RECENT UPDATE ON ANTI-CANCER ACTIVITY OF *GYNURA PROCUMBENS (LOUR.) MERR.*

Mohamed R[1], Lim V[2], Aziz MY[2].
[1]Department of Biomedical Science, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, 13200 Kepala Batas, Pulau Pinang, Malaysia
[2]Department of Toxicology, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, 13200 Kepala Batas, Pulau Pinang, Malaysia

Correspondence:
Rafeezul Mohamed,
Department of Biomedical Science,
Advanced Medical and Dental Institute,
Universiti Sains Malaysia, Bertam,
13200 Kepala Batas, Pulau Pinang, Malaysia
E-mail: rafeezul@usm.my

Abstract
Gynura procumbens (Lour.) Merr. is a traditional medicinal herb that is utilised to treat a wide range of illnesses, such as kidney disease, hypertension, eruptive fever, snake bites, migraines, diabetes, and rheumatism. *Gynura procumbens* was also found to exhibit anti-cancer effects, especially related to cervical, breast, leukemia, and osteosarcoma cancers. Nevertheless, the exact mechanisms of its anti-cancer effects are still under investigation. The aim of this study is to provide updated information on ongoing studies which focused on the benefits of *Gynura procumbens* herb in preventing various types of cancers. Google and PubMed were explored using the keywords "*Gynura procumbens* and cancer" by selecting published journals on the anti-cancer effects of *Gynura procumbens*. This review has highlighted the botanical aspects, chemical constituents, and anti-cancer effects of *Gynura procumbens* on selected types of cancers as have been reported by previous studies. The study findings have implications on future research which pave way for future clinical usage, either alone or in combination with the currently available medicines in treating cancers.

Keywords: Anti-Cancer, *Gynura procumbens*, Medicinal Herbs

Introduction

Gynura procumbens (Lour.) Merr. grows naturally in Southeast Asia regions, specifically in Indonesia, Malaysia, the Philippines, and Thailand (1). The Gynura plant is a member of the *Asteraceae-Senecioneae* family; With 44 species ranging across tropical Africa to South and East Asia and Australasia, this herb plant is known by various local and native names (1). In Malaysia, it is called "*Pokok Sambung Nyawa*" (or translated as "Longevity Spinach"), whereby in China, it is known as "*Bai Bing Cao"* which means "100 Ailments" (2). The plant is also known as "*Paetumpung*" and "*Daun Dewa*" in Thailand and Indonesia, respectively (2). Gynura is typically small in size and can grow up to 1 to 3 m in height, with a purple shade and fleshy stem (2). The shape of its leaves is lanceolate or ovate-elliptic with 3.5 to 8 cm long and 0.8 to 3.5 cm wide (2). Its Flowering heads are panicled, narrow, yellow in color, and 1 to 1.5 cm long (2). *Gynura procumbens* can easily grow on well-drained and fertile moist soil which is used to place the stem cuttings (2). In this study, the most suitable journals in PubMed were explored using keywords "*Gynura procumbens* and cancer" from the year 2011 to the year 2022. All the articles were selected comprising original and review articles. A few outdated related journal articles were also chosen in order to obtain comprehensive evidence on *Gynura procumbens* effects on various types of cancers. Review of related articles was also carried out through Google search, which also included published theses and conference papers in order to explore various forms of studies related to anti-cancer properties of *Gynura procumbens*.

