Antioxidant and Anti-Inflammatory Effects of Genus Gynura: A Systematic Review

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Background: Gynura species have been used traditionally to treat various ailments, such as fever, pain, and to control blood glucose level. This systematic review critically discusses studies regarding Gynura species that exhibited antioxidant and anti-inflammatory effects, thus providing perspectives and instructions for future research of the plants as a potential source of new dietary supplements or medicinal agents.

Methods: A literature search from internet databases of PubMed, Scopus, Science Direct, e-theses Online Service, and ProQuest was carried out using a combination of keywords such as “Gynura,” “antioxidant,” “anti-inflammatory,” or other related words. Research articles were included in this study if they were experimental (in vitro and in vivo) or clinical studies on the antioxidant or anti-inflammatory effects of Gynura species and if they were articles published in English.

Abbreviations: ADI, acceptable daily intake; AOM, azoxymethane; BW, body weight; CAT, catalase; CCl4, carbon tetrachloride; COX-2, cyclooxygenase-2; CRP, c-reactive protein; DCFH-DA, 2′,7′-dichlorofluorescin diacetate; DMSO, dimethyl sulfoxide; EAE, enzyme-assisted extraction; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; EMSA, electrophoretic mobility shift assay; ESI-MS, electrospray ionization-mass spectrometry; ESI-TOF-MS, electrospray ionization-time-of-flight mass spectrometry; ETHOS, e-theSES online service; FRSA, free radical scavenging activity; GC-MS, gas chromatography-mass spectrometer; GPT, glutamate pyruvate transaminase; GR, glutathione reductase; GSH, glutathione; GSH-S-transferase; GSTR, glutathione-S-transferase; GSTR1, human glutathione-S-transferase 1; H, human; H2O2, hydrogen peroxide; HDFs, human dermal fibroblasts; HPLC, high-performance liquid chromatography; HP-TLC, high-performance thin-layer chromatography; HSOS, sinusoidal obstruction syndrome; huvec, human umbilical vein endothelial cell; HVOD, hepatic veno-occlusive disease; i.p., intraperitoneal; IC50, inhibitory concentration 50%; IFN-γ, interferon-gamma; IL, interleukin; iNOS, inducible nitric oxide synthase; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; MBI, matrix metalloproteinase-1; mRNA, messenger ribonucleic acid; NF-κB, nuclear factor kappa B; NMR, nuclear magnetic resonance; NO, nitric oxide; Nrf2, nuclear factor erythroid 2-related factor 2; OHAT, of other related words. Research articles were included in this study if they were experimental (in vitro and in vivo) or clinical studies on the antioxidant or anti-inflammatory effects of Gynura species and if they were articles published in English.

Abbreviations: ADI, acceptable daily intake; AOM, azoxymethane; BW, body weight; CAT, catalase; CCl4, carbon tetrachloride; COX-2, cyclooxygenase-2; CRP, c-reactive protein; DCFH-DA, 2′,7′-dichlorofluorescin diacetate; DMSO, dimethyl sulfoxide; EAE, enzyme-assisted extraction; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; EMSA, electrophoretic mobility shift assay; ESI-MS, electrospray ionization-mass spectrometry; ESI-TOF-MS, electrospray ionization-time-of-flight mass spectrometry; ETHOS, e-theSES online service; FRSA, free radical scavenging activity; GC-MS, gas chromatography-mass spectrometer; GPT, glutamate pyruvate transaminase; GR, glutathione reductase; GSH, glutathione; GSH-S-transferase; GSTR, glutathione-S-transferase; GSTR1, human glutathione-S-transferase 1; H, human; H2O2, hydrogen peroxide; HDFs, human dermal fibroblasts; HPLC, high-performance liquid chromatography; HP-TLC, high-performance thin-layer chromatography; HSOS, sinusoidal obstruction syndrome; huvec, human umbilical vein endothelial cell; HVOD, hepatic veno-occlusive disease; i.p., intraperitoneal; IC50, inhibitory concentration 50%; IFN-γ, interferon-gamma; IL, interleukin; iNOS, inducible nitric oxide synthase; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; MBI, matrix metalloproteinase-1; mRNA, messenger ribonucleic acid; NF-κB, nuclear factor kappa B; NMR, nuclear magnetic resonance; NO, nitric oxide; Nrf2, nuclear factor erythroid 2-related factor 2; OHAT, of other related words. Research articles were included in this study if they were experimental (in vitro and in vivo) or clinical studies on the antioxidant or anti-inflammatory effects of Gynura species and if they were articles published in English.
INTRODUCTION

Inflammation refers to a complex array of defensive immune responses (Arulselvan et al., 2016), and tissue damage is one of the consequences of an exaggerated or uncontrolled prolonged inflammatory process (Biswas, 2016). Inflammatory cells, including neutrophils and macrophages, generate free radicals at the inflammation site, where reactive oxygen species (ROS) (e.g., hydroxyl radicals, superoxide anion radicals, and hydrogen peroxide) act as both signaling molecules and inflammation mediators. Enhancement of pro-inflammatory gene expression can be achieved via the initiation of the intracellular signaling cascade by reactive species. The exaggerated generation of reactive species in pathological inflammatory conditions may induce localized oxidative stress and tissue injury, thus promoting progression of many inflammatory diseases (Mittal et al., 2014). Hence, inflammation and oxidative stress are highly interdependent pathophysiological events in various types of chronic diseases (Vaziri and Rodriguez-Iturbe, 2006; Biswas, 2016).

A total of 46 species are identified in the genus Gynura (The Plant List, 2020). They are distributed from tropical Africa to South and East Asia as well as Australia where the highest specific diversity is found in Southeast Asia (Vanijajiva and Kadereit, 2011). The fresh leaves of G. procumbens (Lour.) Merr. are traditionally consumed to control blood glucose level by Orang Asli in Kampung Bawong, Perak, West Malaysia (Samuel et al., 2010). G. procumbens is also traditionally used to treat kidney discomfort, inflammation, rheumatic fever, and viral ailments (Wiart, 2006). G. pseudochirina (L.) DC. is traditionally used to treat fever and sore eye (Davies, 1980). In Chinese folk medicine, G. segetum (Lour.) Merr. is consumed as a decoction or is soaked in wine and orally taken to promote microcirculation or relieve pain (Chen et al., 2011). In Nepal, the juice of G. nepalensis DC. is applied on cuts and wounds as healing treatment (Manandhar, 2002).

Incorporation of a plant-based natural antioxidant in daily diet can prevent several human illnesses (Knekt et al., 1996) as the prevalence of many illness is inversely linked to the dietary consumption of antioxidant-rich foods (Sies, 1993). For any herb to be developed into a dietary supplement, it is essential that the evidence for the claimed effects of the herb is scientifically demonstrated. Ideally, the mechanisms of action should also be understood. The potential use of Gynura as a phytomedicine with antioxidant and anti-inflammatory properties has been well documented, but we were unable to identify specific review articles that focused on the antioxidant and anti-inflammatory effects of the Gynura species. This review was undertaken to assess published experimental data that investigated the antioxidant and anti-inflammatory activities of the Gynura species to support the design of future studies. It also provided an evaluation of the quality of available information, summarized mechanisms of action data from animal and cell studies following the administration of the Gynura species, and identified research gaps in the literature.

METHODS

This systematic review was carried out according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline (Moher et al., 2010). The quality of
references was determined by referring to the Office Health Assessment and Translation for Conducting a Systematic Review and Evidence Integration (OHAT) guideline (Rooney et al., 2014).

**Search Strategy**

The information was obtained through a comprehensive literature search using the electronic databases of PubMed, Scopus, and Science Direct from 2000 to March 2020 for journal articles and the databases of e-theses Online Service and ProQuest Dissertations and Theses Global for theses. The combination of keywords used in PubMed was as follows: [Gynura (Title/Abstract)] AND [antioxidant (Title/Abstract)] OR [oxidant (Title/Abstract)] OR [antioxidant (Title/Abstract)] OR [antioxidant (Title/Abstract)] OR [antioxidant (Title/Abstract)] OR [antioxidant (Title/Abstract)]. The reference lists of all included papers were checked for other potentially relevant citations. Studies selection was restricted to articles in English because of language barrier, time efficiency, and high cost for translation. However, only four studies were excluded on the basis of not being published in English, which is unlikely to impact our findings. In order to achieve a comprehensive search of relevant studies, university dissertations, and theses were accessed in the selection process. However, the confidentiality of these tools had possibly veiled some important information and results, for example, negative findings.

**Inclusion and Exclusion Criteria**

Studies were included in this review if they were experimental studies (in vitro and in vivo) or clinical studies on the antioxidant or anti-inflammatory effects of Gynura and if they were articles published in English. The Gynura plant can be from any parts of the plant and in any form such as extracts, essential oils, fractions, or isolated compounds. Studies were excluded if they met at least one of the following criteria: 1) study models were not accepted as evidence for pharmacological effects (i.e., antioxidant experiments of FRAP, ABTS, DPPH, and Trolox equivalent antioxidant capacity assays), and 2) intervention was not focused on Gynura, although the antioxidant or anti-inflammatory effects were measured. Review articles and book chapters were excluded from this study, but the references were mined to search for further relevant studies.

**Data Extraction and Handling**

The following details were extracted from each selected study: 1) the species and part of the Gynura used; 2) methods used, including animal species or cell lines, study design, and treatment details; 3) outcome measures; and 4) findings on the antioxidant and anti-inflammatory effects of Gynura.

**Quality Assessment**

The reporting completeness of the material used by each selected study was assessed using the information of the Gynura species material, voucher specimens, report on quality control of extract and chemical analysis. The risk of bias in the included studies was assessed by two investigators based on the OHAT guideline for any potential bias such as selection bias, performance bias, attrition or exclusion bias, detection bias, selection reporting bias, and other sources of bias. However, some of the questions in the bias assessment were excluded as they were not applicable for use in the assessment of in vitro, in vivo, and human randomized control study designs.

**RESULTS AND DISCUSSION**

**Study Selection**

A total of 183 studies were found from the database search, and 11 additional articles were identified from other sources. After removing the duplicates, 125 articles were shortlisted, and after title–abstract screening, 57 articles were excluded because of the following reasons: The articles were published in languages other than English (n = 4), the articles are non-experimental journal articles (n = 10), and the articles reported on the effects of Gynura other than antioxidant and anti-inflammatory (n = 43). By full-text screening of the remaining 68 articles, a total of 41 articles were eliminated based on the following exclusion criteria: study models used are not accepted as evidence for pharmacological effects (n = 25), study intervention does not focus on Gynura (n = 1), and a combination of both exclusion criteria (n = 15). Thus, 27 articles from year 2002 to 2020 were selected in the final qualitative analysis of this systematic review. Three potential dissertations and theses were accessed through the two databases. Only one of the theses was selected, but the result presented in the theses has been published in one of the article selected in this review (Siriwatanametanon et al., 2010). Other two theses were excluded because they did not fulfill the inclusion criteria. A flowchart depicting the search process and study selection is presented in Figure 1.

