Sains Malaysiana 53(2)(2024): 369-381
http://doi.org/10.17576/jsm-2024-5302-11

Anti-Angiogenic Effect of *Polygonum* Species: A Comprehensive Review of Literature

(Kesan Anti-Angiogenik Spesies *Polygonum*: Kajian Komprehensif Kepustakaan)

Najla' Shakirah Ab Halim[1], Sheril June Ankasha[1], Aslah Nabilah Abdull Sukor[1], Nur Najmi Mohamad Anuar[2], Norhazlina Abdul Wahab[1], Shahidee Zainal Abidin[3], Azizah Ugusman[1] & Adila A Hamid[1,*]

[1]*Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia*
[2]*Programme of Biomedical Science, Centre for Toxicology and Health Risk Studies, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia*
[3]*Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21300 Kuala Terengganu, Terengganu, Malaysia*

Received: 13 September 2023/Accepted: 6 February 2024

ABSTRACT

Angiogenesis is a physiological, tightly regulated process which is characterized by the development of new blood vessels. Compounds with the potential to control angiogenesis would be highly valuable as therapeutics, as an imbalance in angiogenesis may lead to several pathological disorders, including cancer, retinopathy, and arthritis. In this study, the anti-angiogenic effect of *Polygonum* sp. has been comprehensively reviewed and this plant also has been known to possess other medicinal benefits such as antioxidative, anti-inflammatory, anti-proliferative, and anti-tumor agents. Hence, this study systematically identified the evidence reporting the anti-angiogenic effects of *Polygonum* sp. Four electronic databases, namely PubMed, Ovid MEDLINE, Scopus and Web of Science were searched for relevant articles. Based on the pre-set eligibility criteria, 50 relevant articles were identified, and ten qualified articles were selected and reviewed. It was demonstrated that four namely *P. cuspidatum*, *P. barbatum*, *P. hydropiper,* and *P. perfoliatum* showed anti-angiogenic activities mainly through inhibition of the vascular endothelial growth factor-signaling pathways. Therefore, these species *Polygonum* have the potential to be developed as natural anti-angiogenic agents for prevention and treatment of various diseases related to pathological angiogenesis.

Keywords: Angiogenesis; anti-angiogenesis; *Polygonum* genus; vascular endothelial growth factor

ABSTRAK

Angiogenesis adalah proses fisiologi yang dikawal ketat yang dicirikan oleh perkembangan saluran darah baharu. Sebatian yang berpotensi untuk mengawal angiogenesis akan sangat berharga sebagai terapeutik, kerana ketidakseimbangan dalam angiogenesis boleh membawa kepada beberapa gangguan patologi, termasuk kanser, retinopati dan arthritis. Dalam kajian ini, kesan anti-angiogenik spesis *Polygonum* telah dikaji secara menyeluruh dan tumbuhan ini juga telah diketahui mempunyai manfaat perubatan lain seperti antioksidan, anti-radang, agen anti-proliferatif dan anti-tumor. Walau bagaimanapun, kesan anti-angiogenik *Polygonum* sp. masih belum diulas. Oleh itu, kajian ini dilakukan secara sistematik untuk mengenal pasti bukti yang melaporkan kesan anti-angiogenik oleh *Polygonum* sp. Empat pangkalan data elektronik, iaitu PubMed, Ovid MEDLINE, Scopus dan Web of Science digunakan bagi pencarian artikel yang berkaitan. Berdasarkan kriteria relevan yang telah ditetapkan, terdapat 50 artikel yang berkaitan telah dikenal pasti dan hanya sepuluh artikel yang layak telah dipilih dan diulas. Hasil kajian menunjukkan bahawa terdapat empat iaitu *P. cuspidatum*, *P. barbatum*, *P. hidropiper* dan *P. perfoliatum* menunjukkan aktiviti anti-angiogenik terutamanya melalui perencatan laluan isyarat faktor pertumbuhan endotelium vaskular. Oleh itu, spesis *Polygonum* mempunyai potensi untuk dibangunkan sebagai agen anti-angiogenik semula jadi dalam pencegahan dan rawatan pelbagai penyakit yang berkaitan dengan patologi angiogenesis.

Kata kunci: Angiogenesis; anti-angiogenesis; faktor pertumbuhan endotelium vaskular; genus *Polygonum*

Introduction

Angiogenesis is a central process for tissue growth and regeneration, involving the formation of new capillaries from pre-existing vascular networks. It plays an important role in various physiological conditions such as embryonic development, wound healing (Ayaz et al. 2016; Cho et al. 2019), corpus luteum formation (Ayaz et al. 2016) and ovarian function (Cho et al. 2019). The process of angiogenesis is tightly controlled by the critical balance of pro- and anti-angiogenic factors. The imbalance of these factors can lead to the formation of pathological angiogenesis, such as in cancer metastases, ischemic and inflammatory diseases such as endometriosis, psoriasis, rheumatoid arthritis and diabetic retinopathy (Hu et al. 2018; Rashidi et al. 2017). As a result, anti-angiogenic therapy is increasingly used to treat diseases related to angiogenesis.

Recently, the anti-angiogenic effect of bioactive compounds from natural sources has gained more attention, as the drugs approved by the Food and Drug Administration (FDA) may cause various side effects and have some clinical limitations such as the development of resistance (Azam, Mehta & Harris 2010). In fact, 60% of anticancer drugs such as paclitaxel, etoposide, vinblastine, and doxorubicin are derived from natural sources (Newman & Cragg 2012; Rayan, Raiyn & Falah 2017). Therefore, the identification of other natural products with anti-angiogenic activity would be beneficial as an alternative strategy for angiogenesis-based treatment due to their low and selective toxicity.

The genus *Polygonum* in the family Polygonoceae includes 300 species around the world, ranging from prostrate herbaceous annuals (4-5 cm tall) to herbaceous perennials (3-4 m tall) and other trees or woody perennial vines that grow to about 20-30 m tall. Most species are aquatic and occur naturally in swampy areas as floating plants, either in rivers or ponds (Ayaz et al. 2016). The name *Polygonum* is derived from the Greek, poly meaning 'many' and gonu meaning 'knee', referring to the swollen, jointed stem of this genus. Many of the plants in the genus *Polygonum* and their active metabolites have been studied for phytochemical, biological, and pharmacological purposes. They are known to produce a variety of secondary metabolites, namely flavonoids (Peng et al. 2003), anthraquinones (Matsuda et al. 2001), coumarins (Sun & Sneden 1999) and sucrose phenylpropanoid esters (Sun & Sneden 1999). Of all of them, flavonoids are the most abundant constituents of the genus *Polygonum* (Nkuété et al. 2015).

