false starwort root | <i>Pseudostellaria heterophylla</i> (Miq.) Pax.                                               | Root (Tuberous root)      | 0.141                   |
| 184       | Plant   | Hibiscus, Roselle                | <i>Hibiscus sabdariffa</i> L.                                                                 | Flower (Calyx)            | 0.030                   |
| 185       | Plant   | Hollyhock                        | <i>Alcea rosea</i> L.                                                                         | Flower                    | 0.906                   |
| 186       | Plant   | Hop strobile                     | <i>Humulus lupulus</i> L.                                                                     | Flower                    | 1.628                   |
| 187       | Plant   | Horse chestnut                   | <i>Aesculus hippocastanum</i> L.                                                              | Leaf                      | 0.397                   |
| 188       | Plant   | Horseradish                      | <i>Armoracia rusticana</i> G.Gaertn., B.Mey. & Scherb.                                        | Root                      | 0.422                   |
| 189       | Plant   | Horsetail, Field hare-tail       | <i>Equisetum arvense</i> L.                                                                   | Stem                      | 0.639                   |
| 190       | Plant   | Houttuynia herb                  | <i>Houttuynia cordata</i> Thunb.                                                              | Whole plant (Aerial part) | 1.880                   |
| 191       | Plant   | Hovenia seed or fruit            | <i>Hovenia dulcis</i> Thunb.                                                                  | Fruit                     | 3.446                   |
| 192       | Plant   | Hyssop                           | <i>Hyssopus officinalis</i> L.                                                                | Stem and Leaf             | 4.945                   |
| 193       | Plant   | Immature citrus unshiu peel      | <i>Citrus × aurantium</i> f. <i>deliciosa</i> (Ten.) M.Hiroe/ <i>Citrus reticulata</i> Blanco | Fruit (Peel)              | 0.091                   |
| 194       | Plant   | Immature orange fruit            | <i>Citrus × aurantium</i> L.                                                                  | Fruit                     | 0.066                   |
| 195       | Plant   | Indian madder root               | <i>Rubia cordifolia</i> L.                                                                    | Root                      | 12.147                  |
| 196       | Plant   | Indigo                           | <i>Isatis tinctoria</i> L.                                                                    | Branch and Leaf           | 9.762                   |
| 197       | Plant   | Inula flower                     | <i>Pentanema britannica</i> (L.) D.Gut.Larr., Santos-Vicente, Anderb., E.Rico & M.M.Mart.Ort  | Flower (Capitula)         | 13.349                  |
| 198       | Plant   | Ipe, Taheebo                     | <i>Handroanthus impetiginosus</i> (Mart. ex DC.) Mattos                                       | Stem (Bark)               | 4.377                   |
| 199       | Plant   | Ipecac                           | <i>Carapichea ipecacuanha</i> (Brot.) L.Andersson                                             | Root                      | 0.577                   |
| 200       | Plant   | Isatis root                      | <i>Isatis tinctoria</i> L.                                                                    | Root                      | 0.373                   |
| 201       | Plant   | Isodon herb                      | <i>Isodon japonicus</i> (Burm.f.) H.Hara                                                      | Stem and Leaf             | 5.270                   |
| 202       | Plant   | Japanese angelica root           | <i>Angelica acutiloba</i> (Siebold & Zucc.) Kitag.                                            | Root                      | 16.284                  |
| 203       | Plant   | Japanese angelica tree           | <i>Aralia elata</i> (Miq.) Seem                                                               | Stem                      | 2.791                   |
| 204       | Plant   | Japanese banana, Hardy banana    | <i>Musa basjoo</i> Siebold & Zucc. ex Iinuma                                                  | Root                      | 94.135                  |
| 205       | Plant   | Japanese bush cherry             | <i>Prunus japonica</i> Thunb.                                                                 | Seed                      | 1.300                   |
| 206       | Plant   | Japanese gentian                 | <i>Gentiana scabra</i> Bunge.                                                                 | Root (Root and Rhizome)   | 2.791                   |
| 207       | Plant   | Japanese horse chestnut          | <i>Aesculus turbinata</i> Blume.                                                              | Fruit                     | 0.098                   |
| 208       | Plant   | Japanese primrose                | <i>Primula sieboldii</i> É.Morren                                                             | Flower                    | 51.176                  |
| 209       | Plant   | Japanese thistle root            | <i>Cirsium japonicum</i> DC                                                                   | Root                      | 13.009                  |
| 210       | Plant   | Japanese thyme                   | <i>Thymus quinquecostatus</i> Čelak                                                           | Leaf                      | 11.359                  |
| 211       | Plant   | Japanese torreyia                | <i>Torreya nucifera</i> (L.) Siebold & Zucc.                                                  | Seed                      | 0.094                   |
| 212       | Plant   | Japanese valerian                | <i>Valeriana fauriei</i> Briq.                                                                | Root (Root and Rhizome)   | 9.203                   |
| 213       | Plant   | Jasmine                          | <i>Jasminum</i> L.                                                                            | Flower                    | 0.044                   |
| 214       | Plant   | Javanese turmeric                | <i>Curcuma zanthorrhiza</i> Roxb.                                                             | Root (Rhizome)            | 13.399                  |
| 215       | Plant   | Jujube                           | <i>Ziziphus jujuba</i> var. <i>inermis</i> (Bunge) Rehder                                     | Fruit                     | 0.123                   |
| 216       | Plant   | Jujube seed                      | <i>Ziziphus jujuba</i> var. <i>spinosa</i> (Bunge) Hu ex H.F.Chow                             | Seed                      | 0.002                   |
| Continued |         |                                  |                                                                                               |                           |                         |

