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ANTIANGIOGENIC ACTIVITY OF AQUEOUS FLOWER EXTRACTS OF ASTER, CALENDULA AND GERBERA PLANTS

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ABSTRACT Angiogenesis is the formation of new blood vessels form pre-existing vessels. Inhibition of angiogenesis is considered to be an important strategy for cancer therapy. Antiangiogenic activity of crude aqueous flower extracts of *Callistephus chinensis*, *Calendula officinalis* and *Gerbera jamesonii* were assessed using in vivo CAM assay. All three plants are from Asteraceae family, an aromatic annual herb, which is used in traditional system of medicine to treat various diseases like anti-inflammatory, antispasmodic and antitumor activity. Phytochemical analysis of the plant extracts was done by TLC. They are rich in Flavanoids, Alkaloids and Glycosides. Aster and Calendula showed maximum inhibition of blood vessels formation as compared to Gerbera. The number of blood vessels formed is (23.83, 24.66 and 30.33) respectively. All three plants extract inhibited angiogenesis by blocking normal vascularisation as compared to normal at 50 mcg/ml concentration. Sorafinib is used as positive anti-angiogenic drug control.

KEYWORDS : Antiangiogeneic activity, *Callistephus chinensis, Calendula officinalis, Gerbera jamesonii.*

INTRODUCTION

Angiogenesis is the process of formation of new blood vessels from the pre-existing ones; it involves migration growth, and differentiation of endothelial cells of blood vessels. It is fundamental step in transition of tumours from benign state to malignant tumours, leading to use of angiogenesis inhibitors in the treatment of cancer **(Kalimuthu, 2013)**. Angiogenesis is viewed as an attractive therapeutic target for the development of novel anticancer agents being assessed in clinical trials **(Ferrara, 2006)**. Chemotherapeutic agents as antiangiogenic drug have serious side effects such as hypertension, bleeding, gastrointestinal perforations, limiting their use **(Kamba, 2007)**. Plants with antiangiogenic activity are therefore of considerable importance for diseases such as cancer, macular degeneration, diabetic retinopathy and others. Consumption of plant based diet has been implicated in the prevention of cancer development **(Fang, 2007)**. A variety of food including Cinnamon (*Cinnamomum zeylanicum*) powder as well as green and black tea (*Camellia chinensis*) is rich in polyphenols that are proposed to have anti-angiogenic activity **(Sartippour, 2008)**.

Callistephus genus is used for the relief of cough and it possess diuretic, antitumour, antibacterial and antitumour activity **(Schulz, 2004 and Shirota, 1997)**. The polyphenols ingredients in the *Callistephus chinensis* have inhibition effect on tumour cells at effective dose of 10mcg/ml. Calendula is used in Ayurveda for treatment of fever and cancer **(Duke, 1985)**. Calendula has antibacterial and antifungal activities and it has been used for the treatment of burns, abarasions, skin inflammation, ulcers, wounds and eczema **(Krag, 1976)**. *Gerbera jamesonii* is ornamental flowering plants known to be planted as cut flowers bedding plants ant pot marigold. It has anticancer activity on A549-Adeno-Carcinoma Human Alveolar Basal epithelial cell **(Agarwal, 2014)**.

MATERIALS AND METHOD

Collection of plant material

Fresh plants were collected from More Nursery (Vangani). The taxanomic identification of these plants was done by Dr. Pravin, Blatter Hebarium, St. Xaviers College, Mumbai. The voucher specimens were preserved.

Method of extraction

The flowers from plant of *Calendula officinalis, Callistephus chinensis* and *Gerbera jamesonii* were collected, cleaned and dried in oven at 40°c. The dried flowers were pulverized by mechanical grinder and passed through mesh sieve. Powdered material were mixed with water and kept on shaker for 24 hours at room temperature. The extracts were filtered, evaporated and concentrated at 45°c **(Sartippour, 2008, Kokate, 2005, Joseph 2005 and Khandelwal, 1998)**.

Chorioallantoic Membrane Assay (CAM in chicken egg)

Antiangiogenic activity of aqueous flower extracts of *Calendula officinalis, Callistephus chinensis* and *Gerbera jamesonii* were conducted on fertilized white Leghorn chicken eggs were obtained from a local hatchery with 3 days incubation. The eggs were incubated at 37oc in humified incubator for 48 hrs. The eggs were grouped as per type and concentration of extracts, negative and positive control. The eggs were surface sterilized with70% ethanol. On day 6, 26 gauze needles were used to puncture a small hole in the air sac of the egg and 2-3 ml of albumen was sucked and sealed. This allows separation of vascularised CAM from the vitelline membrane and the shell. A window was then cut in the shell using a sterile blade and the shell was removed. The window was then closed and returned to the incubator after addition of desired concentration 10mcg/ml, 50 mcg/ml of extracts of each plant Aster, Calendula and Gerbera in CAM on the surface of blood vessels. After 48 hrs of incubation on 8th day photographs of embryo were taken to obtain the image of CAM after treatment with various extracts. Six eggs were used for each extract dose with normal and positive control. Positive control drug used is Sorafinib a standard antiangiogenic drug. Average numbers of blood vessels formed are counted and standard deviation was calculated **(Ean-Jeong, 2013 and Shanshan Wanga)**.

Preliminary phytochemical analysis

Qualitative phytochemical analysis of aqueous flower extracts of all 3 plants was used for determining presence of Tannins, Alkaloids, Flavonoids, Glycosides and Steroids by TLC (Thin Layer Chromatography). Silica gel 60 F254 TLC aluminium, Merck was used to perform analysis **(Khandelwal, 1998 and Harborne, 1998)**.