Several studies have reported the pharmacological effects of *Polygonum* sp. including its antioxidant (Ismail et al. 2012), anti-inflammatory (Bralley et al. 2008; Hamid et al. 2022), antiproliferative, antitumour (Ahmad et al. 2018), antiallergic (Lim et al. 2007), antibacterial, antifungal, and antiviral activities (Brandão et al. 2010). In addition, *Polygonum* sp. has a preventive effect against hepatotoxicity in rats (Rashid et al. 2019) and has the potential to improve mood, cognitive function and quality of life in middle-aged women (Yahya et al. 2017). In addition, there is evidence to suggest that these *Polygonum* sp. could be developed as targeted drugs for Alzheimer's disease or as anti-HIV drugs (Ahmad et al. 2022). Some *Polygonum* sp. such as *P. cuspidatum, P. hydropiper, P. barbatum,* and *P. perfoliatum* have been shown to have anti-angiogenic effects (Ayaz et al. 2016; Farooq et al. 2017; Hu et al. 2018; 2019; Kimura & Okuda 2001; Li et al. 2019; Mahnashi et al. 2021; Ozgur et al. 2018; Wang et al. 2004). The morphology of these *Polygonum* sp. is summarised in Table 1. However, no study has been conducted to systematically investigate the anti-angiogenic activities of these *Polygonum* sp. Therefore, the aim of this study was to comprehensively investigate the anti-angiogenic properties of *Polygonum* sp. to gain better insight into their potential as therapeutics for diseases related to angiogenesis.

Materials and Methods

Search strategy

The study aims to search and identify relevant studies on the anti-angiogenic effect of *Polygonum* species. The relevant studies were retrieved from four databases, namely PubMed, Scopus, OvidMEDLINE and WoS from 1946 to fourth week of August 2023. The following keywords were used: (1) *Polygonum* OR *Persicaria* AND (2) angiogenesis OR angiogenesis inhibitor* OR anti*angiogen*.

Study inclusion and exclusion criteria

Articles retrieved from the database following the search were reviewed independently by two authors (N.S.A.H and A.A.H) according to the following criteria: (1) Full-length original article published in English language, (2) articles that reported the anti-angiogenic activities of *Polygonum* or *Persicaria* sp. Or their isolated compounds, regardless of whether the studies focus on *Polygonum* or *Persicaria* sp. alone or include other plants and *Polygonum* (3) *in vitro*, *in vivo*, clinical trial or any combined studies that reported the angiogenesis-related activities of *Polygonum* or *Persicaria* sp. Review articles, news, case report, book chapters, conference proceedings, and editorial letters were excluded from this study. Any disagreement was resolved by a third reviewer (A.U) or consensus-based discussion.

TABLE 1. The morphology of *P. cuspidatum, P. barbatum, P. hydropiper,* and *P. perfoliatum* plants

P. cuspidatum	*P. barbatum*	*P. hydropiper*	*P. perfoliatum*
			
Stem: hollow like bamboo Leaves: wide heart shaped with rounded lobes at the base Flowers: elongated cluster and small green or white cream	Stem: cylindrical, hollow, thickened at the nodes and thorn Leaves: short oblong to lanceolate with helical shape and acute to obtuse base with acute apex Flowers: imbricate oval of white to green white or pink	Stem: smooth and hairless to fine hair swollen at the nodes, green to red or light brown, and branch towards their tips Leaves: single leaves with short stalks and fine hair glandular blades Flower: green to pink which the outer parts are covered with dark glands	Stems: reddish branched and covered with small, curved spines Leaves: triangular, light green and barbed on the undersurface Flowers: small white Fruit: metallic blue and segmented with each segment containing a single black or reddish black seed

ARTICLE SCREENING

Article screening was conducted in three phases. First, articles that did not fulfill the selection criteria were excluded according to the title alone. Second, articles that were not related to the anti-angiogenic activities of *Polygonum* sp. were excluded by reading through the abstracts. Finally, the remaining articles that did not match the inclusion criteria were excluded by reading the full texts thoroughly. The study design, plant source, plant part, types of extract, phytoconstituents, results, outcomes and reference of each study were recorded.

RESULTS AND DISCUSSION

STUDY SELECTION

Overall, a total of 50 potential articles were retrieved from four online databases, of which seven articles were from Ovid MEDLINE, 17 articles were from PubMed, 12 articles were from Scopus and 14 articles from Web of Science (WoS). Subsequently, 20 articles were removed because of duplication. The full-length articles for the remaining 25 studies were obtained and reviewed thoroughly. However, 15 studies were excluded due to not related to anti-angiogenesis activity. In total, only ten remaining articles were selected to be included for final analysis. The articles were published between the years 2001 and 2021. Summary of the article selection process was shown in Figure 1.