| No  | Species | English name               | Scientific name                                                      | Part                          | Limulus activity (µg/g) |
|-----|---------|----------------------------|----------------------------------------------------------------------|-------------------------------|-------------------------|
| 217 | Plant   | Juniper berry              | <i>Juniperus communis</i> L.                                         | Fruit                         | 0.019                   |
| 218 | Plant   | Kava, Kava pepper          | <i>Piper methysticum</i> G.Forst                                     | Root                          | 54.365                  |
| 219 | Plant   | Kidachi aloe               | <i>Aloe arborescens</i> Mill.                                        | Leaf                          | 1.174                   |
| 220 | Plant   | Kombu                      | <i>Laminariaceae</i>                                                 | Root                          | 1.117                   |
| 221 | Plant   | Kuwagataso                 | <i>Veronica miqueliana</i> Nakai                                     | Stem and Leaf                 | 7.481                   |
| 222 | Plant   | Lady's mantle              | <i>Alchemilla vulgaris</i> L.                                        | Leaf                          | 6.936                   |
| 223 | Plant   | Lemon grass                | <i>Cymbopogon citratus</i> (DC.) Stapf.                              | Stem and Leaf                 | 0.142                   |
| 224 | Plant   | Licorice                   | <i>Glycyrrhiza glabra</i> L.                                         | Root                          | 22.730                  |
| 225 | Plant   | Ligusticum sinense rhizome | <i>Conioselinum anthriscoides</i> (H.Boissieu) Pimenov & Kljuykov    | Root (Rhizome)                | 10.642                  |
| 226 | Plant   | Ligustrum fruit            | <i>Ligustrum lucidum</i> W.T.Aiton                                   | Fruit                         | 8.430                   |
| 227 | Plant   | Lilium bulb                | <i>Lilium lancifolium</i> Thunb.                                     | Leaf (Bulb)                   | 0.056                   |
| 228 | Plant   | Linden, Lime flower        | <i>Tilia cordata</i> Mill.                                           | Leaf                          | 3.891                   |
| 229 | Plant   | Lindera root               | <i>Lindera aggregata</i> var. <i>aggregata</i>                       | Root                          | 61.986                  |
| 230 | Plant   | Lithospermum root          | <i>Lithospermum erythrorhizon</i> Siebold & Zucc.                    | Root                          | 10.530                  |
| 231 | Plant   | Long pepper                | <i>Piper longum</i> L.                                               | Fruit                         | 1.063                   |
| 232 | Plant   | Lonicera flower            | <i>Lonicera japonica</i> Thunb.                                      | Flower (Flower bud)           | 0.256                   |
| 233 | Plant   | Lonicera leaf and stem     | <i>Lonicera japonica</i> Thunb.                                      | Stem and Leaf                 | 2.162                   |
| 234 | Plant   | Lophatherum herb           | <i>Lophatherum gracile</i> Brongn.                                   | Whole plant                   | 1.628                   |
| 235 | Plant   | Loquat leaf                | <i>Eriobotrya japonica</i> (Thunb.) Lindl.                           | Leaf                          | 0.084                   |
| 236 | Plant   | Low-bush cranberry         | <i>Vaccinium macrocarpon</i> Aiton                                   | Leaf                          | 3.268                   |
| 237 | Plant   | Luffa, Vegetable sponge    | <i>Luffa aegyptiaca</i> Mill.                                        | Fruit (Fiber of mature fruit) | 23.892                  |
| 238 | Plant   | Luobuma                    | <i>Apocynum venetum</i> L.                                           | Leaf                          | 5.984                   |
| 239 | Plant   | Lycium bark                | <i>Lycium chinense</i> Mill.                                         | Root (Root bark)              | 5.609                   |
| 240 | Plant   | Lycium leaf                | <i>Lycium chinense</i> Mill.                                         | Branch and Leaf               | 6.155                   |
| 241 | Plant   | Magnolia bark              | <i>Magnolia obovata</i> Thunb.                                       | Stem (Bark)                   | 2.946                   |
| 242 | Plant   | Magnolia flower            | <i>Magnolia kobus</i> DC                                             | Flower (Flower bud)           | 0.131                   |
| 243 | Plant   | Mallotus bark              | <i>Mallotus japonicus</i> (L.f.) Müll.Arg.                           | Stem (Bark)                   | 3.088                   |
| 244 | Plant   | Mallow                     | <i>Malva sylvestris</i> L.                                           | Whole plant                   | 5.469                   |
| 245 | Plant   | Malt                       | <i>Hordeum vulgare</i> L.                                            | Seed                          | 73.500                  |
| 246 | Plant   | Marjoram, Sweet marjoram   | <i>Origanum majorana</i> L.                                          | Leaf                          | 0.141                   |
| 247 | Plant   | Marshmallow, Altea         | <i>Althaea officinalis</i> L.                                        | Leaf                          | 19.486                  |
| 248 | Plant   | Marshmallow, Altea         | <i>Althaea officinalis</i> L.                                        | Root                          | 8.276                   |
| 249 | Plant   | Meadowsweet                | <i>Filipendula ulmaria</i> (L.) Maxim                                | Stem and Leaf                 | 26.486                  |
| 250 | Plant   | Melia fruit                | <i>Melia azedarach</i> L.                                            | Fruit (Mature fruit)          | 0.049                   |
| 251 | Plant   | Melilot                    | <i>Melilotus officinalis</i> (L.) Lam.                               | Whole plant                   | 2.503                   |
| 252 | Plant   | Mentha herb                | <i>Mentha canadensis</i> L.                                          | Leaf                          | 6.199                   |
| 253 | Plant   | Milk thistle               | <i>Silybum marianum</i> (L.) Gaertn.                                 | Whole plant                   | 2.059                   |
| 254 | Plant   | Mountain ash               | <i>Sorbus aucuparia</i> L.                                           | Fruit                         | 0.121                   |
| 255 | Plant   | Moutan bark                | <i>Paeonia × suffruticosa</i> Andrews                                | Root (Root bark)              | 1.424                   |
| 256 | Plant   | Mulberry                   | <i>Morus alba</i> L.                                                 | Leaf                          | 3.101                   |
| 257 | Plant   | Mulberry bark              | <i>Morus alba</i> L.                                                 | Root (Root bark)              | 10.530                  |
| 258 | Plant   | Mulberry fruit             | <i>Morus alba</i> L.                                                 | Fruit                         | 5.264                   |
| 259 | Plant   | Myrobalan fruit            | <i>Terminalia chebula</i> Retz.                                      | Fruit                         | 0.001                   |
| 260 | Plant   | Nandina fruit              | <i>Nandina domestica</i> Thunb.                                      | Fruit                         | 2.649                   |
| 261 | Plant   | Natural indigo (Dye)       | <i>Indigofera tinctoria</i> L.                                       | Leaf                          | 0.020                   |
| 262 | Plant   | Nikko maple                | <i>Acer maximowiczianum</i> Miq.                                     | Stem (Bark)                   | 0.906                   |
| 263 | Plant   | Notopterygium              | <i>Hansenia weberbaueriana</i> (Fedde ex H.Wolff) Pimenov & Kljuykov | Root (Rhizome)                | 2.946                   |
| 264 | Plant   | Nutmeg                     | <i>Myristica fragrans</i> Houtt.                                     | Seed                          | 0.012                   |
| 265 | Plant   | Oat                        | <i>Avena sativa</i> L.                                               | Stem and Leaf                 | 825.541                 |
| 266 | Plant   | Olive                      | <i>Olea europaea</i> L.                                              | Leaf                          | 0.302                   |
| 267 | Plant   | Ophiopogon root            | <i>Ophiopogon japonicus</i> (Thunb.) Ker Gawl.                       | Root                          | 0.220                   |
| 268 | Plant   | Orange daylily             | <i>Hemerocallis fulva</i> var. <i>fulva</i>                          | Flower (Flower bud)           | 0.022                   |
| 269 | Plant   | Orange flower              | <i>Citrus × aurantium</i> L.                                         | Flower                        | 0.919                   |
| 270 | Plant   | Orange leaf                | <i>Citrus × aurantium</i> L.                                         | Leaf                          | 30.878                  |
| 271 | Plant   | Orange peel (bitter)       | <i>Citrus × aurantium</i> L.                                         | Fruit                         | 0.015                   |

