Process Biochemistry 89 (2020) 71–80

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Antiangiogenic, anti-inflammatory and their antioxidant activities of *Turnera subulata* Sm. (Turneraceae)

Mythili Saravanan[a], Palanisamy Senthilkumar[a], Vajjiram Chinnadurai[b], Kunnathur Murugesan Sakthivel[c], Raman Rajeshkumar[d], Arivalagan Pugazhendhi[e,*]

[a] PG and Research Department of Biotechnology, Hindusthan College of Arts and Science, Coimbatore - 641 028, Tamil Nadu, India
[b] PG Department of Botany, Sri Vidya Mandir Arts and Science College, Uthangarai - 636 902, Tamil Nadu, India
[c] Department of Biochemistry, PSG College of Arts and Science, Coimbatore - 641 014, Tamil Nadu, India
[d] Department of Pharmaceutical Biotechnology, JSS College of Pharmacy (A Constituent College of JSS Academy of Higher Education & Research), Udhagamandalam - 643 001, Tamil Nadu, India
[e] Innovative Green Product Synthesis and Renewable Environment Development Research Group, Faculty of Environment and Labour Safety, Ton Duc Thang University, Ho Chi Minh City, Viet Nam

ARTICLE INFO

Keywords:
Turnerasubulata
Antioxidant
Anti-inflammatory
Antiangiogenesis
Chick chorioallantoic membrane (CAM)

ABSTRACT

Turnera subulata is a substantial medicinal plant used in folk medicine to treat various ailments. The current study was assess the total phenolic and flavonoid contents to evaluate the antioxidant and anti-inflammatory activities of the sequentially extracted *T. subulata* plant samples. *In vitro* anti-angiogenic activity was evaluated by chick chorioallantoic membrane (CAM) model for chloroform, ethyl acetate and ethanol extracts. The results obtained revealed that total phenolic content of the chloroform extract (24.13 ± 0.27 mg/g) and total flavonoid content (TFC) of the chloroform extract (22.28 ± 0.40 mg/g) were found to be suggestively higher than the other extracts. A strong antioxidant property was observed for all the six extracts. A study anti-inflammatory activity was observed in chloroform and ethanol extracts, with IC_{50} ranging from 79 ± 1.01 µg/mL to 81 ± 1.01 µg/mL for protein denaturation assay and from 74 ± 0.11 µg/mL to 76 ± 1.11 µg/mL for HRBC membrane stabilization assay, respectively. The chloroform and ethanol extracts have exhibited good anti-angiogenic property. Eventually, these results justified that the chloroform and ethanol extracts of *T. subulata* with great antioxidant, anti-inflammatory and antiangiogenesis potentials could be promising candidates for the development of a cost effective, potent anticancer drug with minimal side effects.

1. Introduction

Turnera subulata is a medium-sized perennial shrub native to Africa, North America, and South America, mainly in Northeastern Brazil. It is used in the treatment of diabetes, influenza, cuts, tumors, hypertension, gastrointestinal, respiratory ailments, chronic pain and inflammation. Some studies have demonstrated the antioxidant effects of few Turnera species [1,2]. Recently, studies have shown that the genus Turnera contains greater concentrations of arbutin, an important biological compound found in different parts of the plant [3,4]. Though *T. subulata* is widely used in folk medicine for ages only few research articles are available about its pharmacological and biological properties [5].

The World Health Organization (WHO) report says that cancer is a leading reason for death in developing and developed countries [6]. In spite of tremendous progress in clinical oncology, the underlying pathological conditions remain a major concern to public health [6]. Several studies have shown that free radical accumulation, chronic inflammatory ailment and angiogenesis play important roles in primary tumor formation, tumor progression and metastasis [7,8]. Reactive oxygen species plays a major role in the maintenance of cell oxidative homeostasis, activation and regulation of cellular signaling pathways [9]. ROS production in mitochondria assimilates cells energy state, metabolic concentration and other upstream signaling process. It plays a vital role in cell signaling, stem cell production, survival and neoplastic transformation [10,11]. The excessive release of reactive oxygen/ nitrogen products, such as hydrogen peroxide, superoxide radical anions, nitric oxide and hydroxyl radicals, may result in subsequent alteration in the intracellular homeostasis, which causes damage to the essential cellular components due to oxidative stress [12–14]. Chronic oxidative stress can induce carcinogenesis,

* Corresponding author.
E-mail address: arivalagan.pugazhendhi@tdtu.edu.vn (A. Pugazhendhi).

https://doi.org/10.1016/j.procbio.2019.10.011
Received 27 July 2019; Received in revised form 16 September 2019; Accepted 10 October 2019
Available online 18 October 2019
1359-5113/

neurodegenerative, diabetes, and cardiovascular diseases [15]. The search for novel naturally derived plant based anticancer drug is the main concern in oncology research.

Based on Rudolf Virchow's hypothesis, the origin of malignancy is at the site of chronic inflammation [16]. Certain groups of irritants during tissue injury cause inflammation and enhance cell proliferation. Now, it has become clear that cell proliferation alone does not cause malignancy. The constant cell proliferation - neoplastic growth is seen in the environment that is rich in inflammatory cells, activated stroma, growth factors and inhibitors [17]. Some types of chronic inflammation promote primary tumor growth via pro-inflammatory cytokine and tumor necrosis factor-α (TNFα), which is significant in the initial phases of tumor formation, regulation, adhesion and pro-biogenesis [16].

Angiogenesis is the development of new blood capillaries from arterial vascularization, which involves transportation, growth and differentiation of endothelial cells lining the inner walls of blood capillaries. The endothelial cells not only aid in new vessel formation but also in supplying oxygen, required nutrients, immune cells and toxic substance removal [18]. Angiogenesis is essential for aggressive tumor growth and metastasis of cancer cells. Angiogenesis is a key factor in malignancy, which is regulated by activators and inhibitors. More than half a dozen of protein molecules and free radicals have been identified as activators and inhibitors such as vascular endothelial growth factor (VEGF) and matrix metalloproteinase (MMPs) [19–21]. Imbalance between the endogenous angiogenic activators and inhibitors is the main reason behind the tumor progression. Tumor cell aggressiveness depends on the expression of cytokines and angiogenic factors [22]. Development of angiogenic inhibitors may be an essential step to reduce both the illness and death caused by malignancy. The biological and healing effect of *T. subulata* has been studied only to lesser extent, on antioxidant, anti-inflammatory. The present study was the first systematic study report on the antiangiogenic potential. Based on traditional claims regarding the usage of *T. subulata* the current study was aimed to quantify the total phenol, flavonol, antioxidant content, and to study the antioxidant, anti-inflammatory and antiangiogenic potentials of various extracts of *T. subulata*.

2. Materials and methods

2.1. Plant collection, identification and extract preparation

The fresh whole plant was collected near the foot hills of Marudhamalai, Coimbatore, Tamil Nadu, India. The collected plant material was identified at the Botanical Survey of India (Reference Number: BSI/SRC/5/23/2016/Tech/213), Coimbatore, Tamil Nadu, India. The whole plant sample was collected during morning time, washed thoroughly with running tap water and shade dried. The shade dried plant was coarsely ground and stored in an air tight container. The sample was successively extracted by hot percolation method (soxhlet apparatus) with n-hexane, petroleum ether, chloroform, ethyl acetate, ethanol and methanol respectively. After evaporation of the excess solvents, the residual yields were stored in airtight brown bottles, respectively.

2.2. In vitro antioxidant assays

DPPH (α, α-diphenyl-β-picrylhydrazyl) radial scavenging assay [23], Nitric oxide scavenging assay, Hydrogen peroxide (H_2O_2) scavenging assay, Scavenging of Hydroxyl Radical by pNDA (p-nitrosodimethyl aniline) method [24], ABTS (2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) scavenging assay [25], Lipid peroxidation assay [26], Superoxide scavenging assay [27] and Deoxyribose degradation assay [28] based antioxidant activities of all the six extracts of *T. subulata* was estimated using standard protocol with ascorbic acid, rutin, α-Tocopherol, (BHA- Butylated hydroxyanisole) as the standard. Various concentrations of all the six extracts (50–1000 μg/mL) were studied for radical scavenging potential. Finally, the percentage of inhibition was calculated.

2.3. Total antioxidant capacity (TAC)

The total antioxidant capacity (TAC) [29] of all the six extracts of *T. subulata* were estimated by phosphomolybdate method. To 0.1 mL of each plant extract, 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added and mixed thoroughly. Then, the reaction mixtures were covered with aluminum foil and kept in a water bath for 75 min at 80 °C. After incubation, the samples were allowed to reach room temperature and finally, the absorbances were recorded at 695 nm. Ascorbic acid was used as standard. The antioxidant capacity was calculated using the following formula:

$$\text{Antioxidant effect}(\%) = \frac{(\text{control absorbance-sample absorbance})}{(\text{control absorbance})} \times 100$$

2.4. Total phenolic content (TPC)

TPC [28] of all the six extracts of *T. subulata* were determined by Folin-Ciocalteu's (FC) method. To 1 mL of each plant extract, 1 mL of Folin-Ciocalteu's phenol reagent was added and the reaction mixture was incubated for 5 min after which, 10 mL of 7% sodium carbonate was added. Finally, 13 mL of deionized distilled water was added to the reaction mixture and incubated in dark at 23 °C for 2 h. After incubation, the absorbance was measured at 765 nm. Gallic acid was used as standard. The results were stated as gallic acid equivalents to dry weight of plant material.