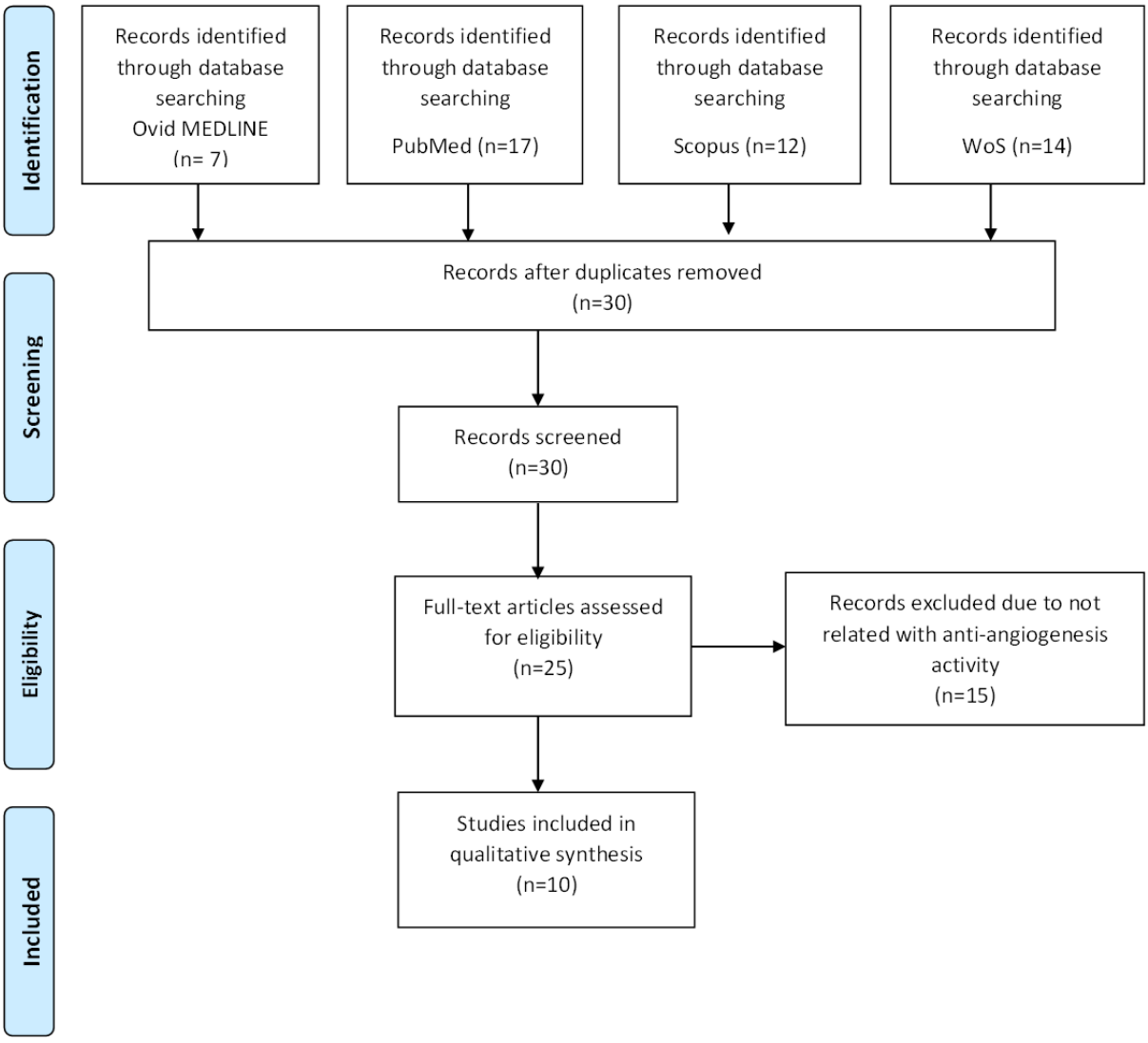

FIGURE 1. PRISMA flow diagram. Summary of the literature search and the study selection process according to the PRISMA guidelines. Overall, ten studies met the search criteria. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses

STUDY DESIGN CHARACTERISTICS

The designs of the selected studies were characterized in Table 2. Based on the study design, this review comprised of an *in vitro* study, an *in vivo* study, five combined *in vitro* and *in vivo* studies, and two combined *in vitro, in vivo* and *in silico* studies. For the *in vitro* studies, most of the studies investigated the anti-angiogenic activity by using human umbilical vein endothelial cells (HUVEC) as the model. It has been used in tube formation assay to measure the tube-like network formation (Hu et al. 2019, 2018; Kimura & Okuda 2001). Another study used bovine aortic endothelial cells (BAEC), where the migration of BAEC was assessed using crystal violet migration assay (Wang et al. 2004). The remaining study utilized non-small human lung carcinoma cells (NCI-H460) to investigate the angiogenic regulation by *Polygonum* extract (Farooq et al. 2017).

For the *in vivo* studies, there were different types of animal used, which were female C57BL/6 mice (Kimura & Okuda 2001), female Wistar rats (Ozgur et al. 2018), clean-grade Institute of Cancer Research (ICR) and BALB/c-nude mice (Li et al. 2019), zebrafish embryo (Hu et al. 2019, 2018) and chorioallantoic membrane (CAM) of chicken embryo (Ayaz et al.2016; Farooq et al. 2019; Mahnashi et al. 2021; Wang et al. 2004). CAM assay is an extraembryonic membrane that is frequently used to study angiogenesis, in which the numbers of blood vessels in each egg are counted under the microscope (Rezzola et al. 2020). Besides, zebrafish embryo was also become one of the relevant models for discovery of any therapeutic drugs related to angiogenesis as it can be observed through the blood flow and development of any vascular-related organ systems without the need of sophisticated instrument (Chávez et al. 2016).

Molecular docking is an *in silico* study used to examine the binding activity and affinity of a protein ligand towards its receptor. By using docking software, the activity and binding modes of *Polygonum*-isolated compounds could be explored to study their anti-angiogenesis activity (Mahnashi et al. 2021) and anti-cancer activity (Farooq et al. 2019).

SELECTED *Polygonum* sp. AND THEIR PHYTOCONSTITUENTS

There were only four species of Polygonum plants shown to have anti-angiogenic activities, namely *P. hydropiper* (Ayaz et al. 2016; Mahnashi et al. 2021), *P. barbatum* (Farooq et al. 2019, 2017), *P. cuspidatum* (Hu et al. 2019, 2018; Kimura & Okuda 2001; Ozgur et al. 2018; Wang et al. 2004) and *P. perfoliatum* (Li et al. 2019). *P. hydropiper* (Ayaz et al. 2016; Mahnashi et al. 2021) and *P. barbatum* (Farooq et al. 2019, 2017) were collected in Khyber Pakhtoonkhwa, Pakistan. *P. cuspidatum* was collected in China (Hu et al. 2019, 2018; Wang et al. 2004), Japan (Kimura & Okuda 2001) and Turkey (Ozgur et al. 2018), while *P. perfoliatum* was collected in China (Li et al. 2019). Three of the ten selected studies used the whole plant (Farooq et al. 2019; Mahnashi et al. 2021; Wang et al. 2004), two studies used root and rhizome parts (Hu et al. 2019, 2018), one study used only the root part (Kimura & Okuda 2001) and another study used the aerial parts of the plant (Farooq et al. 2017). The remaining three studies (Ayaz et al. 2016; Li et al. 2019; Ozgur et al. 2018) did not mention the plant parts used.