Continued

| No        | Species | English name                   | Scientific name                                                                                | Part                    | Limulus activity (µg/g) |
|-----------|---------|--------------------------------|------------------------------------------------------------------------------------------------|-------------------------|-------------------------|
| 272       | Plant   | Oregano                        | <i>Origanum vulgare</i> L.                                                                     | Stem and Leaf           | 7.350                   |
| 273       | Plant   | Oriental arborvitae leafy twig | <i>Platycladus orientalis</i> (L.) Franco.                                                     | Leaf                    | 3.470                   |
| 274       | Plant   | Orris root                     | <i>Iris florentina</i> L.                                                                      | Root                    | 12.327                  |
| 275       | Plant   | Pale butterfly bush flower     | <i>Buddleja officinalis</i> Maxim.                                                             | Flower (Flower bud)     | 0.208                   |
| 276       | Plant   | Panax japonicus rhizome        | <i>Panax japonicus</i> (T.Nees) C.A.Mey                                                        | Root (Rhizome)          | 0.322                   |
| 277       | Plant   | Panax notoginseng root         | <i>Panax notoginseng</i> (Burkill) F.H.Chen                                                    | Root                    | 0.002                   |
| 278       | Plant   | Parsley                        | <i>Petroselinum crispum</i> subsp. <i>crispum</i>                                              | Leaf                    | 0.627                   |
| 279       | Plant   | Patrinia herb                  | <i>Patrinia scabiosifolia</i> Link                                                             | Whole plant             | 0.723                   |
| 280       | Plant   | Peach                          | <i>Prunus persica</i> (L.) Batsch                                                              | Leaf                    | 0.464                   |
| 281       | Plant   | Pennyroyal                     | <i>Mentha pulegium</i> L.                                                                      | Whole plant             | 0.389                   |
| 282       | Plant   | Peony                          | <i>Paeonia lactiflora</i> Pall.                                                                | Flower                  | 0.125                   |
| 283       | Plant   | Peony root                     | <i>Paeonia lactiflora</i> Pall.                                                                | Root                    | 0.529                   |
| 284       | Plant   | Perilla fruit                  | <i>Perilla frutescens</i> var. <i>frutescens</i>                                               | Fruit                   | 0.084                   |
| 285       | Plant   | Perilla herb                   | <i>Perilla frutescens</i> var. <i>crispa</i> (Thunb.) H.Deane                                  | Leaf                    | 4.650                   |
| 286       | Plant   | Perilla, Beefsteak plant       | <i>Perilla frutescens</i> (L.) Britton                                                         | Stem                    | 5.264                   |
| 287       | Plant   | Persimmon                      | <i>Diospyros kaki</i> L.f.                                                                     | Leaf                    | 1.474                   |
| 288       | Plant   | Persimmon calyx                | <i>Diospyros kaki</i> L.f.                                                                     | Fruit (Calyx)           | 0.465                   |
| 289       | Plant   | Peucedanum root                | <i>Kitagawia praeuptora</i> (Dunn) Pimenov.                                                    | Root                    | 1.628                   |
| 290       | Plant   | Phellodendron bark             | <i>Phellodendron amurense</i> Rupr.                                                            | Stem (Bark)             | 6.993                   |
| 291       | Plant   | Pine                           | <i>Pinus</i> L.                                                                                | Leaf                    | 1.019                   |
| 292       | Plant   | Pinellia tuber                 | <i>Pinellia ternata</i> (Thunb.) Makino                                                        | Root (Tuber)            | 1.872                   |
| 293       | Plant   | Plantago herb                  | <i>Plantago asiatica</i> L.                                                                    | Whole plant             | 2.642                   |
| 294       | Plant   | Plantago seed                  | <i>Plantago asiatica</i> L.                                                                    | Seed                    | 0.385                   |
| 295       | Plant   | Platycodon root                | <i>Platycodon grandiflorus</i> (Jacq.) A.DC.                                                   | Root                    | 2.199                   |
| 296       | Plant   | Polygala root                  | <i>Polygala tenuifolia</i> Willd.                                                              | Root                    | 0.075                   |
| 297       | Plant   | Polygonatum rhizome            | <i>Polygonatum falcatum</i> A.Gray                                                             | Root (Rhizome)          | 0.006                   |
| 298       | Plant   | Polygonum root                 | <i>Reynoutria multiflora</i> (Thunb.) Moldenke                                                 | Root (Tuberous root)    | 0.008                   |
| 299       | Plant   | Pomegranate rind               | <i>Punica granatum</i> L.                                                                      | Fruit (Peel)            | 0.777                   |
| 300       | Plant   | Potentilla, Silverweed         | <i>Argentina anserina</i> (L.) Rydb                                                            | Whole plant             | 6.731                   |
| 301       | Plant   | Prickly pear cactus            | <i>Opuntia</i> Mill.                                                                           | Flower                  | 1.174                   |
| 302       | Plant   | Processed ginger               | <i>Zingiber officinale</i> Roscoe                                                              | Root (Rhizome)          | 45.486                  |
| 303       | Plant   | Processed mume                 | <i>Prunus mume</i> (Siebold) Siebold & Zucc.                                                   | Fruit                   | 0.010                   |
| 304       | Plant   | Prunella spike                 | <i>Prunella vulgaris</i> subsp. <i>asiatica</i> (Nakai) H.Hara                                 | Flower (Spike)          | 35.216                  |
| 305       | Plant   | Psoralea corylifolia fruit     | <i>Cullen corylifolium</i> (L.) Medik.                                                         | Fruit                   | 10.605                  |
| 306       | Plant   | Pueraria Root                  | <i>Pueraria montana</i> var. <i>lobata</i> (Willd.) Maesen & S.M.Almeida ex Sanjappa & Predeep | Root                    | 7.547                   |
| 307       | Plant   | Purslane herb                  | <i>Portulaca oleracea</i> L.                                                                   | Whole plant             | 13.055                  |
| 308       | Plant   | Quercus bark                   | <i>Quercus acutissima</i> Carruth.                                                             | Stem (Bark)             | 6.836                   |
| 309       | Plant   | Quercus salicina leaf          | <i>Quercus salicina</i> Blume.                                                                 | Leaf                    | 0.259                   |
| 310       | Plant   | Raspberry                      | <i>Rubus idaeus</i> L.                                                                         | Leaf                    | 9.614                   |
| 311       | Plant   | Red clover                     | <i>Trifolium pratense</i> L.                                                                   | Whole plant             | 7.507                   |
| 312       | Plant   | Red poppy, Corn poppy          | <i>Papaver rhoeas</i> L.                                                                       | Flower                  | 5.001                   |
| 313       | Plant   | Rehmannia root                 | <i>Rehmannia glutinosa</i> (Gaertn.) DC                                                        | Root                    | 0.529                   |
| 314       | Plant   | Rhubarb                        | <i>Rheum palmatum</i> L.                                                                       | Root (Rhizome)          | 1.364                   |
| 315       | Plant   | Rice paper plant               | <i>Tetrapanax papyrifer</i> (Hook.) K.Koch                                                     | Stem (Pith)             | 0.028                   |
| 316       | Plant   | Rooibos                        | <i>Aspalathus linearis</i> (Burm.f.) R.Dahlgren                                                | Leaf                    | 94.135                  |
| 317       | Plant   | Rose                           | <i>Rosa</i> L.                                                                                 | Flower (Flower bud)     | 0.028                   |
| 318       | Plant   | Rose fruit                     | <i>Rosa multiflora</i> Thunb.                                                                  | Fruit                   | 2.196                   |
| 319       | Plant   | Rosehips                       | <i>Rosa</i> L.                                                                                 | Fruit (Peel)            | 0.030                   |
| 320       | Plant   | Rosemary                       | <i>Salvia rosmarinus</i> Spenn.                                                                | Leaf                    | 1.555                   |
| 321       | Plant   | Rosewood                       | <i>Dalbergia cochinchinensis</i> Pierre                                                        | Stem (Heart wood)       | 0.004                   |
| 322       | Plant   | Rugosa rose flower             | <i>Rosa rugosa</i> Thunb.                                                                      | Flower (Flower bud)     | 0.156                   |
| 323       | Plant   | Sacred lotus, Lotus            | <i>Nelumbo nucifera</i> Gaertn.                                                                | Root (Rhizome node)     | 624.459                 |
| 324       | Plant   | Safflower                      | <i>Carthamus tinctorius</i> L.                                                                 | Flower                  | 38.392                  |
| 325       | Plant   | Salvia miltiorrhiza root       | <i>Salvia miltiorrhiza</i> Bunge.                                                              | Root                    | 10.530                  |
| 326       | Plant   | Sambucus wood                  | <i>Sambucus williamsii</i> Hance.                                                              | Stem                    | 2.515                   |
| 327       | Plant   | Saposhnikovia root and rhizome | <i>Saposhnikovia divaricata</i> (Turcz. ex Ledeb.) Schischk                                    | Root (Root and Rhizome) | 4.650                   |
| Continued |         |                                |                                                                                                |                         |                         |

