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Evaluation of phytochemical content and *In vitro* cytotoxic activity of various ornamental plant flower extracts against MCF-7 Cell lines

[1,*]Pusapati Madan Ranjit, [1]Nagarani, T., [2]Swathi, V., [3]Pahni Kumar, K., [1]Chowdary, Y. A., [4]Siva Reddy, C. H. and [5]Girijasankar, G.

[1]NRI College of Pharmacy, Pothavarappadu (V), Agiripalli Mandal, Krishna District, A.P, India
[2]Faculty of pharmacy, Department of pharmacology, JNTUK, Vijayanagarum campus, A.P., India
[3]ANU College o f Pharmaceutical Sciences, Nagarjuna Nagar, Nagarjuna University, Guntur (Dist.), A.P, India
[4]KVSR Siddhartha College of pharmaceutical sciences, Vijayawada, Krishna District, A.P, India
[5]University College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, AP, India

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ABSTRACT

Ornamental flowers have been traditionally used for the treatment of different ailments such as pain, inflammation, dandruff, dermatitis, hair growth and scabies. The present study was aimed to investigate the phytochemical and cytotoxic activity of aqueous and chloroform extracts of various ornamental flowers (*Ixora coccinia, Allamanda cathartica, Hibuscus rosa-sinensis and Tecoma stans*) against MCF-7 cell lines by using MTT assay. Preliminary phytochemical quantitative results indicate the presence of flavonoids equivalent to quercetin. Aqueous extrct of *Allamanda cathartica* (225.5 mg/g) and *Tecoma stans* (195 mg/g) was found to contain more amount of flavoniod in comparison to other aqueous extrcts and chloroform extracts. Dose dependent cytotoxicity and a significant i.e mean % inhibition and IC_{50} was observed in comparison to both tamoxifen and quercetin. aqueous extract of *Allamanda cathartica* and *Tecoma stans* showed cytotoxicity similar to quercetin. Flavoniods are responsible for cytotoxic activity of flower extracts. This study provides scientific support for the use of ornamental flowers as cytotoxic agents.

Key words:

INTRODUCTION

Research on plants as potent medicinal agents to treat various human diseases was increased drastically in this decade (Parekh *et al.,* 2007) and in India certain systems of herbal medicines namely Ayurveda, Siddha, Unani etc., which are mainly associated with medicinal plants (Guha Bakshi *et al.,* 1999). Mostly in India, traditionaly people prefer the whole herbs, leaves, stems and roots for different ailments but the flowers also plays vital role in medicine. The flower medicine was introduced by Dr. Edward Bach, called father of flower medicine. He was a bacteriologist and consultant of homeopathy in London, Not satisfied with homeopathic practice, he turned to flower medicines (Trivedi 2004). A number of researcher reported that various medical application of plant flowers (Thiripura Sundari *et al.,* 2012). Hence, this is a thrust area for evaluation and exploration of flower as medicinal agents. Hence, the present study has been carried out to evaluate medicinal values of various ornamental plant flowers from the different areas of Vijayawada, Andhra Pradesh, India. *Allamanda cathartica* L. commonly known as yellow bell, golden trumpet or butter cup flower is genus of tropical shrubs and vines belonging to the family Apocynaceae (Tiwari *et al.,* 2002). *Allamanda cathartica L.* is perennial shrub used in traditional medicine for treating malaria and jaundice. Almost all parts of the plant contain allamondin, a toxic iridoid lactone. Leaves extract showed wound healing, anti-inflammatory property and flower showed laxative property (Lekshmy *et al.,* 2011; Nithya *et al.,* 2011). *Ixora coccinea L.* is commonly known as 'Jungle-geranium' and 'Flame of the woods'. It is a popular flowering shrub belongs to the family Rubiaceae. The "wild" flower colour is red or red-orange, but ornamental varieties are white, yellow, pink flowers. A decoction of the roots is given for dysentery and as a sedative for hiccoughs, nausea and loss of appetite, fever and gonorrhea. In the traditional medicine the leaves and roots are used to treat a wide variety of ailments like hepatoprotective, chemoprotective, antimicrobial, antioxidant, antinociceptive, anti inflammatory, dysentery, ulcers and gonorrhea (The Wealth of India 2012; Seethadevi 1986). Flowers and bark is used on reddened eyes and eruptions in children. Decoction of flowers is given for hemoptysis, catarrhal bronchitis and dysmenorrhoea. Flowers are used externally to sores, employed for chronic ulcer, scabies and some type of dermatitis and also used internally for cholera, diarrhoea, dysentry, leucorrhoea, antitumor and gonorrhoea, fresh juice of flower have protective action against