**Quality Assessment**

All selected articles were assessed for their quality and risk of bias. Table 1 shows the quality assessment of studies on the antioxidant and anti-inflammatory effects of Gynura species. The reporting completeness of the material in all selected studies was assessed using information of the Gynura species material, voucher specimens, quality control, and chemical analysis. Naming inconsistency between studies was detected as full botanical taxonomic names were not stated in 13 selected studies (Iskander et al., 2002; Siriwanametanon and Heinrich, 2011; Akowuah et al., 2012; Seow et al., 2014; Chao et al., 2015; Wong et al., 2015; Xu et al., 2015; Rerknimitr et al., 2016; Yin et al., 2017; Rahman et al., 2018; Pai et al., 2019; Yang et al., 2019; Chandradevan et al., 2020). All plant species need to be validated taxonomically, and the full names have to be clearly stated as the incomplete name of plant species can lead to confusion of readers and any recorded uses or properties attributed may, in fact, correlate to different species. Such erroneous publications on the use of plant names are a permanent source of confusion for future research, search engines, and databases (Rivera et al., 2014). An incomplete plant name in some contexts may be ambiguous or may even mislead readers. This error is possible to occur within genus Gynura. Gynura divaricata is no guarantee to solely indicate Gynura divaricata (L.) DC. as Gynura divaricata subsp. barbareifolia (Gagneıp.) F.G.Davies.
The flowchart shows the selection process of (A) the journal articles, (B) university dissertations, and theses in this systematic review based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline.
| Study | Species stated in article | Plant source | Authenticated species | Quality control reported? | Chemical analysis reported? |
|-------|--------------------------|--------------|----------------------|---------------------------|----------------------------|
| Iskander et al. (2002) | Gynura procumbens | Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand | – | No | No |
| Akowuah et al. (2012) | Gynura procumbens (Merr., compositae) | Penang Island, Malaysia | + | No | No |
| Kim et al. (2011) | Gynura procumbens (Lour.) Merr | Specialty Natural Products Co. Ltd., Thailand | + | No | Yes—HPLC, electrospray ionization time-of-flight mass spectrometry |
| Shwter et al. (2014) | Gynura procumbens (Lour.) Merr | Ethno Resources Sdn Bhd, Malaysia | + | No | No |
| Wong et al. (2015) | Gynura procumbens | Green House Facility, Faculty of Science and Technology, Universiti Kebangsaan Malaysia (UKM) | + | No | No |
| Huang et al. (2019) | Gynura procumbens (Lour.) Merr | Hainan Province, South China | + | No | Yes—GC-MS |
| Liu M. et al. (2019) | Gynura procumbens (Lour.) Merr | Jing’an, Jiangxi Province, China | – | No | Yes—ultra-high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry |
| Nazi et al. (2019) | Gynura procumbens (Lour.) Merr | Semenyih, Selangor, Malaysia | + | No | Yes—LC-MS/MS |
| Ning et al. (2019) | Gynura procumbens (Lour.) Merr | Brightmark Sdn. Bhd, Semenyih, Malaysia | + | No | No |
| Liu Y. et al. (2019) | Gynura procumbens (Lour.) Merr | Shang Ji Tintai Biol, China | – | No | Yes—LC-MS/MS |
| Chandradevan et al. (2020) | Gynura procumbens | Net house located in the Malaysia Agricultural Research and development institute (MARDI) | + | No | Yes—1H NMR spectroscopy |
| Wu et al. (2013) | Gynura bicolor (Roxb. and Willd.) DC. | Yuanshan Village, Ilan, Taiwan | + | No | Yes—HPLC |
| Chao et al. (2015) | Gynura bicolor DC. | Farms in Puli Town, Nanton county, Taiwan | + | No | No |
| Yin et al. (2017) | Gynura bicolor DC. | Farms in Puli Town, Nanton county, Taiwan | + | No | No |
| Pai et al. (2019) | Gynura bicolor | Farms, unknown location | – | No | No |
| Yang et al. (2019) | Gynura bicolor DC. | Farms, unknown location | – | No | No |
| Sriwatanametanon et al. (2010) | Gynura pseudochina (L.) DC. var. hispida Thn | Farmerland in north-eastern part of Thailand, mainly in Buriram province | + | No | No |
| Sriwatanametanon and Heinrich (2011) | Gynura pseudochina (L.) var. hispida Thn | Not stated | – | No | Yes—electrospray ionization-mass spectrometry, 1H NMR, 13C NMR, 2D-NMR |
| Rekinmitir et al. (2016) | Gynura pseudochina DC. var. hispida Thn | Not stated | – | No | No |
| Sukadeetad et al. (2018) | Gynura pseudochina (L.) DC. | Koeng Sub-district, Mueang District, Maha Sarakham province, Thailand | + | No | Yes—HP-TLC, HPLC, LC-MS/MS |
| Seow et al. (2014) | Gynura segetum | Jabatan Pertanian Relau, Penang, Malaysia | + | No | No |
| Yuanrdani et al. (2017) | Gynura segetum (Lour.) Merr | Yogyakarta, West Java, Indonesia | + | No | Yes—HPLC |
| Yu et al. (2016) | Gynura nepalensis DC. | Suburb of Shanghai, China | + | No | Yes—HPLC |
| Rahman et al. (2018) | Gynura nepalensis | Kendua under Netrokona district of Bangladesh | + | No | Yes—method not stated |
| Xu et al. (2015) | Gynura divaricata L | Silk Biotechnology Laboratory, Soochow University, Suzhou, China | – | No | No |
| Dong et al. (2019) | Gynura divaricata (L.) DC. | Silk biotechnology Lab, Soochow University, Suzhou, China | – | No | Yes—HPLC |
| Ma et al. (2017) | Gynura formosana Kitam | Plant greenhouse of Longyan University, Longyan, China | – | No | No |

HPLC, high-performance liquid chromatography; +, includes a voucher specimen; –, a voucher specimen is missing.
is a synonym of *Gynura barbareifolia* Gagnep and *Gynura divaricata* subsp. *formosana* (Kitam.) FG Davies is a synonym of *Gynura formosana* Kitam (The Plant List, 2020).

A total of 17 selected studies (Siriwatanametanon et al., 2010; Kim et al., 2011; Akowuah et al., 2012; Wu et al., 2013; Seow et al., 2014; Shwter et al., 2014; Chao et al., 2015; Wong et al., 2015; Yu et al., 2016; Yin et al., 2017; Yuandani et al., 2017; Rahman et al., 2018; Sukadeetad et al., 2018; Huang et al., 2019; Nazri et al., 2019; Ning et al., 2019; Chandradevan et al., 2020) provided the full information about the botanical material and authenticated the *Gymura* species by depositing voucher specimens. Six selected studies (Iskander et al., 2002; Xu et al., 2015; Ma et al., 2017; Dong et al., 2019; Liu M. et al., 2019; Liu Y. et al., 2019) reported the information of the *Gymura* species source but did not provide voucher specimens. Four selected studies (Siriwatanametanon and Heinrich, 2011; Rerknimitr et al., 2016; Pai et al., 2019; Yang et al., 2019) were inadequate on material information as the *Gymura* species source was not reported and voucher specimens were missing. The absence of voucher specimens seriously makes the reliability of the article suspicious. Sufficient description of the experimental methods used and citation of voucher specimens as evidence of the plants used are critical factors of the repeatability of the ethnopharmacological or any botanical study. Otherwise, the scientific impact of a study will be drastically diminished. Erroneous identification is a serious problem that may jeopardize any recorded uses or properties that may, in fact, relate to different species (Rivera et al., 2014).

All selected studies showed no data on quality control as none of the Gymura preparation was mentioned to follow the monograph of pharmacopeia. The safety, efficacy, and quality control of medicinal plants are getting more attention from both health authorities and the public. Herbal medicines are in widespread use, and the public believes that natural products are safe and devoid of adverse effects. However, medicinal plants are often used in combination, may be contaminated and adulterated, and may contain toxic compounds. The common misconception among the public often leads to inappropriate use and uncontrolled consumption, where poisoning and acute health problems are possible consequences. Hence, quality control of herbal medicines has a direct impact on their safety and efficacy (Wachtel-Galor and Benzie, 2011). According to good manufacturing practice, the crucial requirements for quality control of starting materials include correct identification of medicinal plant species, special storage, and special sanitation and cleaning methods for various materials (Ekor, 2014).

From harvesting to manufacturing, the quality of herbal medicines can be affected by vast factors. Quality evaluation of medicinal plants is possible by detecting the presence of chemical markers within a sample (Li et al., 2008). Qualitative chemical evaluation covers identification and characterization of phytochemical constituents in the medicinal plants through different analytical techniques. Phytochemical screening techniques involve botanical identification, extraction with suitable solvents, purification, and characterization of the active constituents of pharmaceutical importance (Folashade et al., 2012). Chromatographic methods such as thin-layer chromatography/high-performance TLC (TLC/HP-TLC) and high-performance liquid chromatography (HPLC), which are the most commonly used chemical techniques in the identification and quality assessment of medicinal plant ingredients, have provided characteristic qualitative and quantitative patterns of the constituents. Spectroscopic techniques, including UV, IR, and nuclear magnetic resonance (NMR), allow the quantitation of single or multiple compounds that share similarities in their UV absorbance, thus providing a more holistic view of herbal medicines in contrast to the quantitation of a single compound (Upton et al., 2019). Chemical analysis of Gymura species were reported in studies conducted by Kim et al. (2011), Siriwatanametanon and Heinrich (2011), Wu et al. (2013), Yu et al. (2016), Yuandani et al. (2017), Rahman et al. (2018), Sukadeetad et al. (2018), Dong et al. (2019), Huang et al. (2019), Liu M. et al. (2019), Liu Y. et al. (2019), Nazri et al. (2019), and Chandradevan et al. (2020) using methods such as HP-TLC, HPLC, gas chromatography-mass spectrometer, electrospray ionization-mass spectrometry, electrospray ionization time-of-flight mass spectrometry, liquid chromatography with tandem mass spectrometry, ultra-high-performance liquid chromatography–quadrupole time-of-flight mass spectrometry, 1H and 13C NMR, and 2D-NMR.

On the basis of the risk of bias score in Table 2, under the domain “other sources of bias,” five studies (Siriwatanametanon et al., 2010; Kim et al., 2011; Siriwatanametanon and Heinrich, 2011; Chao et al., 2015; Rahman et al., 2018) were rated as “definitely high” risk of bias because of the unclear or absence of statistical analysis. This finding raised the validity of the interpretations of these studies in question because the claimed significant results are possibly misinterpreted under errors of statistical analysis. Hence, the erroneous statistical methods may contribute to false positive findings and may successively become misleading literature. Under the domain “Detection bias,” Iskander et al. (2002) and Rahman et al. (2018) were rated as definitely high risk of bias because the outcome assessment methods used were insensitive to indicate antioxidant or anti-inflammatory effects.

### Study Characteristics

#### Species of Gymura

Of the 27 selected studies, there are seven species of genus Gymura, which are *G. procumbens*, *G. bicolor*, *G. segetum*, *G. divaricata*, *G. formosana*, *G. nepalensis*, and *G. pseudochina*. Among these Gymura species, 11 out of 27 selected studies focused on *G. procumbens* (Iskander et al., 2002; Kim et al., 2011; Akowuah et al., 2012; Shwter et al., 2014; Wong et al., 2015; Huang et al., 2019; Liu M. et al., 2019; Liu Y. et al., 2019; Nazri et al., 2019; Ning et al., 2019; Chandradevan et al., 2020), five studies on *G. bicolor* (Wu et al., 2013; Chao et al., 2015; Yin et al., 2017; Pai et al., 2019; Yang et al., 2019), four studies on *G. pseudochina* (Siriwatanametanon et al., 2010; Siriwatanametanon and Heinrich, 2011; Rerknimitr et al., 2016; Sukadeetad et al., 2018), two studies on *G. segetum* (Seow et al., 2014; Yuandani et al., 2017), and two studies on *G. nepalensis* (Yu et al., 2016; Rahman et al., 2018), two studies on *G. divaricata* (Xu et al., 2015; Dong et al., 2019), and one study on *G. formosana* (Ma et al., 2017). Identical plant species might exhibit phytochemical or bioactivity variations that can possibly be attributed to intrinsic factors (age of the plant and part of the plant used) and extrinsic factors (age of the plant and part of the plant used).
### Table 2: Risk of bias assessment of each individual study on Gynura species according to OHAT guideline.

| Domain                        | Questions                                                                 | Iskander et al. | Akowuah et al. (2012) | Kim et al. (2011) | Sherer et al. (2014) | Wong et al. (2015) | Huang et al. (2018) | Liu M. et al. (2018) | Nanji et al. (2018) | Ning et al. (2019) | Liu Y. et al. (2015) | Chandraelwan et al. (2010) | Wu et al. (2015) | Chao et al. (2015) | Yin et al. (2017) | Pai et al. (2019) | Yang et al. (2019) | Silvestrano et al. and Heinrich (2010) | Rerknimitr et al. (2016) | Sukadayarat et al. (2018) | Seny et al. (2014) | Yuanrani et al. (2017) | Yu et al. (2016) | Rahman et al. (2018) | Xu et al. (2013) | Dong et al. (2019) | Ma et al. (2017) |
|-------------------------------|---------------------------------------------------------------------------|-----------------|------------------------|-------------------|----------------------|-------------------|----------------------|---------------------|-------------------|-----------------|----------------------|----------------------------|--------------------|-----------------|------------------|----------------|----------------|----------------|----------------|----------------|----------------|------------------|------------------|------------------|----------------|----------------|----------------|----------------|
| **Selection bias**            | 1. Was administered dose or exposure level adequately randomized?           | 2               | 3                      | 3                  | 3                    | 3                  | 3                    | 3                   | 3                 | 3               | 3                    | 3                             | 3                 | 3               | 3               | 3              | 3              | 3              | 3              | 3              | 3               | 3               | 3               | 3              | 3              | 3              |
|                              | 2. Was allocation to study groups adequately concealed?                    | 3               | 3                      | 3                  | 3                    | 3                  | 3                    | 3                   | 3                 | 3               | 3                    | 3                             | 3                 | 3               | 3               | 3              | 3              | 3              | 3              | 3              | 3               | 3               | 3               | 3              | 3              | 3              |
| **Performance bias**          | 5. Were experimental conditions identical across study groups?             | 2               | 2                      | 3                  | 3                    | 3                  | 3                    | 3                   | 3                 | 3               | 3                    | 3                             | 3                 | 3               | 3               | 3              | 3              | 3              | 3              | 3              | 3               | 3               | 3               | 3              | 3              | 3              |
|                              | 6. Were the research personnel and human subjects blinded to the study group during the study? | 3               | 3                      | 3                  | 3                    | 3                  | 3                    | 3                   | 3                 | 3               | 3                    | 3                             | 3                 | 3               | 3               | 3              | 3              | 3              | 3              | 3              | 3               | 3               | 3               | 3              | 3              | 3              |
| **Attrition/exclusion bias**  | 7. Were outcome data complete without attrition or exclusion from analysis? | 3               | 3                      | 3                  | 3                    | 3                  | 3                    | 3                   | 3                 | 3               | 3                    | 3                             | 3                 | 3               | 3               | 3              | 3              | 3              | 3              | 3              | 3               | 3               | 3               | 3              | 3              | 3              |
|                              | 8. Can we be confident in the exposure characterization?                  | 3               | 3                      | 3                  | 3                    | 3                  | 3                    | 3                   | 3                 | 3               | 3                    | 3                             | 3                 | 3               | 3               | 3              | 3              | 3              | 3              | 3              | 3               | 3               | 3               | 3              | 3              | 3              |
| **Detection bias**            | 9. Can we be confident in the outcome assessment?                         | 4               | 2                      | 2                  | 2                    | 2                  | 2                    | 2                   | 2                 | 2               | 2                    | 2                             | 2                 | 2               | 2               | 2              | 2              | 2              | 2              | 2              | 2               | 2               | 2               | 2              | 2              | 2              |
|                              | 10. Were all measured outcomes reported?                                  | 2               | 2                      | 2                  | 2                    | 2                  | 2                    | 2                   | 2                 | 2               | 2                    | 2                             | 2                 | 2               | 2               | 2              | 2              | 2              | 2              | 2              | 2               | 2               | 2               | 2              | 2              | 2              |
| **Selective reporting bias**  | 11. Were there no other sources of potential threats to internal validity? | 3               | 1                      | 4                  | 2                    | 2                  | 2                    | 2                   | 2                 | 2               | 2                    | 2                             | 2                 | 2               | 2               | 2              | 2              | 2              | 2              | 2              | 2               | 2               | 2               | 2              | 2              | 2              |