| No        | Species | English name                    | Scientific name                                                              | Part                      | Limulus activity (µg/g) |
|-----------|---------|---------------------------------|------------------------------------------------------------------------------|---------------------------|-------------------------|
| 328       | Plant   | Sappan wood                     | <i>Biancaea sappan</i> (L.) Tod.                                             | Stem (Heart wood)         | 0.198                   |
| 329       | Plant   | Sargentgloryvine stem           | <i>Sargentodoxa cuneata</i> (Oliv.) Rehder & E.H.Wilson                      | Stem                      | 1.488                   |
| 330       | Plant   | Sarsaparilla                    | <i>Smilax purhampuy</i> Ruiz.                                                | Root (Root and Rhizome)   | 24.176                  |
| 331       | Plant   | Saussurea root                  | <i>Dolomiaea costus</i> (Falc.) Kasana & A.K.Pandey                          | Root                      | 5.609                   |
| 332       | Plant   | Schizonepeta spike              | <i>Nepeta tenuifolia</i> Benth.                                              | Flower (Spike)            | 0.596                   |
| 333       | Plant   | Scisandra fruit                 | <i>Schisandra chinensis</i> (Turcz.) Baill                                   | Fruit                     | 0.022                   |
| 334       | Plant   | Scrophularia root               | <i>Scrophularia ningpoensis</i> Hemsl.                                       | Root                      | 1.916                   |
| 335       | Plant   | Scutellaria root                | <i>Scutellaria baicalensis</i> Georgi                                        | Root                      | 2.485                   |
| 336       | Plant   | Sea buckthorn, Argasse          | <i>Hippophae rhamnoides</i> L.                                               | Fruit                     | 0.024                   |
| 337       | Plant   | Seaweed                         | <i>Sargassum fusiforme</i> (Harvey) Setchell                                 | Whole plant               | 2.668                   |
| 338       | Plant   | Senna Leaf                      | <i>Senna alexandrina</i> var. <i>alexandrina</i>                             | Leaf                      | 0.206                   |
| 339       | Plant   | Sesame                          | <i>Sesamum indicum</i> L.                                                    | Seed                      | 2.004                   |
| 340       | Plant   | Sheep sorrel                    | <i>Rumex acetosella</i> subsp. <i>pyrenaicus</i> (Pourr. ex Lapeyr.) Akeroyd | Whole plant               | 6.392                   |
| 341       | Plant   | Shiny bugleweed                 | <i>Lycopus lucidus</i> Turcz. ex Benth.                                      | Stem and Leaf             | 1.364                   |
| 342       | Plant   | Siberian cocklebur fruit        | <i>Xanthium strumarium</i> L.                                                | Fruit                     | 0.098                   |
| 343       | Plant   | Siberian ginseng                | <i>Eleutherococcus senticosus</i> (Rupr. & Maxim.) Maxim.                    | Root                      | 35.216                  |
| 344       | Plant   | Silktree Albizia bark           | <i>Albizia julibrissin</i> Durazz.                                           | Stem (Bark)               | 0.927                   |
| 345       | Plant   | Sinomenium stem                 | <i>Sinomenium acutum</i> (Thunb.) Rehder et E.H.Wilson                       | Stem                      | 9.065                   |
| 346       | Plant   | Smilax rhizome                  | <i>Smilax glabra</i> Roxb.                                                   | Root (Rhizome)            | 0.047                   |
| 347       | Plant   | Snowbell-leaf tickclover herb   | <i>Grona styracifolia</i> (Osbeck) H.Ohashi & K.Ohashi                       | Stem and Leaf             | 13.399                  |
| 348       | Plant   | Songaria cynomorium herb        | <i>Cynomorium coccineum</i> subsp. <i>songaricum</i> (Rupr.) J.Léonard       | Stem (Fleshy stem)        | 0.075                   |
| 349       | Plant   | Sophora japonica flower         | <i>Styphnolobium japonicum</i> (L.) Schott.                                  | Flower (Flower bud)       | 7.350                   |
| 350       | Plant   | Sophora root                    | <i>Sophora flavescens</i> Aiton                                              | Root                      | 4.822                   |
| 351       | Plant   | Sophora subprostrata root       | <i>Sophora tonkinensis</i> var. <i>tonkinensis</i>                           | Root                      | 7.001                   |
| 352       | Plant   | Sour cherry                     | <i>Prunus cerasus</i> L.                                                     | Fruit                     | 1.608                   |
| 353       | Plant   | Sparganium rhizome              | <i>Sparganium stoloniferum</i> (Buch.-Ham. ex Graebn.) Buch.-Ham. ex Juz.    | Root (Rhizome)            | 0.018                   |
| 354       | Plant   | Spatholobus suberectus stem     | <i>Spatholobus suberectus</i> Dunn.                                          | Stem (Vine)               | 0.927                   |
| 355       | Plant   | Spearmint                       | <i>Mentha spicata</i> L.                                                     | Whole plant (Aerial part) | 2.389                   |
| 356       | Plant   | Spicebush                       | <i>Lindera umbellata</i> Thunb.                                              | Stem                      | 0.168                   |
| 357       | Plant   | Spreading Hedyotis herb         | <i>Scleromitron diffusum</i> (Willd.) R.J.Wang                               | Whole plant               | 36.730                  |
| 358       | Plant   | Star anise                      | <i>Illicium verum</i> Hook.f.                                                | Fruit                     | 0.010                   |
| 359       | Plant   | Stellaria herb                  | <i>Stellaria media</i> (L.) Vill. L.                                         | Whole plant               | 22.730                  |
| 360       | Plant   | Stevia                          | <i>Stevia rebaudiana</i> (Bertoni) Bertoni                                   | Whole plant               | 1.488                   |
| 361       | Plant   | Stinging nettle, Nettle         | <i>Urtica dioica</i> L.                                                      | Leaf                      | 61.986                  |
| 362       | Plant   | Summer savory                   | <i>Satureja hortensis</i> L.                                                 | Leaf                      | 2.649                   |
| 363       | Plant   | Sunflower                       | <i>Helianthus annuus</i> L.                                                  | Flower                    | 0.120                   |
| 364       | Plant   | Sweet flag root                 | <i>Acorus calamus</i> L.                                                     | Root (Rhizome)            | 1.364                   |
| 365       | Plant   | Sweet hydrangea leaf            | <i>Hydrangea serrata</i> (Thunb.) Ser.                                       | Leaf                      | 0.853                   |
| 366       | Plant   | Sweet tea vine, Gospel herb     | <i>Gynostemma pentaphyllum</i> (Thunb.) Makino                               | Stem                      | 0.399                   |
| 367       | Plant   | Sweet violet                    | <i>Viola odorata</i> L.                                                      | Whole plant               | 61.986                  |
| 368       | Plant   | Sweet woodruff                  | <i>Galium odoratum</i> (L.) Scop.                                            | Leaf                      | 9.203                   |
| 369       | Plant   | Sweet wormwood herb             | <i>Artemisia annua</i> L.                                                    | Whole plant (Aerial part) | 1.138                   |
| 370       | Plant   | Tansy                           | <i>Tanacetum vulgare</i> L.                                                  | Whole plant               | 14.878                  |
| 371       | Plant   | Tarragon                        | <i>Artemisia dracunculus</i> L.                                              | Leaf                      | 2.860                   |
| 372       | Plant   | Tetragonia herb                 | <i>Tetragonia tetragonoides</i> (Pall.) Kuntze.                              | Whole plant               | 18.716                  |
| 373       | Plant   | Tokoro rhizome                  | <i>Dioscorea tokoro</i> Makino ex Miyabe                                     | Root (Rhizome)            | 3.107                   |
| 374       | Plant   | Tokyo violet herb               | <i>Viola philippica</i> var. <i>philippica</i>                               | Whole plant               | 11.377                  |
| 375       | Plant   | Tribulus fruit                  | <i>Tribulus terrestris</i> L.                                                | Fruit                     | 1.104                   |
| 376       | Plant   | Trichosanthes fruit             | <i>Trichosanthes kirilowii</i> Maxim.                                        | Fruit                     | 0.238                   |
| 377       | Plant   | Trichosanthes peel              | <i>Trichosanthes kirilowii</i> Maxim.                                        | Fruit (Peel)              | 2.418                   |
| 378       | Plant   | Trichosanthes root              | <i>Trichosanthes kirilowii</i> Maxim.                                        | Root                      | 0.180                   |
| 379       | Plant   | Trichosanthes seed              | <i>Trichosanthes kirilowii</i> Maxim.                                        | Seed                      | 0.029                   |
| 380       | Plant   | Trifoliate orange, Hardy orange | <i>Citrus trifoliata</i> L.                                                  | Fruit                     | 0.058                   |
| 381       | Plant   | Turmeric                        | <i>Curcuma longa</i> L.                                                      | Root (Rhizome)            | 21.095                  |
| Continued |         |                                 |                                                                              |                           |                         |