Corresponding author: Pusapati Madan Ranjit,
NRI College of Pharmacy, Pothavarappadu (V), Agiripalli Mandal, Krishna District, A.P, India.

electroconvulsions (Saha *et al.,* 2008; Kholkute *et al.,* 1976). *Hibiscus rosa sinensis (Malvaceae)* is an ornamental plant, The flowers have been reported to possess anti-implantation and antispermatogenic activities (Jonadet *et al.,* 1990; Gilani *et al.,* 2005) traditionally the plant is attributed to antifertility activity in Ayurvedic literature (Jadhav *et al.,* 2009). The extracts of *Hibiscus rosa sinensis* have also been shown a protective effect against the tumor promotion stage of cancer development. The leaves and flowers are observed to be promoters of hair growth and aid in healing of ulcer (Gilani *et al.,* 2005) Aerial part of *H. rosa sinensis* has calcium channel blocking action (Khare *et al.,* 2007).

Tecoma stans (L.) Kunth. Commonly known as yellow bell and yellow strumpet bush belongs to the family bignoniaceae . The leaves of the plant contain the alkaloids tecomine and tecostamine responsible for hypoglycemic activity. The roots of plant exhibit a powerful diuretic and vermifuge activity (The useful plants of India *et al.,* 2006). Our previous studies on preliminary phytochemical analysis and cytotoxic activity of various flower extracts, *Delonix regia* (Ranjit *et al.,* 2014), *Couroupita Guianensis* (Pusapati Madan Ranjit *et al.,* 2014), *Calotropis procera* (Pusapati Madan Ranjit *et al.,* 2014) on different cell line. Hence, the present study has been carried out to evaluate the preliminary phytochemical screening, quantitative estimation of total flavonoids content and in vitro cytotoxic effect of various ornamental plant flower extracts against MCF-7 (Breast cancer cell line).

Table 1: Showed the preliminary phytochemical constituents of various extracts

Test for	*Ixora coccinia*		*Allamanda cathartica*		*Hibuscus rosa-sinensis*		*Tecoma stans*	
	Aqueous extract	Chloroform extracts	Aqueous extract	Chloroform extracts	Aqueous extract	Chloroform extracts	Aqueous extract	Chloroform extracts
Alkaloids	-	+	-	+	-	+	-	-
Tannins	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+
Sugars	+	-	+	-	+	-	+	-
Glycosides	-	+	-	+	-	+	-	+
Proteins	-	-	-	+	-	-	-	-
Saponins	+	-	+	-	+	-	+	-
Sterols	-	+	-	+	-	+	-	+
Terpenoids	+	-	+	-	+	+	+	+

MATERIALS AND METHODS

Collection of plant material

The selected plant flowers were collected in the month of July to August from the different areas of Vijayawada, Andhra Pradesh, India. The collected flowers were authenticated by Dr. K. Madhava Chetty, Asst. Professor, Dept. of Botany, Sri Venkateswara University, Tirupati. The herbarium specimens were deposited in the department of Pharmacognosy with specimen numbers of 003.NRI/COL/P.COG (*Ixora coccinia* L.*Rubiaceae*), 004.NRI/COL/P.COG (*Allamanda cathartica* L. *Apocynaceae*), and 005.NRI/COL/P.COG (*Hibiscusrosa-sinensis* L. *Malvaceae*), 006.NRI/COL/P.COG (*Tecoma stans* (L.) Kunth. Bignoniaceae).

Preparation of extracts and Qualitative Preliminary Phytochemical Analysis

The shade-dried powders of flowers were subjected to extraction for 48 hours by using soxhlet extractor, distilled water (Aqueous), chloroform used as solvents and extracts were collected. The collected extracts were evaporated to dryness using a rotary evaporator; the dry residues were stored in desiccators until use. Preliminary qualitative phytochemical analysis was performed to all extracts by using standard methods for identification of reducing sugars, protein, fats, tannins, steroids, alkaloids, glycosides, flavonoids, Saponins, and phenols (Oyedemi *et al.,* 2011) (William C Evan, 2000).

Determination of total flavonoid content

To estimate the total flavonoid content of the extracts Oyedemi *et al.* (2011) method was used. Briefly a volume of 0.5 ml of 2% $AlCl_3$ ethanol solution was added to 0.5 ml of each extract solution. After one hour of incubation at room temperature, the absorbance was measured at 420nm using UV-Visible spectrophotometer (ELICO SL 244). Yellow color indicated the presence of flavonoids. The total flavonoid was estimated by using standard Quercetin graph (20 to 200µg/ml) and all determinations were done in triplicate. Total flavonoid content was calculated from the equation ($y=0.0052_x$, $R^2=0.9977$) obtained from the quercetin standard curve. The result was expressed as Quercetin equivalent in milligrams per ml.Fig1 showed standard Quercetin curve (Concentration µg/ml).

