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Research paper

"Evaluation of anti-breast cancer, anti-angiogenic and antioxidant properties of selected medicinal plants"

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ABSTRACT

Introduction: Historically, medicinal plants and natural products have been used to treat a variety of human health issues and there has been renewed interest in their use for integrated cancer management. The present investigation was aimed to evaluate the anti-breast cancer, anti-angiogenic and antioxidant potential of selected local botanicals.

Methods: The methanolic extracts of *Cassia occidentalis*, *Callistemon viminalis*, *Cleome viscosa* and *Mimosa hamata* were assessed for their cytotoxic properties against a human breast cancer cell line MCF-7 by using the Sulforhodamine B (SRB) assay. The anti-angiogenic potential of all plant extracts was assessed by using an *in vivo* chorioallantoic membrane (CAM) model. Furthermore, the antioxidant potential of plant samples was evaluated using 2, 2-diphenyl-1-picryl hydrazine (DPPH), hydroxyl (OH) and superoxide radical (SOR) scavenging assays.

Results: The results of the present investigation revealed that all the selected plant extracts: *C. occidentalis* (IC_{50} = 70 ± 0.11 µg/ml), *C. viminalis* (IC_{50} = 44 ± 0.19 µg/ml), *C. viscosa* leaves (IC_{50} = 70 ± 0.22 µg/ml), *C. viscosa* root (IC_{50} = 73.2 ± 0.36 µg/ml) and *M. hamata* (IC_{50} = 65.8 ± 0.25 µg/ml) demonstrated an effective cytotoxic effect against MCF-7 cells. In the CAM model, the plant extracts exhibited significant anti-angiogenic activity by inhibiting the blood vessels density. Amongst the tested samples, the most efficient anti-angiogenic effect was demonstrated by extract of *C. viminalis* (67.76 ± 0.77%). Additionally, all the studied plant extracts were found to possess considerable antioxidant activity.

Conclusion: The selected botanicals with their anti-angiogenic and antioxidant potential could be considered as natural resources in the identification of possible therapeutic agents for breast cancer.

1. Introduction

Breast cancer continues to be one of the major causes of mortality amongst women across the world [1]. Increasing tumor heterogeneity, drug resistance, and the high cost of present therapeutic approaches are some of the current issues associated with effective management of breast cancer [2]. Despite great strides that have been made towards the understanding of disease pathophysiology and the progress towards conventional chemotherapy, the effective management of breast cancer is yet to be achieved [3]. Therefore, there is need to invent novel, target oriented, safe, and low cost therapeutic approaches as well as identify new drug candidates. Since ancient times, traditional medicinal plants have played a pivotal role in treatment of variety of human cancers [4]. Medicinal plants and drug discovery has remained a significant hope for treating various human degenerative diseases including cancer. Plants have been explored as essential resource of medicinally important bioactive compounds and majority of them like Taxol, Camptothecin, Vincristine, Vinblastine,Vinorelbine, Vindesine, Vinflunine etc. have apparently established as a strong therapeutic importance for the treatment of variety of cancers [5]. A range of active components acquired from medicinal plants have earlier evaluated for their potency against several human cancers. Several plant species like *Podophyllum peltatum*, *Catharanthus roseus*, *Taxus baccata* and *Taxus brevifolia* have been greatly acknowledged and appreciated for their therapeutic activity against various cancers including breast cancer [6].

Angiogenesis is the production of new blood vessels from the pre-existing ones and the process is robustly involved in the development of solid tumor [7]. Angiogenesis is a crucial step obligatory for the growth as well as metastasis of developing tumor as the vascular support is necessary for the tumor to grow ahead of 2 mm^3 size. Therefore tumor angiogenesis has been considered as a hallmark of cancer and has been identified as drug target against which a battery of over 11 drugs has

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been approved by the FDA [8]. Emergence of tumor angiogenesis is the result of angiogenic disturbance because of imbalance amongst the endogenous angiogenic activators and inhibitors as the proangiogenic factors dominate over antia-ngiogenic factors during tumor progression [9]. Additionally, in tumor growth and tumor metastasis an unrestrained angiogenic state often aggravates the disease. The growth of tumors is reliant on their competence to induce angiogenesis as the blood vessels are a basic requisite to deliver the nutrients and oxygen to the growing tumor [10]. Currently a variety of angiogenesis activators and corresponding inhibitors are reported. Presently, vascular endothelial growth factor (VEGF) is considered as the foremost proangiogenic factor for recruiting angiogenesis [11]. The circumstantial literature clearly advocates the development of anti-angiogenic agents to suppress the tumor growth and metastasis. Anti-angiogenic therapy has been recognized as an efficient strategy for the treatment of solid tumor as well as non-tumor cancers [12]. Therefore finding such novel anti-angiogenic agents have attracted the immense attention by the research fraternity in recent times.