2.5. Total flavonoid content (TFC)

TFC (Total flavonoid content) [30] of all the six extracts of *T. subulata* were estimated using 100 mg of each extract. All the plant extracts were dissolved in 10 mL of distilled water. From the stocks, 0.5 mL of extracts were mixed with 1.5 mL of distilled water (D. H_2O); 0.1 mL of 10% of aluminum chloride, 0.1 mL of 1 m of potassium acetate and 2.8 mL of D.H_2O. Then, the reaction mixtures were kept at room temperature for about 40 min. Finally, the absorbance was read at 415 nm in an UV–vis spectrophotometer. Rutin was used as standard. The total flavonol content was expressed as rutin equivalents per gram of dried fraction.

2.6. In vitro anti-inflammatory activity

2.6.1. Inhibition of albumin denaturation

Protein denaturation assay was carried out for all the six extracts of *T. subulata*. The 5 mL solution contained 2.8 mL of phosphate buffer saline, 2 mL of test sample (plant extracts) and 0.2 mL of hen egg albumin. Aspirin was used as (standard drug) positive control and double distilled water as negative control. All the reaction mixtures were incubated at room temperature for 15 min and then, heated at 70 °C for 10 min. Later, the samples were allowed to cool to room temperature, and then, the absorbances were read at 660 nm [31]. The inhibition of protein denaturation was estimated using following equation.

$$\text{Percentage of inhibition}\ (\%) = \frac{\text{control-sample}}{\text{control}} \times 100$$

2.6.2. HRBC membrane stabilization assay

The anti-inflammatory ability of all the six extracts of *T. subulata* was assessed by HRBC (Human Red Blood Cell) membrane stabilization method. From the local blood bank, blood samples were collected. To

that blood samples, an equal volume of sterilized Alsever solution (dextrose 2%, sodium chloride 0.42%, citric acid 0.05%, sodium citrate 0.8%, and distilled water 100 mL) was added and then, the sample mixtures were spun at 3000 rpm for 8 min. The packed cells obtained from the sample mixtures were washed with isosaline solution for three times. The packed cells were weighed, and then, 10% suspensions were made from the pellets with isosaline. To 0.5 mL of the suspension samples, an equal volume of test drug in different concentrations was added. Aspirin was used as the standard drug. To the blood control, 2 mL of distilled water was added instead of hyposaline. After incubation at room temperature for 15 min, all the reaction mixtures were then spun at 3000 rpm for 10 min. The supernatant solutions were read at 550 nm using the spectrophotometer for hemoglobin content estimation [32]. The hemolysis percentage was calculated. The HRBC membrane stabilization was calculated.

$$\text{Percentage of inhibition (\%)} = \frac{(\text{Absorbance control} - \text{Absorbance sample})}{\text{Absorbance control}} \times 100$$

2.7. In vivo antiangiogenesis by CAM assay

To study the *in vivo* antiangiogenic potential of chloroform, ethyl acetate and ethanol extracts of *T. subulata* Chick chorioallantoic membrane (CAM) model was employed. Three days old fertilized chick eggs were incubated at 37 °C in a laboratory incubator with 55–60% humidity. After 2 days of incubation, all the dirt's were carefully removed from the eggshells with 70% ethanol. A small round hole was made on the outer shell of the eggs and 0.75–1 mL egg whites were removed with a hypodermic needle, which could help in detachment of the CAM during embryo development; the outer hole was carefully sealed with cellophane tape [33]. The prepared eggs were kept in incubator for 2 days. On the 7th day, sterile discs containing different concentrations of plant extract *viz*., chloroform, ethyl acetate and ethanol (50 µg/mL and 100 µg/mL) extracts were placed on the top of the target area in CAM. The embryos were incubated for 24 h and then the number of blood vessels inhibited in CAM of each treated group was counted in the intervals of 0, 2, 12 and 24 h, respectively.

2.8. Statistical analysis

All experiments were done in triplicates and data's were expressed as the mean ± standard deviation of triplicates. Minimum inhibitory concentration IC_{50} were estimated with Graph Pad Prism software (v. 7.00) by the curve fitted method using the mean values of three quotients. Differences between groups were compared by the one-way analysis of variance (ANOVA) using SPSS Version 22 and considered significant at P values < 0.05.

3. Results and discussion

Medicinal plants contain a remarkable amount of biologically active phytocompounds such as alkaloids and polyphenols. Polyphenols such as phenols, flavonoids and tannins always play a vital role in promotion and maintenance of good health [34]. In this study, six different extracts from *T. subulata* were evaluated for different types of antioxidant, anti-inflammatory and antiangiogenesis activities, TPC, TFC, and TAC, respectively.

Different kinds of antioxidants from plant sources have always been potential drugs in treating and reducing the risk of various ailments such as acute and chronic inflammation and tumor formation [35]. Plant based antioxidant substances always have the ability to scavenge reactive oxygen species that can cause impairment to DNA, lipids and proteins. The antioxidants may destroy cancer cells by initiating apoptosis or by affecting the enzyme activities (eg. cyclooxygenase-2 enzyme) or inhibiting the expression of oncogenes. For evaluation of the antioxidant efficiency of an endogenous substance, a particular assay method alone will not be sufficient because the antioxidants may be polar or non-polar; they can scavenge radicals by donating an electron or hydrogen ions, which may protect the antioxidant defense mechanisms. Hence, several techniques have been used to determine the antioxidant potential of extracts based on their rate and time of scavenging [36].

3.1. In vitro antioxidant activity

In vitro antioxidant capacities of all the six extracts of *T. subulata* were examined using various assays.

3.1.1. DPPH radical scavenging assay

The electron donation capability of the natural substance can be evaluated using DPPH assay. DPPH is a steady free radical that can accept an electron or hydrogen radical and convert it into a stable diamagnetic molecule. The electron or hydrogen donated by the natural substance will be scavenged by DDPH molecule [37]. This method is based on the scavenging of DPPH through the addition of a radical species that decolorizes the DPPH solution. The amount of colour change in the reaction mixture is proportional to the concentration and effectiveness of the antioxidants present in the reaction mixture. DPPH assay is the frequently used method for antioxidant screening because they detect active ingredients at low concentration [7,8]. In the present study, almost all the extracts have shown scavenging activities but ethanol, methanol and chloroform extracts have shown stronger inhibitions (Table 1) at very low concentrations with IC_{50} values of 10.27 ± 0.03 µg/mL (ethanol), 10.48 ± 0.01 µg/mL (methanol) and 45.6 ± 2.5 µg/mL (chloroform), respectively as compared with the standard ascorbic acid (10.28 ± 0.01 µg/mL). These differences were

Table 1
Radical scavenging activity of various solvent extracts of *Turnera subulata* at different concentration.

Plant extract chemical	IC_{50} values (µg/mL) of radical scavenging							
	DPPH	Nitric Oxide	Hydrogen peroxide	Hydroxyl radical	Lipid peroxide	ABTS	Alkaline DMSO	Deoxyribose
n-Hexane	991.6 ± 10.1^{a}	111.00 ± 1.00^{b}	102.0 ± 1.0^{d}	30.80 ± 1.05^{f}	< 1000	93.75 ± 0.9^{e}	< 1000	< 1000
Petroleum ether	358.00 ± 2.0^{d}	338.00 ± 1.0^{a}	425.0 ± 1.0^{a}	42.50 ± 1.2^{c}	< 1000	450.00 ± 1.0^{b}	355.0 ± 1.0^{b}	500.00 ± 1.0^{a}
Chloroform	45.60 ± 2.50^{c}	24.60 ± 0.01^{ef}	91.21 ± 1.0^{e}	23.75 ± 0.9^{b}	< 1000	415.00 ± 1.0^{c}	250.0 ± 1.0^{c}	< 1000
Ethyl acetate	847.30 ± 2.5^{b}	31.90 ± 0.04^{d}	120.0 ± 1.0^{b}	24.44 ± 1.15^{e}	< 1000	250.00 ± 1.0^{d}	680.0 ± 1.0^{a}	250.00 ± 2.0^{b}
Ethanol	10.27 ± 0.03^{e}	36.12 ± 0.04^{de}	106.2 ± 0.8^{c}	21.87 ± 0.95^{f}	< 1000	250.00 ± 3.0^{d}	11.56 ± 1.2^{e}	117.50 ± 2.0^{c}
Methanol	10.48 ± 0.01^{e}	25.12 ± 0.26^{f}	25.00 ± 1.0^{g}	120.00 ± 1.0^{a}	97.5 ± 1.0^{a}	500.00 ± 2.0^{a}	15.62 ± 0.8^{d}	118.70 ± 0.9^{c}
Ascorbic acid	10.28 ± 0.01^{e}	24.42 ± 0.25^{ef}	23.50 ± 1.2^{g}	–	–	67.60 ± 0.98^{f}	13.92 ± 0.9^{d}	–
Rutin	13.14 ± 0.02^{e}	53.31 ± 0.03^{c}	34.10 ± 1.0^{f}	32.06 ± 1.0^{d}	–	56.24 ± 0.87^{g}	14.38 ± 1.1^{d}	–
α-Tocopherol	–	–	–	–	89.03 ± 1.03^{b}	–	–	–
BHA	–	–	–	–	–	–	–	58.12 ± 0.98^{d}

Each value in the table is represented as Mean ± SD.
Values in the same column followed by different letter are significantly different ($P < 0.05$).