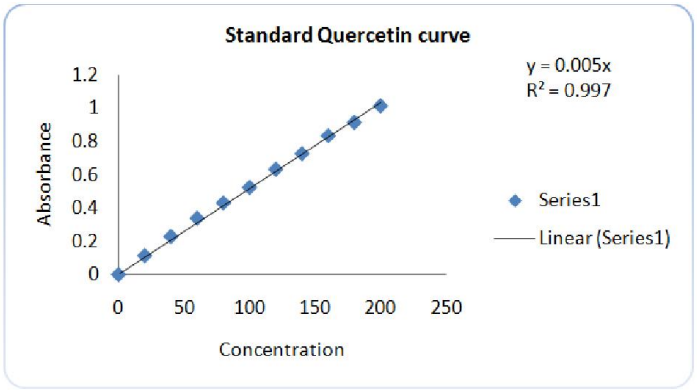


Fig 1. Showed the standard Quercetin curve (Concentration µg/ml)

In vitro Anticancer Activity

Cell culture

Carcinoma of breast cancer (Michigan Cancer Foundation-7 (MCF-7)) cell lines are used in this study were procured from

National Centre for Cell Science, Pune. Cancer cells was maintained in Dulbecco's modified essential medium (DMEM) supplemented with grown in Minimal essential medium (MEM, GIBCO) supplemented with 4.5 g/L glucose, 2 mM L-glutamine, antibiotics (50U/mL of Benzyl penicillin, 50µg/mL of Streptomycin and 50µg/mL of Amphotericin-B) and 5% fetal bovine serum (FBS) (growth medium) at 37°C in 5% CO2 incubator.

RESULTS AND DISCUSSION

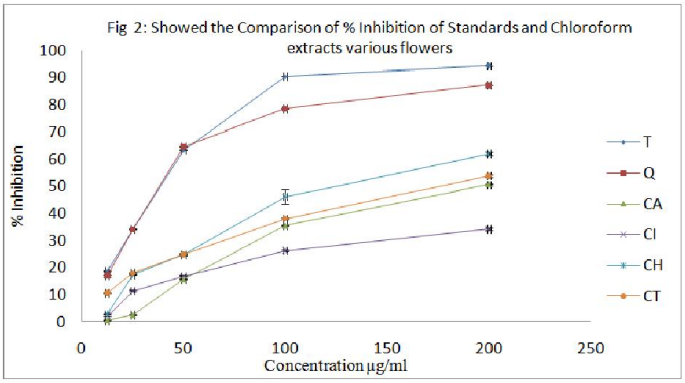
Qualitative phytochemical analysis of extracts showed presence of flavonoid, tannins, phenolic compounds, steroids and saponins. Table 1 showed presence of various chemical constituents of aqueous and chloroform extracts of plants mentioned above.

Table 2. Showed *Mean of triplicate determination, total flavonoid content was calculated form equation ($y=0.0052_x$, $R^2=0.9977$) obtained from the Quercetin standard curve

S.no	Type of extract	Quercetin equivalent (mg/gm)*
1.	*Allamanda cathartica Chloroform extract (CA)*	155.5
2.	*Ixora coccinea Chloroform extract (CI)*	125.5
3.	*Hibuscus Chloroform extract (CH)*	165
4.	*Tecoma stans Chloroform extract (CT)*	160.5
5.	*Allamanda cathartica aqueous extract (AA)*	225.5
6.	*Ixora coccinea aqueous extract (AI)*	175.5
7.	*Hibuscus aqueous extract (AH)*	165.5
8.	*Tecoma stans aqueous extract (AT)*	195