Oxidative stress has been often implicated as one of the key players in the initiation of carcinogenesis. Nevertheless, the upcoming cancer therapies have been designed to combat the oxidative stress. Antioxidant supplementation has been employed as an adjuvant therapy for patients from cancer; however such adjuvant treatment modalities are still uncertain owing to the lack of proper integrative and personalized clinical approach. Reactive oxygen species (ROS) generated byproducts of lipid peroxidation are not only cytotoxic, but also act as regulators of signal transduction in cells. Therefore, pro-oxidants and antioxidants are studied as modulators of specific cellular redox signaling mechanisms. Hence, it is imperative to identify novel sources of antioxidants and evaluate the possible benefits of antioxidant adjuvant therapy in cancer patients during therapy and drug holidays [13].

It is well established fact that, plants retain numerous phytochemicals that include phenols, phenolic acids, flavonoids, terpenoids, alkaloids, tannins and other components which are responsible for the distinct pharmacological activities [14]. As a result of tumor heterogeneity and drug resistance, the use of traditional medicinal plants against these evolving pathological conditions is still considered as a ray of hope for the effective management of variety of human degenerative diseases including cancer.

In the context of above situation, we were inspired to conduct the present study to assess the cytotoxic, anti-angiogenic and antioxidant effects of *Cassia occidentalis* (Caesalpiniaceae), *Callistemon viminalis* (Myrtaceae), *Cleome viscosa* (Cleomaceae) and *Mimosa hamata* (Fabaceae) against MCF-7 breast cancer cells. In addition, the samples were also analyzed for their antioxidant potentials.

2. Materials and methods

The human breast cancer cell line (MCF-7) was procured from National Centre for Cell Science (NCCS: a National Cell Line Facility) Pune (MS), India. Fertilized chicken eggs were collected from the regional marketplace at Nanded city (MS), India. RPMI 1640 medium, SRB and DPPH was obtained from Sigma-Aldrich Co. (St. Louis MO, USA). Further necessary chemicals such as ascorbic acid, solvents, reagents used were of analytical grade and were acquired from commercial sources.

2.1. Instrumentation

Rotary evaporator (Heidolph-HEI-Vap Advantage 36000130, Germany), Automatic Ex-Micro plate reader (M 51118170, USA), Microscope (Olympus make SZ 61TR Zoom Trinocular Microscope, Japan), CO_2 incubator (Eppendorf, Germany), Spectrophotometer (Uv–vis Helios Omega 9423, USA).

2.2. Plant collection, identification and extraction

The plant samples were collected from the different localities of Nanded district (MS) India. The botanical identification and authentication of all plant specimens was confirmed by Prof. R. N. Gacche, at Department of Botany, SRTM University, Nanded (MS), India. The voucher specimens of the collected plants viz 245, 258, 289 & 297 were deposited in the herbarium of the Botany Department of SRTM University, Nanded (MS), India. The collected plants were washed and air-dried in shade and afterward powdered mechanically. Extracts of the powdered plant samples were prepared in methanol using the Soxhlet apparatus for 8 h at 60 °C. Thereafter, solvent containing extracts were filtered and concentrated under vacuum in a rotary evaporator. The extracted plant samples were stored at -20 °C for the further use. All the prepared extracts were initially dissolved in 1% Dimethyl sulfoxide (DMSO) solution and subsequently dilutions were made to attain final concentrations.

2.3. Phytochemical screening of plant extracts

Plant extracts were tested for the presence of various phytochemicals such as alkaloids, tannins, terpenoids and saponins by using the different qualitative tests as per the standard procedures reported earlier [15].

2.4. Estimation of total phenol content

The concentration of total phenol present in selected plant extracts was determined by Folin-Ciocalteu (FC) reagent method [16]. In brief, 500 µl of methanolic extracts of individual plant sample (1 mg/ml) was mixed with 1.5 ml of 10% FC reagent. After 5 min, 3 ml of 7.5% Na_2CO_3 solution was added. The reaction mixture was incubated at 30 °C for 2 h. The absorbance of the solution was measured at 760 nm. The phenol content was verified from a gallic acid standard curve and demonstrated as gallic acid equivalents per gram of dry weight of individual extract (mg GAE/g DW).