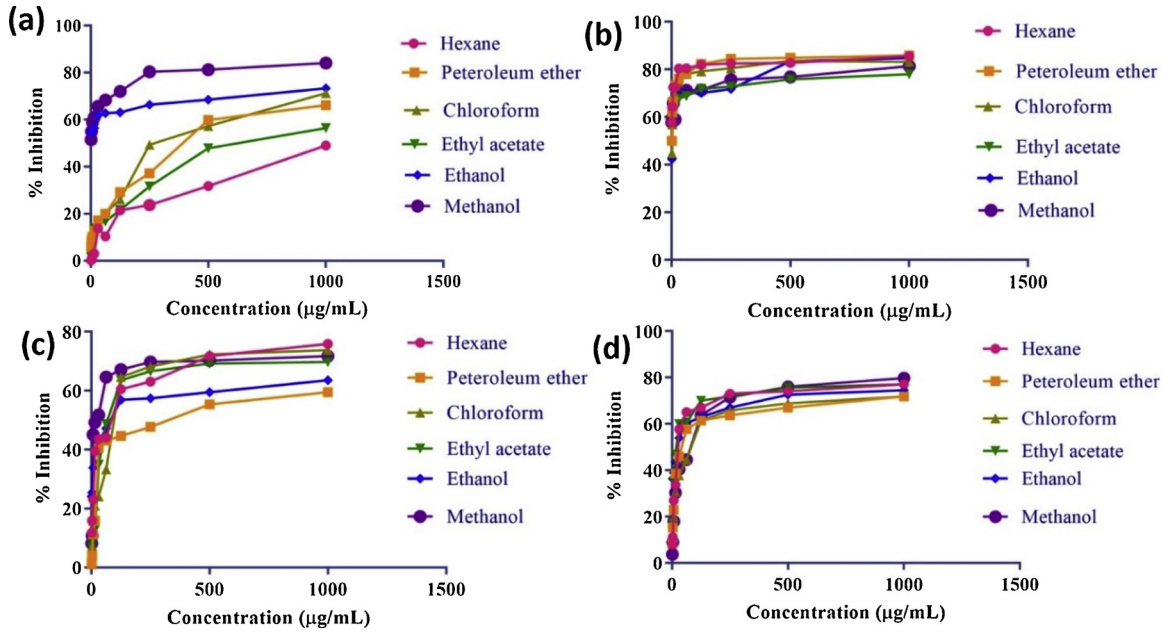

Fig. 1. Antioxidant activities of various solvent extracts and concentration of *Turnera subulata*. (a) DPPH radical scavenging ability; (b) Nitric oxide radical scavenging ability; (c) Hydrogen peroxide scavenging ability; (d) Hydroxyl radical scavenging ability of *Turnera subulata*.

because of the differences in the concentrations of the secondary metabolites such as polyphenols and flavonoids. The percentages of inhibition are shown in Fig. 1a. The outcomes of the present study recommend that the plant extracts containing phytochemical constituents are capable of donating hydrogen to a free radical to scavenge the potential damages.

3.1.2. Nitric oxide scavenging assay

The therapeutic ability of medicinal plants mainly depends on their antioxidant capacities [38]. It is a known fact that nitric oxide plays a vital role in numerous acute and chronic inflammation processes [39]. Uncontrolled production of this free radical is straight away toxic to tissues, which finally contributes to the vascular breakdown [40]. The building up of nitrite radical in the living cell could be highly toxic, may induce mutagenic reactions. The NO scavenging abilities of all the extracts were measured based on their abilities to scavenge free NO radicals compared to the standards, ascorbic acid and rutin. It was witnessed that all the test extracts had higher scavenging activities but significant results were observed for chloroform (24.60 ± 0.01 µg/mL), methanol (24.12 ± 0.26 µg/mL) as well for ethyl acetate (31.90 ± 0.04 µg/mL) extracts. The formation of nitrite radical was inhibited by plant extract by competing with the oxygen. These results specified that based on their proton-donating abilities, these extracts could either serve as a free radical inhibitors or hunters [41]. The percentages of inhibition exhibited by all the six extracts of *T. subulata* are shown in the Fig. 1b and IC_{50} values of all the extracts are provided in Table 1.

3.1.3. Hydrogen peroxide radial scavenging assay

Hydrogen peroxide is usually present at very low concentrations in plants, humans and microorganisms [8]. H_2O_2 radical is considered as a ROS because it will quickly disintegrate into oxygen and water. Hydroxyl radical produced by H_2O_2 can initiate oxidative degradation of lipids; hence, the free radicals easily diffuse through cells, which causes damage to protein, enzyme and DNA. The human body produces more H_2O_2 free radical through the enzyme Superoxide dismutase (SOD). SOD enzyme removes O_2, which is found in cell cytoplasm, mitochondria, extracellular and intracellular spaces. Hence, it is very important to know H_2O_2 radical scavenging capabilities of plant extracts. In hydrogen peroxide radial scavenging assay, most of the extracts have exhibited radical scavenging ability. This might have been due to the presence of phenolic groups in those extracts, which could donate to electrons to neutralize the hydrogen peroxide in to water. Among the six extracts tested, chloroform and methanol have showed potent activity with IC_{50} values of 25 ± 1.0 µg/mL and 91.21 ± 1.0 µg/mL, respectively [42] when compared to that of standard ascorbic acid (23.5 ± 1.2 µg/mL) (Table 1 and Fig. 1c).

3.1.4. Hydroxyl radical scavenging activity by pNDA method

Decomposition of oxygen peroxide through iron catalyzation is considered the most predominant reaction in biological systems and this process acts as a source for numerous deleterious lipid peroxidation products [43]. It must be noted that an essential part of hydroxyl ions is also created along with NO_2 radical by the decay of peroxinitrite or peroxynitrous acid. Hydroxyl radical scavenging activity was measured by the inhibition of pNDA bleaching process [8]. In this study, the maximum scavenging ability was observed for ethanol and chloroform extracts with IC_{50} values of 21.87 ± 0.95 µg/mL and 23.75 ± 0.9 µg/mL, respectively. Meanwhile, minimum activity was observed for methanol and petroleum ether extracts (120 ± 1.0 µg/mL and 42.5 ± 1.2 µg/mL), respectively. All other samples sensibly exhibited scavenging g abilities as compared with rutin (32.06 ± 1.0 µg/mL) (Table 1 and Fig. 1d).

3.1.5. Lipid peroxide assay

Lipid peroxidation process is a very interesting area of research. The process of lipid peroxidation occurs by the free radicals in the cell stealing electrons from the lipids present in the cell membrane, lipoproteins, and other molecules, which cause oxidative stress resulting in the production of secondary oxidation products *viz*., ketones, dialdehydes, and acids leading to cell damage [44,45]. In LPO method, most of the extracts exhibited very low activities with IC_{50} values above 1000 µg/mL (Table 1 and Fig. 2a). A strong scavenging activity was observed in methanol alone with an IC_{50} value of 97.5 ± 1.0 µg/mL compared to the standard α-Tocopherol (89.03 ± 1.03 µg/mL). Thus, the production of secondary oxidation products during lipid peroxidation could have been reduced [46].

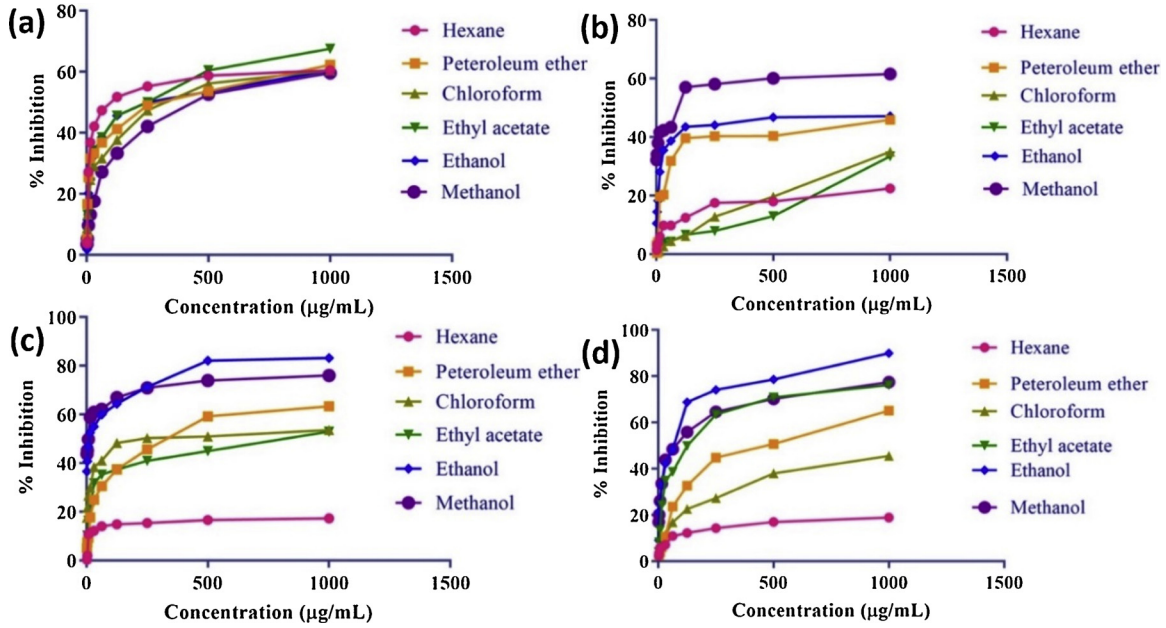

Fig. 2. Antioxidant activities of various solvent extracts and concentration of *Turnera subulata*. (a) ABTS radical scavenging ability (b) Lipid peroxide scavenging ability; (c) Alkaline DMSO scavenging ability; (d) Deoxyribose scavenging ability of *Turnera subulata*.