Ethnobotanical and pharmacological aspects of Gynura procumbens

Gynura procumbens is a species of plant that can be

consumed and utilised as a safe alternative medicine to chemical-based medication due to its wide range of natural health benefits. This herb plant is utilised as folk medicine to treat multiple illnesses, such as kidney disease, hypertension, constipation, diabetes mellitus, eruptive fever, urinary tract infection, skin viral diseases, and rheumatism (3). Additionally, due to their mild flavour, its leaves are also served as an appetiser in which they can be consumed raw in salads, soups ingredient, rich dishes, and other meals as the leaves have been proven to be safe to consume and do not cause any harm to human health (3). Furthermore, *Gynura procumbens* leaves can be processed and transformed into topical cream which can be used to reduce inflammation as well as skin infection due to viral, rheumatism, and general body pain (3). Some of these traditional practices have been proven scientifically in pharmacological studies, including anti-herpes virus, anti-inflammatory, anti-hyperlipidaemic, anti-hyperglycemic, and anti-hypertensive activities (4). *Gynura procumbens* becomes a promising natural source with a variety of potent chemical contents that contribute to the evolution of innovative therapeutic use. The plant extract has been described as a no-observed-adverse-effect level (NOAEL) crude drug which does not cause any harm to the human body, following its evaluation based on currently available standard guidelines. In a previous study, the toxicological evaluation of 1000 to 5000 mg/kg of *Gynura procumbens* leaves on rats demonstrated zero mortality rate and no significant alteration in the animals' general health performance, organ gross presentation, or body weight (5). Another *in vivo* study showed that 25% of *Gynura procumbens* ethanol extract was safe for oral consumption by both male and female Sprague Dawley rats, with the lethal dose-50 (LD_{50}) higher than 2000 mg/kg (6). Based on the results of this study, *Gynura procumbens* leaves were suggested as NOAEL drug or substance with a daily consumption of 700 mg/kg/day (6).

Chemical constituents of Gynura procumbens

Different parts of *Gynura procumbens,* such as leaves, stem and entire plant, contain a wide range of chemical constituents. Parts of this plant can be extracted and fractionated using various types of solvents, including polar and non-polar solvents. As reported in a study, the less polar or non-polar compounds are usually extracted or fractionated using non-polar solvents such as chloroform, whereas polar compounds are typically extracted or fractionated using polar solvents like ethyl acetate (7). The introduction of new technology and instrument, including liquid chromatography with tandem mass spectrometry (LCMS/MS), nuclear magnetic resonance (NMR), and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QToF-MS), has facilitated the detection of various compounds in crude extracts or fractions from different parts of *Gynura procumbens* which were collected from various countries, as listed in Table 1.

Mechanisms of anti-cancer effects of Gynura procumbens extracts

Overview of cancer

In 2015, cancer was ranked at either the first or second leading cause of mortality among people in many countries with age below 70 years, as reported by the World Health Organisation (WHO) (11). The number of new cases of cancer and cancer-associated deaths by 2030 were estimated about 21.4 and 13.2 million yearly, respectively (12). The rapid increase in the number of newly reported cancer cases and death rates globally are due to several reasons, including the complexity of cancer mechanism, growth of aging population, as well as alteration to the main cancer risk factors, in which these factors are closely linked with socioeconomic development in the countries (11). Generally, cancer occurs when a normal cell converts into a dysplastic cell, and then evolves into a malignant cell. The uncontrolled proliferation of the malignant cell will cause the cell dissemination and penetration into various body parts via blood circulation (13). The initiation of cancer takes place at the cellular level due to various factors, such as metabolic, genetic, and carcinogen, leading to DNA molecule impairment (14). Nevertheless, an uncomplicated genetic defect is not able to stimulate the cancer growth. Cancer development progresses in the promotion stage in which the defective cells undergo proliferation and multiple division (14). The final cancer stage involves the spreading of cancer cells into various sites of the body, as described by Nordling and later by Knudson (15,16). There are six indications that illustrate the nature of cancer cells, namely self-reliance in growth signals, unresponsiveness to anti-growth signals, escapism from the apoptosis mechanism, infinite replication capabilities, continuous angiogenesis process, as well as tissue intrusion and transition (17).