Different solvents can be used for extraction and the solvents can be divided into three groups, namely polar, semi-polar and non-polar solvents. Regarding polar extracts, two studies used ethanol extract (Hu et al. 2019, 2018), one study used methanol extract (Kimura & Okuda 2001) and one study used boiled aqueous extract (Wang et al. 2004). Ethyl acetate is a semi-polar solvent, while n-hexane, n-butanol, and chloroform are non-polar solvents. Essential oils are considered solvents with a mixture of polar and non-polar molecules. In other studies, several extracts were used and compared. For example, one study used aqueous, methanol, ethyl acetate, chloroform, n-hexane, n-butanol, and saponin extracts (Ayaz et al. 2016), while another study used petroleum ether, ethyl acetate, ethanol, and aqueous extracts (Li et al. 2019). The other two studies used methanol, ethyl acetate, n-hexane, and n-butanol extracts (Farooq et al. 2019, 2017). All these studies used mixed polar, semi-polar, and non-polar solvents for their plant extractions. However, one study compared chloroform and ethyl acetate extracts (Mahnashi et al. 2021), which are nonpolar and semipolar solvents, while another study did not mention the type of extracts used (Ozgur et al. 2018).

Polygonum sp. is known for its diverse medicinal properties. This is due to the phytoconstituents identified in the plants such as resveratrol, piceid, polydatin, saponin, sesquiterpenes and several new fractions. In studies with *P. cuspidatum*, stilbenoid phytoconstituents such as resveratrol (Ozgur et al. 2018), resveratrol with polydatin or piceid (Hu et al. 2018; Kimura & Okuda 2001) and polydatin alone (Hu et al. 2019) were extracted and tested for their anti-angiogenesis activities. However, one study did not analyse the phytoconstituent content in their *P. cuspidatum* extract (Wang et al. 2004). Meanwhile, saponin (Ayaz et al. 2016) and two different bioactive compounds; 4-methyl-5-oxo-tetrahydrofuran-3-yl acetate and methyl 4-hydroxy-3-methoxybenzoate (Mahnashi et al. 2021) were extracted from *P. hydropiper*. Several different fractions of sesquiterpenes were isolated from ethyl acetate fractions (n-hexane:ethyl acetate) of *P. barbatum* (Farooq et al. 2019, 2017). In addition, new fractions from different solvent ratios of ethyl acetate:methanol were isolated from *P. perfoliatum* (Li et al. 2019). Interestingly, all phytoconstituents in the ten selected studies showed positive anti-angiogenic activities.

TABLE 2. Characteristics of the included studies

Plants	Study design	Plant source (s)	Plant part (s)	Type (s) of Extract	Phyto-constituent	Results	Outcomes	Reference
P. cuspidatum	*In vitro* cell culture study by using HUVEC *In vivo* study by using female C57BL/6 mice administered intraperitoneally twice daily	Japan	Root	Methanol	Resveratrol, piceid	Inhibition of tumor-induced neovascularization at 2.5 and 10 mg/kg Inhibition of capillary-like tube formation (52.6% at 100 µmol/L and 45.5% at 10 µmol/L) ▲inhibition of VEGF binding to HUVEC (53.2% at 50 µmol/L) ▼inhibition of VEGF binding to HUVEC (16.9% at 10 µmol/L)	Resveratrol in *P. cuspidatum* showed an anti-angiogenic activity by inhibiting the capillary-like tube formation and binding of VEGF to HUVEC	Kimura & Okuda (2001)
P. cuspidatum	*In vitro* cell culture study by using BAECs *In vivo* study by using CAM model	Shanghai, China	Whole plants	Boiled aqueous	-	Inhibition of angiogenesis in CAM model and BAECs at 1 g herb/mL	*P. cuspidatum* showed an anti-angiogenic effect	Wang et al. (2004)
P. cuspidatum	*In vitro* cell culture study by using HUVEC *In vivo* study by using zebrafish	Kunming, Yunnan, China	Root and rhizome	Ethanol	Resveratrol, polydatin	Inhibition of VEGF-induced angiogenesis in a dose-dependent manner.	*P. cuspidatum* showed anti-angiogenic activity by suppressing VEGF activity through VEGF signalling	Hu et al. (2018)

continued to the next page

from the previous page

P. cuspidatum	*In vitro* cell culture study by using HUVEC *In vivo* study by using zebrafish embryos model	Kunming, Yunnan, China	Root and rhizome	Ethanol	Polydatin	Inhibition of VEGF-induced angiogenesis in a dose-dependent manner Suppression of VEGF-induced phosphorylations of VEGFR2 and JNK Inhibition of VEGF-mediated phosphorylations of Erk, Akt and eNOS	Polydatin in *P. cuspidatum* showed anti-angiogenic activity by suppressing VEGF-induced angiogenesis	Hu et al. (2019)
P. cuspidatum	*In viv*o study by using female Wistar rats	Turkey	-	Commercial supplement	Resveratrol	↓ total VEGF scores in resveratrol-treated group (60 mg/kg/d) ↓ intense VEGF immunoreactivity in the ovaries ↓ peritoneal fluid VEGF levels	Resveratrol in *P. cuspidatum* showed anti-angiogenic activity by lowering VEGF activity	Ozgur et al. (2018)
P. hydropiper L.	*In vitro* study by using mouse embryonic fibroblast NIH/3T3cell line and potato disc anti-tumor assay I*n vivo* study by using CAM model	Talash Valley, Khyber Pakhtoonkhwa, Pakistan	-	Methanol, N-hexane, Chloroform, Ethyl acetate, N-Butanol, Aqueous, Saponin	Crude extract, saponins	▲ anti-angiogenic activity exerted by saponins, followed with chloroform, ethyl acetate and methanol extracts with IC_{50} of 19.21 µg/mL, 28.65 µg/mL, 88.75 µg/mL and 461.53 µg/mL, respectively	Different extracts of *P. hydropiper* demonstrated anti-angiogenic activity, with saponins showing the highest activity	Ayaz et al. (2016)