RESULT AND DISCUSSION

Antiangiogenic activity of aqueous flower extract of *Callisthephus chinensis, Calendula officinalis* and *Gerbera jamesonii* were tested using in vivo CAM model using 5 day old egg chicken. After inoculation of drug, the 8th day old CAM was used to count the number of blood vessels formed and there reduction was calculated. The evaluation of antiangiogenic activity was done by measuring the number of blood vessels inhibited. The average number of blood vessels in Callistephus, Calendula and Gerbera is (23.83 ± S.D7, 24.66 ± S.D5.87, 30.33± S.D2.49) respectively (Plate 1 B, D and F). The Calendula and Callistephus showed maximum inhibition as compared to Gerbera at concentration of 50mcg/ml, shown in Table 2. At 10mcg/ml concentration the number of blood vessels formed is as same as normal i.e untreated CAM. The results were compared with standard antiangiogenic drug Sorafinib as positive control.

The anti-angiogenic effect of Callistephus, Calendula and Gerbera may be due to phytoconstituents present. All three plants are rich in Flavanoids, glycosides and alkaloids, as shown in Table 1. The *Boucerosia diffusa* chloroform extract and methanol extract of *B. truncato* showed higher inhibition of angiogenesis **(Hafez, 2012)**. This property may be attributed due to its phytoconstituents, glycosides exhibiting antitumour and anticancerous effect **(Deepak, 1997)**. *Calendula officinalis* extract showed a potent invitro inhibition of

tumour cell proliferation when tested on human and murine tumour cell lines which can be correlated with antiangiogenic effect **(Eva Jimenez, 2006)**. *Aster thomsonii* flower and leaves extract showed antitumor and cytotoxic activity **(Bivi, 2011)**. Cinnamon, Black and green Tea extracts showed anti-angiogenic activity used in the treatment of Breast cancer in rats. *Gerbera jamesonii* have anticancer activity on Adeno carcinomic human alveolar basal epithelial cellline. This justifies that plant extracts having antiangiogenic potential can be used in the treatment of cancer **(Hafez, 2012)**.

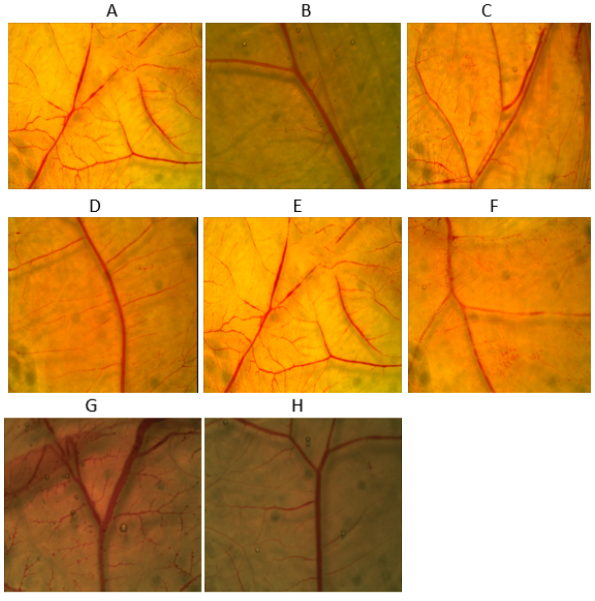
Table: 1 Phytochemical analysis by TLC

	Family	Parts used	Phytochemical Analysis					
			Saponins	Alkaloids	Flavonoids	Glycosides	Tannins	Steroids
Callistephus chinensis	Asteraceae	Flower	+	+	+	+	+	+
Calendula officinalis	Asteraceae	Flower	-	+	+	+	+	-
Gerbera jameisonii	Asteraceae	Flower	-	+	+	+	-	-

Table 2: Antiangiogenic activity of aqueous flower extracts of *Callistephus chinensis, Calendula officinalis* and *Gerbera jamesonii*.

No. Of eggs	Normal Control	A10	A50	C10	C50	G10	G50	Positive Control
1	47	36	10	36	21	45	30	21
2	41	38	28	37	26	48	30	16
3	49	35	30	41	15	40	28	9
4	56	40	25	42	28	48	27	8
5	44	36	30	42	34	47	34	25
6	49	43	20	59	24	41	33	15
Mean	47.66	38	23.83	42.83	24.66	44.83	30.33	15.66
Standard Deviation	4.67	2.76	7.0	7.60	5.87	3.23	2.49	6.04

Plate 1. Antiangiogenic activity of aqueous flower extracts of *Callistephus chinensis, Calendula officinalis* and *Gerbera jamesonii* in chick CAM.



Note: A=Normal Control, B=positive Control (Sorafinib drug), C=*Callistephus chinensis* (10mcg/ml), D=*Callistephus chinensis* (50mcg/ml), E=*Calendula officinalis* (10mcg/ml), F= *Calendula officinalis* (50mcg/ml), G= *Gerbera jamesonii* (10mcg/ml), H= *Gerbera jamesonii*(50mcg/ml).

CONCLUSION

In the review, aqueous flower extracts of *Callistephus chinensis, Calendula officinalis* and *Gerbera jamesonii* showed almost 50% inhibition of blood vessel formation as compared to normal. All three plants contain flavanoids, alkaloids and glycosides. These promising results open up new avenues and possibility for cancer therapy after confirmation of anticancerous activity.

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