Table 1: Chemical constituents detected in different parts of *Gynura procumbens*

Parts of Gynura procumbens	Types of extracts/fractions	Methods of detection	Chemical constituents	Location	Reference
Leaves	Ethanol extract	LC-MS/MS	α-9(10)-EpODE, Cpd 45: 9Z,12Z,15E-octadecatrienoic acid, 6E,9E-octadecadienoic acid, Pheophorbide a	Malaysia	8
	Aqueous fraction		Isovitexin 2''-O-xyloside, Homoesperetin 7-rutinoside, 9Z,12Z,15E-octadecatrienoic acid, Ipolamiide, 6,8-Di-C-beta-D-arabinopyranosylapigenin		
	Chloroform fraction		4-(2-hydroxypropoxy)-3,5-dimethyl-Phenol, 11-hydroperoxy-12,13-epoxy-9-octadecenoic acid, Decenedioic acid, (-)-12-hydroxy-9,10-dihydrojasmonic acid, 5,8,12-trihydroxy-9-octadecenoic acid, (6S)-dehydrovomifoliol		
	Ethyl acetate fraction		p-Salicylic acid (4-Hydroxybenzoic acid), Luteolin 7-rhamnosyl(1->6)galactoside, 6-Hydroxyluteolin 5-rhamnoside, 2,3-dinor Thromboxane B1, Formononetin 7-O-glucoside-6''-O-malonate		
	Hexane fraction		Harderoporphyrin, 6E,9E-octadecadienoic acid, Pheophorbide a, 9Z,12Z,15E-octadecatrienoic acid		
Stem	Ethanol extract	NMR and ESI-MS	hexacosanoic acid (1), [beta]-sitosterol (2), daucosterol (3), [beta]-stigmasterol (4), 5[alpha]-stigmastan-3-one (5), methyl linoleate (6), 1-methoxyheneicosan-1-ol (7), homoorientin (8), kaempferol (9), and eriocitrin (10).	China	9
Whole plant	Ethanol extract	Silica gel column chromatography, Sephadex LH-20 gel column chromatography, medium pressure column chromatography, and semi-	quercetin, apigenin, luteolin, kaempferol, astragaline, kaempferol-5-O-(6”-O-acetyl)-β-D-glucopyranoside, negletein, 4-methoxycinnamic acid, benzyl-O-β-D-glucopyranoside, 2-phenylethyl-O-β-D-glucopyranoside, 3,5-	China	10

	preparative HPLC	dicaffeoylquinic acid methyl ester, 3,5-dicaffeoylquinic acid ethyl ester, 3,4-dicaffeoylquinic acid methyl ester, 4,5-dicaffeoylquinic acid methyl ester, protocatechuic acid, eugenol glucoside

Additionally, Hanahan and Weinberg proposed two new hallmarks of cancer, namely reorganisation of cellular metabolism and hindrance from immune system destruction (17). Tumor progression is regulated by various signaling pathways, including distortion of DNA repair, redox balance, apoptosis, and cell cycle (18). Most of the cancers can be classified into three main groups, namely carcinomas, sarcomas, and lymphomas. Carcinomas, which encompass about 90% of human cancers, are defined as malignant tumors that derive from epithelial tissues (13). Sarcomas are infrequent solid tumors which originate from skeletal and connective tissues (13,19). Lymphomas emerge from immune cells and bone marrow stem cells (13,20). Mainly, there are four most frequently occurred types of cancers which constitute more than half of total identified cancer cases, namely breast, colorectal, lung, and prostate cancers (14).

Anti-cancer effects of Gynura procumbens

To date, numerous efforts have been made by research practitioners and scholars to discover novel cancer drugs as medicinal herbs are becoming more widely accepted by the communities as an alternative medicine used for hindering cancer progression. Recently, the anti-cancer effects of *Gynura procumbens* have similarly received a lot of attention, as the plant has long being used by traditional medical practitioners to treat various types of cancers. The anti-cancer effects of *Gynura procumbens* in selected types of cancers were summarised in the following sub-sections.