3.1.6. ABTS assay

ABTS radical scavenging assay is a smart method to investigate the antioxidant potential of hydrogen-donating antioxidants and chain breaking antioxidants *via* generation of blue/green $ABTS^+$ chromophore by chemical oxidation [47]. In ABTS radical scavenging, cation is produced by the oxidation of ABTS with potassium persulfate and the antioxidants present in the plant sample decrease the absorbance of ABTS [48]. In ABTS radial scavenging assay, n-hexane extract has exhibited a potent antioxidant activity with an IC_{50} value 93.75 ± 0.9 µg/mL and all the other extracts have shown low to moderate activities [49]. These results were comparable with the standards, ascorbic acid and rutin, which showed IC_{50} values of 67.6 ± 0.98 µg/mL and 56.24 ± 0.87 µg/mL, respectively (Table 1 and Fig. 2b).

3.1.7. Alkaline DMSO assay

Scavenging capability of superoxide radical can be assessed using alkaline DMSO. In this assay, intracellular superoxide radical is produced by chemical reaction *in vitro* [50]. The nitroblue tetrazolium (NBT) reduction by O_2 was determined in the presence and absence of plant extracts [43]. The current results showed that ethanol and methanol extracts of *T. subulata* showed potent activities with IC_{50} values of 11.56 ± 1.2 µg/mL and 15.62 ± 0.8 µg/mL, respectively. The other extracts have shown low to moderate activities with IC_{50} from 250 ± 0.10 µg/mL to 680 ± 1.0 µg/mL as compared with ascorbic acid (13.92 ± 0.9 µg/mL) and rutin (14.38 ± 1.1 µg/mL) and n-hexane extract had IC_{50} above 1000 µg/mL (Table 1, Fig. 2c).

3.1.8. Deoxyribose assay

Hydroxyl radical will be formed in the reaction between iron-EDTA (Ethylenediaminetetraacetic acid) in the existence of ascorbic acid [40]. Deoxyribose is attacked by ascorbic acid on heating with TBA and forms a pink chromogen. The hydroxyl radical that got escaped from EDTA scavenging will be available to scavenge the deoxyriobose and any compound present in reaction mixture; will try to compete for the hydroxyl radical [51]. In Deoxyribose assay, BHA was used as standard (IC_{50} 58.12 ± 0.98 µg/mL) (Table 1, Fig. 2d). Ethanol, methanol and ethyl acetate extracts have shown moderate activity with IC_{50} ranging from 117.5 ± 2.0 µg/mL to 250 ± 2.0 µg/mL. However, n-hexane and chloroform extracts showed no scavenging activity against deoxyriobose radical, even with IC_{50} above 1000 µg/mL.

3.2. Total phenolic content (TPC)

TPC of all the six plant extracts was estimated by Folin- Ciocalteau assay using gallic acid as standard. Almost all the extracts have shown a good quantity of phenolic compounds. However, compared to all the extracts, chloroform (24.13 ± 0.27 mg/g) and ethanol (21.24 ± 0.33 mg/g) extracts have shown higher quantities of phenolic compounds (Table 2 and Fig. 3a). Phenolic compounds and their derivatives present in plants are responsible for therapeutic properties [7]. Hence, higher phenolic contents in general emphasize the medicinal importance of the selected plant. Natural phenolic compounds plays a vital role in malignancy prevention and treatment [52]. Plant based phenolic compounds from important medicinal herbs and dietary plants include phenolic acids, tannins, flavonoids, curcuminoids, coumarins, lignans and others. Different bioactivities of phenolic compounds are responsible for their chemopreventive properties (*e.g.*, anticarcinogenic, antioxidant and anti-inflammatory effects) and also for apoptosis induction by arresting cell cycle, ontogenesis expression, migration, proliferation or differentiation and blocking signaling pathways [53].

Table 2
Total phenolics, flavonoids contents and total antioxidant capacity of *Turnera subulata* various solvent extracts.

Plant extract	Yield value in mg/g		
	Total phenolics content (TPC) (mg/g)	Total flavonoid content (TFC) (mg/g)	Total antioxidant capacity (TAC) (mg/g)
n-Hexane	12.03 ± 0.52^{e}	11.27 ± 0.54^{e}	14.23 ± 0.44^{e}
Petroleum ether	8.02 ± 0.19^{f}	9.10 ± 0.28^{f}	13.14 ± 0.10^{f}
Chloroform	24.13 ± 0.27^{a}	24.13 ± 0.27^{a}	20.13 ± 0.27^{c}
Ethyl acetate	18.05 ± 0.28^{c}	17.38 ± 0.41^{c}	15.31 ± 0.44^{d}
Ethanol	21.24 ± 0.33^{b}	20.26 ± 0.46^{b}	19.14 ± 0.20^{a}
Methanol	17.11 ± 0.22^{d}	16.11 ± 0.31^{d}	18.29 ± 0.23^{b}

Each value in the table is represented as Mean ± SD.
Values in the same column followed by different letter are significantly different ($P < 0.05$).

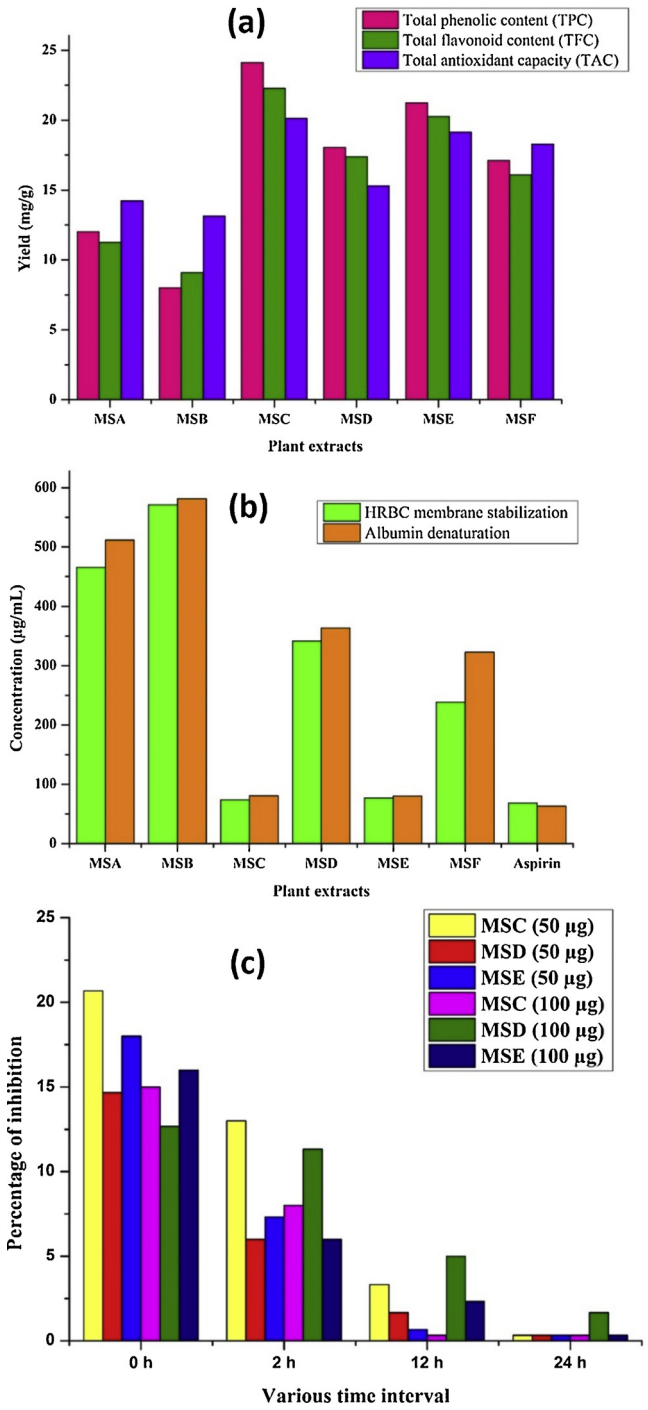

Fig. 3. Phytochemical quantification, anti-inflammatory and antiangiogenic activity of various solvent extracts of *Turnera subulata*. (a) Total phenolics, flavonoids contents and total antioxidant capacity of different solvent extracts of *Turnera subulata*; (b) Effect of various solvent extracts of *Turnera subulata* on *in vitro* HRBC membrane stabilization and Albumin denaturation assay; (c) Effect of various solvent extracts on antiangiogenic ability of *Turnera subulata*.

3.3. Total flavonoid content (TFC)

TFC of all the six extracts of *T. subulata* was determined by using rutin as standard. The quantity of flavonoids presents in all the extracts ranged from 9.10 ± 0.28 mg/g to 22.28 ± 0.40 mg/g and TFC was found abundant in the chloroform extract. Whereas other extract contains satisfactory amount of flavonoid content (Table 2; Fig. 3a). The varied concentration of TFC in all the extracts depends polarity nature of the solvents used for the extraction [54]. The flavonoid fraction present in different extracts of the *T. subulata* exhibits significant correlation between. The total phenolic contents (TPC) (24.13 ± 0.27 mg/g) of MSC and total flavonoid contents (TFC) of MSC (24.13 ± 0.27 mg/mL), which were found significantly higher as compared to other solvent fractions [8].