1Definitely low risk of bias.
2Probably low risk of bias.
3Probably high risk of bias or Not Reported.
4Definitely high risk of bias.
important parameters in improving herbal product safety. Stability testing and toxicity pro
duced from plants originating from Nanjing were shown to
Gynura are crucial to gain regulatory approval of genus
et al., 2015). Hence, standardization and quality control of genus
of a drug can be used as a con
mortality or signi
G. procumbens
orally administered
model orally administered 1.0 g/kg BW G. segetum extract for 48 h
(Xiong et al., 2018). Hence, any contraindications caused by long-
term consumption of genus Gynura must be determined prior to the
commercialization of genus Gynura products. Appropriate clinical
studies are needed to determine the optimal efficacy and minimum
Toxicity of genus Gynura leaves is caused by the presence of hepatotoxic pyrrolizidine
Gynura Antioxidant and Anti-inflammatory Effects

Part of Plant Used in Studies
Twenty selected studies used the leaves of Gynura to investigate their
pharmacological effects. Nazri et al. (2019) and Ning et al. (2019)
used the whole plant of G. procumbens, Xu et al. (2015) and Huang
et al. (2019) used the leaf and stem parts of Gynura, Iskander et al.
(2002) and Dong et al. (2019) used the aerial part of Gynura, and Liu
M. et al. (2019) did not mention the part of Gynura used in their
study. Hence, the leaf is the dominant part of the plant used in all
selected research studies. Notably, the part of the plant that is usually
consumed is the leaf. However, compared to the other parts of the
plant, the root extract of both G. procumbens and G. bicolor
exhibited the highest phenolic content, flavonoid content,
ascorbic acid content, and antioxidant capacities
(Krishnan et al., 2015). Mice with intraperitoneal injection of G. procumbens leaf
extract at 25, 50, 100, and 250 mg/kg/day for four days survived until
day 30 of post-injection with no signs of toxicity such as diarrhea,
excess urination, and lethargy (Vejanan et al., 2012). On the basis of the
study by Zahra et al. (2011) and Shwter et al. (2014), no
mortality, or organ toxicity was detected in the animals that were
orally administered G. procumbens leaf extract at doses of 2 and
5 g/kg for 14 days. The study carried out by Algariri et al. (2014)
demonstrated that the leaf extract of G. procumbens was safe, with no
observed acute toxicity effects as there was no treatment-related
mortality at 2 g/kg throughout the 14 days observation period.
Hence, the oral lethal dose (LD_{50}) of G. procumbens extract for
rats was determined to be greater than 2,000 mg/kg, and
the acceptable daily intake was 700 mg/kg/day. The same study also
showed that consumption of 250, 500, and 1,000 mg/kg of G. procumbens extract for 28 days did not lead to substantial toxicity
as no abnormal behavior, disease, or death was observed in the
animal model that received the plant extract. Administration of G. procumbens leaf extract at 1,000–5,000 mg/kg did not cause
mortality or significant changes in the general behavior, bodyweight,
or organ gross appearance of rats (Rosidah et al., 2009). At the same
time, G. procumbens and G. bicolor leaf extracts both showed a
negligible level of toxicity when administered orally at 300, 2,000,
and 5,000 mg/kg for 14 days (Teoh et al., 2013, 2016).

However, the use of genus Gynura requires extra caution on
Toxic risk because of the presence of hepatotoxic pyrrolizidine
alkaloids which was determined in the leaves of G. pseudochina
(Siriwatanametanon and Heinrich, 2011; Sukadeetad et al., 2018)
as well as the aerial part of G. bicolor and G. divaricata (Chen et al.,
2017). Hepatic injury caused by genus Gynura has been reported in
few other studies. By daily administration of the root of G. segetum at
1.0 g extract/kg for 40 successive days, hepatic veno-occlusive
disease, also called hepatic sinusoidal obstruction syndrome
(HSOS), was induced in the mouse model where liver fibrosis-
related factors and pro-inflammatory cytokines were upregulated
(Zhang et al., 2019). The mouse model in which a 30 g/kg decoction
of G. segetum dried rhizome was administered by gavage in the
morning for 30 days showed features of HSOS, including increased
weight ratio of the liver and body, serum transaminase, bilirubin,
decreased albumin (Chen et al., 2011), bulging abdomen, a large
number of clear ascites, floating bowels, jelly omentum in the
abdominal cavity, and congested and swollen or contracted
and hard liver with a grainy surface and blunt edges (Zhu et al., 2011).
The potential markers of hepatotoxicity induced by the decoction of
G. segetum dried rhizome are verified in the animal model as
differential metabolites of arginine, creatine, valine, glutamine,
and citric acid, which are involved in the regulation of multiple
metabolic pathways, primarily amino acid metabolism and energy
metabolism (Qiu et al., 2018). G. segetum dried rhizome also
caused liver injury by dysregulating mitochondrial ROS generation through the SIRT3-SOD2 pathway (Li et al., 2019). The roots and
aerial parts of G. segetum were proven to contain large amounts of
pyrrolizidine alkaloids, including seneceionine, which were
responsible for the impaired bile acid homeostasis in the animal
model orally administered 1.0 g/kg BW G. segetum extract for 48 h
(Xiong et al., 2018). Hence, any contraindications caused by long-
term consumption of genus Gynura must be determined prior to the
commercialization of genus Gynura products. Appropriate clinical
studies are needed to determine the optimal efficacy and minimum
toxicity of genus Gynura. The application of genus Gynura must be
closely monitored in term of doses and qualities, especially the
species with reported cases of hepatotoxicity.

Solvents Used for Extraction
Five studies used the methanol extracts of G. procumbens, G.
pseudochina and G. segetum to investigate their antioxidant or
anti-inflammatory activities in HeLa cells (Siriwatanametanon et al.,
2010), human leukocytes (Siriwatanametanon et al., 2010; Yuandani et al., 2017), murine macrophages (Yuandani et al., 2017),
oxidative stress model rats (Akowuah et al., 2012), and granuloma
model rats (Seow et al., 2014). As a continuation study of
Siriwatanametanon et al. (2010), Siriwatanametanon and Heinrich
(2011) as well as Yuandani et al. (2017) isolated compounds from the
methanol extract of Gynura and determined their antioxidant or anti-
flammatory effects in HeLa cells or macrophages. The extraction of
G. procumbens with 80% methanol at a temperature below 60°C
would give greater retention of the total phenolic content (TPC) and
greater expression of free radical scavenging activity (FRSA)
(Akowuah et al., 2009). Methanol was also identified as a more
effective extraction solvent of G. procumbens compared to 95%
ethanol and water extracts based on its higher TPC and FRSA
(Akowuah et al., 2012). Sukadeetad et al. (2018) demonstrated a
higher chlorogenic acid content in G. pseudochina leaf extract by
microwave drying as well as good efficiency for recovering phenolic
compounds by extraction with 50% (v/v) methanol. However,
### TABLE 3  | List of studies on the antioxidant effects of genus Gynura.

| Plant species | Part, *Gynura* form | Cell line/Animal study model | Concentration/dose, control groups | Parameter measured and technique used | Findings | Reference |
|---------------|----------------------|-----------------------------|-----------------------------------|--------------------------------------|----------|-----------|
| *Gynura procumbens* (lour.) Merr | Leaf | Human HaCaT keratinocytes | 1, 10, 50 μg/ml | Intracellular ROS production level by dichlorofluorescein (DCF) content | GP extract treatment inhibited UV-induced ROS generation levels about 36% at 50 μg/ml | Kim et al. (2011) |
| | Extract (ethanol) | In vitro study stimulated by UV irradiation | 200 μg/ml vitamin C | | Intracellular ROS production level | ↓ROS level by 80 and 160 μg/ml GP extract | Liu Y. et al. (2019) |
| | Leaf | Mouse normal liver cell line NCTC-1469 | 80 and 160 μg/ml (24 h) | | | |
| | Extract (aqueous) | In vitro study stimulated by palmitic acid and oleic acid | Normal control (culture medium) | Normal control | 80 and 160 μg/ml GP extract | ↓ROS level by 80 and 160 μg/ml GP extract | Liu Y. et al. (2019) |
| | Leaf | 18 Sprague-Dawley rats (6 rats/group) | 1.0 g/kg body weight | Plasma lipid peroxidation levels using thiobarbituric acid reactive substances (TBARS) assay | ↓plasma TBARS level | Akowuah et al. (2012) |
| | Extract (methanol) | In vivo study using carbon tetrachloride (CCl₄)-induced oxidative stress rats | Daily single dose for 14 days | Plasma total antioxidant status (TAS) | | |
| | Leaf | 30 adult male Sprague-Dawley rats (6 rats/group) | 250 and 500 mg/kg body weight | Lipid peroxidation levels using TBARS assay | | |
| | Extract (ethanol) | In vivo study using carcinogen-induced colon cancer rats | Normal control (normal saline subcutaneous injections, 10% tween 20 oral administration) | 250 and 500 mg/kg body weight | | |
| | Whole plant | 48 female Sprague-Dawley rats (6 rats/group) | 250 and 500 mg/kg body weight | Plasma malondialdehyde (MDA) level using high-performance liquid chromatography (HPLC) | ↑ MDA level in treatment groups 250 and 500 mg/kg GP. | Nazri et al. (2019) |
| | Extract (ethanol) | In vivo study using postmenopausal rats fed with cholesterol diet enriched with repeatedly heated palm oil | Oral route | Superoxide dismutase (SOD) activity | | |

(Continued on following page)
| Plant species | Part, *Gynura* form | Cell line/Animal study model | Concentration/dose, control groups | Parameter measured and technique used | Findings | Reference |
|---------------|---------------------|-----------------------------|-----------------------------------|--------------------------------------|----------|-----------|
| Leaf          | Extract (aqueous)   | 32 male C57BL/6 J mice (8 mice/group) | 500 and 1,000 mg/kg body weight Oral route Daily for 6 weeks | Hepatic malondialdehyde (MDA) level Glutathione peroxidase (GSH-Px) activity Catalase (CAT) activity Heme oxygenase 1 (HO-1) activity | \[ \text{↓↓ MDA level in treatment groups} \] \[ \text{↓↓ GSH-Px, CAT and HO-1 activities in treated mice (500 and 1,000 mg/kg)} \] | Liu Y. et al. (2019) |
|               |                     | In vivo study using non-alcoholic steatohepatitis (NASH) mice | Control groups Normal control (methionine- and choline-sufficient (MCS) diet) Model control (methionine- and choline-deficient (MCD) diet) | | | |
|               |                     | | | | | |
| *Gynura* bicolor (roxb.) | Leaf | Human umbilical vein endothelial cells Extract (aqueous, ethanol) | Aqueous or ethanol extract at 1, 2 or 4% (v/v) 12 h pre-treatment | ROS production level by 2',7'-dichlorofluorescein (DCF) content Catalase (CAT) activity | | Chao et al. (2015) |
| (Ex willd.) DC. | | In vitro study treated by high glucose Control groups Control (5.5 mM glucose) Model control (33 mM glucose) | | Glutathione (GSH) content Glutathione peroxidase activity (GSH-Px) activity | | |
| | Extract (aqueous) | | | Glutathione peroxidase activity (GSH-Px) activity | Pre-treatments with aqueous or ethanol extract dose-dependently ↓ GSH-Px, CAT and HO-1 activities in treated mice (500 and 1,000 mg/kg) | | |
| | PC12 cell line (rat adrenal gland pheochromocytoma) | | | | | |
| | Leaf | Extract (aqueous) | Aqueous extract at 0.25, 0.5 or 1% 48 h pre-treatment | ROS level by 2',7'-dichlorofluorescein diacetate (DCFH-DA) | | Yang et al. (2019) |
| | | In vitro study of H$_2$O$_2$ induced injury Control groups | Normal control Model control (H$_2$O$_2$ stimulation) Liquid diet group (without ethanol) Ethanol diet group | | | |
| | | Extract (aqueous) | | | | |
| | Male C57BL/6 mice (8 mice/group) | In vivo study using chronic ethanol consumption-induced hepatic injury mice model | 0.25 or 0.5% *G. bicolor* aqueous extract diet Oral route 6 weeks | | | |
| | | | Control groups Normal control Model control (standard mouse basal diet) | | | |
| | | | Extract (aqueous) | | | |
| | | | | | | |
| *Gynura divaricata* (L.) | Leaf, stem | Lyophilized into powder | 60 male imprinting control region mice (15 mice/group) | | | |
| DC. | | In vivo study using high-fat diet and streptozotocin (STZ) induced type 2 diabetic mice | Diets with 1.2% and 4.8% GD Oral route Daily for 4 weeks | Glutathione peroxidase activity (GSH-Px) activity Total superoxide dismutase activity (SOD) Lipid peroxidation level by MDA level assay | 1.2% GD ↑ T-SOD levels, ↓ MDA level 4.8% GD ↑ T-SOD level, ↓ MDA level | Xu et al. (2015) |
| | | | Control groups Normal control (normal diet) Diabetic model control (high-fat diet 18% lard, 20% sugar, 3% egg yolk, 59% basal diet) and 100 mg/kg STZ | | | |