Breast cancer

The ethyl acetate fraction of *Gynura procumbens* leaves (FEG) was found to prevent breast cancer resistance due to prolonged doxorubicin (DOX) exposure (21). As reported in the study, it significantly suppressed the growth of MCF-7 breast cancer cells which were exposed to DOX (MCF-7/DOX), compared to the MCF-7 cells alone (21). The chemoresistance of the MCF-7/DOX was indicated by high expression of P-gp, i.e. an adenosine triphosphatase (ATPase) which is a member of ATP-binding cassette (ABC) transporter encoded by the MDR1 gene (21). Moreover, the FEG was also found to reduce the expression of P-gp in MCF-7/DOX in a dose-dependent manner which caused the reversal effect of resistancy and induced the sensitivity of the exposed breast cancer cells towards the DOX treatment (21). Another study by Nurulita et al. suggested that kaempferol and quercetin detected in the FEG could potentially play a role in modulating the MDR-1 gene and P-gp protein level which influenced the breast cancer drug resistance (22). A year later, the anti-cancer effects of protein extracted from *Gynura procumbens* leaves (i.e. SN-F11/12 fraction) on human metastatic breast cancer cell line, MDA-MB231, was demonstrated by Hew et al. (23). As observed in their study, the active protein fraction, SN-F11/12, hindered the proliferation of the MDA-MB231, reduced the Ki67 and PCNA mRNA expressions that involved in the cancer cell proliferation, and suppressed the invasion marker, CCL2 expression, in the treated cancer cells (23). A recent study by Ashraf et al. showed that the methanol extract of *Gynura procumbens* displayed the highest anti-proliferative effects on the MCF-7 cells in comparison to other extracts of the plant such as ethyl acetate, chloroform, and butanol (24).

Colon cancer

In a study, the FEG showed moderate inhibitory effects against colon cancer cell line (WiDr) whereby comparatively, quercetin displayed more cytotoxicity effects on the cancer cells, thus indicating that there are other compounds inside the FEG, other than quercetin, which may contribute to the cytotoxicity effects on the WiDr (25). Besides, the FEG worked in synergy with 5-fluorouracil (5-FU) to inhibit the growth of the WiDr [25]. However, the combination of FEG and cisplatin (FEG-cisplatin) exhibited an opposite effect in which it did not hinder the WiDr cell proliferation (25). This finding seemed to suggest that the combinational use of *Gynura procumbens* and various chemotherapy medicines might produce different outcomes of treatment efficacy (25). Furthermore, the effects of the combined FEG and 5-FU treatment on the induced cell death of the WiDr cells were indicated by increasing chromatin condensation,

membrane shrinkage, and nuclear fragmented on the cancer cells (25). Another study involving rats revealed that the ethanolic extract of *Gynura procumbens* reduced about 80% of azoxymethane-induced aberrant crypt foci in the animals, which suggests the promising role of this plant extract in hindering colon cancer (26). Teoh et al. investigated the cytotoxicity effects of various *Gynura procumbens* extracts on selected human colon cancer cells (Caco2, HCT-116, HCT-15, HT-29 and SW480) and normal colon cells (CCD-18Co) (27). It was observed that only the ethyl acetate extract moderately inhibited the growth of six colon cancer cell lines at 72 hours of treatment with low cytotoxicity against the CCD-18Co, thus indicated the efficacy of this extract in destroying the cancer cells selectively (27).

Liver cancer

In a study where *Gynura procumbens* ethanol extract (GPE) was treated in the male rat with carcinogenesis induced by 7,12- dimethylbenz(a)antracene (DMBA), the findings indicated that the GPE hindered the exacerbation of liver cancer as elicited by the DMBA (28). On the other hand, a recent study by Ashraf et al. showed that the *Gynura procumbens* methanol extract significantly suppressed the cell viability of human liver cancer line, HepG2, in a dose-dependent manner and also led to the cancer cells towards cytoplasmic compression, shrinkage, declined size, and inclination of floating in the medium (24). The most recent study carried out by Zhang et al. revealed that the ethanol extract of *Gynura procumbens* stem (EEGS) hindered the exacerbation of liver cancer in a mouse model which was induced by nanodiethylnitrosamine (nanoDEN) (29). The EEGS altered the tumor microenvironment triggered by the nanoDEN by reducing the aging, hypoxia-associated protein, and proliferation of the mouse liver tissue as indicated by the immunohistochemistry analysis, inhibited inflammatory, fatty and fibrosis-associated factors genes expression, as well as the retransformation of the deficient hepatocyte nanostructure of the hepatocyte into the normal structure (29).