continued to the next page

from the previous page

P. hydropiper L.	*In vitro* study by using Agrobacterium culture, breast cancer cells (MCF-7), cervical cancer cells (HeLA) and NIH/3T3 fibroblasts cells cultures *In vivo* study by using CAM model *In silico* molecular docking study	District Talash, Khyber Pakhtunkhwa (KP)	Whole plants	Ethyl acetate, Chloroform	4-Methyl-5-oxo-tetrahydrofuran-3-yl acetate (PH-1) Methyl 4-hydroxy-3-methoxybenzoate (PH-2)	↑ inhibition of blood vessels formation with increasing PH-1 and PH-2 concentrations IC_{50} value of PH-1 and PH-2 were 340 μgmL^{-1} and 500 μgmL^{-1}, respectively compared to dexamethasone IC_{50} of 37.50 μgmL^{-1} Tested compounds of PH-1 and PH-2 have strong inter-molecular interactions with Lys868, Val916 and Asp1046	Two bioactive compounds of *P. hydropiper* L. (PH-1 and PH-2) exhibited anti-angiogenic potentials and may have the ability to inhibit EGFR, HER2 and VEGFR receptors	Mahnashi et al. (2021)
P. barbatum	*In vitro* study by using oral cancer (CAL-27) and lung cancer (NCI H460) cell lines *In vivo* study by using CAM model *In silico* molecular docking study	Khyber Pakhtunkhwa, Pakistan	Whole plant	Methanol, N-hexane, Ethyl acetate N-butanol	Three new sesquiterpenes were isolated from Ethyl acetate fractions: (n-hexane: ethyl acetate) - compound 1 (65:35), - compound 2 (58:42) - compound 3 (55:45)	▼ blood vessels formed in CAM assay with compound 1, followed by compounds 2 and 3 ▼ IC_{50} value of compound 1 (8.2 ± 1.1 μM) compared to compounds 2 (13.4 ± 1.1 μM) and 3 (57.7 ± 0.3 μM)	N-hexane: ethyl acetate fractions of *P. barbatum* extract showed an anti-angiogenic activity with compound 1 having the most excellent activity	Farooq et al. (2019)

continued to the next page

from the previous page

P. perfoliatum L.	*In vitro* study by using human cervical cancer cell line (Hela), human gastric cancer cell line (SGC-7901), human prostate cancer cell line (PC-3), human lung cancer cell line (A549), human glioma cell line (BT-325), and human pancreatic cancer cell line (PANC-1) *In vivo* study by using equal numbers of male and female of clean-grade ICR mice and BALB/c-nude mice	China	-	Petroleum ether, Ethyl acetate, Ethanol, Aqueous	Five different fractions from ethyl acetate-methanol (40:1), (35:1), (25:1), (15:1), and (5:1), which were recorded as PEA, PEB, PEC, PED, and PEE, respectively	▲ concentration of PEC (100 and 120 µg/mL) exerted cytotoxicity against various cancer cell lines with inhibition ratio of over 70% ▼ number of VEGF-positive cells in the PEC-treated groups ↓ endothelial cells and CD31-positive intensity in the PEC-treated tumors compared to control ↓ positive vascular endothelial cells in the highest dose group compared to control	Ethyl acetate extracts of *P. perfoliatum* L. exerted anti-angiogenesis effect, with PEC being the isolated compound with the highest activity	Li et al. (2019)
P. barbatum	*In vitro* study by using non-small cell lung carcinoma (NCI-H460), breast cancer (MCF-7), cervical cancer (HeLa) and normal mouse fibroblast (NIH-3 T3) cell lines	Northern areas (Mansehra), Khyber Pakhtunkhwa, Pakistan	Aerial	Methanol, N-hexane, Ethyl acetate N-butanol	Three new sesquiterpenes were isolated from Ethyl acetate fractions: (n-hexane:ethyl acetate) - compound 1 (40:60), - compound 2 (36:64) - compound 3 (27:73)	Compound 3 at 20 µM and 40 µM concentrations delayed the rate of wound healing as compared to untreated cells Compound 3 slows the scratch closing with only 17% and 24% at 20 and 40 µM concentrations, respectively compared to control Compound 3 inhibited angiogenesis by downregulating the angiogenic genes (VEGF and COX-2) in NCI-H460 cells	*P. barbatum* exerts an anti-angiogenic effect by downregulating VEGF and COX-2	Farooq et al. (2017)

↑: Increased; ↓: Decreased; ▲: Highest; ▼: Lowest: BAEC: Bovine aortic endothelial cells; COX-2: cyclooxygenase 2; CAM: chorioallantoic membrane; HUVEC: Human umbilical vein endothelial cells; IC_{50}: inhibition concentration at 50%; VEGF: Vascular endothelial growth factor; VEGFR: vascular endothelial growth factor receptor; VEGFR2: vascular endothelial growth factor receptor 2; JNK: C-Jun N-terminal kinases; EGFR: Epidermal growth factor receptor; HER2: Human epidermal growth factor receptor 2

ANTI-ANGIOGENIC ACTIVITIES OF *Polygonum* sp.

Angiogenesis is a complex and dynamic process controlled by a variety of mediators with pro- or anti-angiogenic effects. A variety of signals mediated by growth factors and their receptors have been shown to have a significant impact on the mechanisms underlying angiogenesis. Among the important signalling pathways, the vascular endothelial growth factor (VEGF) protein family is a critical regulator of the angiogenesis process and is highly selective for endothelial cells. Other factors such as the matrix metalloproteinases (MMPs) and basic fibroblast growth factor (bFGF) have a wider range of target cells. The VEGF family consists of six members that bind to their respective receptors: Vascular Endothelial Growth Factor Receptor (VEGFR)-1, VEGFR2 and VEGFR3, depending on their purpose. VEGFR2 is the receptor that promotes migration, proliferation and angiogenesis and triggers several downstream signalling cascades (Rafii et al. 2002; Rajasekar, Perumal & Vallikannan 2019). Through their inhibition, the VEGF family and its receptor (VEGFR) have emerged as an attractive molecular therapeutic target for anti-angiogenic agents, particularly in cancer treatment (Yahya et al. 2017).

Resveratrol, a polyphenol from the phenolic group of stilbenes isolated from *P. cuspidatum*, showed anti-angiogenic activity by inhibiting neovascularisation in mice and HUVEC tube formation. This was achieved by inhibition of VEGF binding to HUVEC by resveratrol (Kimura & Okuda 2001). Resveratrol also decreased VEGF levels and immunoreactivity in rats with ovarian hyperstimulation syndrome (OHSS) (Ozgur et al. 2018). Moreover, polydatin isolated from *P. cuspidatum* showed its anti-angiogenic activity by binding to VEGF. This consequently suppressed VEGF-induced angiogenesis, cell proliferation, migration and invasion, and inhibited VEGFR1 and VEGFR2 signalling pathways. Polydatin also inhibited other downstream events of VEGF-mediated VEGFR2 signalling pathways, including activation of protein kinase B (Akt), c-Jun N-terminal kinase (JNK) and endothelial nitric oxide synthase (eNOS) (Hu et al. 2018). The results showed that the phytoconstituents of *P. cuspidatum* have the potential to be anti-angiogenic agents, as they could suppress the VEGF signalling pathway, which is essential for endothelial cell proliferation, sprouting and tube formation.