(Continued on following page)
TABLE 3 | (Continued) List of studies on the antioxidant effects of genus Gynura.

| Plant species | Part, Gynura form | Cell line/Animal study model | Concentration/dose, control groups | Parameter measured and technique used | Findings | Reference |
|---------------|------------------|-----------------------------|-----------------------------------|---------------------------------------|----------|-----------|
| Aerial part   | Lyophilized into powder | Male ICR mice (15 mice/group) | Diet with 1%, 5% and 10% GD Oral route Daily for 4 weeks | Hepatic glutathione peroxidase activity (GSH-Px) | 1% GD: ↑ GSH-Px level, ↑ T-SOD level 5% GD: ↑ GSH-Px level, ↑ T-SOD level, ↓ 8-OHdG level 10% GD: ↑ GSH-Px level, ↑ T-SOD level, ↓ MDA level, ↓ 8-OHdG level | Dong et al., (2019) |
| Gynura segetum | Leaf (lour.) Merr | Polymorphonuclear cells neutrophils (PMN) | Extract: 6.25–100 μg/ml Compounds: 3.125–50 μg/ml | ROS production level by luminol method | GS extract exhibited inhibitory activity upon activation by PMA (IC50 = 1.41 ± 0.63 μg/ml) and zymosan (IC50 = 2.63 ± 0.89 μg/ml) 8,8'- (ethene-1,2-diyl)-dinaphtalene-1,4,5-triol revealed ROS inhibitory activity upon activation by PMA (IC50 = 0.13 μM) and zymosan (IC50 = 0.05 μM) Rutin inhibited ROS activated by PMA (IC50 = 0.08 μM) and zymosan (IC50 = 0.13 μM) | Yuandani et al. (2017) |
| Gynura nepalensis | Leaf DC. | H9c2 cardiomyoblasts | 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50, 100 μM 1 h pre-treatment | Intracellular ROS production by DCF content | Compound 6 (3,5-dicaffeoylquinic acid ethyl ester) exhibited a more potent cytoprotective effect thus selected for further evaluation 1) ROS production in H2O2-treated cells, even at a concentration of 0.78 μM 2) H2O2-induced decrease in CAT activity at doses of 25 and 50 μM | Yu et al. (2016) |
| Gynura formosana | Leaf kitam | Nine caffeoylquinic acid analogs (1–9) isolated from ethanol extract | In vitro study stimulated by H2O2 | CAT activity assay | ↑↑↑ ROS production in H2O2-treated cells, even at a concentration of 0.78 μM | Ma et al. (2017) |
| Gynura formosana | Leaf Extract (ethyl acetate) | 4B male sprague-dawley rats (8 rats/group) | 100 mg/kg, 250 mg/kg, 500 mg/kg body weight Oral route Once daily for 7 days | CAT activity assay | Total superoxide dismutase activity (SOD) Glutathione level (GSH) Lipid peroxidation level | Ma et al. (2017) |

↑ indicates significantly induce (p < 0.05), ↑↑ indicates significantly induce (p < 0.01), ↑↑↑ indicates significantly induce (p < 0.001), ↓ indicates significantly inhibit (p < 0.05), ↓↓ indicates significantly inhibit (p < 0.01), and ↓↓↓ indicates significantly inhibit (p < 0.001).
ethanol extract was used in this study for successive bioassays by considering ethanol as a safer solvent for health product application.

Eleven studies tested the antioxidant or anti-inflammatory activities of the ethanol extract of *G. procumbens*, *G. bicolor*, *G. pseudochinina* and *G. nepalensis* in human keratinocytes (Kim et al., 2011; Sukadeetad et al., 2018), human dermal fibroblasts (Kim et al., 2011), human endothelial cells (Chao et al., 2015), murine macrophages (Chandradevan et al., 2020; Liu M. et al., 2019; Ning et al., 2019), ear-inflamed mice (Iskander et al., 2002; Rahman et al., 2018), rat colon cancer model (Shwter et al., 2014), parasite-infected mice (Wong et al., 2015), and hypercholesterolemic rats (Nuriz et al., 2019). Liu M. et al. (2019) simultaneously studied the anti-inflammatory effect of the ethanol extract and its fractions, including the petroleum ether fraction, ethyl acetate fraction, n-butanol fraction, and water fraction. Yu et al. (2016) studied the antioxidant and anti-inflammatory effects of nine caffeoylquinic acid analogs isolated from the ethanol extract on cardiomyoblasts. The optimal extraction of *G. divaricata* was determined with 45% ethanol for 30 min at 90°C, where the increase in the extraction temperature (from 40 to 100°C) led to a significant elevation of the TPC, TPG, and FRSA (Van et al., 2011). Another study on the extraction method optimization of *G. bicolor* showed that 40% ethanol, 40°C, 30 min sonication time, and 50:1 liquid-to-solid ratio were the optimal conditions for a higher extraction yield of TPC (Qiu X. L. et al., 2018).

Five studies used the aqueous extract of *G. procumbens* and *G. bicolor* to determine their antioxidant or anti-inflammatory activities in human endothelial cells (Chao et al., 2015), murine adrenal gland pheochromocytoma (Yang et al., 2019), murine hepatocytes (Liu Y. et al., 2019), diabetic mice (Pai et al., 2019), and liver-injured mice (Yin et al., 2017; Liu Y. et al., 2019). The ethyl acetate extract of *G. formosana* was studied for its antioxidant and anti-inflammatory activities in granuloma model rats (Ma et al., 2017). Meanwhile, the ether extract of *G. bicolor* was studied for its anti-inflammatory activity in murine macrophages (Wu et al., 2013). *G. divaricata* was lyophilized into powder and tested in diabetic mice (Xu et al., 2015; Dong et al., 2019). The study by Huang et al. (2019) determined the anti-inflammatory effect of *G. procumbens* essential oil and its active ingredients in murine macrophage and ear-inflamed mice. One randomized controlled study on patients with moderate plaque psoriasis was conducted to study the anti-inflammatory effect of *G. pseudochinina* ointment from an ethanol extract with a ratio of one extract to 10 vehicles (Rerknimitr et al., 2016).

### Antioxidant Parameters

In general, ROS production level was the major parameter measured in vitro antioxidant studies (Kim et al., 2011; Chao et al., 2015; Yu et al., 2016; Yuandani et al., 2017; Liu Y. et al., 2019; Yang et al., 2019). The other in vitro parameters included glutathione (GSH) content and GSH peroxidase (GSH-Px) or catalase (CAT) activity (Chao et al., 2015; Yu et al., 2016; Yang et al., 2019). The main focus of in vivo antioxidant studies of *Gynura* species was lipid peroxidation (Akowuah et al., 2012; Shwter et al., 2014; Xu et al., 2015; Ma et al., 2017; Dong et al., 2019; Liu Y. et al., 2019; Nazri et al., 2019). The other in vivo parameters measured were plasma total antioxidant status (TAS) (Akowuah et al., 2012), CAT activity (Ma et al., 2017; Liu Y. et al., 2019; Nazri et al., 2019; Pai et al., 2019), ROS production (Yin et al., 2017; Pai et al., 2019), superoxide dismutase (SOD) activity (Shwter et al., 2014; Xu et al., 2015; Ma et al., 2017; Dong et al., 2019; Nazri et al., 2019), heme oxygenase 1 (HO-1) (Liu Y. et al., 2019), 8-hydroxy-2′-deoxyguanosine (8-OHdG) level (Dong et al., 2019), and GSH-related parameters, including GSH-Px activity (Xu et al., 2015; Yin et al., 2017; Dong et al., 2019; Liu Y. et al., 2019; Nazri et al., 2019; Pai et al., 2019), GSH-S-transferase (GST) activity (Shwter et al., 2014), GSH reductase (GR) activity (Yin et al., 2017; Pai et al., 2019), GSH disulfide (GSSG) content (Yin et al., 2017), and GSH content (Ma et al., 2017; Yin et al., 2017; Pai et al., 2019).

### Anti-inflammatory Parameters

Overall, in vitro anti-inflammatory studies on *Gynura* species focused on the secretion or expression of pro-inflammatory mediators, including interleukin-1 (IL-1) (Siriwatametanon et al., 2010; Yuandani et al., 2017; Yang et al., 2019), IL-6 (Siriwatametanon et al., 2010; Kim et al., 2011; Chao et al., 2015; Liu M. et al., 2019; Yang et al., 2019), IL-8 (Kim et al., 2011; Sukadeetad et al., 2018), tumor necrosis factor alpha (TNF-α) (Siriwatametanon et al., 2010; Chao et al., 2015; Yuandani et al., 2017; Liu M. et al., 2019; Yang et al., 2019), prostaglandin E2 (PGE2) (Siriwatametanon et al., 2010; Wu et al., 2013; Chao et al., 2015), cyclooxygenase-2 (COX-2) (Wu et al., 2013; Chao et al., 2015), inducible nitric oxide synthase (iNOS) (Wu et al., 2013; Ning et al., 2019), nitric oxide (NO) (Wu et al., 2013; Yuandani et al., 2017; Liu M. et al., 2019; Ning et al., 2019; Chandradevan et al., 2020), lactate dehydrogenase (LDH) activity, mitochondrial membrane potential (Δψm) (Yu et al., 2016; Yang et al., 2019), matrix metalloproteinase-1 and 9 (MMP-1 and MMP-9) (Kim et al., 2011), and infiltration of inflammatory cells (Huang et al., 2019). Moreover, in vitro studies included investigations on the anti-inflammatory effect of the *Gynura* species on the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway (Liu Y. et al., 2019), nuclear factor kappa B (NF-κB) signaling pathway (Siriwatametanon et al., 2010; Siriwatametanon and Heinrich, 2011; Wu et al., 2013; Sukadeetad et al., 2018; Yang et al., 2019), and mitogen-activated protein kinases (MAPK) signaling pathway, including c-Jun N-terminal kinase (JNK) (Yu et al., 2016; Liu Y. et al., 2019), p38 (Yu et al., 2016; Yang et al., 2019), and extracellular signal-regulated kinase (ERK) (Yu et al., 2016). Similarly, the in vivo studies also measured the parameters of IL-1 (Seow et al., 2014; Ma et al., 2017; Yin et al., 2017; Pai et al., 2019), IL-6 (Yin et al., 2017; Pai et al., 2019), and TNF-α (Seow et al., 2014; Wong et al., 2015; Ma et al., 2017; Yin et al., 2017; Dong et al., 2019; Pai et al., 2019). The other anti-inflammatory effect parameters of in vivo studies were interferon-γ (IFN-γ), IL-10, phosphorylation of glycogen synthase kinase-3 (GSK3β) (Wong et al., 2015; Dong et al., 2019), LDH, alamine aminotransferase (GPT), c-reactive protein (CRP) (Ma et al., 2017), Nrf2 (Liu Y. et al., 2019), peroxisome proliferator-activated receptor gamma (PPARγ) (Dong et al., 2019; Liu Y. et al., 2019), COX-2 (Huang et al., 2019), inflamed-ear thickness (Iskander et al., 2002; Rahman et al., 2018; Huang et al., 2019), cotton pellet granuloma (Seow et al., 2014; Ma et al., 2017), paw edema (Rahman et al., 2018; Huang et al., 2019), signaling pathways of phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) (Xu et al., 2015; Dong et al., 2019), JNK (Liu Y. et al., 2019), p38 (Pai et al., 2019).
The effect of Gynura on NF-κB phosphorylation was also studied in a randomized controlled study (Rerknimitr et al., 2016).

**Antioxidant Effects of Genus Gynura**

Table 3 shows the list of studies on the antioxidant effects of genus Gynura. Several potential mechanisms for the antioxidant activity of Gynura are suggested as follows: inhibition of ROS, inhibition of lipid peroxidation, modulation of enzymatic antioxidant production or activities, and modulation of GSH-related parameters. Figure 2 illustrates the proposed signaling pathways of antioxidant effects by Gynura species.

**Gynura Inhibits Reactive Oxygen Species**

At physiological concentrations, ROS is involved in many cellular activities, including gene transcription, signaling transduction, and immune response. However, overproduction of ROS can result in oxidative damage to biomolecules, including lipids, proteins, and DNA, which is the underlying cause of various diseases (Liu et al., 2018). Inhibition of ROS production using G. procumbens extracts was determined in human HaCaT keratinocytes at 50 μg/ml (inhibitory effect comparable to 200 μg/ml vitamin C) (Kim et al., 2011) and in murine hepatocytes at 80 and 160 μg/ml (Liu Y. et al., 2019); that of G. bicolor in human umbilical vein endothelial cell (huvec) at 1–4% v/v (Chao et al., 2015), in adrenal gland pheochromocytoma in a concentration dependent manner (Yang et al., 2019), and in liver-injured mice at 0.25 and 0.5% diet (Yin et al., 2017); and that of G. segetum for zymosan = 2.63 ± 0.89 μg/ml; IC₅₀ for PMA = 1.41 ± 0.63 μg/ml) in polymorphonuclear cells (PMNs) neutrophils. The compound isolated from G. segetum extract, 8,8′-(ethene-1,2-diyl)-dinaphtalene-1,4,5-triol, possessed ROS inhibitory effect upon the activation by PMA (IC₅₀ = 0.13 μM) and zymosan (IC₅₀ = 0.05 μM).