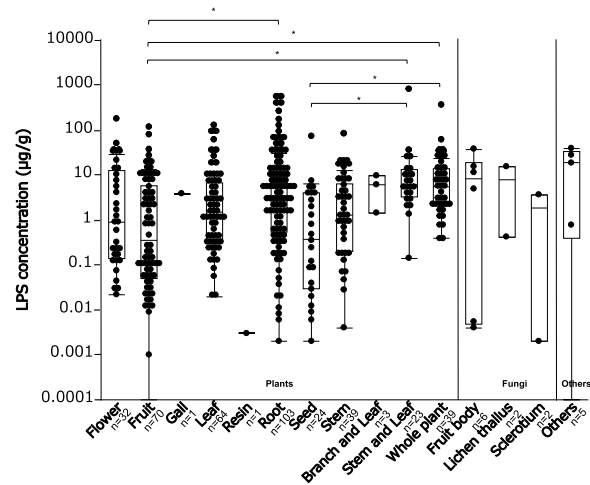
| No  | Species           | English name               | Scientific name                                                      | Part           | Limulus activity (µg/g) |
|-----|-------------------|----------------------------|----------------------------------------------------------------------|----------------|-------------------------|
| 382 | Plant             | Uncaria hook               | <i>Uncaria rhynchophylla</i> (Miq.) Miq.                             | Stem (Hook)    | 3.891                   |
| 383 | Plant             | Violet                     | <i>Viola</i> L.                                                      | Stem and Leaf  | 2.059                   |
| 384 | Plant             | Walnut                     | <i>Juglans</i> L.                                                    | Fruit (Hull)   | 11.472                  |
| 385 | Plant             | Walnut                     | <i>Juglans</i> L.                                                    | Leaf           | 6.561                   |
| 386 | Plant             | Walnut                     | <i>Juglans regia</i> L.                                              | Seed           | 0.033                   |
| 387 | Plant             | Water chestnut             | <i>Trapa natans</i> var. <i>bispinosa</i> (Roxb.) Makino             | Fruit          | 2.953                   |
| 388 | Plant             | Watercress                 | <i>Nasturtium officinale</i> R.Br.                                   | Stem           | 20.041                  |
| 389 | Plant             | Wheat                      | <i>Triticum aestivum</i> L.                                          | Seed           | 0.244                   |
| 390 | Plant             | White dead-nettle          | <i>Lamium album</i> subsp. <i>barbatum</i> (Siebold & Zucc.) Mennema | Stem and Leaf  | 25.365                  |
| 391 | Plant             | White horehound            | <i>Marrubium vulgare</i> L.                                          | Whole plant    | 7.782                   |
| 392 | Plant             | White sandalwood           | <i>Santalum album</i> L.                                             | Stem (Xylem)   | 0.176                   |
| 393 | Plant             | White willow               | <i>Salix alba</i> L.                                                 | Stem (Bark)    | 0.373                   |
| 394 | Plant             | Wild strawberry            | <i>Fragaria vesca</i> L.                                             | Leaf           | 2.286                   |
| 395 | Plant             | Witch hazel, Hamamelis     | <i>Hamamelis virginiana</i> L.                                       | Leaf           | 1.256                   |
| 396 | Plant             | Wormwood, Mugwort          | <i>Artemisia princeps</i> Pamp.                                      | Whole plant    | 2.059                   |
| 397 | Plant             | Yarrow                     | <i>Achillea millefolium</i> L.                                       | Whole plant    | 1.306                   |
| 398 | Plant             | Yerbadetajo herb           | <i>Eclipta prostrata</i> (L.) L.                                     | Stem and Leaf  | 6.155                   |
| 399 | Plant             | Yew                        | <i>Taxus brevifolia</i> Nutt.                                        | Leaf           | 0.487                   |
| 400 | Fungus            | Agaricus                   | <i>Agaricus blazei</i> Murill.                                       | Fruit body     | 0.005                   |
| 401 | Fungus            | Baikisei, Artist's bracket | <i>Ganoderma applanatum</i> (Pers.) Pat.                             | Fruit body     | 12.312                  |
| 402 | Fungus            | Ganoderma                  | <i>Ganoderma lucidum</i> P.Karsten                                   | Fruit body     | 0.004                   |
| 403 | Fungus            | Iceland moss               | <i>Cetraria islandica</i> (L.) Ach.                                  | Lichen thallus | 15.527                  |
| 404 | Fungus            | Jelly ear                  | <i>Auricularia auricula-judae</i> (Bull.) Quél                       | Fruit body     | 38.392                  |
| 405 | Fungus            | Meshima                    | <i>Tropicoporus linteus</i> (Berk. & M.A.Curtis) L.W.Zhou & Y.C.Dai  | Fruit body     | 4.945                   |
| 406 | Fungus            | Polyporus sclerotium       | <i>Polyporus umbellatus</i> Fries                                    | Sclerotium     | 3.673                   |
| 407 | Fungus            | Poria sclerotium           | <i>Wolfiporia cocos</i> Ryvarden & Gilbertson (Poria cocos Wolf)     | Sclerotium     | 0.002                   |
| 408 | Fungus            | Snow tea                   | <i>Thamnia vermicularis</i> (Sw.) Ach. ex Schaer                     | Lichen thallus | 0.422                   |
| 409 | Fungus            | Turkey tail                | <i>Trametes versicolor</i> (L.) Lloyd.                               | Fruit body     | 11.472                  |
| 410 | Other (non-plant) | Abalone shell              | <i>Haliotis diversicolor</i> Reeve, 1846                             | Shell          | 0.778                   |
| 411 | Other (non-plant) | Earthworm                  | <i>Pheretima aspergillum</i> Perrier                                 | Whole body     | 18.716                  |
| 412 | Other (non-plant) | Spirulina                  | <i>Arthrospira platensis</i> Gomont.                                 | Algae          | 39.662                  |
| 413 | Other (non-plant) | Troglodytes feces          | <i>Troglodytes xanthipes</i> (Milne-Edwards)                         | Feces          | 28.014                  |
| 414 | Other (non-plant) | Water buffalo horn         | <i>Bubalus bubalis</i>                                               | Horn           | 0.000                   |

**Table 1.** LPS concentrations of 414 samples of herbal extracts. For herbal extracts with multiple scientific names, the scientific names listed in this table are those most used in Japan.

(*Zingiber officinale* Roscoe) was comparable, and that of *Artemisia* leaf (*Artemisia princeps* Pamp.) and Bitter melon (*Momordica charantia* L.) was lower than that of LPSP. The Pearson correlation between the amount of LPS and phagocytosis showed a clear positive correlation at  $R = 0.474$ . This suggests that LPS in crude drugs may increase the phagocytosis ability of macrophages, but other factors may also be involved.

To compare the NO production ability of the 12 herbal extracts with that of the positive control LPSP, the dose–response curves of the 12 herbal extract samples are presented in Fig. 3. The 12 herbal extracts were divided based on the amount of LPS required to induce 5 µM more nitrite than LPSP. Oat, (*Avena sativa* L.), Sacred lotus (*Nelumbo nucifera* Gaertn.), Fortune's drynaria rhizome (*Drynaria roosii* Nakaike), and Couch grass (*Elytrigia repens* (L.) Gould) required a fewer samples per LPS content to induce 5 µM NO compared with LPSP (Fig. 3a). Corn silk (*Zea mays* L.), Bupleurum root (*Bupleurum falcatum* L.), Angelica dahurica root (*Angelica dahurica*), Common duckmeat (*Spirodela polyrrhiza* (L.) Schleid.), and Angelica dahurica root (*Angelica dahurica*) required equivalent amounts of LPSP (Fig. 3b), whereas Ginger (*Zingiber officinale* Roscoe), *Artemisia* leaf (*Artemisia princeps* Pamp.), and Bitter melon (*Momordica charantia* L.) required more samples per LPS content to induce 5 µM Nitrite compared with LPSP (Fig. 3c). Table 3 shows the amount of LPS content in each herbal extract required to induce 5 µM NO and the relative NO induction strength compared with LPSP.

NO production results suggested that herbal extracts containing high LPS levels can activate macrophages. Moreover, NO production was significantly inhibited by the reaction with polymyxin B, an LPS inhibitor. In addition, an LPS inhibitor was used by Montenegro et al. as a way to verify that NO-inducing activity is obtained from LPS. The 12 samples exhibited significant inhibition of NO production, with inhibition rates of 71–95% (Fig. 4). The decrease in NO production when polymyxin was added suggests that it is mostly the LPS content that is involved in the macrophage-activating capacity of these herbal extracts.



**Figure 1.** The distribution of the LPS concentration of the 414 samples measured using the Limulus reaction. The samples were divided into plants, fungi, and others. The plant samples were further categorized according to their parts. \*p-value < 0.05 for Steel–Dwass test.