2.5. Estimation of total flavonoid content

Estimation of total flavonoid content in selected plant extracts was determined by using aluminium chloride ($AlCl_3$) colorimetric method [16]. Briefly, 1 ml of methanolic plant extracts (1 mg/ml) were mixed with 3 ml of methanol, 0.2 ml of 10% $AlCl_3$, 0.2 ml of 1 M potassium acetate and 3.8 ml of distilled water. The reaction mixture was incubated for 30 min and the absorbance was recorded at 420 nm. The flavonoid content was verified from a catechin standard curve and demonstrated as catechin equivalents per gram of dry weight of individual extract (mg CAE/g DW).

2.6. In-vitro cytotoxic activity by SRB assay

To determine the effects of selected plant extracts on breast cancer cells, *in vitro* SRB assay was conducted as described earlier [17]. MCF-7 cells were treated individually with methanolic plant extracts dissolved in dimethyl sulfoxide (0.1% DMSO) whereas the control sets (untreated) received the vehicle (DMSO 0.1%) only. The cell lines were inoculated in 96 well culture plates at density of 1×10^5 cells/ml. The cells were incubated for 24 h at 37 °C in 5% CO_2 and humidified atmosphere. After incubation the media containing plant extracts at various concentrations were added and cells were further incubated for 48 h. The cells were fixed with ice cold trichloro acetic acid (TCA; 50 µl per well) and stained for 60 min at 4 °C, after which the plates were washed repeatedly and stained with SRB solution (0.4% w/v in 1% acetic acid, 50 µl per well) for 20 min. The excess dye was removed by washing with 1% acetic acid and SRB was solubilized with 10 Mm Tris base and absorbance was measured at 570 nm. Finally the

concentration required for 50% inhibition of cell viability (IC_{50}) was calculated.

2.7. In-vivo chorioallantoic membrane (CAM) assay

The CAM assay was performed as described previously in our reports [18,19]. In brief, fertilized eggs were placed in a humidified incubator at 37 °C. The IC_{50} concentrations of the individual methanolic plant extracts were prepared in dimethyl sulfoxide (DMSO, 0.05%, v/v) and air dried on sterile glass discs. The discs were placed over the CAM and eggs were returned to incubator for 48 h. After incubation the eggs were reopened and the CAMs were examined for changes in vessel density in the area of glass disc under a microscope and images were captured. The anti-angiogenic effect was expressed by using an equation 1-T/C × 100, where T, indicates the no. of blood vessels intersecting the disc treated with plant extracts, while C indicates the no. of blood vessels intersecting the disc in control. The results obtained were expressed in percent values.

2.8. Antioxidant activity

Antioxidant capability of plant extracts was assessed by using DPPH [20], OH [21], and SOR radical scavenging spectrometric protocols [22].

2.8.1. DPPH radical scavenging activity

In brief, the reaction mixture comprises equal volumes of 10^{-4} M ethanol solution of DPPH with individual concentrations of plant extract (100 µg). After 20 min incubation time the absorbance was recorded at 517 nm.

2.8.2. OH radical scavenging activity

OH radicals were introduced in reaction mixture by using the Fenton reaction system. The reaction mixture contained 60 µl of 1 mM $FeCl_3$, 90 µl of 1, 10-phenanthroline, 2.4 ml 0.2 M phosphate buffer (pH 7.4), 150 µl of 0.17 M H_2O_2 and 100 µg individual plant extract solution. Reaction was initiated upon the addition of H_2O_2. After the 5 min incubation, the absorbance of the mixture was read at 412 nm.

2.8.3. SOR scavenging activity

The reaction mixture contained 1 ml of NBT (300 µM), NADH (936 µM) respectively and the individual concentrations of plant extract (100 µg) in Tris−HCl buffer (100 mM, pH 7.4). The reaction was started by adding PMS (120 µM) to the mixture. The reaction cocktail was incubated at 25 °C for 5 min and the absorbance was read at 560 nm.

The percent DPPH, OH, SOR radical scavenging activities were calculated using following equation.

$$\text{Activity}(\%) = 1 - \frac{T}{C} \times 100$$

Where T = Absorbance of the test sample and
C = Absorbance of the control sample.

3. Results

3.1. Phytochemical screening of plant extracts

The results of the phytochemical analysis of plant samples are presented in Table 1. Preliminary screening of individual methanolic plant extracts revealed the presence of different phytoconstituents like alkaloids, tannins, terpenoids and saponins which could be responsible for their pharmacological activities.