Besides resveratrol and polydatin, there are many other compounds isolated from *Polygonum* sp. extracts that exert anti-angiogenesis effects. For example, saponin extracted from *P. hydropiper* had the strongest anti-angiogenic effect compared to chloroform, ethyl acetate and methanol (Ayaz et al. 2016). A molecular docking study showed that two components known as PH -1 (4-methyl-5-oxo-tetrahydrofuran-3-yl acetate) and PH -2 (methyl-4-hydroxy-3-methoxybenzoate) in *P. hydropiper* are known to have anti-angiogenic potential through their binding affinity to epidermal growth factor receptors (EGFR), human epidermal growth factor 2 (HER2), and VEGFR (Mahnashi et al. 2021).

Other studies conducted by Farooq et al. (2019) showed that compound **1** isolated from ethyl acetate fractions of *P. barbatum* had the highest anti-angiogenic activity compared to compounds **2** and **3**. This result contrasts with their previous study in 2017, where chicken embryos treated with compound **3** showed the best anti-angiogenic effect by downregulating VEGF and COX -2 in lung carcinoma cells (Farooq et al. 2017). Meanwhile, a fraction of ethyl acetate methanol extract of *P. perfoliatum*, known as PEC, showed the most potent cytotoxic activities against cancer cell lines and xenograft models of various cancers through its anti-tumour and anti-angiogenesis activities (Li et al. 2019). PEC reduced VEGF expression, thereby affecting the proliferation, growth, migration, and permeability of vascular endothelial cells.

A summary of the mechanisms underlying the anti-angiogenic effects of *Polygonum* sp. is shown in Figure 2. The studies investigated the underlying anti-angiogenic mechanisms of *Polygonum* sp. via the VEGF signalling pathways. The effect of these *Polygonum* sp. on angiogenesis-related factors such as bFGF and MMPs was not investigated. Most of the studies demonstrating the anti-angiogenic effect of *Polygonum* sp. were performed *in vivo* on experimental animal models. No clinical studies on the anti-angiogenic effect of *Polygonum* sp. have been conducted in humans. Furthermore, only a few studies have identified the phytoconstituents of *Polygonum* sp. This highlights the need to conduct studies that evaluate the specific phytoconstituents of *Polygonum* sp. with anti-angiogenic activity. Furthermore, the genus *Polygonum* comprises about 300 species. The four *Polygonum* sp. with anti-angiogenic effects discussed in this review highlight the potential of other *Polygonum* sp. with similar effects that have not yet been studied.

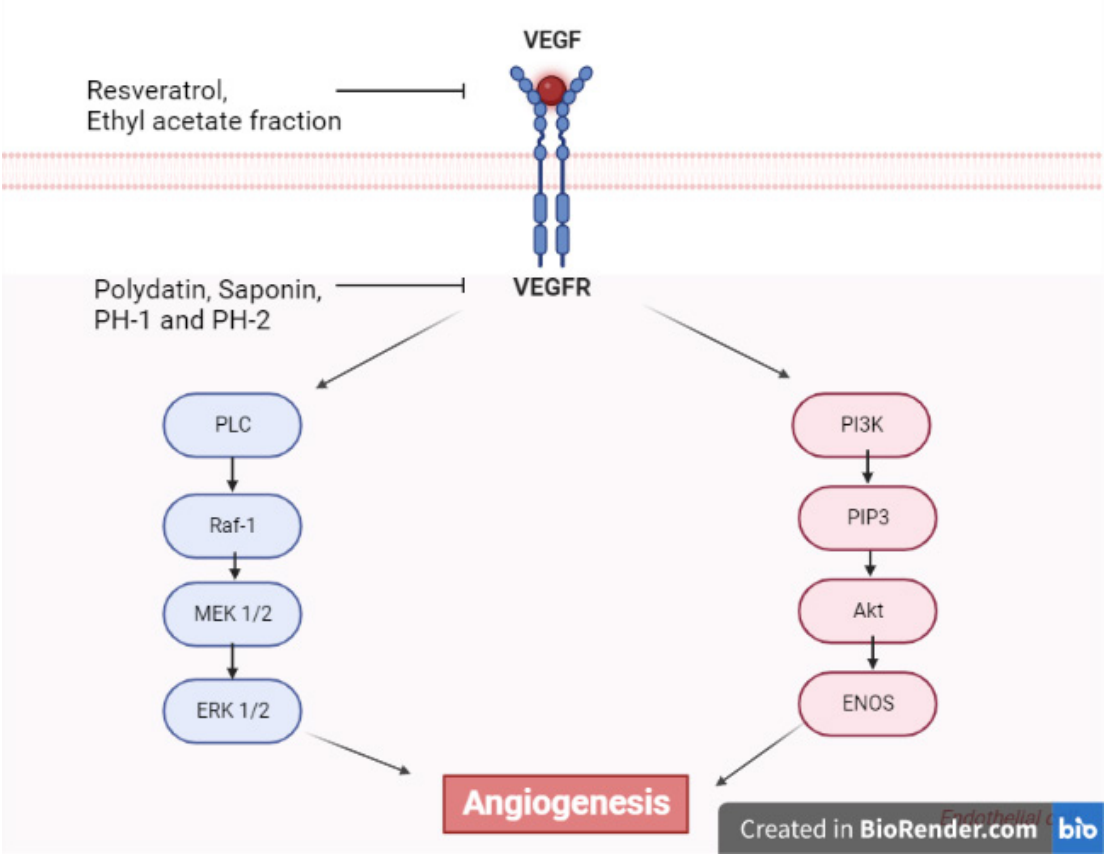

FIGURE 2. Schematic representation of the anti-angiogenic mechanisms of *Polygonum* sp. and their active constituents. Resveratrol suppresses the binding of VEGF to HUVEC by attenuating VEGF-induced phosphorylation of VEGFR2, as well as Erk and eNOS pathway that lead to angiogenesis. VEGF activates three MAPKs, namely JNK, ERK and p38 MAPK. Polydatin also suppresses VEGF-induced angiogenesis through VEGFR2 and inhibits their receptor signalling, particularly the JNK, Erk and eNOS pathways; VEGF: vascular endothelial growth factor, VEGFR2: vascular endothelial growth factor receptor 2, FGF: fibroblast growth factor, FGFR2: fibroblast growth factor receptor 2, PI3K: phosphatidylinositol-3-kinase, MEKK: mitogen-activated protein kinase kinase kinase, JNK: C-Jun N-terminal kinases, PLC: phospholipase C, PKC: protein kinase C, MEK: mitogen-activated protein kinase, Erk: extracellular regulated kinase, PIP3: phosphatidylinositol-3,4,5-triphosphate, eNOS: endothelial nitric oxide synthase.