MTT assay

The MTT assay developed by (Mosmann *et al.,* 1983), used to determine the inhibitory effects of test compounds on cell growth *in vitro*. Human cancer cell lines used in this study were procured from National Centre for Cell Science, Pune. All cells were grown in Minimal essential medium (MEM, GIBCO) supplemented with 4.5 g/L glucose, 2 mM L-glutamine, antibiotics (50U/ml of Benzyl penicillin, 50 µg/ml of streptomycin and 50 µg/ml of Amphotericin – B) and 5% fetal bovine serum (FBS) (growth medium) at 37°C in 5% CO_2 incubator. In brief, the trypsinized cells from T-25 flask were seeded in each well of 96-well flat-bottomed tissue culture plate not the same concentration but minimum of 5000 cells per well were seeded l in growth medium and cultured at 37°C in 5% CO_2 to adhere. After 48hr incubation, the supernatant was discarded and the cells were pretreated with growth medium and were subsequently mixed with different concentrations of extracts and quercetin (12.5, 25, 50, 100, and 200 µg/ml) in triplicates to achieve a final volume of 100 µl and then cultured for 48 hours. The compound was prepared as 1.0 mg/ml concentration stock solutions in PBS. Each well then received 5 µl of fresh MTT (0.5mg/ml in PBS) followed by incubation for 2hr at 37°C. The supernatant growth medium was removed from the wells and replaced with 100 µl of DMSO to solubilize the colored formazan product. Tamoxifen is taken as positive control in order to compare IC_{50} of extract against standard drug used. Culture medium and solvent used as negative controls. After 30 min incubation, the absorbance (OD) of the culture plate was read at a wavelength of 570 nm on an ELISA reader, Anthos 2020 spectrophotometer.



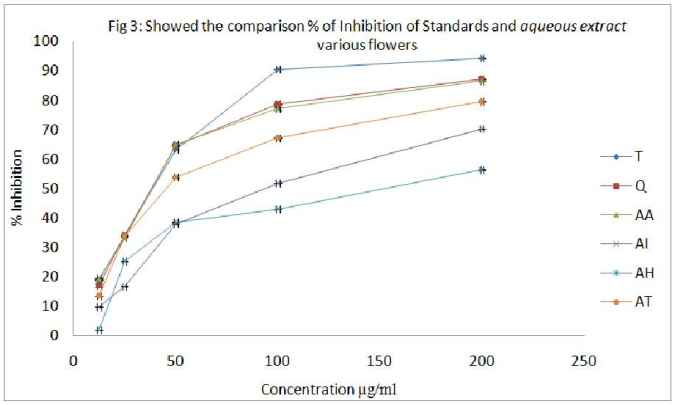
Total flavonoid content was calculated form equation ($y=0.0052_x$, $R^2=0.9977$) obtained from the Quercetin standard curve. Fig 1 showed the standard Quercetin curve. Total flavonoid content of extracts was expressed as quercetin equivalent in milligrams per gram of dry sample. Based on the estimation of flavonoids we are performed the cytotoxic activity using MTT assay against MCF-7 cell line. Tamoxifen and Quercetin used as standard to know cytotoxic activity of extracts. Table 3&4, fig.3&4 represented % inhibition against MCF-7 cell lines of both chloroform and aqueous extracts. Table 5 and 6 showed the IC_{50} values (µg/ml) of standards (Tamoxifen and Quercetin) and different extracts against MCF7 Cell line. Tamoxifen showed anticancer activity through protein kinase C inhibition, facilitate apoptosis in cancer cell, generation of oxidative stress resulting in thiol depletion and activation of the transcriptional factor NF-kappaβ.

Table 3. Showed the Mean ±SEM % of Inhibition of Standards and Chloroform extracts various flowers

Conc. In µg/mL	Mean ±SEM % of Inhibition of Standards		Mean ±SEM % of Inhibition of *Chloroform extracts various flowers*			
	Tamoxifen(T)	*Quercetin(Q)*	*Allamanda cathartica (CA)*	*Ixora coccinea (CI)*	*Hibuscus rosa sinensis(CH)*	*Tecoma stans (CT)*
12.5	18.75 ± 0.252	16.9 ±0.368	0.54 ±0.064	2 ±0.047	2.75 ±0.131	10.66 ±0.141
25	34.15 ±0.127	34.06 ±0.195	2.55 ±0.145	11.36 ±0.391	17.4 ±0.263	17.97 ±0.135
50	63.28 ±0.062	64.57 ±0.189	15.65 ±0.102	16.91 ±0.18	24.65 ±0.304	24.92 ±0.245
100	90.5 ±0.049	78.63 ±0.117	35.57 ±0.218	26.28 ±0.266	46.1±2.816	38.11 ±0.42
200	94.32 ±0.115	87.25 ±0.124	50.62 ±0.397	34.19 ±0.413	61.88 ±0.662	53.86 ±0.494

Table 4. Showed the Mean ±SEM % of Inhibition of standards and *aqueous extract* various flowers