3.4. Total antioxidant capacity (TAC)

Total antioxidant capacity was tested for all the six extract of *T. subulata*. Since it was a quantitative method, ascorbic acid was used as standard. The results indicated that the chloroform extract has shown the highest TCA (20.13 ± 0.27 mg/g) among the extracts (Table 2 and Fig. 3a).

The total antioxidant capacity increased proportionally to the total phenol and flavonoid content. Total antioxidant capacity results indicated that the ethanol (20.14 ± 0.20 mg/g) and chloroform extracts (19.14 ± 0.20 mg/g) have shown higher TCA among the other extracts. In recent times, several researches have revealed the strong relationship between TCA, TPC and TFC, endorsing the importance of polyphenols as a sturdy antioxidant biomolecule [55,56]. As per recent reports, phenol and flavonoid molecules in medicinal plants play a major role in maintaining the stability of lipid oxidation in association with antioxidant activity [57,58]. In this study, the chloroform and ethanol extracts with higher phenol (24.13 ± 0.27 mg/g and 21.24 ± 0.33 mg/g) and flavonoid contents (22.28 ± 0.40 mg/g and 20.26 ± 0.46 mg/g) showed higher antioxidant capabilities. The variations in the antioxidant capacities of the extracts might have been be due to the redox potential of phenol content, which could act as a free radical scavenger, hydrogen donor, oxygen, quenchers, metal chelators and reducing agents [59].

3.5. In vitro anti-inflammatory activity

3.5.1. Protein denaturation test

Protein denaturation is a well-defined reason for inflammatory process. The anti-inflammatory potential of all the six extracts of *T. subulata* and their potentials to inhibit protein denaturation were studied. The observed results indicated that chloroform and ethanol extracts exhibited good antidenaturation potential with IC_{50} from 80.67 ± 2.5 µg/mL to 80.0 ± 2.6 µg/mL, when compared to that of the reference drug aspirin (IC_{50} 63.33 ± 2.5 µg/mL) (Table 3 and Fig. 3b). The other four extracts (n-hexane, petroleum ether, ethyl acetate and methanol) have shown moderate to low anti-denaturation potentials with IC_{50} from 322.67 ± 2.5 µg/mL to 581.33 ± 3.0 µg/mL. Inflammation is a complex response towards harmful stimuli like pathogens and irritants characterized by warmth, swelling, redness and pain [60]. Denaturation of proteins is a known fact of inflammation. The commercial inflammatory drugs (salicylic acid) have shown

Table 3
In vitro anti-inflammatory activity of different solvent extracts of *Turnera subulata*.

Plant extract	HRBC membrane assay (µg/mL)	Albumin denaturation assay (µg/mL)
n-Hexane	465.67 ± 2.08^{b}	511.33 ± 3.03^{b}
Petroleum Ether	571.33 ± 3.05^{a}	581.33 ± 3.0^{a}
Chloroform	74.00 ± 2.0^{e}	80.67 ± 2.5^{e}
Ethyl Acetate	341.67 ± 3.5^{c}	363.33 ± 2.5^{c}
Ethanol	77.00 ± 2.6^{e}	80.00 ± 2.6^{e}
Methanol	238.67 ± 3.05^{d}	322.67 ± 2.5^{d}
Aspirin	68.43 ± 2.08^{f}	63.33 ± 2.5^{f}

Each value in the table is represented as Mean ± SD.
Values in the same column followed by different letter are significantly different ($P < 0.05$).

Table 4
Effect of various solvent extracts on antiangiogenesis ability of *Turnera subulata*.

Treatment (h.)	Test concentration	Plague formation	No. of vessels inhibition	Anti-angiogenic activity	Status of embryos
0	MSC – 50 µg/mL	–	20.67 ± 1.50^{a}	No	Alive
2		++	13.0 ± 2.00^{a}	Weak	Alive
12		+	3.33 ± 0.50^{b}	Good	Alive
24		–	0.33 ± 0.50^{c}	Strong	Alive
0	MSC – 100 µg/mL	–	15.0 ± 1.00^{cd}	Weak	Alive
2		–	8.0 ± 1.00^{b}	Good	Alive
12		–	0.33 ± 0.50^{d}	Strong	Alive
24		–	0.33 ± 0.50^{c}	Strong	Alive
0	MSD – 50 µg/mL	–	14.67 ± 0.50^{cd}	No	Alive
2		–	6.0 ± 2.00^{b}	Weak	Alive
12		+	1.67 ± 0.50^{cd}	Good	Alive
24		+	0.33 ± 0.50^{c}	Strong	Alive
0	MSD – 100 µg/mL	–	12.67 ± 1.50^{d}	No	Alive
2		++	11.33 ± 1.50^{a}	Weak	Alive
12		+	5.0 ± 1.00^{a}	Fair	Alive
24		–	1.67 ± 0.50^{a}	Strong	Alive
0	MSE – 50 µg/mL	–	18.0 ± 1.00^{b}	No	Alive
2		–	7.33 ± 1.50^{b}	Fair	Alive
12		–	0.67 ± 0.50^{d}	Good	Alive
24		–	0.33 ± 0.50^{c}	Strong	Alive
0	MSE – 100 µg/mL	–	16.0 ± 2.00^{bc}	No	Alive
2		++	6.0 ± 1.00^{b}	Fair	Alive
12		++	2.33 ± 1.50^{bc}	Good	Alive
24		+	0.33 ± 0.50^{c}	Strong	Alive

MSC - *T. subulata* chloroform extract; MSD - *T. subulata* ethyl acetate extract; MSE - *T. subulata* ethanol extract.
Each value in the table is represented as Mean ± SD.
Values in the same column followed by different letter are significantly different ($P < 0.05$).

thermally induced protein denaturation in dose dependent manner [61,62]. The denaturation, the change of proteins nature from a soluble to an insoluble form are due to chemical and physical agents. Mechanism of denaturation is alteration of electrostatic force, hydrogen, hydrophobic and disulphide bonds. Similar results were observed by [63] in *Pedalium murex*.

3.5.2. Membrane stabilization test

All the six extracts of *T. subulata* were tested for HRBC membrane stability against hypotonic solution induced lysis. The results observed are illustrated in Table 3 and Fig. 3b. All the extracts have exhibited inhibition against red blood cell lysis. The chloroform and ethanol extracts have potent anti-inflammatory potentials with IC_{50} values from 74.0 ± 2.0 µg/mL to 77.0 ± 2.6 µg/mL as compared with the reference standard drug aspirin (IC_{50} 68.43 ± 2.08 µg/mL). These anti-inflammatory responses were related to the polyphenol contents, especially flavonoids of the plant [64]. The polyphenol contents were high in both the chloroform and ethanol extracts leading to potential anti-inflammatory responses for those extracts. The other four extracts namely, n-hexane, petroleum ether, ethyl acetate and methanol have displayed moderate to low anti-inflammatory potentials with IC_{50} ranging from 238.67 ± 3.05 µg/mL to 571.33 ± 3.05 µg/mL.

Inflammatory conditions are still the main health concern of the world population. Proteins denaturation is a known cause for inflammation. Reactive oxygen kindle this process, some studies demonstrate that anti-inflammatory drug has ability to induce thermal protein denaturation based on a dose-dependent manner by ROS generation. Proteolytic enzymes, such as pancreatin, trypsin, chymotrypsin, rutin, are important regulators and modulators of the inflammatory response, which was also triggered by ROS [65]. In current scenario synthetic drugs are more dominant in the market, in spite of its worst side effects like peptic ulcers and gastrointestinal bleeding [66,67]. So there is urgent need for plant based drug with fewer side effects. To know the anti-inflammatory effect of the all the six extracts of *T. subulata, in vitro* albumin denaturation and HRBC membrane stability assays were done. The *T. subulata* chloroform and ethanol extract has shown the good anti-inflammatory response in protein denaturation and membrane stabilization assay, compare to that of standard drug aspirin. Similar studies have reported that by Chowdhury *et al.* [68] by using methanolic extracts of *Gardenia coronaria* leaves and Saleem *et al.* [67] observed anti-inflammatory activity of leaf extracts of *Gendarussa vulgaris* Nees.

3.6. In vivo antiangiogenesis by CAM assay

Angiogenesis is a vital step in solid tumor growth, vascular invasion, and metastasis. There are two ways to control angiogenesis by direct and indirect pathways. Direct way is suppressing the process of proliferation and migration of vascular endothelial cells in response to angiogenic protein, such as (Vascular endothelial growth factor) VEGF [69]. The indirect way is to modify the angiogenic protein expression and their activities such as regulation of receptors on endothelial cells [70]. The tumor blood vessels are the potential targets in cancer therapy because of their genetic instabilities. Plant based anti-angiogenic substances are gaining more attention in the prevention and treatment of inflammatory diseases and tumor formation because of less side effects [71]. The CAM (Chick chorioallantoic membrane) assay is an accurate and economic *in vivo* investigation to test the anti-angiogenic ability of individual compounds and plant extracts [72]. The assay provides information on the effectiveness of test samples *in vivo* and also their toxicities. Plant based antiangiogenesis drug with less side effects may be a prospective target for pharmacological intervention on angiogenesis based diseases [71].