Lung cancer

In the same study above, the *Gynura procumbens* methanol extracts exhibited significant anti-growth effects on lung adenocarcinoma cell line, A549 cell, in a dose-dependent manner whereby the plant's ethyl acetate and butanol extracts did not reduce the viability of the A549 cells (24). Confocal microscopic images of the A549 cells treated with highly concentrated methanol extract indicated the alteration of the cells' morphology: transformation of shape from the epithelial-like to rounded and spindle form, cytoplasmic compression, shrinkage, declined size, and inclination of floating in the medium (24).

Other cancers

The studies on the effects of *Gynura procumbens* extracts towards other types of cancers are summarised in Table 2.

Future perspective

At present, the anti-cancer effects of *Gynura procumbens* as discussed above were observed via various forms of phenotypic screening which aim to recognise the chemical constituents for the desired anti-cancer effects without a proper understanding on the exact mechanisms involved. This is due to the fact that cancer is derived from the intricate interaction of a wide arrays of internal and environmental determinants which can lead to DNA, metabolites, protein, and RNA modifications (17,33). Therefore, suppression of a cancer-inducing receptor may not produce the desired anti-cancer therapeutic effects; Yet, at the same time, it may tend to elicit cancer resistance. *Gynura procumbens* contains a variety of bioactive compounds that contribute to multiple mechanisms of anti-cancer effects. Thus, the incorporation of phenotypic and targeted assays by utilising various stages of cancer models, starting with cancer cell lines, organoids, cancer animal model, and patient-derived xenografts, is expected to potentially disclose the exact therapeutic benefits of *Gynura procumbens* in cancer treatments (34). In addition, the emergence of new cutting-edge methods, namely multi-omics technologies like genomics, metabolomics and transcriptomics, has allowed the prediction of drug-herb interaction using bioinformatic software.

This would be helpful in identifying molecular pathways and measuring a variety of expressed molecules, either under influence or in the absence of the *Gynura procumbens* treatment, that contributes to a precise profiling of drug effects. The combination of these multi-omics methods in the research of *Gynura procumbens* from the cancer's perspective has also stimulated the advancement of the precision medicine technique for cancer treatments. Moreover, the effects of *Gynura procumbens* extract on gut microbiota, especially related to their composition, metabolites and mucosal immunology, also need further verification as gut microbiome was reported to play a critical role in cancer progression (35).

Table 2: Biological activities of *Gynura procumbens* in selected cancers.

Cancer types	Cancer cells	*Gynura procumbens* extract types	Biological activities	Reference
Bone	Osteosarcoma (OS) cell line, U2-OS	Ethanol	- GPE suppressed U2-OS cell proliferation and metastasis and elicited cell apoptosis - GPE inhibited the expression of the NF-κBp65 protein	30
Brain	Glioblastoma multiforme cell lines (U-87)	Methanol, ethanol and ethyl acetate extracts	- Ethanol extract had the highest anti-proliferative effects on the U-87 cell, followed by methanol and ethyl acetate extracts - Methanol and ethanol extracts displayed anti-proliferative effects in time- dependent manner but ethyl extract only inhibited proliferation of U-87 after 48 hours	31
Canine mammary	CHMp-13a and CHMp-5b	Ethanol	- GPE reduced cell proliferation of both CHMp-13a and CHMp-5b cells in concentration and time-dependent manner - GPE hindered cancer cell migration, elicited caspase 3/7 dependent apoptosis, reduced EGFR mRNA and protein expression levels in both cell lines in a dose-dependent manner.	32

Conclusion

In conclusion, based on *in vitro* and a few *in vivo* studies, *Gynura procumbens* extracts can be seen as a potent chemotherapeutic agent to prevent various types of cancers due to the composition of a wide range of chemical constituents that can induce multi-faceted anti-cancer mechanisms. However, there are still many issues which remain to be addressed, such as in terms of the selection of an appropriate model to fully understand the anti-cancer effects of *Gynura procumbens* and the exact anti-cancer mechanisms elicited by this plant. In addition, with the varying alteration of cancer therapy prospects, numerous new cutting-edge technologies can potentially be utilised in accelerating the progress and success rate of future research exploring the anti-cancer properties of *Gynura procumbens*.