At the same time, rutin, which was also isolated from G. segetum extract, inhibited the ROS activated by PMA (IC₅₀ = 0.08 μM) and zymosan (IC₅₀ = 0.13 μM). Both isolated compounds from G. segetum showed a higher antioxidant effect than the positive control aspirin (Yuan dani et al., 2017). One of the compounds isolated from G. nepalensis extract, 3,5-dicaffeoylquinic acid ethyl ester, showed an inhibitory effect on intracellular ROS production in cardiomyoblasts at a concentration of 0.78 μM and above (Yu et al., 2016). In nuclear and mitochondrial DNA, the interaction of the hydroxyl radical (HO•) with the nucleobases of the DNA strand will produce 8-hydroxy-2′-deoxyguanosine (8-OhdG), a predominant form of a free radical-induced oxidative lesion, which is a critical biomarker of oxidative stress (Valavanidis et al., 2009). G. divaricata diet (5 and 10%) showed the inhibition of the hepatic 8-OhdG level in a diabetic mice model (Dong et al., 2019). G. procumbens also improved the plasma TAS in rats with induced oxidative stress (Akowuah et al., 2012).

**Gynura Inhibits Lipid Peroxidation**

Lipid peroxidation is a reaction of oxygen with unsaturated lipids that successively results in the production of oxidation products, including lipid peroxyl radicals and hydroperoxides. Among a wide variety of oxidation products from lipid peroxidation, malondialdehyde (MDA) appears to be the most mutagenic product (Ayala et al., 2014). G. procumbens, G. divaricata, and G. formosana exhibited inhibition of lipid peroxidation by lowering the MDA level in carbon tetrachloride-induced oxidative stress rats (Akowuah et al., 2012), carcinogen-induced colon cancer rats (Shwter et al., 2014), high-fat-diet and streptozotocin (STZ)-induced diabetic mice (Xu et al., 2015; Dong et al., 2019), cotton pellet-induced granuloma rats (Ma et al., 2017), postmenopausal rats fed with cholesterol diet enriched with repeatedly heated palm oil (Nazri et al., 2019), and non-alcoholic steatohepatitis mice (Liu Y. et al., 2019). The reduction in the MDA level of G. procumbens extract-treated rats was comparable with treatments of anticancer drug, 5-fluorouracil (Shwter et al., 2014), and lipid-lowering drug atorvastatin (Nazri et al., 2019). It is worth noticing that Xu et al. (2015) and Dong et al. (2019) both studied on G. divaricata using identical treatment methods and diabetic mice models. The results of the study by Xu et al. (2015) showed a significant inhibitory effect of G. divaricata lipophilized powder on lipid peroxidation at treatment doses as low as 1.2% of diet. However, only 10% lipophilized powder diet was able to exert significant inhibition of lipid peroxidation in the study by Dong et al. (2019). This variation between findings could be due to the phytochemical or bioactivity variations that possibly correlated to intrinsic factors (age of the plant and part of the plant used) and extrinsic factors (geographical climate, nature of soil, season, and processing methods).

**Gynura Modulates Enzymatic Antioxidant Production or Activities**

The antioxidant mechanism of heme oxygenase-1 (HO-1) is found to be associated with an increase in superoxide dismutase (SOD) and CAT (Turkseven et al., 2005). HO-1 is responsible for the oxidative cleavage of heme groups, which generates biliverdin, carbon monoxide, and ferrous iron. Biliverdin is converted to bilirubin, and both of these bile pigments are potent scavengers of singlet oxygen (Stocker et al., 1990). Oral administration of G. procumbens extract was shown to increase HO-1 activity in liver-injured mice model (Liu Y. et al., 2019). SOD is an antioxidant enzyme that is responsible for the catalytic conversion of the superoxide radical (O₂⁻) or singlet oxygen radical (¹O₂) to hydrogen peroxide (H₂O₂) and molecular oxygen (O₂). However, the accumulation of H₂O₂ causes toxicity to body tissues or cells. Successfully, CAT breaks down H₂O₂ into water and molecular oxygen, thus minimizing free radical-induced damage (Ighodaro and Akinloye, 2018). G. procumbens, G. divaricata, and G. formosana showed an antioxidant effect by inducing in vivo total SOD activity (Shwter et al., 2014; Xu et al., 2015; Ma et al., 2017; Dong et al., 2019; Nazri et al., 2019). The in vitro CAT activity was induced by G. bicolor (Chao et al., 2015; Yang et al., 2019) and a compound isolated from G. nepalensis, which were 25 and 50 μM of 3,5-dicaffeoylquinic acid ethyl ester (Yu et al., 2016). At the same time, in vivo CAT activity was increased by G. procumbens (Liu Y. et al., 2019; Nazri et al., 2019), G. bicolor (Pai et al., 2019), and G. formosana (Ma et al., 2017). Notably, the induction of SOD and CAT by the G. procumbens extract was comparable to the effects of atorvastatin (Nazri et al., 2019).

**Gynura Modulates Glutathione-Related Parameters**

Owing to the absence of CAT in the mitochondria, the reduction of H₂O₂ and lipid peroxides is carried out by GSH peroxidase.
(GSH-Px). Therefore, protection of cells against oxidative stress is reinforced by increasing the activity of GSH-Px, which plays a crucial role in inhibiting the lipid peroxidation process (Ighodaro and Akinloye, 2018). GSH-Px activity was induced by G. procumbens (Liu Y. et al., 2019; Nazri et al., 2019), G. bicolor (Chao et al., 2015; Yin et al., 2017; Pai et al., 2019; Yang et al., 2019), and G. divaricata (Xu et al., 2015; Dong et al., 2019). G. procumbens showed higher efficacy in inducing GSH-Px activity as compared to atorvastatin (Nazri et al., 2019). GSH is a crucial low-molecular-weight antioxidant that protects cells from oxidative damage via reduction, conjugation, and interaction with other non-enzymatic antioxidants (Forman et al., 2009). G. formosana extract diet (250 and 500 mg/kg) was found to increase hepatic GSH activity of Gynura pseudochina var. Meanwhile, ethyl acetate extract of G. procumbens (0.25, 0.5, and 1% of diet) maintained GR activity (Yin et al., 2017; Pai et al., 2019) and suppressed the hepatic GSSG content in the liver-injured mice model (Yin et al., 2017). GSH-S-transferase (GST) exerts protection on cellular macromolecules from damage of reactive electrophiles by catalyzing the conjugation of GSH to various endogenous and exogenous electrophilic compounds (Townsend and Tew, 2003). G. procumbens extract diet showed antioxidative potential by increasing in vivo GST activity (Shwter et al., 2014).

**Anti-inflammatory Effects of Genus Gynura**

Table 4 shows the studies on the anti-inflammatory effects of Gynura. The potential mechanisms for the anti-inflammatory activity of Gynura are suggested as follows: modulation of inflammatory cytokine production, inhibition of PGE2 and NO production, inhibition of cellular inflammatory-related parameters, and inhibition of inflammation on animal models. As shown in Figure 2, the several potential anti-inflammatory signaling pathways of Gynura are PI3K/Akt, Nrf2, PPARγ, GS3K, NF-κB, and MAPK.

**Gynura Modulates Inflammatory Cytokine Production**

Cytokines are released by cells for signaling, where critical pro-inflammatory cytokines, including IL-1, IL-6, IL-8, IFN-γ, and TNF-α, are involved in the upregulation of inflammatory reactions (Turner et al., 2014) and IL-10 is a potent anti-inflammatory cytokine (Zhang and An, 2007). G. procumbens inhibited the IL-6 and IL-8 production in UV-induced HaCaT keratinocytes (Kim et al., 2011). G. procumbens extract also dose-dependently suppressed the IL-6 production in macrophages and inhibited the TNF-α level. At the same time, the ethyl acetate fraction of G. procumbens showed the best inhibitory effect on IL-6 and TNF-α (Liu M. et al., 2019). In a parasite-infected mice model, G. procumbens reduced the levels of the liver and serum TNF-α and IFN-γ as well as increased IL-10 level (Wong et al., 2015). G. bicolor possessed an anti-inflammatory effect by inhibiting the production of IL-1β, IL-6, and TNF-α both in vitro (Chao et al., 2015; Yang et al., 2019) and in vivo (Yin et al., 2017; Pai et al., 2019). G. pseudochina var. hispida methanol extract also caused inhibition of IL-1β production (IC50 = 2.40 μg/ml). Meanwhile, ethyl acetate extract of G. pseudochina var. hispida showed strong inhibition of the release of IL-6 (IC50 = 8.14 μg/ml) and TNF-α (IC50 = 1.49 μg/ml) in monocytes (Siriwanatametanon et al., 2010). The extract of G. pseudochina and its marker compounds of chlorogenic acid, caffeic acid, rutin, and p-coumaric acid also showed significant inhibition of IL-8 production in keratinocytes (Sukadeetad et al., 2018). G. segetum extract inhibited the release of TNF-α (IC50 = 16.20 ± 3.94 μg/ml) and IL-1β (IC50 = 2.72 ± 1.84 μg/ml) in macrophages. Among the isolated compounds of G. segetum, 4,5,4′-trihydroxyxalcone was the most potent sample in inhibiting IL-1β (IC50 = 6.69 μM), and another isolated compound, rutin, demonstrated the strongest inhibition against TNF-α release in macrophages (IC50 = 16.96 μM) (Yuanadi et al., 2017). The anti-inflammatory effects of G. segetum and G. formosana have been demonstrated by the in vivo inhibition of TNF-α and IL-1, where the inhibitory effects of 500 mg/kg of G. segetum and G. formosana extracts were comparable to that of the nonsteroidal anti-inflammatory drug indomethacin (Seow et al., 2014; Ma et al., 2017). G. divaricata also suppressed TNF-α production in a diabetic mice model (Dong et al., 2019).

**Gynura Inhibits Prostaglandin E2 and Nitric Oxide Production**

Prostaglandin E2 (PGE2) is a bioactive lipid that physiologically mediates the regulation of immune responses, blood pressure, gastrointestinal integrity, and fertility. However, the sequential actions of cyclooxygenase-2 (COX-2) catalyze PGE2 synthesis. Consequently, dysregulated PGE2 production has been correlated to a variety of pathological conditions, such as chronic inflammation (Legler et al., 2010). Inhibition of COX-2 expression in mice model was exerted by G. procumbens essential oil and its active ingredients. Both G. procumbens essential oil and its active ingredients were showing similar COX-2 inhibitory effects as that of the positive control drug diclofenac diethylamine emulgel (Huang et al., 2019). The use of the G. bicolor extract inhibited PGE2 production and COX-2 protein expression and activity in activated macrophages (Wu et al., 2013) and endothelial cells (Chao et al., 2015). PGE2 production was also inhibited using the extract of G. pseudochina var. hispida with an IC50 value of 25.23 μg/ml (Siriwanatametanon et al., 2010).

Nitric oxide (NO) is a signaling molecule that physiologically exerts an anti-inflammatory effect and acts as a pro-inflammatory mediator in the overproduction of the enzyme iNOS (Sharma et al., 2007). G. procumbens extract had shown an inhibitory effect on NO production in macrophages (Liu M. et al., 2019; Ning et al., 2019; ChandraDevan et al., 2020). This significant NO inhibitory effect by 250 μg/ml G. procumbens was comparable to the positive control of the non-selective NOS inhibitor Nω-nitro-l-arginine methyl ester hydrochloride (L-NAME) (Ning et al., 2019). G. procumbens fractions (petroleum ether, ethyl acetate, n-butanol, and water) at concentrations of 0.3–0.8 mg/ml also exerted 2–64% inhibition of NO production in macrophages. Among the fractions, the ethyl acetate fraction possessed the highest NO inhibitory effect (Liu M. et al., 2019). The protein expression of iNOS in macrophages was shown to be inhibited by the G. procumbens extract at a concentration of 250 μg/ml (Ning et al., 2019). Similarly, G. bicolor showed concentration dependent...
suppression on iNOS protein expressions and NO production (30% decrease with 120 μg/ml extract) in macrophages (Wu et al., 2013). *G. segetum* extract also inhibited NO production in macrophages, with IC50 = 0.16 ± 0.03 μg/ml. Meanwhile, one of the compounds isolated from *G. segetum* extract, 8,8′-(ethene-1,2-diyl)-dinaphtalene-1,4,5-triol, depicted the strongest NO inhibitory activity with an IC50 value of 0.15 μM (Yuandani et al., 2017).

**Gynura Inhibits Cellular Inflammmatory-Related Parameters**

Matrix metalloproteinases (MMPs) act as crucial regulatory enzymes in both pro- and anti-inflammatory pathways through the actions of cytokine or chemokine activation and antagonism (Manicone and McGuire, 2008). *G. procumbens* dose-dependently inhibited MMP-1 and MMP-9 expression in human dermal fibroblasts (HDFs), where the inhibition of the MMP-1 expression by the *G. procumbens* extract was even more effective than that of the positive control drug retinoic acid (Kim et al., 2011).