Conc. In µg/mL	Mean ±SEM % of Inhibition of Standards		Mean ±SEM % of Inhibition of *aqueous extract various flowers*			
	Tamoxifen(T)	*Quercetin(Q)*	*Allamanda cathartica (AA)*	*Ixora coccinea (AI)*	*Hibuscus rosa sinensis (AH)*	*Tecoma stans (AT)*
12.5	18.75 ± 0.252	16.9 ±0.368	19.33 ±0.107	9.67 ±0.093	1.83 ±0.141	13.31 ±0.046
25	34.15 ±0.127	34.06 ±0.195	33.21 ±0.079	16.58 ±0.128	25.2 ±0.045	33.66 ±0.125
50	63.28 ±0.062	64.57 ±0.189	64.89 ±0.105	38.01 ±0.442	38.41±0.071	53.77 ±0.227
100	90.5 ±0.049	78.63 ±0.117	77.07 ±0.638	51.73 ±0.069	42.91 ±0.104	67.11 ±0.148
200	94.32 ±0.115	87.25 ±0.124	86.51 ±0.052	70.23 ±0.054	56.29 ±0.083	79.57 ±0.124



Allamanda cathartica aqueous extract (37.5 ± 0.35), *Ixora coccinia* L.aqueous extract (184.26 ± 2.8), *Hibiscusrosa-sinensis* L.aqueous extract (129.33 ± 0.54) and *Tecoma stans* (L.) Kunth.aqueous extract (49.77 ± 0.43) against MCF-7 cell lines. *Allamanda cathartica* L.aqueous extract and *Tecoma stans* aqueous extract showed better cytotoxicity than other groups and their IC_{50} values nearer to standards. We are observed that, all these extracts are showed the % inhibition values are dose dependent and % of flavonoidal content. This study reveals the cytotoxic effect of ornamental plants.

Table 5. Showed IC_{50} Values of standers and chloroform extracts various flowers

IC_{50} Values of standers		IC_{50} Values of *Chloroform extracts various flowers*			
Tamoxifen (T)	*Quercetin(Q)*	*Allamanda cathartica (CA)*	*Ixora coccinea (CI)*	*Hibuscus rosa sinensis (CH)*	*Tecoma stans (CT)*
34.52	37.39	180.96	429.9	124.1	173

Table 6. Showed IC_{50} Values of standers and *aqueous extract* various flowers

IC_{50} Values of standers		IC_{50} Values of *aqueous extract various flowers*			
Tamoxifen (T)	*Quercetin (Q)*	*Allamanda cathartica (AA)*	*Ixora coccinea (AI)*	*Hibuscus rosa sinensis (AH)*	*Tecoma stans (AT)*
34.52	37.39	37.5	184.26	129.33	49.77

Novotny *et al.,* reviews the application of tamoxifen in various cancers like melanoma, small cell lung carcinoma, pancreatic, other endocrine and soft tissue cancers (Novotny *et al.,* 2000). Tamoxifen is clinically used for treatment of breast cancer, so it was used as a standard against MCF-7 cancer cell lines (Felix Adje *et al.,* 2008). Earlier reports explained that, phenolic compounds and its congeners are known to showed cytotoxicity on various cancer cell lines and capable to induce caspase-mediated apoptosis activity (Owen *et al.,* 2000; Nandi *et al.,* 2007).

Quercetin was a natural phenolic compound have antitumor, antioxidant, anti mutagenic and broad spectrum pharmacological activities in both in *vitro* and *vivo* studies (Aly *et al.,* 2011). Based on the review of literature tamoxifen and quercetin used as standards in this experiment. Flavonoids are available either in a free form or glycosidal form. Based on that, we are used chloroform and aqueous extracts. Extracts showed marked % inhibition of cell viability against MCF-7 cell lines in dose dependent manner. The mean IC_{50} values of standards tamoxifen (34.52 ± 0.14), and quercetin (37.39 ± 0.23), and mean IC_{50} values of extracts *Allamanda cathartica* L.chloroform extract (180.96 ± 3.58), *Ixora coccinia* L.chloroform extract (429.9 ± 27.7), *Hibiscusrosa-sinensis* L.chloroform extract (124.1 ± 10.84), *Tecoma stans* (L.) Kunth. chloroform extract (173 ± 6.82),

Our conclusion is all the extracts have cytotoxic properties, aqueous extract of *Allamanda cathartica* L. contains 225.5 mg/gm (Quercetin equivalent) and showed better activity than the other extracts. Further studies are required to identify, which chemical constituents is responsible for *in vitro* cytotoxic activity of extracts and establish the molecular mechanism of action with regard to their cytotoxic activity.

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