CONCLUSIONS

P. cuspidatum, P. barbatum, P. hydropiper, P. perfoliatum and their bioactive compounds exert anti-angiogenic activities through various mechanisms, mainly by inhibiting vascular endothelial growth factor signalling pathways. Therefore, these *Polygonum* sp. have the potential to be developed as natural anti-angiogenic agents for the prevention and treatment of various diseases associated with pathological angiogenesis. However, further studies are needed to understand the underlying mechanisms and to explore other *Polygonum* sp. with similar effects.

ACKNOWLEDGEMENTS

This research was funded by Faculty of Medicine, Universiti Kebangsaan Malaysia (project code: FF-2021-058).

REFERENCES

Ahmad, R., Rosandy, A.R., Sahidin, I., Ab Ghani, N.S., Noor, N.M. & Baharum, S.N. 2022. Bioassay analysis and molecular docking study revealed the potential medicinal activities of active compounds polygonumins B, C and D from *Polygonum minus* (*Persicaria minor*). *Plants (Basel)*. 12(1): 59. https://doi.org/10.3390/plants12010059

Ahmad, R., Sahidin, I., Taher, M., Low, C., Noor, N. M., Sillapachaiyaporn, C., Chuchawankul, S., Sarachana, T., Tencomnao, T., Iskandar, F., Rajab, N.F. & Baharum, S.N. 2018. Polygonumins A, a newly isolated compound from the stem of *Polygonum minus* Huds with potential medicinal activities. *Scientific Reports* 8: 4202. doi:10.1038/s41598-018-22485-5

Ayaz, M., Junaid, M., Ullah, F., Sadiq, A., Subhan, F., Khan, M.A., Ahmad, W., Ali, G., Imran, M. & Ahmad, S. 2016. Molecularly characterized solvent extracts and saponins from *Polygonum hydropiper* L. show high anti-angiogenic, anti-tumor, brine shrimp, and fibroblast NIH/3T3 cell line cytotoxicity. *Frontiers in Pharmacology* 7: 74. doi:10.3389/fphar.2016.00074

Azam, F., Mehta, S. & Harris, A.L. 2010. Mechanisms of resistance to antiangiogenesis therapy. *European Journal of Cancer (Oxford, England: 1990)* 46(8): 1323-1332. doi:10.1016/j.ejca.2010.02.020

Bralley, E.E., Greenspan, P., Hargrove, J.L., Wicker, L. & Hartle, D.K. 2008. Topical anti-inflammatory activity of *Polygonum cuspidatum* extract in the TPA model of mouse ear inflammation. *Journal of inflammation (London, England)* 5: 1. doi:10.1186/1476-9255-5-1

Brandão, G.C., Kroon, E.G., Duarte, M.G.R., Braga, F.C., de Souza Filho, J.D. & de Oliveira, A.B. 2010. Antimicrobial, antiviral and cytotoxic activity of extracts and constituents from *Polygonum spectabile* Mart. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology* 17(12): 926-929. doi:10.1016/j.phymed.2010.03.004

Chávez, M.N., Aedo, G., Fierro, F.A., Allende, M.L. & Egaña, J.T. 2016. Zebrafish as an emerging model organism to study angiogenesis in development and regeneration. *Front. Physiol.* 7: 56. https://doi.org/10.3389/fphys.2016.00056

Cho, H-D., Lee, K-W., Won, Y-S., Shin, D-Y. & Seo, K-I. 2019. Studies on the anti-angiogenic activities of wild and cultivated *Orostachys japonicus* extracts in human umbilical vein endothelial cells. *Journal of Food Science* 84(7): 1764-1775. doi:10.1111/1750-3841.14675

Farooq, U., Naz, S., Shams, A., Raza, Y., Ahmed, A., Rashid, U. & Sadiq, A. 2019. Isolation of dihydrobenzofuran derivatives from ethnomedicinal species *Polygonum barbatum* as anticancer compounds. *Biological Research* 52: 1. doi:10.1186/s40659-018-0209-0

Farooq, U., Naz, S., Zehra, B., Khan, A., Ali, S.A., Ahmed, A., Sarwar, R., Bukhari, S.M., Rauf, A., Ahmad, I. & Mabkhot, Y.N. 2017. Isolation and characterization of three new anti-proliferative Sesquiterpenes from *Polygonum barbatum* and their mechanism via apoptotic pathway. *BMC Cancer* 17: 694. doi:10.1186/s12885-017-3667-9

Hamid, A.A., Aminuddin, A., Anuar, N.N.M., Mansor, N.I., Ahmad, M.F., Saleh, M.S. & Ugusman, A. 2022. *Persicaria minor* (Huds.) opiz prevents *in vitro* atherogenesis by attenuating tumor necrosis factor-α-induced monocyte adhesion to human umbilical vein endothelial cells. *Life* 12(10): 1462. https://doi.org/10.3390/life12101462

Hu, W.H., Wang, H.Y., Kong, X.P., Xiong, Q.P., Poon, K.K.M., Xu, L., Duan, R., Chan, G.K.L., Dong, T.T.X. & Tsim, K.W.K. 2019. Polydatin suppresses VEGF-induced angiogenesis through binding with VEGF and inhibiting its receptor signaling. *FASEB Journal* 33(1): 532-544. doi:10.1096/fj.201800750R

Hu, W.H., Chan, G.K.L., Lou, J.S., Wu, Q.Y., Wang, H.Y., Duan, R., Cheng, M.Y.T., Dong, T.T.X. & Tsim, K.W.K. 2018. The extract of *Polygoni cuspidati* Rhizoma et Radix suppresses the vascular endothelial growth factor-induced angiogenesis. *Phytomedicine* 42: 135-143. doi:10.1016/j.phymed.2018.03.029