Mitochondrial membrane potential (Δψm), the energy provider to generate ATP, is produced when free energy is used to pump protons out of the mitochondrial matrix via oxidative phosphorylation. Under a stress condition, Δψm can be reduced and can possibly lead to mitochondrial dysfunction in inflammatory responses (Yue and Yao, 2016). In a concentration dependent manner, Δψm was increased, and LDH activity was inhibited by *G. bicolour* extract (Yang et al., 2019) and the isolated compound from the *G. nepalensis*, 3,5-dicaffeoylquinic acid ethyl ester (Yu et al., 2016). The induction of Δψm by the isolated compound of *G. nepalensis* was more effective than that of carbonyl cyanide m-chlorophenyl hydrazone, a chemical inhibitor of oxidative phosphorylation (Yu et al., 2016).

*G. formosana* also suppressed plasma inflammatory biomarker (LDH, GPT, and CRP) activities in the treated group, where these inhibitory effects were comparable to indomethacin (Ma et al., 2017). The essential oil of *G. procumbens* and its active ingredient, limonene, showed an inhibitory effect on inflammatory cell infiltration (Huang et al., 2019).

**Gynura Inhibits Inflammation in Animal Models**

Tissue swelling (edema) is one of the cardinal signs of inflammation, where the increased fluid filtration is further enhanced by the arteriolar vasodilator action of the inflammatory mediators (Amelang et al., 1981). *G. nepalensis* extract diet (Rahman et al., 2018), topical administration of *G. procumbens* extract (Iskander et al., 2002), *G. procumbens* essential oil, and the active ingredients mixture from the *G. procumbens* essential oil (Huang et al., 2019) showed anti-
## TABLE 4 | List of studies on the anti-inflammatory effects of genus Gynura.

| Plant species   | Part, Gynura form | Cell line/Animal study model | Concentration/dose, control groups | Parameter measured and technique used | Findings | Reference |
|-----------------|-------------------|------------------------------|------------------------------------|--------------------------------------|----------|-----------|
| Gynura procumbens (Lour.) Merr. | Leaf | Human HaCaT keratinocytes | 100, 500 µg/ml (24 h) | IL-6 and IL-8 production level using ELISA | By 100 and 500 µg/ml GP extract, IL-6 and IL-8 were inhibited. | Kim et al. (2011) |
| | Extract (Ethanol) | Human dermal fibroblasts (HDFs) | 1, 10, 20 µg/ml (48 h) | MMP-1 expression using western blotting | GP extract dose-dependently inhibited MMP-1 and MMP-9 expression in UV-B irradiated HDFs. | |
| | | In vitro study stimulated by UV irradiation | Control groups: Normal control, Model control (UV 40 mJ/cm²), Positive control (10 µM Retinoic acid) | MMP-9 expression using Zymography | | |
| | Leaf, stem | RAW 264.7 macrophages | GP: 0.003, 0.01, 0.03 µg/ml Active ingredients: concentration not stated Treatment time not stated | Inhibition on inflammatory cell infiltrates using migration assay | GPED ↓↓↓ LPS-induced cell migration, Limonene, but not α-pinene, 3-carene, or their components mixture, ↓↓ cell migration. | Huang et al. (2019) |
| | Essential oil and its active ingredients | In vitro study stimulated by LPS | 0.4, 0.6, 0.8 mg/ml 24 h incubation with LPS | TNF-α and IL-6 production level using ELISA | GP extract dose-dependently ↓ IL-6, GP extract at 0.8 mg/ml ↓ TNF-α. Ethyl acetate fraction showed the best inhibitory effect on IL-6 and TNF-α. | Liu M. et al. (2019) |
| | Extract (Ethanol) | Fractions (Petroleum ether, ethyl acetate, n-butanol, water) | 3.9, 15.63, 62.5 and 250 µg/ml | Nitric oxide production level using Griess assay | Pre-treatment of 250 µg/ml GP: ↓ NO production (dose dependent) ↓ INOS protein expression. | Ning et al. (2019) |
| | Whole plant | RAW 264.7 macrophages | 1 h pre-treatment | INOS protein expression using western blotting | | |
| | Extract (Ethanol) | In vitro study stimulated by LPS | Control groups: Normal control Vehicle control (0.1% DMSC) Model control (1 µg/ml LPS) | NO production (dose dependent) | | |
| | Leaf | Mice normal liver cell line NCTC-1469 | 80 and 160 µg/ml (24 h) | Nrf2 and p-JNK protein expressions using western blotting | 80 µg/ml GP treatment ↓ Nrf2 protein level, ↓ p-JNK 160 µg/ml GP treatment ↓ Nrf2 protein levels, ↓↓ p-JNK | Liu Y. et al. (2019) |
| | Extract (Aqueous) | In vitro study transfected with Ad-shCFLAR | Normal control (Ad-shCtrl) Model control (pre-treated with Ad-shCFLAR for 24 h) | Nrf2 and p-JNK protein expressions using western blotting | | |
| | Leaf | RAW 264.7 macrophages | 15.63, 31.25, 62.5, 125, 250, 500 µg/ml | Nitric oxide production level using Griess assay | GP extract showed anti-inflammatory activity by inhibiting NO production | Chandradevan et al. (2020) |
| | Extract (Ethanol) | In vitro study stimulated by LPS | Normal control Model control (IFN-γ + LPS) | | | |
| | Aerial part | Bab/c white mice (5 mice/group) | 0.75 mg/20 µl | Ear thickness (anti-inflammatory activity) | Original organic crude extract ↓↓↓ croton oil-induced ear inflammation. | Iskander et al. (2002) |

(Continued on following page)
| Plant species | Part, Gynura form | Cell line/Animal study model | Concentration/dose, control groups | Parameter measured and technique used | Findings | Reference |
|---------------|------------------|-----------------------------|------------------------------------|--------------------------------------|----------|-----------|
| Leaf          | Male BALB/c mice (5 mice/group) | 50 mg/kg body weight | GSK3β phosphorylation using western blotting | GP ↑ phosphorylation of liver GSK3β (Ser9) compared with non-treated control | Wong et al. (2015) |
| Extract (Ethanol) | In vivo study using parasite-infected mice model | Intraperitoneally treated 1 day pre-infection | TNF-α, IFN-γ and IL-10 production level using ELISA | ↓ TNF-α and IFN-γ levels in liver and serum by administration of GP ↑ IL-10 level in serum. |
| Leaf, stem    | 50 male Kunming mice (5 mice/group) | GPEO: 0.433, 0.865, 1.73 mg/ml | Ear thickness by xylene-induced ear edema | GPEO ↓↑ xylene-induced ear and hind paw edema at all doses throughout experiment. Treatment with active ingredients mixture ↓ ear and hind paw edema. |
| Essential oil and its active ingredients | In vivo study using xylene and formalin-induced inflammation mice model | Active ingredients: α-pinene (0.174 mg/ml), 3-carene (0.153 mg/ml), limonene (0.036 mg/ml) and mixture of all 3 ingredients | COX-2 expression using immunohistochemical staining and multispectral imaging analysis | ↓ COX-2 expression by 0.865 mg/ml GPEO and all 3 individual and mixture of active ingredients. ↓↓ COX-2 expression by 1.73 mg/ml GPEO. |
| Leaf          | 32 Male C57BL/6J mice (8 mice/group) | 500 and 1,000 mg/kg body weight | Nrf2 and p-JNK protein expressions using western blotting | 500 mg/kg GP treatment ↑ Nrf2 protein level, no obvious effect on p-JNK, ↑ PPARγ mRNA expression. 1000 mg/kg GP treatment ↓ protein levels of hepatic Nrf2, ↓ protein level of hepatic p-JNK, ↑ PPARγ mRNA expression. |
| Extract (Aqueous) | In vivo study using non-alcoholic steatohepatitis (NASH) mice | Oral route Daily for 6 weeks | mRNA expression of PPARγ using RT-qPCR | ↑↑ PPARγ mRNA expression. |
| Gynura bicolor (Roob. ex Wild.) DC | RAW 264.7 macrophages | 15, 30, 60, or 120 μg/ml | Nitric oxide production level using Griess assay PGE2 production using competitive enzyme immunoassay (EIA) kit iNOS, COX-2, phosphorylated-iBa and p65 protein expression using immunoblot analysis | ↓ NO production (30% decrease with 120 μg/ml GB) ↓ PGE2 production (dose dependent) ↓ iNOS and COX-2 protein expressions (concentration dependent) ↓ p-iBa protein (47% decrease with 120 μg/ml GB) ↓ nucleic p65 protein levels (3–41% decrease with 30, 60, 120 μg/ml GB) ↓ translocation of NF-κB from the cytosol to nuclei ↓ DNA-binding activity of NF-κB nuclear protein by 30, 60, 120 μg/ml GB |

(Continued on following page)
| Plant species | Part, *Gynura* form | Cell line/Animal study model | Concentration/dose, control groups | Parameter measured and technique used | Findings | Reference |
|---------------|---------------------|-------------------------------|------------------------------------|--------------------------------------|----------|-----------|
| Leaf          | Human umbilical vein endothelial cells (HUVEC) | Aqueous or ethanol extract at 1, 2 or 4% (v/v) | IL-6 and TNF-α production using cytoscreen immunoassay | Pre-treatments with aqueous or ethanol extract of GB at 2 and 4% ↓ IL-6, TNF-α formation ↓ PGE2 formation ↓ COX-2 activity | Chao et al. (2015) |
| Extract (Aqueous, Ethanol) | In vitro study treated by high glucose | 12 h pre-treatment; Control groups: Control (5.5 mM glucose); Model control (33 mM glucose) | Prostaglandin E (PGE2) production; Cyclooxygenase (COX)-2 activity | | |
| Leaf          | PC12 cell line (rat adrenal gland pheochromocytoma) | Aqueous extract at 0.25, 0.5 or 1% LDH activity | Mitochondrial membrane potential (Δψm) using fluorescent dye Rh123 IL-6, IL-1β and TNF-α production level by cytoscreen assay NF-κB and p38 mRNA expression using RT-PCR | ↓ LDH activity ↓ Δψm in concentration dependent manner ↓ IL-6, IL-1β and TNF-α production level Test concentrations (0.25, 0.5 and 1%) ↓ NF-κB mRNA expression 0.5 and 1% extract ↓ p38 mRNA expression. | Yang et al. (2019) |
| Extract (Aqueous) | In vitro study of H2O2 induced injury | 48 h pre-treatment; Control groups: Normal control Model control (H2O2 stimulation) | | | |
| Leaf          | Male C57BL/6 mice (8 mice/group) | 0.25 or 0.5% G. bicolor aqueous extract diet Hepatic levels of IL-1β, IL-6 and TNF-α using cytoscreen immunoassay kits | | GB dose-dependently ↓ hepatic levels of inflammatory cytokines of IL-1β, IL-6 and TNF-α. | Yin et al. (2017) |
| Extract (Aqueous) | In vivo study using chronic ethanol consumption-induced hepatic injury mice model | Oral route; 6 weeks Control groups: Normal control Liquid diet group (without ethanol) Ethanol diet group | | | |
| Leaf          | 50 male Balb/cA mice (10 mice/group) | 0.25, 0.5, 1% GB diet Cardiac or renal level of IL-1β, IL-6 or TNF-α using cytoscreen immunoassay kits | | GB at 3 doses: ↓ level of IL-1β, IL-6, and TNF-α in heart and kidney ↓ p38 and NF-κB mRNA expression in heart or kidney | Pai et al. (2019) |
| Extract (Aqueous) | In vivo Streptozotocin-induced type 1 diabetic mice model | Oral route; 8 weeks Control groups: Normal control (basal diet) Diabetic model control (40 mg/kg BW streptozotocin in citrate buffer, 0.1 M) via i.p. injection for 5 days | | | (Continued on following page) |
### TABLE 4 (Continued) List of studies on the anti-inflammatory effects of genus *Gynura*.

| Plant species | Part, *Gynura* form | Cell line/Animal study model | Concentration/dose, control groups | Parameter measured and technique used | Findings | Reference |
|---------------|----------------------|------------------------------|-----------------------------------|---------------------------------------|----------|-----------|
| *Gynura* pseudochina (L.) DC. | Leaf | HeLa cells | 0.2–200 μg/ml | IL-6/luciferase assay (NF-κB assay) | Gynura pseudochina var. hispida (MeOH) showed the strongest NF-κB inhibitory effects, as well as inhibition on release of IL-1, IL-6, TNF-α and PGE2. | Siriwatanametanon et al. (2010) |
| | Extract (MeOH, Ethyl acetate, Petroleum ether) | In vitro study stimulated by PMA | 1, 10 and 50 μg/ml; 24 h | IL-6, IL-1, TNF-α and PGE2 production level using ELISA and EIA | | |
| | | Monocytes from healthy human donors | | | | |
| | | In vitro study stimulated by LPS | | | | |
| | | Control groups: | | | | |
| | | Positive control (ethanol) | | | | |
| | | Negative control (Unstimulated cells) | | | | |
| | | Reference group (Parthenolide, hydrocortisone) | | | | |
| | Leaf | HeLa cells | Non-toxic concentrations (using the MTT assay) | IL-6/luciferase assay (NF-κB assay) | Quercetin 3-rutinoside showed the highest NF-κB inhibitory effect. NF-κB inhibitory activities IC50: Quercetin 3-rutinoside: 24.1 ± 0.1 μg/ml; 3,5-di-cafeoylquinic acid: 42.8 ± 0.2 μg/ml; 4.5-di-cafeoylquinic acid: 49.1 ± 0.1 μg/ml; 5-mono-cafeoylquinic acid: 83.0 ± 0.1 μg/ml | Quercetin 3-rutinoside, 3,5-di-cafeoylquinic acid, 4.5-di-cafeoylquinic acid, 5-mono-cafeoylquinic acid | Siriwatanametanon and Heinrich (2011) |
| | Isolated compound from methanol extract | In vitro study stimulated by PMA | Control groups: | | | |
| | | Positive control (ethanol) | | | | |
| | | Negative control (Unstimulated cells) | | | | |
| | | Reference group (Parthenolide) | | | | |
| | Leaf | Human HaCaT keratinocytes | Extract: 375 and 750 μg/ml | IL-8 production level using ELISA | Extract at both tested concentrations and some concentration of each marker compounds: ↓ IL-8 production level ↓ translocation of RelB S573 into nucleus | Sukadeetad et al. (2018) |
| | Extract (Ethanol), marker compounds | In vitro study stimulated by TNF-α | Chlorogenic acid: 140 and 280 μg/ml | RelA and RelB localization by immunofluorescence assay | | |
| | | Caffeic acid: 30 and 60 μg/ml | | | | |
| | | Rutin: 750 and 1,500 μg/ml | | | | |
| | | p-coumaric acid: 1,400 and 2,800 μg/ml | 24 h | | | |
| | | Control groups: | | | | |
| | | Normal control | | | | |
| | | Model control (50 ng/ml TNF-α with/ without 0.7% DMSO) | | | | |
| | | Positive control (50 μg/ml curcumin) | | | | |
| | Leaf | 25 patients with mild to moderate plaque psoriasis | Mixture of extract and vehicle (1:10) | Phosphorylation of NF-κB p65 using immunohistochemistry (skin sections from two patients) | Immunohistochemical staining revealed diminution of phosphorylated NF-κB p65 in the lesions treated with the GP ointment. | Rerknimitr et al. (2016) |
| | Ointment from ethanol extract | Randomized controlled study | Twice daily for 4 weeks | Control: 0.1% triamcinolone cream | | |