Table 1
Phytochemical screening of plant samples.

Name of plants	Qualitative tests			
	Alkaloid	Tannin	Terpenoid	Saponin
C. occidentalis	+	+	+	–
C. viminalis	+	–	–	+
C. viscosa (Leaves)	+	+	–	+
C. viscosa (Root)	+	–	–	+
M. hamata	+	+	–	+

Results are expressed as the mean values from three independent experiments ± SD, + = Positive, − = Negative.

3.2. Estimation of total phenol content

The phenol content in plant extracts was estimated using gallic acid as standard and demonstrated as gallic acid equivalents per gram of dry weight of extract (mg GAE/g DW). Significant amount of phenol was found in all plant extracts. The highest total phenol content was found in the *C. viminalis* (78.79 ± 1.92 mg of GAE/g DW) and *M. hamata* (78.5 ± 0.76 mg of GAE/g DW) followed by *C. occidentalis* (66.5 ± 0.94 mg of GAE/g DW), *C. viscosa* leaves (66.38 ± 0.82 mg of GAE/g DW) and *C. viscosa* root (45.39 ± 0.94 mg of GAE/g DW) respectively (Table 2).

3.3. Estimation of total flavonoid content

The flavonoid content in plant extracts was estimated using catechin as standard and demonstrated as catechin equivalents per gram of dry weight of extract (mg CAE/g DW). A considerable amount of flavonoid was found in all plant extracts. The highest total flavonoid content was found in *C. viminalis* (46.41 ± 2.23 mg of CAE/g DW) and *M. hamata* (40.33 ± 1.16 mg of CAE/g DW) followed by *C. viscosa* leaves (36.22 ± 0.74 mg of CAE/g DW), *C. occidentalis* (35.32 ± 0.70 mg of CAE/g DW) and *C. viscosa* root (33.63 ± 1.25 mg of CAE/g DW) respectively (Table 2).

3.4. In-vitro cytotoxic activity by SRB assay

In-vitro cytotoxic activity of plant extracts against human breast cancer cell line MCF-7 was assessed by using SRB assay. The results are summarized in Table 3 and expressed as the IC_{50} values. Adriamycin was utilized as a positive control. The represented microscopic images in Fig. 1 show how the morphology of cells after the treatment with plant extracts was affected. The results of the study further demonstrate that, all plant extracts exhibited cytotoxicity against the MCF-7 cells when compared with the reference drug Adriamycin. *C. viminalis* demonstrated the promising cytotoxic activity against MCF-7 cells with IC_{50} value of 44 ± 0.09 µg/ml compared with reference compound Adriamycin (3 ± 0.16 µg/ml). While the remaining plants *C.*

Table 2
Estimation of total phenol & flavonoid content.

Name of plants	Quantitative tests	
	Phenol mg GAE/g DW	Flavonoid mg CAE/g DW
C. occidentalis	66.5 ± 0.94	35.32 ± 0.70
C. viminalis	78.7 9 ± 1.92	46.41 ± 2.23
C. viscosa (Leaves)	66.38 ± 0.82	36.22 ± 0.74
C. viscosa (Root)	45.39 ± 0.94	33.63 ± 1.25
M. hamata	78.5 ± 0.76	40.33 ± 1.16

Results are expressed as the mean values from three independent experiments ± SD, mg GAE/g DW = gallic acid equivalents per gram of dry weight of extract, mg CAE/g DW = catechin equivalents per gram of dry weight of extract.

Table 3
In-vitro cytotoxic activity of plant extracts against breast cancer cell line MCF-7.

Name of Plant	IC_{50} value (µg/ml)
Cassia occidentalis	70 ± 0.11
Callistemon viminalis	44 ± 0.09
Cleome viscosa (Leaves)	70 ± 0.22
Cleome viscosa (Root)	73.2 ± 0.36
Mimosa hamata	65.8 ± 0.25
Adriamycin	3 ± 0.16

Adriamycin = Positive control. Results are expressed as the mean values from three independent experiments ± SD.

occidentalis (70 ± 0.11 µg/ml), *C. viscosa* leaves (70 ± 0.22 µg/ml), *C. viscosa* root (73.2 ± 0.36 µg/ml) and *M. hamata* (65.8 ± 0.25 µg/ml) showed the significant cytotoxicity.