Based on the antioxidant and anti-inflammatory results, the chloroform, ethyl acetate and ethanol extracts were chosen for anti-angiogenic ability test by the CAM assay. Seven day old eggs were treated with 50 µg/mL and 100 µg/mL of chloroform, ethyl acetate and ethanol extracts as an *in vivo* model. The number of vessels inhibited, plague formation, embryo survival rates and status of eggs after anti-angiogenesis were recorded and provided in Table 4 and Fig. 3c.

The biological and healing effect of *T. subulata* has been studied only to lesser extent, on antioxidant, anti-inflammatory. The present study was the first systematic study report on the antiangiogenic potential of the chloroform, ethyl acetate and ethanol extracts of *T. subulata*. The

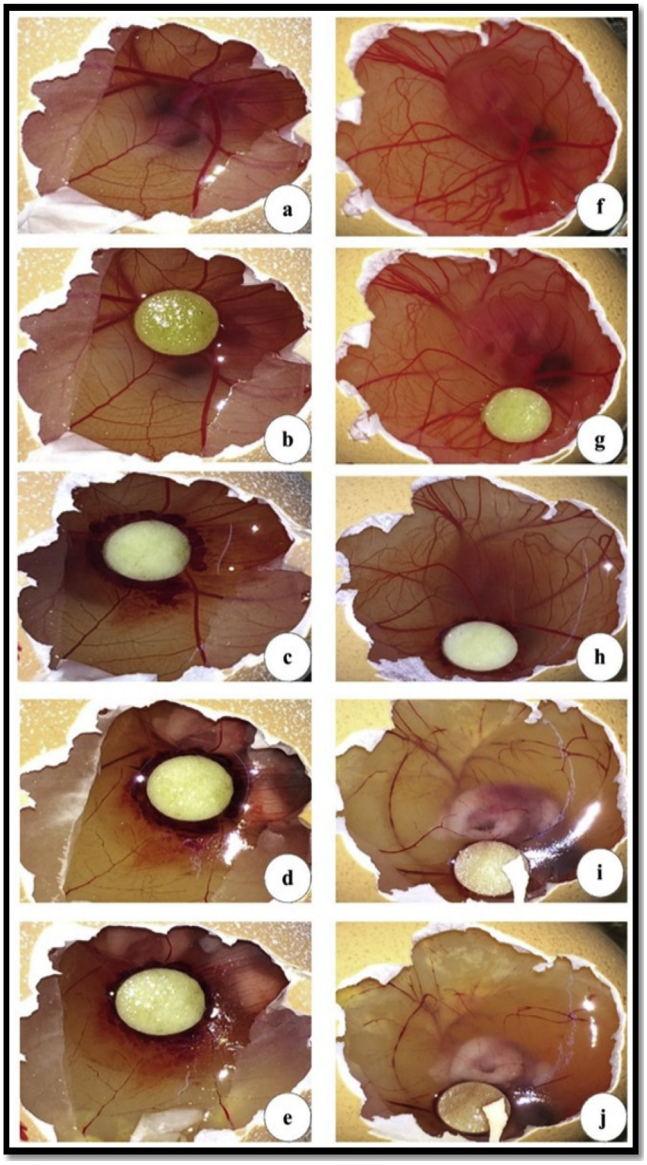

Fig. 4. Anti-angiogenesis activity of MSC extract of *Turnera subulata*. (a–f) untreated (control) - 50 and 100 µg/mL; (b–g) – 0 h of 50 and 100 µg/mL; (c–h) – 2 h of 50 and 100 µg/mL; (d–i) – 12 h of 50 and 100 µg/mL; (e–j) – 24 h of 50 and 100 µg/mL.

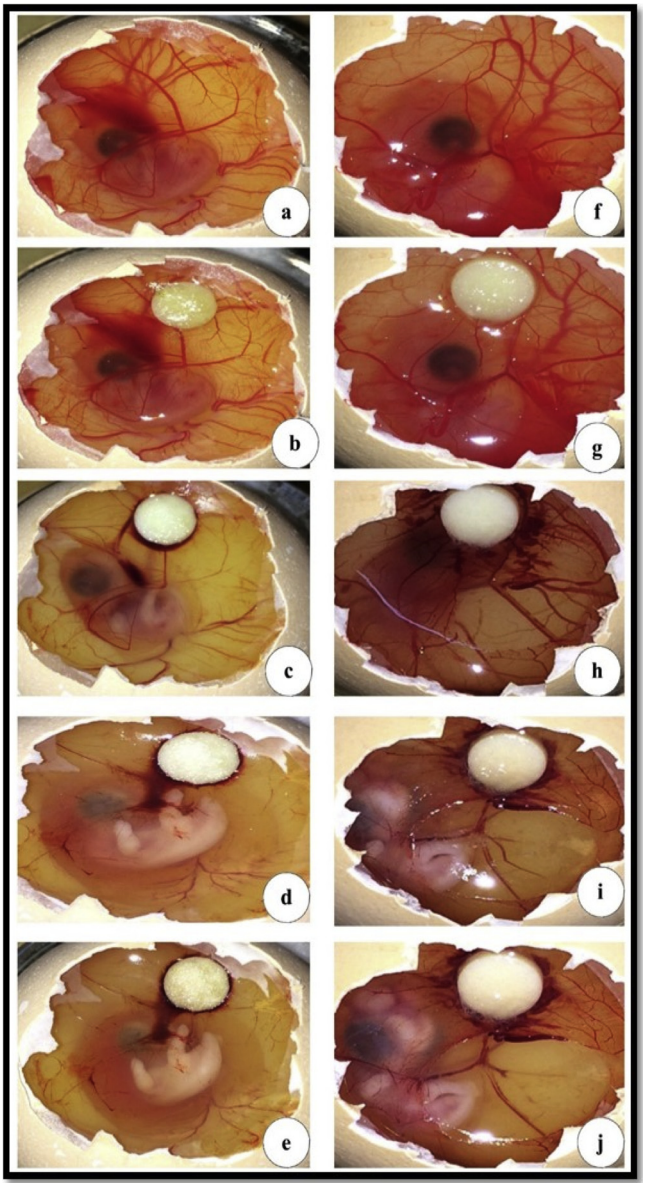

Fig. 5. Anti-angiogenesis activity of MSD extract of *Turnera subulata*. (a–f) – untreated (control) - 50 and 100 µg/mL; (b–g) – 0 h of 50 and 100 µg/mL; (c–h) – 2 h of 50 and 100 µg/mL; (d–i) – 12 h of 50 and 100 µg/mL; (e–j) – 24 h of 50 and 100 µg/mL.

potential antiangiogenic activity was observed in all the three extracts of *T. subulata.* The CAM treated with 50 and 100 µg of all the three extracts not only displayed distorted primary, secondary and tertiary micro vessels but also perturbation on existing vasculatures [7]. In the low concentration (50 µg) of chloroform extract reduces the minor and major vessels in CAM assay reduced from 20.67–0.33 in a regular time interval (Fig. 4a–e), whereas the high concentration (100 µg) of a same extract registered the vessel inhibition in the range between 15-0.33 (Fig. 4f–j). The vessel inhibition rate of ethyl acetate low concentration (50 µg) sample is 14.67-0.33 (Fig. 5a–e), while in high concentration (100 µg) the number of vessels inhibited by the plant extract is ranged between 12.67–1.67 (Fig. 5f–j). The vessel inhibition range of low concentration ethanol is 18–0.33 (Fig. 6a–e) where in high concentration, it various between 16–0.33 (Table 4; Fig. 6f–j). The results reveal that all the three tested extracts registered enhancing anti-angiogenesis property.

In addition, it also observed the plague formation during anti-angiogenesis activity of plant extracts. Among the three tested extracts the ethanol (100 µg) registered high amount of plague formation after 2 h of incubation (Table 4), while the other tested concentration of remaining extracts registered satisfactory level of plague formation. However, no plague formation was observed for the 100 µg concentration of methanol and 50 µg of ethanol (Table 4). In all the tested CAM model the embryo was alive in condition. The antiangiogenic activity of both concentration (50 µg and 100 µg) of three extracts is increased in proportion to increased incubation hours.

Though all the extracts have exhibited activities, chloroform and ethanol extracts with higher concentrations of total phenols, total flavonoids, and total antioxidants have demonstrated better antioxidant, anti-inflammatory and antiangiogenesis activities. These extracts should be studied systematically in future to develop a potent anticancer drug from the important medicinal plant, *T. subulata*.