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Conflict of Interest

No potential conflict of interest was reported by the authors.

References

1. Mou KM, Dash PR. A comprehensive review on *Gynura procumbens*. Inter J Pharmacogn. 2016;3(4):167-174.
2. Mohammad Yusoff M, Misran A, Ahmed OA, Wan Majid WHD, Megat Wahab PE, Ahmad NF. *Gynura procumbens: agronomic practices and future prospects in Malaysia.* Pertanika J Trop Agric Sc. 2019;42(2):421-434.

3. Kaewseejan N, Sutthikhum V, Siriamornpun S. Potential of *Gynura procumbens* leaves as source of flavonoid-enriched fractions with enhanced antioxidant capacity. J Funct Foods 2015;12:120-8.
4. Tan HL, Chan KG, Pusparajah P, Lee LH, Goh BH. *Gynura procumbens*: An overview of the biological activities. Front Pharmacol. 2016;7(52):1-14.
5. Rosidah, Yam MF, Ahmad M, Sadikun A, Akowuah GA, Asmawi MZ. Toxicology evaluation of standardized methanol extract of *Gynura procumbens*. J Ethnopharmacol. 2009;123(2):244–249.
6. Algariri K, Atangwho IJ, Meng KY, Asmawi MZ, Sadikun A, Murugaiyah V. Antihyperglycaemic and toxicological evaluations of extract and fractions of *Gynura procumbens* leaves. Trop Life Sci Res. 2014;25(1):75-93.
7. Ashraf K. An updated phytochemical and pharmacological review on *Gynura procumbens*. Asian J Pharm Clin Res. 2019;12:9–14.
8. Manogaran M, Lim V, Mohamed R. Phytoconstituents of the *Gynura procumbens* ethanol leaf extract and its fractions and their effects on viability of macrophages. J Herbmed Pharmacol. 2019; 8(3):224-230
9. Hu JW, Wu J, Zhang Y, Huang BH, Hu JP, Wu L, Xu G. Chemical constituents of the stems of *Gynura procumbens*. Chem Nat Compd. 2019;55(3):583–585.
10. Gong SS, Liu YL, Li Y, Li XR, Feng YL, Xu QM, Yang SL. Chemical constituents from whole plant of *Gynura procumbens*. Chin Tradit Herb Drugs 2016;24: 1856-1860.
11. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394-424.
12. Center M, Siegel R, Jemal A. Global Cancer Facts and Figures 2010. American Cancer Society, Atlanta, GA, 2011.
13. Cooper GM. The Cell: A molecular approach. In: The development and causes of cancer. 2nd edition. Sinauer Associates, Sunderland, MA, 2000.
14. Alzahrani SM, Al Doghaither HA, Al-Ghafari AB. General insight into cancer: An overview of colorectal cancer (Review). Mol Clin Oncol. 202;15(6):271.
15. Nordling CO. A new theory on the cancer-inducing mechanism. Br J Cancer 1953;7:68-72.
16. Knudson AG Jr. Mutation and cancer: Statistical study of retino- blastoma. Proc Natl Acad Sci USA 1971;68:820-823.
17. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell 2011;144: 646-674.
18. Chaudhary S, Devkar RA, Bhere D, Setty MM, Pai KSR. Selective cytotoxicity and pro-apoptotic activity of stem bark of wrightia tinctoria (Roxb.) R. Br. in cancerous cells. Pharmacogn Mag. 2015;11(3):S481-S487.
19. Hoang NT, Acevedo LA, Mann MJ, Tolani B. A review of soft-tissue sarcomas: Translation of biological advances into treatment measures. Cancer Manag Res. 2018;10:1089-1114.
20. Davis AS, Viera AJ, Mead MD. Leukemia: An overview for primary care. Am Fam Physician 2014;89:731-738.
21. Nurulita NA, Meiyanto E, Sugiyanto, Matsuda E, Kawaichi M. *Gynura procumbens* prevents chemoresistance through inhibition MDR1 expression on MCF-7 breast cancer cell line and sensitizes the cells to Doxorubicin. Indo J Biotech. 2012;17(1):51-60.
22. Nurulita NA, Meiyanto E, Sugiyanto, 2012, Selectivity of ethyl acetate fraction of *Gynura procumbens* on colon cancer and breast cancer, Indo J Cancer Chemoprev. 2012;3(4):274-280.
23. Hew CS, Khoo BY, Gam LH. The anti-cancer property of proteins extracted from *Gynura procumbens (Lour.) Merr*. PLoS One. 2013;8(7):e68524.
24. Ashraf K, Halim H, Lim SM, Ramasamy K, Sultan S. *In vitro* antioxidant, antimicrobial and antiproliferative studies of four different extracts of *Orthosiphon stamineus*, *Gynura procumbens* and *Ficus deltoidea*. Saudi J Biol Sci. 2020;27(1):417-432.
25. Nurulita NA, Meiyanto E, Matsuda E, Kawaichi M, Mada G. The ethyl acetate fraction of *Gynura procumbens* sensitizes widr colon cancer cell line against 5-fluorouracil but shows antagonism with cisplatin. Inter J Phytomed. 2011;3:392-405.
26. Shwter AN, Abdullah NA, Alshawsh MA, Alsalahi A, Hajrezaei M, Almaqrami AA, *et al*. Chemoprevention of colonic aberrant crypt foci by *Gynura procumbens* in rats. J Ethnopharmacol. 2014;151(3):1194-1201.
27. Teoh WY, Abdul Wahab N, Richardson JSM,Sim KS. Evaluation of antioxidant properties, cytotoxicity and acute oral toxicity of *Gynura*