3.5. In-vivo chorioallantoic membrane (CAM) assay

The report of anti-angiogenic activities of selected plant extracts using the CAM model is summarized in Table 4. The IC_{50} concentrations of plant extracts were used to evaluate the efficiency of plants as anti-angiogenic agents in CAM model. The inhibition of angiogenesis by using plant extracts clearly exhibited the potential of all plants as anti-angiogenic agents in CAM model. Amongst the tested plants, *C. viminalis* (67.76 ± 0.77%), demonstrated the most promising anti-angiogenic activity. However, the plant extracts of *C. occidentalis* leaves (55.56 ± 0.69%), *C. viscosa* leaves (58.82 ± 0.91%), *C. viscosa* root (54.54 ± 0.87%), and *M. hamata* leaves (51.66 ± 0.88%) demonstrated the significant anti-angiogenic activity. The representative digitized images of the CAMs indicating the reduced vascularization by treated plant extracts are shown in Fig. 2. The digitized control and CAMs treated with the IC_{50} concentrations of the individual plant extracts were further subjected to image analysis software AngioQuant v 1.33 (a MATLAB-based software tool for quantification of angiogenesis) for the analysis of number, length, size, and the junctions of the tubule complexes (data not shown). The results acquired from analysis clearly revealed the progressive reduction in length, size, number and junctions of tubule complexes.

Table 4
Anti-angiogenic activities (%) of plant extracts.

Name of Plant	Anti-angiogenic activity (%)
Cassia occidentalis	55.56 ± 0.69
Callistemon viminalis	67.76 ± 0.77
Cleome viscosa (Leaves)	58.82 ± 0.91
Cleome viscosa (Root)	54.54 ± 0.87
Mimosa hamata	51.66 ± 0.88

Results are expressed as the mean values from three independent experiments ± SD.

3.6. Antioxidant activity

The antioxidant potential of selected plant samples were evaluated by using DPPH, OH and SOR scavenging spectrophotometric methods. The results obtained are summarized in Table 5.

3.6.1. DPPH radical scavenging activity

The extracts of selected plants exhibited prominent DPPH radical scavenging activity. The extract of *C. occidentalis* (78.06 ± 0.58%), *C. viminalis* (75.4 ± 0.18%), *C. viscosa* leaves (75.86 ± 1.03%), *C. viscosa* root (71.54 ± 0.28%), and *M. hamata* (76.41 ± 0.88%) has the potent radical scavenging activity as compared to reference compound ascorbic acid (82.52 ± 0.55%).

3.6.2. OH radical scavenging activity

As per the OH radical scavenging activities it was observed that about all selected plant samples showed effective OH radical stabilizing potential. The extract of *C. viscosa* leaves (68.90 ± 0.69%) and *M. hamata* (69.52 ± 0.37%) was found to be hyper reactive towards OH radical as compared to ascorbic acid (65.35 ± 1.20%) whereas the extracts of *C. occidentalis* (66.2 ± 1.04%), *C. viminalis* (65.8 ± 0.58%) and *C. viscosa* root (56.29 ± 0.60%) showed effective OH neutralizing activity.

3.6.3. SOR scavenging activity

The results of SOR scavenging activities showed that, *C. viminalis* (57.08 ± 0.75%) exhibited better activity as compared to ascorbic acid (72.58 ± 0.54%). While other plant extracts *C. occidentalis* (48.75 ± 0.44%), *C. viscosa* leaves (49.84 ± 0.64%), *C. viscosa* root