(Continued on following page)
TABLE 4 | (Continued) List of studies on the anti-inflammatory effects of genus *Gynura*.

| Plant species | Part, *Gynura* form | Cell line/Animal study model | Concentration/dose, control groups | Parameter measured and technique used | Findings | Reference |
|---------------|---------------------|-------------------------------|-----------------------------------|---------------------------------------|----------|-----------|
| *Gynura divaricata* (L.) DC. | Leaf, stem Lyophilized into powder | 60 male imprinting control region mice (15 mice/group) In vivo study using high-fat diet and streptozotocin (STZ)-induced type 2 diabetic mice | Diets with 1.2 and 4.8% GD Oral route Daily for 4 weeks | Pancreatic Akt, PI3K, and PDK-1 mRNA expressions using qPCR Pancreatic p-Akt, PI3K, and PDK-1 protein expressions using western blotting | ↑ Akt mRNA, ↑ p-Akt protein expression, ↑ PI3K mRNA and protein expression, ↑ PDK-1 mRNA and protein expression 1.2% GD: ↑ Akt mRNA, ↑ p-Akt protein expression, ↑ PI3K mRNA and protein expression, ↑ PDK-1 mRNA, ↑ PDK-1 protein expression. | Xu et al. (2015) |
| Aerial part | Male ICR mice (15 mice/group) | Diets with 1, 5 and 10% GD Oral route Daily for 4 weeks | Western blotting to determine hepatic protein expression of phosphatidylinositol 3-kinase (PI3K) phosphorylated protein kinase B (p-Akt) | 1% GD: ↑ p-GSK3β, ↑ PPARγ 5% GD; ↑ PI3K, ↑ p-Akt, ↑ p-GSK3β, ↑ PPARγ, ↑ TNF-α 10% GD: ↑ PI3K, ↑ p-Akt, ↑ p-GSK3β, ↑ PPARγ, ↑ TNF-α, ↑ NF-κB | Dong et al. (2019) |
| *Gynura segetum* (Lour.) Merr | Leaf RAW 264.7 macrophages | Extract: 6.25–100 μg/ml | Nitric oxide production level using Griess assay | GS extract showed inhibition on NO production with IC50 = 0.16 ± 0.03 μM; IC50 IL-1β = 2.72 ± 1.84 μM; IC50 TNF-α = 6.69 μM. Rutin was the most potent sample against TNF-α release with IC50 = 16.96 μM. | Yu andani et al. (2017) |
| Extract (Methanol), isolated compound from extract | Peripheral blood mononuclear cells (PBMCs) | Compounds: 3.125–50 μg/ml 3 h (Griess assay) or 12 h pre-treatment (ELISA) Positive control (0.025 μM Dexamethasone) | IL-1β and TNF-α production level using ELISA | GS extract showed inhibition on NO production with IC50 = 0.16 ± 0.03 μM; IC50 IL-1β = 2.72 ± 1.84 μM; IC50 TNF-α = 6.69 μM. Rutin was the most potent sample against TNF-α release with IC50 = 16.96 μM. | Seow et al. (2014) |
| Extract (Methanol) | Male Sprague-Dawley rats | 125, 250 and 500 mg/kg body weight Oral route Once daily for 7 days | Cotton pellet granuloma assay TNF-α and IL-1 production level using ELISA | GS dose-dependently ↓ formation of granuloma tissues (17.1, 39.7, and 47.2% inhibition by 125, 250 and 500 mg/kg GS), ↓↓ TNF-α and IL-1 levels in circulating pro-inflammatory cytokine levels. | | (Continued on following page) |
| Plant species | Part, Gynura form | Cell line/Animal study model | Concentration/dose, control groups | Parameter measured and technique used | Findings | Reference |
|---------------|------------------|-----------------------------|-----------------------------------|-----------------------------------|----------|-----------|
| **Gynura nepalensis DC.** | Leaf | H9c2 cardiomyoblasts | 1.56, 3.12, 6.25, 12.5, 25, 50, 100 μM | LDH production level | Compound 6 (3,5-dicaffeoylquinic acid ethyl ester) exhibited a more potent cytoprotective effect thus selected for further evaluation. ↓ LDH leakage at 6.25, 12.5, 25, 50 and 100 μM compound 6. ↑↑↑ Δψm in cells cultured with compound 6 (6.25, 12.5, 25.0 μM) | Yu et al. (2016) |
| | Nine caffeoylquinic acid analogs (1–9) isolated from ethanol extract | In vitro study stimulated by H2O2 1 h pre-treatment | Control groups: Normal control, Model control (0.3 mM H2O2), Positive control (Carbonyl cyanide m-chlorophenylhydrazone (CCCP) for Δψm) | Mitochondrial membrane potential (Δψm) Phosphorylation of ERK, JNK, and p38 | Compound 6 (3,5-dicaffeoylquinic acid ethyl ester) exhibited a more potent cytoprotective effect thus selected for further evaluation. ↓ LDH leakage at 6.25, 12.5, 25, 50 and 100 μM compound 6. ↑↑↑ Δψm in cells cultured with compound 6 (6.25, 12.5, 25.0 μM). | |
| | Leaf | 24 Swiss albino mice (6 mice/group) In vivo study using mice model with induced inflammation | 250 and 500 mg/kg body weight Oral route 1 h before infection Control groups: Model control (distilled water + 20 μl xylene, saline + injection of 0.1 ml 1% carrageenan) Positive control (100 mg/kg diclofenac sodium) | Xylen-induced ear edema test Carrageenan-induced paw edema test | Xylene-induced ear edema and carrageenan-induced models of inflammation. | Rahman et al. (2018) |
| **Gynura formosana Kitam.** | Leaf | 48 male Sprague-Dawley rats (8 rats/group) In vivo study using cotton pellet-induced granuloma rat model | 100 mg/kg, 250 mg/kg, 500 mg/kg body weight Oral route Once daily for 7 days Control groups: Normal control Model control (0.5% carboxymethylcellulose, 1 ml/kg) Standard drug (4 mg/kg indomethacin) | Cotton pellet granuloma assay LDH, GPT, CRP, TNF-α and IL-1β production level using ELISA | GF dose-dependently ↓ granuloma formation. 100, 250, and 500 mg/kg GF ↓ levels of plasma inflammatory biomarkers (LDH, GPT and CRP) activities. GF dose-dependently ↓ plasma pro-inflammatory cytokines (TNF-α and IL-1β). | Ma et al. (2017) |

† indicates significantly increase (p < 0.05), †† indicates significantly increase (p < 0.01), ††† indicates significantly increase (p < 0.001), ↓ indicates significantly inhibit (p < 0.05), ↓↓ indicates significantly inhibit (p < 0.01), and ↓↓↓ indicates significantly inhibit (p < 0.001).
inflammatory effects by significantly reducing ear thickness and paw edema in inflammatory mice model. Granulomatous inflammation is a special variety of chronic inflammation in which cells of the mononuclear phagocyte system are aggregated into well-demarcated focal lesions called granulomas (Williams and Williams, 1983). The anti-inflammatory effect of G. segetum and G. formosana had been demonstrated by significant inhibition of granuloma tissue formation in a rat model (Seow et al., 2014; Ma et al., 2017).

Anti-inflammatory Signaling Pathways of Gynura
Mitogen-activated protein kinases (MAPK) consist of three signaling pathways: ERK, JNK, and p38 MAPK, which mediate fundamental cellular processes by regulating immunomodulatory cytokine expression (Kaminska, 2005). G. bicolor extract possessed an anti-inflammatory effect by inhibiting p38 mRNA expression both in vitro (Yang et al., 2019) and in vivo (Pai et al., 2019). The isolated compound from G. nepalensis extract, 3,5-dicaffeoylquinic acid ethyl ester, exhibited inhibitory effects on the phosphorylation of JNK and ERK in cardiomyoblasts (Yu et al., 2016). Similarly, in vitro both pathway (Dong et al., 2019). In a randomized controlled study, NF-κB phosphorylation with IC₅₀ effect with IC50 compounds of chlorogenic acid, caffeic acid, rutin, and ferulic acid also inhibited RelB S573 (protein transcription factors of NF-κB) (Siriwatanametanon et al., 2010). Successively, quercetin 3,5 diketochina var. hispida, which was one of the isolated compounds from (Siriwatanametanon et al., 2010), showed a strong NF-κB phosphorylation in the lesions treated with chlorogenic acid. Successively, quercetin 3,5 diketochina var. hispida, which was one of the isolated compounds from (Siriwatanametanon et al., 2010), showed a strong NF-κB phosphorylation in the lesions treated with chlorogenic acid. Successively, quercetin 3,5 diketochina var. hispida, which was one of the isolated compounds from (Siriwatanametanon et al., 2010), showed a strong NF-κB phosphorylation in the lesions treated with chlorogenic acid.

GSK3 acts as the central regulator of the inflammatory response to bacterial infections and other insults. Hence, inactivation of GSK3 by the phosphorylation of GSK3β has been proposed as a potential therapeutic target in the control of bacterial-driven inflammatory diseases (Wang et al., 2014). The anti-inflammatory effect of G. procumbens was correlated to the increased phosphorylation of liver GSK3β (Ser9), which inhibited the activities of GSK3 (Wong et al., 2015). G. divaricata lyophilized powder diet increased GSK3β phosphorylation in a diabetic mice model (Dong et al., 2019). G. procumbens treatment showed anti-inflammatory potential through the induction of the Nrf2 protein level in murine hepatocytes and in a mice model (Liu Y. et al., 2019). Akt activation is also essential for the transcriptional activation of peroxisome proliferator-activated receptor-γ (PPARγ) (Kim et al., 2010), a nuclear receptor that inhibits the expression of inflammatory cytokines and directs the differentiation of immune cells toward anti-inflammatory phenotypes (Tyagi et al., 2011). G. procumbens extract in diet significantly induced in vivo PPARγ mRNA expression (Liu Y. et al., 2019), and G. divaricata lyophilized powder diet increased in vivo hepatic protein expression of PPARγ (Dong et al., 2019).