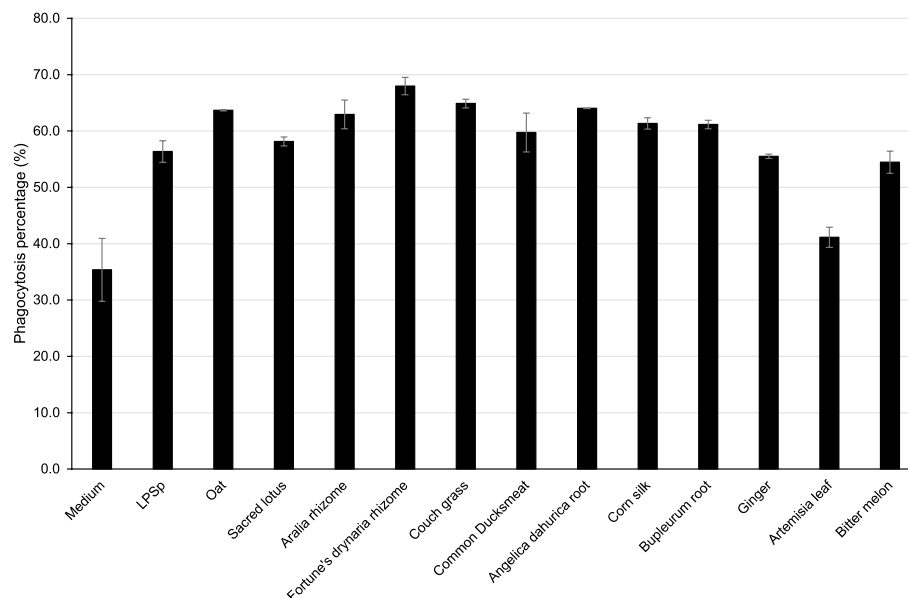
| Sample name                | Scientific name                                                      | Part          | Limulus activity (µg/g) |
|----------------------------|----------------------------------------------------------------------|---------------|-------------------------|
| Oat                        | <i>Avena sativa</i> L.                                               | Stem and leaf | 825.5                   |
| Sacred lotus, Lotus        | <i>Nelumbo nucifera</i> Gaertn.                                      | Root          | 624.5                   |
| Aralia rhizome             | <i>Aralia cordata</i> Thunb.                                         | Root          | 504.0                   |
| Fortune's drynaria rhizome | <i>Drynaria roosii</i> Nakaike                                       | Root          | 423.0                   |
| Couch grass, Quack grass   | <i>Elytrigia repens</i> (L.) Gould                                   | Root          | 392.6                   |
| Common ducksmeat           | <i>Spirodela polyrrhiza</i> (L.) Schleid.                            | Leaf          | 366.6                   |
| Angelica dahurica root     | <i>Angelica dahurica</i> (Hoffm.) Benth. & Hook.f. ex Franch. & Sav. | Root          | 266.6                   |
| Corn silk                  | <i>Zea mays</i> L.                                                   | Flower        | 180.1                   |
| Bupleurum root             | <i>Bupleurum falcatum</i> L.                                         | Root          | 148.5                   |
| Ginger                     | <i>Zingiber officinale</i> Roscoe.                                   | Root          | 122                     |
| Artemisia leaf             | <i>Artemisia princeps</i> Pamp.                                      | Leaf          | 121.8                   |
| Bitter melon               | <i>Momordica charantia</i> L.                                        | Fruit         | 118.5                   |

**Table 2.** Twelve herbal extract samples with significantly higher LPS content than the other samples. The samples are listed in the order of high concentration.

Discussion

Herbal extracts have several health-benefiting effects, such as hemostatic<sup>44,45</sup>, antifebrile<sup>46,47</sup>, detoxifying<sup>48</sup>, sweating<sup>49</sup>, and immunostimulating effects<sup>50</sup>, most of which are low molecular weight substances and have significantly contributed to the development of pharmaceuticals as the beginning of numerous medicines. LPS in herbal extracts supposedly causes this immunostimulating effect because previous LPS screening study revealed that some herbal extracts contain high LPS amounts (> 100 µg/g)<sup>32</sup> and previous studies have shown that the oral intake of LPS enhances immunity and effectively prevents and improves various diseases, including cancer, viral infection, atopic dermatitis, diabetes, atherosclerosis, and Alzheimer's disease<sup>38,51–53</sup>. Although there are more than several hundred herbal extracts worldwide and the possibility that the LPS in these herbal extracts playing a role in their functions is high, the LPS amount in them has never been measured or compared among the parts of plants from which they were obtained. Therefore, this study aimed to create a database of LPS levels in herbal extracts by measuring LPS levels in over 400 herbal extract samples stored at the Faculty of Pharmaceutical Sciences, Hokkaido University of Science, and to provide a basis for research to assess the immunostimulatory effects of herbal extracts and LPS's contribution to these effects.

Table 1 shows the amount of LPS in 414 herbal extracts. LPS concentrations were widely distributed from a few µg/g to several hundred µg/g (Fig. 1). LPS content was shown to be significantly higher in roots (107 samples) than in fruits (69 samples) or seeds (22 samples) in terms of LPS concentration. Of the 414 herbal extracts measured in this study, approximately 100 herbal extracts contained ≥ 10 µg/g of LPS. Twelve of the herbal extracts exhibited very high LPS levels of over 100 µg/g. Comparison among vascular plant parts showed that the overall LPS level in root-derived herbal extracts was high and significantly higher than that in seed- and fruit-derived herbal extracts. Over half (seven) of the 12 high-LPS-content herbal extracts were root-derived. Most vascular plants are symbiotic with soil bacteria in their roots<sup>54–56</sup>. Symbiotic bacteria in soil promote plant growth through



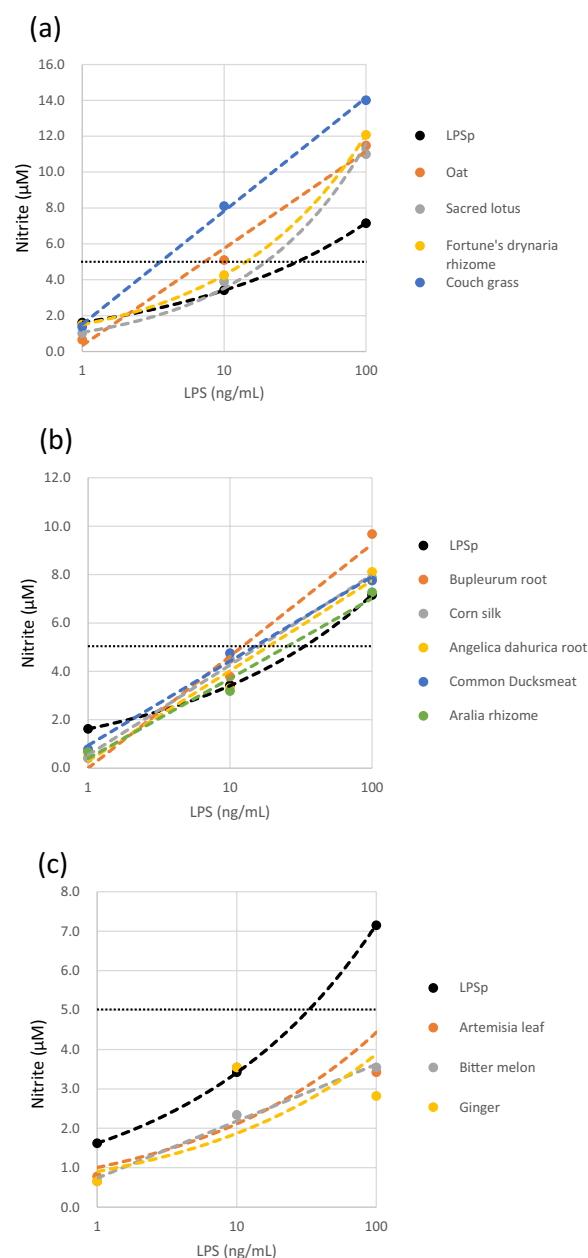
**Figure 2.** The percentage of phagocytic activity of RAW 264.7 cells stimulated by the 12 herbal extract samples containing the highest LPS levels are listed in Table 2. The concentrations of herbs and LPS added were adjusted so that the LPS concentration was 100 ng/ml. The dotted line represents the phagocytosis percentage of RAW 264.7 cells without any external stimulation (medium only). Each bar represents the mean of two independent measurements, and the error bars represent the standard deviation.