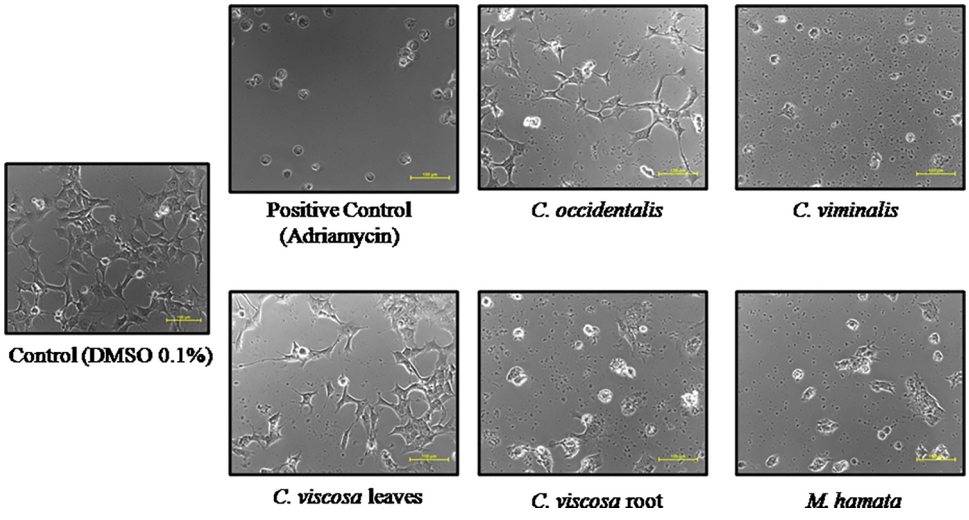

Fig. 1. Representative images showing the effect of tested plant extracts on the morphology of breast cancer (MCF-7) cells. Morphology was affected when treated with plant extracts as compared to control.

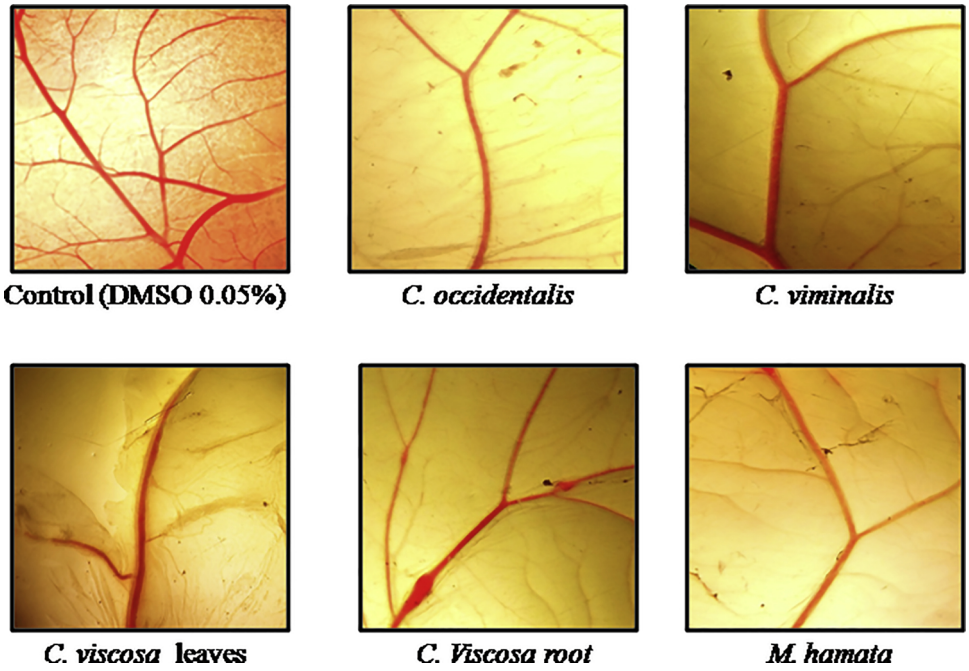

Fig. 2. Representative digitized illustrations of the CAM_S exposed to IC_{50} concentrations of selected plant extracts. The images of CAMS were digitized using Olympus make SZ61TR Zoom Trinocular Microscope with CCD attached camera and an image capturing software Pinnacle v. 6.0.2 (build 152).

Table 5
DPPH, OH, SOR radical scavenging activities of plant extracts.

Name of plants	Anti-oxidant activity (%)		
	DPPH	OH	SOR
Cassia occidentalis	78.06 ± 0.58	66.2 ± 1.04	48.75 ± 0.44
Callistemon viminalis	75.4 ± 0.18	65.8 ± 0.58	57.08 ± 0.75
Cleome viscosa (Leaves)	75.86 ± 1.03	68.90 ± 0.69	49.84 ± 0.64
Cleome viscosa (Root)	71.54 ± 0.28	56.29 ± 0.60	47.11 ± 1.04
Mimosa hamata	76.41 ± 0.88	69.52 ± 0.37	44.66 ± 0.81
Ascorbic acid	82.52 ± 0.55	65.35 ± 1.20	72.58 ± 0.54

Results are expressed as the mean values from three independent experiments ± SD.

(47.11 ± 1.04%), and *M. hamata* (44.66 ± 0.81%) showed the moderate SOR radical scavenging activity.