Interplay Between Physiological, Biochemical and Immunological Aspects
The antioxidant and anti-inflammatory effects of Gynura species are corroborated to each other. As aforementioned, inflammation and oxidative stress are highly interdependent pathophysiological events. Hence the antioxidant effects of Gynura species has supported anti-inflammatory effects by these plants. In general, Gynura species depict anti-inflammatory effects by inhibiting inflammatory signaling pathways, cellular pathogenicity, inflammatory biomolecules secretion, and clinical manifestation of inflammatory diseases. Granulomatous inflammation is the end result of a prolonged complex interplay among causal agents, mononuclear phagocytes activity, circulating immune complexes, and a vast array of biological mediators (Zumla and James, 1996). Inflammatory cell infiltration is the critical process in granuloma formation while tissue swelling is one of the possible associated clinical manifestation. The pathogenesis of granulomatous inflammation is manipulated by the vital player of macrophages along with secretion of cytokines and chemokines by immune cells through stimulation of inflammatory signaling pathways (Facco et al., 2007). Not only granulomatous inflammation, the signaling pathways, biomolecules and cellular responses also contribute...
| Species | Identified phytochemicals | References |
|---------|--------------------------|------------|
| Gynura procumbens (Lour.) Merr. | 15,16-Dihydroxy-9Z, 12Z-octadecadienoic acid (PubChem CID: 16061068) | Akowuah et al. (2002), Rosidah et al. (2008), Kim et al. (2011), Kaewseejan and Siriamornpun (2015), Murugesu et al. (2017), Li et al. (2018), Murugaiyah et al. (2018), Nazir et al. (2019), Huang et al. (2019), Liu M. et al. (2019), Liu Y. et al. (2019), Chandradevan et al. (2020) |
| | 3,4-Dicaffeoylquinic acid (Isochlorogenic acid B) (PubChem CID: 5281789) |  |
| | 3,5-Dicaffeoylquinic acid (Isochlorogenic acid A) (PubChem CID: 6474310) |  |
| | 3,5-O-Dicaffeoylquinic acid (PubChem CID: 13604868) |  |
| | 3-O-Methyl gallic acid sulfate (PubChem CID: not found) |  |
| | 3-Carene (PubChem CID: 26049) |  |
| | 4,5-Dicaffeoylquinic acid (Isochlorogenic acids C) (PubChem CID: 6474309) |  |
| | 4-O-Methyl gallic acid sulfate (PubChem CID: not found) |  |
| | 5-O-(E)-Caffeoyl-galactaric acid (PubChem CID: not found) |  |
| | Apigenin (PubChem CID: 5280443) |  |
| | Caffeic acid (PubChem CID: 689043) |  |
| | Chlorogenic acid (PubChem CID: 1794427) |  |
| | Choline (PubChem CID: 305) |  |
| | Citric acid (PubChem CID: 311) |  |
| | Cynarine (PubChem CID: 5281769) |  |
| | Dicaffeoylquinic acids (PubChem CID: 6474310) |  |
| | Eriocitrin (PubChem CID: 83489) |  |
| | Ferulic acid (PubChem CID: 445858) |  |
| | Feruloylquinic acid (PubChem CID: 10133609) |  |
| | Gallic acid (PubChem CID: 370) |  |
| | Genkwanin isomer (PubChem CID: 5281617) |  |
| | Isobioquercetin (PubChem CID: not found) |  |
| | Kaempferol (PubChem CID: 52880863) |  |
| | Kaempferol 3-O-glucoside (Astragalin) (PubChem CID: 5282102) |  |
| | Kaempferol 3-O-rhamnosyl-(1→6)-glucoside (PubChem CID: not found) |  |
| | Kaempferol-3-O-rutinoside (Nicotiflorin) (PubChem CID: 5318767) |  |
| | Limonene (PubChem CID: 22311) |  |
| | Malic acid (PubChem CID: 525) |  |
| | Myricetin (PubChem CID: 5281672) |  |
| | Neochlorogenic acid (PubChem CID: 5280633) |  |
| | Oxoocadecanoic acid (PubChem CID: 439332) |  |
| | p-Coumaric acid (PubChem CID: 637542) |  |
| | p-Coumaryloquinic acid (PubChem CID: 6441280) |  |
| | p-Hydroxybenzoic acid (PubChem CID: 135) |  |
| | Phenylalanine (PubChem CID: 6140) |  |
| | Protocatechuic acid (PubChem CID: 72) |  |
| | Quercetin (PubChem CID: 5280343) |  |
| | Quercetin 3-O-rhamnosyl-(1→2)-galactoside (PubChem CID: 44259099) |  |
| | Quercetin 3-O-rhamnosyl-(1→6)-glucoside (PubChem CID: not found) |  |
| | Quercetin 3-O-rutinoside (Rutin) (PubChem CID: 5280805) |  |
| | Sinapic acid (PubChem CID: 637775) |  |
| | Syringic acid (PubChem CID: 10742) |  |
| | Trimethyl gallic acid glucuronide (PubChem CID: not found) |  |
| | Vanillic acid (PubChem CID: 8468) |  |
| | α-Pinene (PubChem CID: 6654) |  |

(Continued on following page)
TABLE 5 | (Continued) Phytochemicals of Gynura species.

| Species | Identified phytochemicals | References |
|---------|---------------------------|------------|
| Gynura bicolor (Roxb. ex Willd.) DC. | 3,5-Di-O-caffeoylquinic acid (Isochurogenic acid A) (PubChem CID: 6474310) | Lu et al. (2012), Wu et al. (2015), Qiu X. L. et al. (2018) |
| | 3-O-Feruloylquinic acid (PubChem CID: 9799388) | | |
| | 3-O-p-Coumaroylquinic acid (PubChem CID: 9945785) | | |
| | 4,5-Di-O-Caffeoylquinic acid (Isochurogenic acid C) (PubChem CID: 6474309) | | |
| | 5-O-Caffeoylquinic acid (Neochurogenic Acid) (PubChem CID: 5280633) | | |
| | 5-O-p-Coumaroylquinic acid (PubChem CID: 9945785) | | |
| | Anthocyanin (PubChem CID: 145858) | | |
| | Caffeoyl glucose (PubChem CID: 129715972) | | |
| | Citric acid (PubChem CID: 311) | | |
| | Dihydro-phellopterin (PubChem CID: not found) | | |
| | Gallic acid (PubChem CID: 370) | | |
| | Geniposide (PubChem CID: 107848) | | |
| | Guanosine (PubChem CID: 135398635) | | |
| | Isobavachalcone (PubChem CID: 5281255) | | |
| | Kaempferol-3-O-catecholate (PubChem CID: not found) | | |
| | Kaempferol-3-O-glucoside (Astragalin) (PubChem CID: 5282102) | | |
| | Malic acid (PubChem CID: 525) | | |
| | Phenylalanine (PubChem CID: 6140) | | |
| | Protocatechuic acid-O-glucoside (PubChem CID: not found) | | |
| | Quercetin (PubChem CID: 5280343) | | |
| | Quercetin-3-acetylhexose (PubChem CID: not found) | | |
| | Quercetin-3-O-galactoside (PubChem CID: 5281643) | | |
| | Quercetin-3-O-rutinoside (Rutin) (PubChem CID: 5280805) | | |
| | Tryptophan (PubChem CID: 6305) | | |
| | Uridine (PubChem CID: 6029) | | |
| | β-Carotene (PubChem CID: 5280489) | | |
| Gynura pseudochina (L.) DC. | (+)-Tephropurpurin (PubChem CID: 10047971) | Sirivatanametanon and Heinrich (2011), Ferlinahayati et al. (2017), Sukadietad et al. (2018) |
| | 1-(9Z-octadecenoyl)-sn-glycero-2,3-cyclic phosphate (PubChem CID: 52922109) | | |
| | 1,3,6-Trihydroxy-4-methyl-2,7-diprenylxanthe (PubChem CID: 67261902) | | |
| | 2-(2,4-Dihydroxyphenyl)-5-hydroxy-8-methyl-8-(4-methyl-3-penten-1-yl)-2,3-dihydro-4H,8H-pyrano[2,3-f]chromen-4-one (PubChem CID: not found) | | |
| | 3,4-Dihydroxycinnamoyl-(Z)-2-(3,4-Dihydroxyphenyl) Ethanol (PubChem CID: 14353342) | | |
| | 3,5-Dicaffeoyl quinic acid (Isochurogenic acid A) (PubChem CID: 6474310) | | |
| | 3-O-Caffeoyl-1-O-methylquinic acid (PubChem CID: 131752768) | | |
| | 4,5-Dicaffeoyl quinic acid (PubChem CID: 13887346) | | |
| | 5-Hydroxy-2′-methoxy-6,7-methylenedioxyisoflavone (PubChem CID: 5491329) | | |
| | 5-Caffeoyl quinic acid (Chlorogenic acid) (PubChem CID: 1794427) | | |
| | Caffeic acid (PubChem CID: 689043) | | |
| | Isochurogenic acid B (PubChem CID: 5281780) | | |
| | Isochurogenic acid C (PubChem CID: 6474309) | | |
| | Kaempferol rutinoside (Nicotiflorin) (PubChem CID: 5318767) | | |
| | Quercetin (PubChem CID: 5280343) | | |
| | Quercetin 3-rutinoside (Rutin) (PubChem CID: 5280805) | | |
| | Stigmastanol (PubChem CID: 5280794) | | |
| | β-Sitosterol (PubChem CID: 222284) | | |

(Continued on following page)
to pathogenesis of various diseases. Thus the plants in genus *Gynura* are having high potential to be explored for pharmacological evidence of different ailments.

### FUTURE PERSPECTIVE

Previous studies on *Gynura* species have indicated that several *Gynura* species possessed strong antioxidant and anti-inflammatory effects that are fundamental to various therapeutic purposes. Hence, *Gynura* species are potential continual source of new and useful bioactive compounds. Identification of the bioactive compounds in the plants contributing to the bioactivities, along with their mechanisms of action responsible for the pharmacological activities, is needed. Table 5 shows the list of phytochemicals identified in *Gynura* species. There is a need to investigate whether the crude extracts or isolated pure compounds are more effective. Synergistic effects of multiple active compounds in the extract may lead to a stronger pharmacological effect than that achievable by a single compound. At this stage, limited clinical studies on the antioxidant and anti-inflammatory effects of genus *Gynura* have been conducted. Human clinical trials with clearly defined symptomology to evaluate the therapeutic value of genus *Gynura* are necessary because animal experiments cannot be a substitute for clinical trials in the evaluation of therapeutic efficacy. To the best of our knowledge, there is only one non-systematic randomized controlled trial that has been carried out. Rerknimitr et al. (2016) investigated the efficacy of *G. pseudochina* DC. var. *hispida* Thv. ointment in treating chronic plaque psoriasis in a randomized controlled trial.

The bioavailability of bioactive molecules from genus *Gynura* also has limited investigation. On the basis of the study by Wu et al. (2015), the use of *G. bicolor* extract showed improvement on *in vivo* iron absorption and storage protein, which might be related to its rich phytoactive ingredients. Although the traditional uses of genus *Gynura* are supported by scientific evidence, the processing, and application or consumption methods by the public may alter the phytochemical profile, thus leading to pharmacological effect variations. Herbal combination is possible to provide better effects or benefits in developing therapeutic drugs. The study by Sari et al. (2015) showed that the combination of *Andrographis paniculata* (Burm. f.) Ness and *G. procumbens* could be a potential candidate for the development of an antidiabetic agent. The optimum therapeutic effect could be attributed to the combination of the potent hypoglycemic effect of *A. paniculata* and the potent antioxidant effect of *G. procumbens*. Hence, further studies on the therapeutic effects of the combinations of *Gynura* species with other medicinal plants are potentially producing more effective disease remedies. However, the potential issues of herb–herb and herb–drug interactions should be given due consideration, and further studies are needed to ensure no adverse interactions in polypharmacy and polyherbacy conditions.

### Table 5 (Continued) Phytochemicals of *Gynura* species.

| Species | Identified phytochemicals | References |
|---------|---------------------------|------------|
| *Gynura divaricata* (L.) DC. | 3,4-Dicaffeoylquinic acid (Isochlorogenic acid B) (PubChem CID: 5281780) 3,5-Dicaffeoylquinic acid (Isochlorogenic acid A) (PubChem CID: 6474310) 4,5-Dicaffeoylquinic acid (Isochlorogenic acid C) (PubChem CID: 6474309) 3-Caffeoylquinic acid (Chlorogenic acid) (PubChem CID: 1794427) Cubenol (PubChem CID: 519857) Spathulenol (PubChem CID: 92231) | Jiangseubchatveera et al. (2015), Dong et al. (2019) |
| *Gynura segetum* (Lour.) Merr. | Rutin (PubChem CID: 5280805) Gallic acid (PubChem CID: 370) 4,5,4′-Trihydroxychalcone (PubChem CID: 468135) 8,8′-(Ethen-1,2-diyl)-dinaphtalene-1,4,5-triol (PubChem CID: not found) | Yuandani et al. (2017) |
| *Gynura nepalensis* DC. | 3,4-Dicaffeoylquinic acid methyl ester (PubChem CID: not found) 3,5-Dicaffeoylquinic acid ethyl ester (PubChem CID: not found) 3,5-Dicaffeoylquinic acid methyl ester (PubChem CID: 10075681) 3-O-cis-p-Coumaroylquinic acid (PubChem CID: 9945785) 4,5-Dicaffeoylquinic acid methyl ester (PubChem CID: not found) Chlorogenic acid (PubChem CID: 1794427) Isochlorogenic acid A (PubChem CID: 6474310) Isochlorogenic acid B (PubChem CID: 5281780) Isochlorogenic acid C (PubChem CID: 6474309) Saponins (PubChem CID: 6540709) Tannins (PubChem CID: 250395) | Yu et al. (2016), Aktar et al. (2019) |
CONCLUSION

The extracts and phytochemicals of several Gynura species, particularly G. procumbens, G. bicolor, G. segetum, G. divaricata, G. formosana, G. nepalensis, and G. pseudochina, have been reported to exhibit strong antioxidant and anti-inflammatory effects. However, in vitro and in vivo studies and clinical studies that have been carried out on the species are by no means free of methodological flaws. All 27 studies selected in this systematic review depicted risk of bias at different extent. The lack of randomization, lack of blinding, and unclear methodology explanation are some prevalent limitations. Further preclinical studies, including toxicity and pharmacokinetic studies on Gynura extracts and their bioactive compounds, are necessary before they can be subjected to clinical studies. Genus Gynura has high potential to be developed into medicinal agents for prophylactic supplements of diseases related to oxidative stress or inflammation.

AUTHOR CONTRIBUTIONS

TJN, ZJ, and NMF designed the study. IJ and KH provided information on the ethnopharmacology of Gynura and sources of Gynura research. TJN conducted the literature search, extracted the data, and wrote the first draft. ZJ and NMF oversaw the research project, including checking the research work, reviewing, and interpreting the results. SMS and FB provided methodological advice on the project (literature search, screening, and selection). All authors are involved in reviewing and approval of the final manuscript.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.