their involvement in nitrogen fixation, nutrient supply, and disease defense. Such bacteria are called plant growth-promoting rhizospheric microorganisms (PGPR)<sup>57</sup>; among them, bacteria of the genera *Pseudomonas*, *Azospirillum*, *Bradyrhizobium*, and *Rhizobium* are particularly essential. These bacteria are gram-negative bacteria and, therefore, may contribute to the high-LPS-content in the roots of herbal extracts. Montenegro et al. reported that 519 genera of bacteria are found in *Angelica sinensi*, a root-derived herbal extract that constitutes Juzen Daihoto, a Chinese herbal medicine known for its immunostimulating properties<sup>43</sup>. Among them, *Rahnella*, a gram-negative bacterium found in soil and fresh water, is abundant in *Angelica sinensi*. It was stipulated that the LPS content in *Angelica sinensi* is involved in the immunity-enhancing effects of Juzen Daihoto. The LPS content of *Angelica sinensi* (also called *Angelica acutiloba* Kitag. in Japan) was also measured in this study and it was shown that it contained 16 µg/g LPS, the 61st highest LPS content among all 414 samples in Table 1 (herb sample no. 202). These results suggest that the LPS amount in the root-derived herbal extract correlates with the number of soil-derived microorganisms that symbiotically coexist with the root-derived microorganisms during growth. These microorganisms are mostly gram-negative bacteria that contain a high LPS amount. On the other hand, the variation within each part group is large, suggesting that the high or low LPS content may not so much dependent on the part of the sample.

The amount of LPS contained in plants is considered to be derived from symbiotic bacteria. Therefore, the type and amount of symbiotic bacteria may vary depending on the origin of the plant, time of collection, variety, and cultivation method. Consequently, it is meaningful to measure multiple samples, but it is difficult to obtain multiple lots of crude drugs because most of them are imported. Therefore, we decided to use the variation in LPS content of one crude drug, brown rice, as a model for the variation in a single crude drug sample. In a previous study, we obtained brown rice from 15 different locations in Japan and measured LPS content in the  $10.9 \pm 4.3$  µg/g range<sup>58</sup>. Although the LPS content of brown rice may not necessarily be universalizable to other crude drugs, we believe that this can be used as a reference value for the degree of variation in LPS content. The range of LPS content in this one sample was relatively stable compared to the range of 0.001–100 µg/g in the LPS content data (Table 1, Fig. 1) obtained for individual crude drugs. Therefore, based on this fact, we conducted the experiment with the belief that the approximate degree of LPS content could be evaluated with a single sample.

In this study, Limulus amoebocyte lysate (LAL) test was used to detect LPS in the herbal extracts. However, it has been reported that β-1,3-glucan also reacts with LAL, so, there is a possibility of measuring plant-derived β-1,3-glucan contaminant with ordinary LAL. In this study, this contamination is prevented by using an LAL test kit containing a carboxymethylated curdlan which has reported act as a blocker of β-1,3-glucan mediated coagulation pathway<sup>59</sup>. Therefore, the limulus activity detected in this study were specific to LPS.

The macrophage-activating ability of LPS is a fundamental LPS action<sup>34</sup>. Therefore, the macrophage activation potential of herbal extracts by phagocytosis and NO production was assessed using macrophage-like RAW 264.7 cells. RAW264.7 cells transduce LPS signaling via TLR4<sup>60</sup>. In addition, many mammalian innate immune system cells, including humans, express TLR4<sup>61</sup>. Therefore, even though this study used mouse macrophage cells as a representative model, it is safe to assume that LPS contained in crude drugs is functional for mammals in general, including humans. However, further research is needed to determine the effects of LPS in humans,

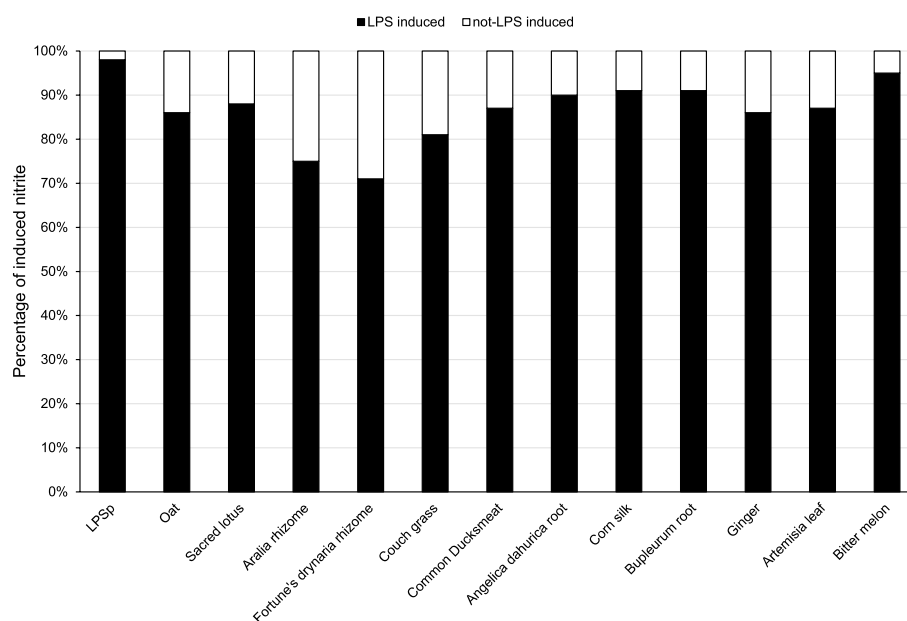


**Figure 3.** Dose–response curve of macrophage activation capacity determined by measuring the amount of NO produced as the amount of nitrite produced by RAW 264.7 cells stimulated by adding 1, 10, and 100 ng/ml per LPS to the 12 herbal extract samples containing the highest LPS content listed in Table 2. The amount of LPS needed to induce 5 μM more nitrite than LPSp used as control is (a) less than LPSp, (b) equivalent to LPSp, and (c) more than LPSp in this group. The dotted lines represent 5 μM Nitrite. The trendline equations (dashed lines) and R<sup>2</sup> of each line are listed in Table 3.

especially when administered orally. Twelve samples containing particularly high amounts of LPS (100 μg/g) were examined using these methods. The results showed that herbal extracts increased the phagocytosis capability of RAW 264.7 cells (Fig. 2). The NO production by RAW 264.7 cells caused by these samples was found to be higher, similar, or lower than purified LPSp, depending on the 12 herbal extracts (Fig. 3). The LPS itself in the group that exhibited higher activity may display high macrophage activation. However, it is speculated that a synergistic effect with macrophage activators, such as bacterial-derived nucleic acids, peptidoglycans, and flagellin, may be observed. Conversely, those that exhibited weaker activity than LPSp derived from *Enterobacteriaceae* may be because of the nature of the symbiotic gram-negative bacteria, as some LPSs, such as Bacteroides, are weak in biological activity, which depends on their lipid A structure<sup>62,63</sup>. Additionally, NO production was significantly (> 70%) reduced in all RAW 264.7 cells stimulated with 12 herbal extracts when polymyxin B, an LPS inhibitor, was added (Fig. 4). These results suggest that LPS is responsible for most of the macrophage activation potential of herbal extracts. However, the strength of the macrophage-activating ability of the herbal extracts is not

| Samples                    | LPS (ng/mL)/nitrite 5 $\mu$ M | Relative NO induction strength (LPSp) | Trendline equation          | R <sup>2</sup> value |
|----------------------------|-------------------------------|---------------------------------------|-----------------------------|----------------------|
| LPSp                       | 32.8                          | 1.0                                   | $y = 1.6218x^{0.3226}$      | 1.00                 |
| Oat                        | 7.3                           | 4.5                                   | $y = 2.3505\ln(x) + 0.3357$ | 0.99                 |
| Sacred lotus               | 19.7                          | 1.7                                   | $y = 1.0709x^{0.517}$       | 0.99                 |
| Aralia rhizome             | 24.7                          | 1.3                                   | $y = 1.4364\ln(x) + 0.3958$ | 0.98                 |
| Fortune's drynaria rhizome | 14.3                          | 2.3                                   | $y = 1.4994x^{0.4533}$      | 1.00                 |
| Couch grass                | 3.6                           | 9.2                                   | $y = 2.7422\ln(x) + 1.5183$ | 1.00                 |
| Common Ducksmeat           | 14.6                          | 2.2                                   | $y = 1.5147\ln(x) + 0.937$  | 0.99                 |
| Angelica dahurica root     | 18.3                          | 1.8                                   | $y = 1.6192\ln(x) + 0.2956$ | 0.97                 |
| Corn silk                  | 15.8                          | 2.1                                   | $y = 1.6192\ln(x) + 0.5361$ | 1.00                 |
| Bupleurum root             | 12.0                          | 2.7                                   | $y = 2.011\ln(x) - 0.0051$  | 0.98                 |
| Ginger                     | 222.7                         | 0.1                                   | $y = 0.903x^{0.3166}$       | 0.64                 |
| Artemisia leaf             | 145.2                         | 0.2                                   | $y = 1.006x^{0.3221}$       | 0.73                 |