4. Discussion

Breast cancer is the most frequently diagnosed cancer and a major cause of female deaths worldwide [23]. Adverse side effects of current synthetic chemotherapy drugs, drug resistance and high cost of breast cancer therapy have prompted the need to search novel, safe, effective and cost affordable natural drugs. Medicinal plants are the enormous source of bioactive molecules; thus the practice of using plants for developing the novel anticancer agents has been running for many years. Therefore various plants and their respective compounds are established as the potent anticancer agents [24].

In the current study, an attempt has been made to investigate the anti-breast cancer, anti-angiogenic and antioxidant effects of selected botanicals like *Cassia occidentalis*, *Callistemon viminalis*, *Cleome viscosa* and *Mimosa hamata*. Initially, the methanolic extracts of plants were analyzed for the presence of phytochemicals. Preliminary phytochemical examination confirmed the presence of different bioactive constituents in plant extracts. The presence of alkaloid, tannin, terpenoid was observed in the extract of *C. occidentalis*. *C. viminalis* had a positive test for alkaloid and saponin. *C. viscosa* leaves extract showed the presence of alkaloid, tannin, and saponins, while the *C. viscosa* root extract showed the presence of alkaloid and saponin. *M. hamata* showed the positive test for alkaloid, tannin and saponins (Table 1). Furthermore, in quantitative estimation, a significant amount of phenol and flavonoids was found in all plant extracts (Table 2). All these secondary metabolites have been shown to have numerous therapeutic properties and are renowned as pharmacologically active constituents. They are precisely responsible for diverse activities including anticancer [25]. Amongst these phytoconstituents, the phenols and flavonoids have been extensively reported for anticancer properties against variety of anticancer targets [26,27] while some alkaloids have been reported to demonstrate antiproliferation effects against various types of cancers [28].

Cytotoxic potential of plant extracts against the human breast cancer cell line MCF-7 was assessed by using SRB which is broadly used in cytotoxicity and cell viability assays. Amongst the selected plants, *C. viminalis* (IC_{50} = 44 ± 0.19 µg/ml) and *M. hamata* (IC_{50} = 65.8 ± 0.25 µg/ml) showed the effective cytotoxicity while the remaining plants demonstrated the significant anti-breast cancer activity. The plant extracts have revealed the presence of main phytoconstituents such as phenol, flavonoids and alkaloids which may possibly associated with their influence on cytotoxicity against MCF-7. Perhaps, the presence of major phytoconstituents in selected plant extracts might be responsible for their cytotoxic activity against MCF-7 breast cancer cells. Previous reports have demonstrated the significant anticancer activity of *C. occidentalis* alcoholic, hydro alcoholic and aqueous extract on human cancer cell line HCT-15, SW-620, COLO 205, OVCAR-5, PC-3, MCF-7 and SiHa. The aqueous extract exhibited the strong activity as compared to hydro-alcoholic and alcoholic extract [29]. In another study, authors have shown moderate cytotoxic effects of essential oils extracted from the leaves and flowers of *C. viminalis* against melanoma cultures (HT144) [30]. Earlier reports have indicated that methanol extract of *C. viscosa* revealed significant antitumor activity in Ehrlich ascites carcinoma (EAC) bearing mice. The extract was evaluated for antitumor activity against EAC bearing swiss albino mice [31]. However, *M. hamata* has not yet been studied for their anticancer activities.

Tumor angiogenesis is a critical step required for the tumor growth. Development of new blood vessels is essential for metastasis as the lack

of angiogenesis eventually leads to the dormant tumor [32]. Numerous pro as well as anti-angiogenic agents are recognized to regulate the process of angiogenesis. The proangiogenic agents include vascular endothelial growth factor (VEGF), transforming growth factor (TGF), basic fibroblast growth factor (bFGF) and tumor necrosis factor-α (TNFα). Amongst the all agents, VEGF is established as the crucial angiogenic factor to control the angiogenesis process [33]. The balance amidst both pro and anti-angiogenic agents restrain the formation of new blood vasculature [34]. Consequently, inhibition of tumor angiogenesis through obstructing angiogenic agents has become a fundamental therapeutic approach for treatment of various cancers. Great efforts have been made in last few decades to discover the novel anti-angiogenic agents by using the natural resources.

So taking this scenario into consideration we have examined the anti-angiogenic ability of the test plant extracts by using the CAM assay which is principally used for the assessment of anti-angiogenic potential of agents. Amongst the selected plant extracts, *C. viminalis* (67.76 ± 0.77%) and *C. viscosa* leaves (58.82 ± 0.91%) illustrated the potent anti-angiogenic activity by inhibiting the branching of blood vessels in CAM model, whereas the remaining plant extracts also showed the efficient inhibition. The anti-angiogenic properties of plant extracts might be attributed to the high content of phenols and flavonoids present in them. Several polyphenols and flavonoids have reported for their anti-angiogenic effects [35]. In particular; the flavonoids have shown to inhibit angiogenesis [36].