Ismail, I.F., Golbabapour, S., Hassandarvish, P., Hajrezaie, M., Abdul Majid, N., Kadir, F.A., Al-Bayaty, F., Awang, K., Hazni, H. & Abdulla, M.A. 2012. Gastroprotective activity of *Polygonum chinense* aqueous leaf extract on ethanol-induced hemorrhagic mucosal lesions in rats. *Evidence-Based Complementary and Alternative Medicine* 2012: 404012. doi:10.1155/2012/404012

Kimura, Y. & Okuda, H. 2001. Resveratrol isolated from *Polygonum cuspidatum* root prevents tumor growth and metastasis to lung and tumor-induced neovascularization in lewis lung carcinoma-bearing mice. *Journal of Nutrition* 131(6): 1844-1849. doi:10.1093/jn/131.6.1844

Li, Q., Fu, X., Ge, X., Tao, F., Huang, P., Ge, M. & Jin, H. 2019. Antitumor effects and related mechanisms of ethyl acetate extracts of *Polygonum perfoliatum* L. *Frontiers in Oncology* 9: 578. doi:10.3389/fonc.2019.00578

Lim, B.O., Lee, J.H., Ko, N.Y., Mun, S.H., Kim, J.W., Kim, D.K., Kim, J.D., Kim, B.K., Kim, H.S., Her, E., Lee, H.Y. & Choi, W.S. 2007. *Polygoni cuspidati* radix inhibits the activation of Syk kinase in mast cells for antiallergic activity. *Experimental Biology and Medicine (Maywood, N.J.)* 232(11): 1425-1431. doi:10.3181/0705-RM-118

Mahnashi, M.H., Alqahtani, Y.S., Alyami, B.A., Alqarni, A.O., Ullah, F., Wadood, A., Sadiq, A., Shareef, A. & Ayaz, M. 2021. Cytotoxicity, anti-angiogenic, anti-tumor and molecular docking studies on phytochemicals isolated from *Polygonum hydropiper* L. *BMC Complementary Medicine and Therapies* 21(1): 239. doi:10.1186/s12906-021-03411-1

Matsuda, H., Shimoda, H., Morikawa, T. & Yoshikawa, M. 2001. Phytoestrogens from the roots of *Polygonum cuspidatum* (Polygonaceae): Structure-requirement of hydroxyanthraquinones for estrogenic activity. *Bioorganic & Medicinal Chemistry Letters* 11(14): 1839-1842. doi:10.1016/s0960-894x(01)00318-3

Newman, D.J. & Cragg, G.M. 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *Journal of Natural Products* 75(3): 311-335. doi:10.1021/np200906s

Nkuété, A.H.L., Kuete, V., Gozzini, D., Migliolo, L., Oliveira, A.L., Wabo, H.K., Tane, P., Vidari, G., Efferth, T. & Luiz Franco, O. 2015. Anti-leukemia activity of semi-synthetic phenolic derivatives from *Polygonum limbatum* Meisn. *Chemistry Central Journal* 9: 40. doi:10.1186/s13065-015-0115-2

Ozgur, S., Oktem, M., Altinkaya, S.O., Oktem, E.O., Cenksoy, C., Erdem, O., Elbeg, S., Helvaci, A., Erdem, A. & Erdem, M. 2018. The effects of resveratrol on ovarian hyperstimulation syndrome in a rat model. *Taiwanese Journal of Obstetrics and Gynecology* 57(3): 383-388. doi:10.1016/j.tjog.2018.04.010

Peng, Z.F., Strack, D., Baumert, A., Subramaniam, R., Goh, N.K., Chia, T.F., Tan, S.N. & Chia, L.S. 2003. Antioxidant flavonoids from leaves of *Polygonum hydropiper* L. *Phytochemistry* 62(2): 219-228. doi:10.1016/s0031-9422(02)00504-6

Rafii, S., Lyden, D., Benezra, R., Hattori, K. & Heissig, B. 2002. Vascular and haematopoietic stem cells: Novel targets for anti-angiogenesis therapy? *Nature Reviews. Cancer* 2(11): 826-835. doi:10.1038/nrc925

Rajasekar, J., Perumal, M.K. & Vallikannan, B. 2019. A critical review on anti-angiogenic property of phytochemicals. *Journal of Nutritional Biochemistry* 71: 1-15. doi:10.1016/j.jnutbio.2019.04.006

Rashid, N.A., Hussan, F., Hamid, A., Ridzuan, N.R., Teoh, S.L. & Budin, S.B. 2019. Preventive effects of *Polygonum minus* essential oil on cisplatin-induced hepatotoxicity in sprague Dawley Rats. *Sains Malaysiana* 48(9): 1975-1988. https://doi.org/0.17576/jsm-2019-4809-19.

Rashidi, B., Malekzadeh, M., Goodarzi, M., Masoudifar, A. & Mirzaei, H. 2017. Green tea and its anti-angiogenesis effects. *Biomedicine & Pharmacotherapy* 89: 949-956. doi:10.1016/j.biopha.2017.01.161

Rayan, A., Raiyn, J. & Falah, M. 2017. Nature is the best source of anticancer drugs: Indexing natural products for their anticancer bioactivity. *PLoS ONE* 12(11): e0187925. doi:10.1371/journal.pone.0187925

Rezzola, S., Loda, A., Corsini, M., Semeraro, F., Annese, T., Presta, M. & Ribatti, D. 2020. Angiogenesis-inflammation cross talk in diabetic retinopathy: Novel insights from the chick embryo chorioallantoic membrane/human vitreous platform. *Frontiers in Immunology* 11: 581288. doi:10.3389/fimmu.2020.581288

Sun, X. & Sneden, A.T. 1999. Neoflavonoids from *Polygonum perfoliatum*. *Planta medica* 65(7): 671-673. doi:10.1055/s-2006-960846

Wang, S., Zheng, Z., Weng, Y., Yu, Y., Zhang, D., Fan, W., Dai, R. & Hu, Z. 2004. Angiogenesis and anti-angiogenesis activity of Chinese medicinal herbal extracts. *Life Sciences* 74(20): 2467-2478. doi:10.1016/j.lfs.2003.03.005

Yahya, H.M., Shahar, S., Ismail, S.N.A., Aziz, A.F., Che Din, N. & Abdul Hakim, B.N. 2017. Mood, cognitive function and quality of life improvements in middle aged women following supplementation with *Polygonum minus* extract. *Sains Malaysiana* 46(2): 245-254. https://doi.org/10.17576/jsm-2017-4602-09

*Corresponding author; email: adilahamid@ppukm.ukm.edu.my