**Table 3.** The equivalent amount of herbal extracts per LPS content needed to induce 5  $\mu$ M nitrite, which is the relative nitrite induction strength compared with LPSp. The trendline equations and R<sup>2</sup> of each line in Fig. 3 are also listed.



**Figure 4.** Macrophage activation potential determined by measuring the NO production of RAW 264.7 cells stimulated by the 12 herbal samples containing the highest LPS levels listed in Table 2. The percentage of NO produced by RAW 264.7 cells stimulated by LPS content (black bars) and other components (white) in the herbal extract samples. The concentrations of herbs and LPSp added were adjusted, making the LPS content 10 ng/mL. The black area represents the percentage of induced NO<sub>2</sub> being decreased following polymyxin B addition, representing the percentage of NO<sub>2</sub> induced by the LPS content in the herb samples. Each bar represents the mean of two independent measurements, and the error bars represent the standard deviation.

proportional to the amount of LPS contained and may significantly differ among various symbiotic bacteria. Therefore, in studying the innate immune activation potential of herbal extracts, it is necessary to assess and clarify their unique qualities.

Herbal extracts are often prescribed in daily doses of 1–10 g<sup>64,65</sup>. Of the 414 herbal extracts for which LPS levels were measured in this study, 98 contained over 10  $\mu$ g/g LPS, and oral intake of LPS increased the phagocytic activity of abdominal macrophages in mice at 10  $\mu$ g/kg BW for 7 days<sup>34</sup>, induced increase in capillary vascularity at 10  $\mu$ g/kg BW in human randomized control trial studies<sup>66</sup>, and in fish, 5–20  $\mu$ g/kg BW increased the ability to prevent infection<sup>67</sup>. Based on these studies, 10  $\mu$ g/kg BW of LPS can activate innate immunity, which is 500  $\mu$ g/day for a 50 kg human. Therefore, consuming a daily dose of herbal extracts may mean taking in an effective amount of LPS, meaning that LPS may contribute to the medicinal effects of the herbal extracts. Juzen Daiho-to, a combination of herbal extracts, reportedly has preventive and ameliorative effects against diabetes and cancer partly because LPS is one of its ingredients<sup>68,69</sup>. The 414 herbal extract samples measured in this study are much

greater than the 157 listed in the Japanese Pharmacopoeia. These should be sufficient populations for primary screening based on the efficacy of oral LPS intake over immune functions and the activation of immune cells using macrophages and other cells in herbal extracts. However, because the LPS content of plants is obtained from the symbiotic gram-negative bacterial population and may differ greatly depending on the time of collection, variety, cultivation method, etc., the LPS content of the samples to be studied should be analyzed with caution on a sample-by-sample basis.

## Methods

### Sample preparation

All dried samples were purchased from Tochimoto Tenkaido Co., Ltd. (Osaka, Japan). The dried samples were pulverized, and 100 mg powdered samples were extracted in 1 ml distilled water for 20 min at 90 °C. Subsequently, the samples were sonicated for 20 min and vortexed for two minutes to extract LPS. Next, the supernatants were obtained after centrifugation at 830 × g for 15 min. All methods involving the dried samples were carried out in accordance with relevant guidelines<sup>70</sup>.

### Measurement of the LPS contents of herbal extracts

The LPS concentration in the samples were assayed using the kinetic–turbidimetric method. All samples were diluted 10,000-fold using pyrogen-free distilled water. Sample supernatants (0.2 ml) were added to LAL-ES in a glass tube (Limulus ES-II single test; Wako Pure Chemical Industries Ltd., Osaka, Japan). After a few seconds of vortexing, the gelation time was measured using a Toxinometer ET-6000 (Wako Pure Chemical Industries Ltd.), and the specific activity was calculated using an LS Toximaster (Wako Pure Chemical Industries Ltd.), a data acquisition program for the Toxinometer.

The LAL test kits of Wako contain carboxymethylated curdlan in freeze-dried reagents, which stops  $\beta$ -D-glucans from triggering an interference in the test. Therefore, this test kit used in this study is specific to LPS<sup>59</sup>.

### Phagocytosis assay

Phagocytic activity was measured using flow cytometry as previously described with minor modifications<sup>71</sup>. Briefly, the mouse macrophage/monocyte cell line RAW 264.7 cells (obtained from TIB-71; ATCC, Manassas, VA, USA) were treated for 18 h with extracts in a 48-well plate. The extract concentrations were measured so that the LPS content was 100 ng/ml. Next, fluorescent latex beads (Fluoresbrite® YG Microspheres 1.0  $\mu$ m; Polysciences, Warrington, PA) at a cell: bead ratio of 1:10 were added and incubated for one hour. Cells were washed to eliminate non-internalized particles and detached from the well plate with 0.25% trypsin treatment (Life Technologies, Carlsbad, CA, USA). The phagocytosis rate of the cells was measured using a Beckman Coulter Gallios flow cytometer and Kaluza software (Beckman Coulter, Indianapolis, IN).

### Nitric oxide (NO) production by murine macrophages

In a 48-well plate, cells from the mouse macrophage/monocyte cell line RAW 264.7 were plated at  $8 \times 10^5$  cells/ml and treated with herbal extracts. The added extract concentrations were measured, so that the LPS content was 1, 10, and 100 ng/ml. The plate was incubated at 37 °C and 5% CO<sub>2</sub>. After 24-h incubation with extracts, the supernatants were collected, and the concentrations of nitrite (NO<sup>2-</sup>) released into the culture media were measured using Griess reagent. In addition, 100  $\mu$ l Griess reagent was added to 100  $\mu$ l diluted culture media in the wells of microtiter plates. After incubation at room temperature for ten minutes, absorbance at 570 nm was determined using an automated microplate reader (BIO-RAD, Hercules, CA, USA). The NO assay was conducted in duplicate. To determine the percentage of NO produced by the LPS in the herbal extracts, the concentrations of the extracts were measured, so that the LPS content was 10 ng/ml, and polymyxin B (Sigma-Aldrich, St. Louis, MO, USA) was added to each culture at a final concentration of 10  $\mu$ g/ml.

### Statistical analysis

Data are presented as mean  $\pm$  standard deviation (SD). Statistical analyses (Steel–Dwass test and Pearson's correlation) were performed using the JMP statistical software, version 17.0.0 (SAS Institute Inc., Cary, NC, USA). Statistical differences between multiple groups in the box-and-whisker plot were calculated using the Steel–Dwass test. A p-value < 0.05 was considered statistically significant. The line equation and its R<sup>2</sup> value in Table 3 were performed using Microsoft Excel.

### Data availability

All data generated or analyzed during this study are included in this published article.

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## Author contributions

Conceptualization, K.W., H.I. and G.S.; methodology, H.I.; software, M.O. and M.Y.; validation, M.Y.; formal analysis, M.Y.; investigation, M.Y.; resources, K.W.; data curation, M.Y., T.I., Y.F.; writing—original draft preparation, V.T. and M.O.; writing—review and editing, V.T., M.O., T.I., K.W., H.I. and G.S.; visualization, M.Y.; supervision, H.I. and G.S. All authors have read and agreed to the published version of the manuscript.

## Competing interests

The authors declare no competing interests.

## Additional information

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