ROS are mostly recognized for their role in diverse human disorders including cancer. Of note, ROS induced oxidative stress has been implicated in the initiation and progression of cancer [37]. It has been demonstrated that severe oxidative stress generates free radicals which may indiscriminately reacts with biologically important biomolecules especially DNA and ultimately leads to carcinogenesis [38]. Therefore free radical scavenging actions of drug candidates are crucial to defend the adverse physiological effects of free radicals at cellular levels. Antioxidant actions of medicinal plants have attracted a great deal of research attention. Antioxidants are well known to prevent the oxidative damage induced by ROS and other free radicals [39]. The plant extracts were assessed for their antioxidant activities using spectrophotometric *in vitro* methods. Initially the free radical scavenging actions of plant extracts were assessed by DPPH assay. DPPH is a stable free radical and the method is widely used to assess the antioxidant capability of the test samples, wherein the sample ingredients acts as DPPH radical scavengers [40]. The experimental data revealed that the selected plant extracts demonstrated impressive DPPH radical scavenging activity. The results obtained are in agreement with previously published data since similar kind of DPPH radical scavenging actions are reported for tested plant samples [41–43]. Furthermore the antioxidant potential of extracts was confirmed by using the OH (hydroxyl) and SOR (super oxide radical) scavenging assays. Superoxide radical is a weak oxidant however it leads to the production of vigorous plus hazardous hydroxyl radicals. Both OH and SOR robustly give rise to oxidative stress [44]. Hydroxyl radical is recognized to be strong hyper reactive oxygen species which have potent ability to react with cellular biomolecules [45]. In case of OH radical, extract of *C. viscosa* leaves (68.90 ± 0.69%) and *M. hamata* (69.52 ± 0.37%) clearly specified radical scavenging action whereas rest of plant extracts showed the efficient activity. On the other hand *C. viminalis* (57.08 ± 0.75%) revealed the significant super oxide radical scavenging action, while residual plants exhibited moderately SOR scavenging activities. The observed antioxidant activity of selected plant extracts is probably because of their phenol and flavonoid contents. The phenolic compounds are impressive antioxidant and free radical scavengers owing to their hydrogen donating ability to free radicals and thereby capable of inhibiting the damaging effects of free radicals on biomolecules [46]. Flavonoids are also broadly reported to possess powerful antioxidant activities [47].

Overall, present study suggest that the selected plants such as *Cassia occidentalis*, *Callistemon viminalis*, *Cleome viscosa* and *Mimosa hamata* exhibited significant cytotoxicity against MCF-7 breast cancer cells along with inhibition of angiogenesis in CAM model and scavenging of free radicals.

4.1. Limitations of study

While describing the limitations of the present study, it is important to note that this is a preliminary screening of the selected botanicals against MCF-7 cells. A complete GS-MS or LC–MS chemo-profile data of the selected samples would be required if the precise ingredients responsible for studied biological activities are to be attributed. Bioactivity guided fractionation of promising botanicals which could lead to the isolation of active ingredients and their biological testing might establish the evidence for the therapeutic value of these selected medicinal plants. However, *in vivo* pharmacological investigations of promising samples or lead compounds are needed to authenticate the efficiency of the selected plants.

5. Conclusion

The results of the present investigation revealed the potential of selected plants as effective anti-breast cancer, anti-angiogenic and antioxidant agents. The results of the SRB assay in current study signify the potential of methanolic plant extracts on growth inhibition of MCF-7 breast cancer cells while *C. viminalis* and *M. hamata* found to be lead anti-breast cancer agents. Furthermore, the study showed that, plant extracts were capable to inhibit the angiogenesis by suppressing the blood vascularization in the *in vivo* CAM assay. Additionally it has been showed that the selected plant samples are potent antioxidant agents when it is compared to standard compound ascorbic acid. The results obtained support the use of these selected medicinal plants against MCF-7 breast cancer cells. Nevertheless additional studies are required for isolation, purification and identification the bioactive compounds present in plant extracts that grant the anti-breast cancer and anti-angiogenic properties.

Author's contribution

RNG designed the research plan, edited the MS and SSK performed all the experiments and wrote the MS.

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Conflict of interest

The authors declare there are no conflicts of interest.

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