4. Conclusion

The biochemical nature of bioactive plant based secondary

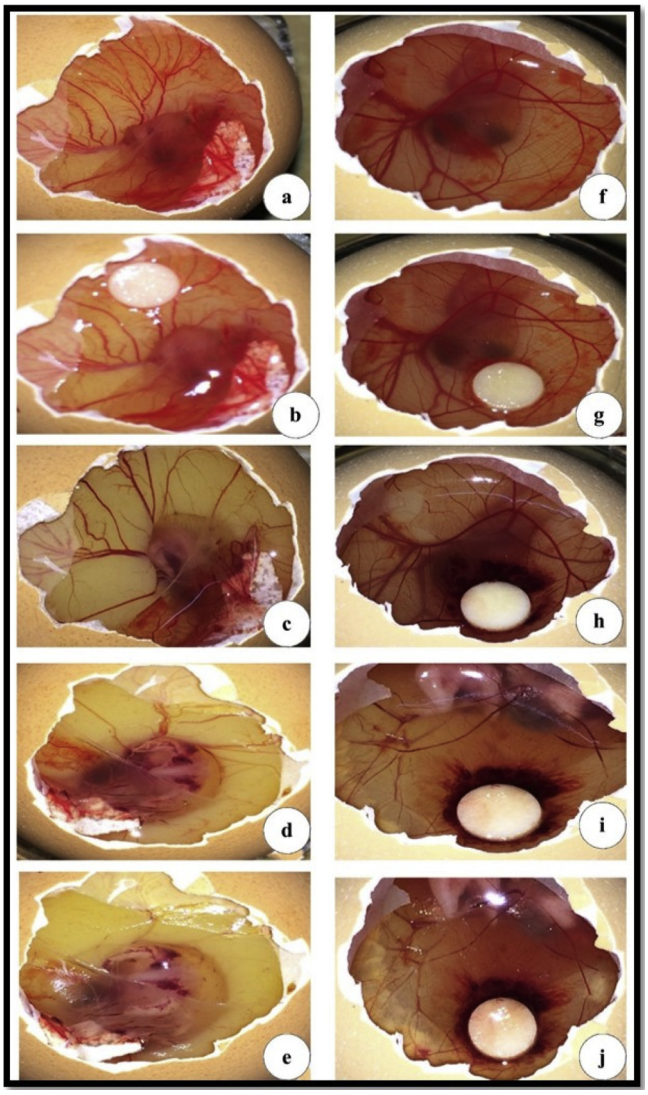

Fig. 6. Anti-angiogenesis activity of MSE extract of *Turnera subulata*. (a–f) – untreated (control) 50 and 100 µg/mL; (b–g) – 0 h of 50 and 100 µg/mL; (c–h) – 2 h of 50 and 100 µg/mL; (d–i) – 12 h of 50 and 100 µg/mL; (e–j) – 24 h of 50 and 100 µg/mL.

metabolites appears to be a promising valuable alternate and has fascinated many researchers to search for novel pharmacologically active phytocompounds, mainly to improve the treatment and quality of patients life after recovery. In this current study, we could demonstrate the quantification of total phenolic and flavonol compounds present in various solvent extracts of *T. subulata*. These extracts as serve as free radical scavenger or inhibitors perhaps by acting as primary oxidants. Which also inhibited the protein denaturation and HRBC membrane stabilization, in more effective way similar to that of the commercial drug aspirin. To the best of our knowledge present study on anti-angiogenesis is first report on *T. subulata.* The chloroform and ethanol extracts exhibited the potent antiangiogenic property by inhibiting the sprouting and branching of arterial vascularization by preventing signals of angiogenic factors; cytokinins from epithelial cells or by initiation of apoptosis. Hence it is inferred that the chloroform and ethanol extracts of *T. subulata* with higher concentrations of secondary metabolites such as polyphenols and flavonoids and with good antioxidant, anti-inflammatory and antiangiogenic activities are the best candidates for the development of a cost effective and potent anticancer drug with less side effects.

Conflict of interest

None.

Acknowledgement

The authors would like to thank IGPRED (www.igpred.com) for providing insight and expertise on the research topic and for the assistance that greatly improved the manuscript.

References

[1] T.-T. Chai, F.-C. Wong, Whole-plant profiling of total phenolic and flavonoid contents, antioxidant capacity and nitric oxide scavenging capacity of Turnera subulata, J. Med. Plants Res. 6 (9) (2012) 1730–1735.
[2] A. Garza-Juárez, M. de la Luz Salazar-Cavazos, R. Salazar-Aranda, J. Pérez-Meseguer, N.W. de Torres, Correlation between chromatographic fingerprint and antioxidant activity of Turnera diffusa (Damiana), Planta Med. 77 (9) (2011) 958–963.
[3] J. Takebayashi, R. Ishii, J. Chen, T. Matsumoto, Y. Ishimi, A. Tai, Reassessment of antioxidant activity of arbutin: multifaceted evaluation using five antioxidant assay systems, Free Radic. Res. 44 (4) (2010) 473–478.
[4] J. Zhao, A.K. Dasmahapatra, S.I. Khan, I.A. Khan, Anti-aromatase activity of the constituents from damiana (Turnera diffusa), J. Ethnopharmacol. 120 (3) (2008) 387–393.
[5] M. Saravanan, P. Senthilkumar, K. Kalimuthu, V. Chinnadurai, S. Vasantharaj, A. Pugazhendhi, Phytochemical and pharmacological profiling of Turnera subulata Sm., a vital medicinal herb, Ind. Crops Prod. 124 (2018) 822–833.
[6] K. Sidhu, K. Pannu, Indigenous use of medicinal plants for health care, Stud. Ethno-Med. 4 (2) (2010) 145–148.
[7] N.S. Muslim, Z.D. Nassar, A.F. Aisha, A. Shafaei, N. Idris, A. Majid, Z. Ismail, Antiangiogenesis and antioxidant activity of ethanol extracts of Pithecellobium jiringa, BMC Complement. Altern. Med. 12 (1) (2012) 210.
[8] N. Saeed, M.R. Khan, M. Shabbir, Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L, BMC Complement. Altern. Med. 12 (1) (2012) 221.
[9] M. Yang, Y. Ma, Z. Wang, A. Khan, W. Zhou, T. Zhao, J. Cao, G. Cheng, S. Cai, Phenolic constituents, antioxidant and cytoprotective activities of crude extract and fractions from cultivated artichoke inflorescence, Ind. Crops Prod. (2019), https://doi.org/10.1016/j.indcrop.2019.05.082.
[10] D.B. Zorov, M. Juhaszova, S.J. Sollott, Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release, Physiol. Rev. 94 (3) (2014) 909–950.
[11] S. Kumari, A.K. Badana, R. Malla, Reactive oxygen species: a key constituent in cancer survival, Biomark. Insights 13 (2018) 1177271918755391.
[12] P.J. Vernon, D. Tang, Eat-me: autophagy, phagocytosis, and reactive oxygen species signaling, Antioxid. Redox Signal. 18 (6) (2013) 677–691.
[13] R.R. Sonani, N.K. Singh, J. Kumar, D. Thakar, D. Madamwar, Concurrent purification and antioxidant activity of phycobiliproteins from Lyngbya sp. A09DM: an antioxidant and anti-aging potential of phycoerythrin in Caenorhabditis elegans, Process. Biochem. 49 (10) (2014) 1757–1766.
[14] Y. Wang, G. Hou, J. Li, M.M. Surhio, M. Ye, Structure characterization, modification through carboxymethylation and sulfation, and in vitro antioxidant and hypoglycemic activities of a polysaccharide from Lachnum sp, Process. Biochem. 72 (2018) 177–187.
[15] B. Khodr, Z. Khalil, Modulation of inflammation by reactive oxygen species: implications for aging and tissue repair, Free Radic. Biol. Med. 30 (1) (2001) 1–8.
[16] F. Balkwill, A. Mantovani, Inflammation and cancer: back to Virchow? Lancet 357 (9255) (2001) 539–545.
[17] G. Finak, N. Bertos, F. Pepin, S. Sadekova, M. Souleimanova, H. Zhao, H. Chen, G. Omeroglu, S. Meterissian, A. Omeroglu, Stromal gene expression predicts clinical outcome in breast cancer, Nat. Med. 14 (5) (2008) 518.
[18] R.H. Adams, K. Alitalo, Molecular regulation of angiogenesis and lymphangiogenesis, Nat. Rev. Mol. Cell Biol. 8 (6) (2007) 464.
[19] Y.-W. Kim, T.V. Byzova, Oxidative stress in angiogenesis and vascular disease, Blood 123 (5) (2014) 625–631.
[20] M. Bhatia, T.C. Karlenius, G. Di Trapani, K.F. Tonissen, The Interaction Between Redox and Hypoxic Signalling Pathways in the Dynamic Oxygen Environment of Cancer Cells, InTech, 2013.
[21] S.S. Kamble, R.N. Gacche, Evaluation of anti-breast cancer, anti-angiogenic and antioxidant properties of selected medicinal plants, Eur. J. Integr. Med. 25 (2019) 13–19.
[22] M. De Palma, D. Biziato, T.V. Petrova, Microenvironmental regulation of tumour angiogenesis, Nat. Rev. Cancer 17 (8) (2017) 457.
[23] M. Szabo, C. Idițoiu, D. Chambre, A. Lupea, Improved DPPH determination for antioxidant activity spectrophotometric assay, Chem. Pap. 61 (3) (2007) 214–216.
[24] R.J. Ruch, S.-j. Cheng, J.E. Klaunig, Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea, Carcinogenesis 10 (6) (1989) 1003–1008.
[25] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radic. Biol. Med. 26 (9–10) (1999) 1231–1237.
[26] A.R. Verma, M. Vijayakumar, C.V. Rao, C.S. Mathela, In vitro and in vivo