procumbens (Compositae). Sains Malaysiana 2016;45(2):229–235.

28. Nisa F, Hermawan A, Murwanti R, Meiyanto E. Antiproliferative effect of *Gynura procumbens (lour.) Merr.* leaves etanolic extract on 7,12 - dimethylbenz(a)antracene induced male rat liver. Adv Pharm Bull. 2012;2(1):99-106.
29. Zhang T, Gu HW, Gao JX, Li YS, Tang HB. Ethanol supernatant extracts of *Gynura procumbens* could treat nanodiethylnitrosamine-induced mouse liver cancer by interfering with inflammatory factors for the tumor microenvironment. J Ethnopharmacol. 2022;285:114917.
30. Wang H, Zhou JW, Fu DH, Zhou Y, Cheng WZ, Liu ZL. *Gynura procumbens* ethanolic extract suppresses osteosarcoma cell proliferation and metastasis *in vitro*. Oncol Lett. 2013;6(1):113-117.
31. Rohin MAK, Jumli MN, Ridzwan N, Baig AA, Latif AZA, Hadi NA. Effect of *Gynura procumbens* extracts on anti- proliferative activity and its associated morphological changes of human Glioblastoma multiforme cell line (U-87). Pharmacogn J. 2018;10(3):492-496.
32. Jermnak U, Supsavhad W, Kunakornsawat S, Jaroensong T, Watcharasit P, Visitnonthachai D, *et al*. Anti-cancer potential of *Gynura procumbens* leaves extract against two canine mammary cell lines. Vet Med Sci. 2022;8:69–84.
33. Du W, Elemento O. Cancer systems biology: embracing complexity to develop better anticancer therapeutic strategies. Oncogene. 2015;34(25):3215-25.
34. Huang M, Lu JJ, Ding J. Natural products in cancer therapy: Past, present and future. Nat Prod Bioprospect. 2021 Feb;11(1):5-13.
35. Gopalakrishnan V, Helmink BA, Spencer CN, Reuben A, Wargo JA. The influence of the gut microbiome on cancer, immunity, and cancer immunotherapy. Cancer Cell. 2018;33(4):570-580.