antioxidant properties and DNA damage protective activity of green fruit of Ficus glomerata, Food Chem. Toxicol. 48 (2) (2010) 704–709.
[27] K. Hyland, E. Voisin, H. Banoun, C. Auclair, Superoxide dismutase assay using alkaline dimethylsulfoxide as superoxide anion-generating system, Anal. Biochem. 135 (2) (1983) 280–287.
[28] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, 14] Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-ciocalteu Reagent, Methods in Enzymology, Elsevier, 1999, pp. 152–178.
[29] M. Umamaheswari, T. Chatterjee, In vitro antioxidant activities of the fractions of Coccinia grandis L. leaf extract, Afr. J. Tradit. Complement. Altern. Med. 5 (1) (2008) 61–73.
[30] S. Chae, G. Kang, S. Ma, K. Bang, M. Oh, S. Oh, Standard food analysis, Jigumoonwha Sa (2002) 381–382.
[31] S. Sakat, A.R. Juvekar, M.N. Gambhire, In vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn, Int. J. Pharm. Pharm. Sci. 2 (1) (2010) 146–155.
[32] M. Varadarasu, S. Mounisamy, V. Kavimani, S. Balu, D. Quine, Evaluation of anti-inflammatory and membrane stabilizing properties of ethanol extract of Cansjera rheedii J. Gmelin (Opiliaceae), J. Pharmacol. Ther. (2007) 235–237.
[33] L.M. Kirchner, S.P. Schmidt, B.S. Gruber, Quantitation of angiogenesis in the chick chorioallantoic membrane model using fractal analysis, Microvasc. Res. 51 (1) (1996) 2–14.
[34] M.E. Olsson, K.-E. Gustavsson, S. Andersson, Å. Nilsson, R.-D. Duan, Inhibition of cancer cell proliferation in vitro by fruit and berry extracts and correlations with antioxidant levels, J. Agric. Food Chem. 52 (24) (2004) 7264–7271.
[35] J.M. Matés, F.M. Sánchez-Jiménez, Role of reactive oxygen species in apoptosis: implications for cancer therapy, Int. J. Biochem. Cell Biol. 32 (2) (2000) 157–170.
[36] M.M. Rahman, M.B. Islam, M. Biswas, A.K. Alam, In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh, BMC Res. Notes 8 (1) (2015) 621.
[37] S.B. Kedare, R. Singh, Genesis and development of DPPH method of antioxidant assay, J. Food Sci. Technol. 48 (4) (2011) 412–422.
[38] S.R. Kanatt, R. Chander, A. Sharma, Antioxidant potential of mint (Mentha spicata L.) in radiation-processed lamb meat, Food Chem. 100 (2) (2007) 451–458.
[39] P. Tripathi, P. Tripathi, L. Kashyap, V. Singh, The role of nitric oxide in inflammatory reactions, FEMS Immunol. Med. Microbiol. 51 (3) (2007) 443–452.
[40] B. Hazra, S. Biswas, N. Mandal, Antioxidant and free radical scavenging activity of Spondias pinnata, BMC Complement. Altern. Med. 8 (1) (2008) 63.
[41] K. Marxen, K.H. Vanselow, S. Lippemeier, R. Hintze, A. Ruser, U.-P. Hansen, Determination of DPPH radical oxidation caused by methanolic extracts of some microalgal species by linear regression analysis of spectrophotometric measurements, Sensors 7 (10) (2007) 2080–2095.
[42] A.J. Ruby, G. Kuttan, K.D. Babu, K. Rajasekharan, R. Kuttan, Anti-tumour and antioxidant activity of natural curcuminoids, Cancer Lett. 94 (1) (1995) 79–83.
[43] F. Boora, E. Chirisa, S. Mukanganyama, Evaluation of nitrite radical scavenging properties of selected Zimbabwean plant extracts and their phytoconstituents, J. Food Process 2014 (2014), https://doi.org/10.1155/2014/918018.
[44] A. Ayala, M.F. Muñoz, S. Argüelles, Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal, Oxid. Med. Cell. Longevity 2014 (2014), https://doi.org/10.1155/2014/360438.
[45] G. Barrera, Oxidative stress and lipid peroxidation products in cancer progression and therapy, ISRN Oncol. 2012 (2012), https://doi.org/10.5402/2012/137289.
[46] F.M. Awah, Antioxidant activity, nitric oxide scavenging activity and phenolic contents of Ocimum gratissimum leaf extract, J. Med. Plants Res. 4 (23) (2010) 2479–2487.
[47] K. Sowndhararajan, S.C. Kang, Free radical scavenging activity from different extracts of leaves of Bauhinia vahlii Wight & Arn, Saudi J. Biol. Sci. 20 (4) (2013) 319–325.
[48] N.S. Rajurkar, S. Hande, Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants, Indian J. Pharm. Sci. 73 (2) (2011) 146.
[49] S. Sahreen, M.R. Khan, R.A. Khan, Evaluation of antioxidant activities of various solvent extracts of Carissa opaca fruits, Food Chem. 122 (4) (2010) 1205–1211.
[50] R.S. Kumar, B. Rajkapoor, P. Perumal, Antioxidant activities of Indigofera cassioides Rottl. Ex. DC. using various in vitro assay models, Asian Pac. J. Trop. Biomed. 2 (4) (2012) 256.
[51] S. Sannigrahi, U.K. Mazuder, D.K. Pal, S. Parida, S. Jain, Antioxidant potential of crude extract and different fractions of Enhydra fluctuans lour, Iran. J. Pharm. Res.: IJPR 9 (1) (2010) 75.
[52] S. Jafari, S. Saeidnia, M. Abdollahi, Role of natural phenolic compounds in cancer chemoprevention via regulation of the cell cycle, Curr. Pharm. Biotechnol. 15 (4) (2014) 409–421.
[53] W.-Y. Huang, Y.-Z. Cai, Y. Zhang, Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention, Nutr. Cancer 62 (1) (2009) 1–20.
[54] M. Pinelo, M. Rubilar, M. Jerez, J. Sineiro, M.J. Núñez, Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace, J. Agric. Food Chem. 53 (6) (2005) 2111–2117.
[55] A.S. Rao, S.G. Reddy, P.P. Babu, A.R. Reddy, The antioxidant and antiproliferative activities of methanolic extracts from Njavara rice bran, BMC Complement. Altern. Med. 10 (1) (2010) 4.
[56] M. Alía, C. Horcajo, L. Bravo, L. Goya, Effect of grape antioxidant dietary fiber on the total antioxidant capacity and the activity of liver antioxidant enzymes in rats, Nutr. Res. 23 (9) (2003) 1251–1267.
[57] A. Panche, A. Diwan, S. Chandra, Flavonoids: an overview, J. Nutr. Sci. 5 (2016), https://doi.org/10.1017/jns.2016.41.
[58] M.N. Sarian, Q.U. Ahmed, M. So'ad, S. Zaiton, A.M. Alhassan, S. Murugesu, V. Perumal, S. Mohamad, S.N. Akilah, A. Khatib, Antioxidant and antidiabetic effects of flavonoids: a structure-activity relationship based study, Biomed Res. Int. 2017 (2017), https://doi.org/10.1155/2017/8386065.
[59] D. Amić, D. Davidović-Amić, D. Bešlo, N. Trinajstić, Structure-radical scavenging activity relationships of flavonoids, Croat. Chem. Acta 76 (1) (2003) 55–61.
[60] L. Chen, H. Deng, H. Cui, J. Fang, Z. Zuo, J. Deng, Y. Li, X. Wang, L. Zhao, Inflammatory responses and inflammation-associated diseases in organs, Oncotarget 9 (6) (2018) 7204.
[61] Y. Mizushima, M. Kobayashi, Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins, J. Pharm. Pharmacol. 20 (3) (1968) 169–173.
[62] N.I. Osman, N.J. Sidik, A. Awal, N.A.M. Adam, N.I. Rezali, In vitro xanthine oxidase and albumin denaturation inhibition assay of Barringtonia racemosa L. and total phenolic content analysis for potential anti-inflammatory use in gouty arthritis, J. Intercult. Ethnopharmacol. 5 (4) (2016) 343.
[63] G. Sangeetha, R. Vidhya, In vitro anti-inflammatory activity of different parts of Pedalium murex (L.), inflammation, J. Herb. Med. Res. 4 (2016) 5.
[64] C. Marrassini, I. Peralta, C. Anesini, Comparative study of the polyphenol content-related anti-inflammatory and antioxidant activities of two Urera aurantiaca specimens from different geographical areas, Chin. Med. 13 (1) (2018) 22.
[65] M.B. Alam, M.S. Hossain, M.E. Haque, Antioxidant and anti-inflammatory activities of the leaf extract of Brassica nigra, Int. J. Pharm. Sci. Res. 2 (2) (2011) 303.
[66] C.A. Anosike, O. Obidoa, L.U. Ezeanyika, Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (Solanum aethiopicum), Daru J. Pharm. Sci. 20 (1) (2012) 76.
[67] T.M. Saleem, A. Azeem, C. Dilip, C. Sankar, N. Prasanth, R. Duraisami, Anti-inflammatory activity of the leaf extacts of Gendarussa vulgaris Nees, Asian Pac. J. Trop. Biomed. 1 (2) (2011) 147.
[68] A. Chowdhury, S. Azam, M.A. Jainul, K.O. Faruq, A. Islam, Antibacterial activities and in vitro anti-inflammatory (membrane stability) properties of methanolic extracts of Gardenia coronaria leaves, Int. J. Microbiol. 2014 (2014), https://doi.org/10.1155/2014/410935.
[69] D.R. Bielenberg, B.R. Zetter, The contribution of angiogenesis to the process of metastasis, Cancer J. (Sudbury, Mass.) 21 (4) (2015) 267.
[70] R. Kerbel, J. Folkman, Clinical translation of angiogenesis inhibitors, Nat. Rev. Cancer 2 (10) (2002) 727.
[71] I. Jantan, W. Ahmad, S.N.A. Bukhari, Plant-derived immunomodulators: an insight on their preclinical evaluation and clinical trials, Front. Plant Sci. 6 (2015) 655.
[72] R. Chopra, S. Nayar, I. Chopra, Glossary of Indian Medicinal Plants (Including The Supplement), Council of Scientific and Industrial Research, New Delhi, 1986, pp. 2–79.