AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Evaluation of Leaves of Aqueous Extract of *Coleus Aromaticus* and Methanolic Extract of *Annona Squamosa* Extracts on Cell Viability

Himesh Soni [1*], A. K. Singhai[2], Sarvesh Sharma[2], Jitender K Malik[2]

1. *Suresh Gyan Vihar University, Jaipur-302025, India*
2. *Lakshmi Narain College of Pharmacy, Raisen Road, Bhopal (M.P.)-462021, India*

ABSTRACT

Medicinal plants play a vital role to preserve human health. Herbs valued for centuries as a traditional medicine, has been used to treat various human ailments. The leaves of *Coleus aromaticus* are useful in cephalagia, otalgia, anorexia, dyspepsia, flatulence, colic, diarrhoea, cholera, halitosis, convulsions, epilepsy, cough, asthma, hiccough, bronchitis, strangury, hepatopathy and malarial fever. *Annona squamosa Linn* is used as an antioxidant, antidiabetics, hepatoprotective, cytotoxicactivity, genetoxicity, antitumor activity, antilice agent. We report here the effects of various plants extracts on the cell viability. The cell viability assay revealed that the aqueous extract of *Coleus aromaticus* leaves and methanolic extract of *Annona squamosa* leaves showed significant results. Further phytochemicals screening of the extracts of both the plants revealed presence of Flavonoids on the above extracts. The total flavonoids content were determined by aluminum chloride colorimetric method. The total flavonoids contents of aqueous extract *Coleus aromaticus* & methanolic extracts of *Annona squamosa* leaves were found to be 2.60% and 2.4% respectively.

Key words: *Coleus aromaticus, Annona squamosa,*Cell viability , Spectrophotometric.

*Corresponding Author Email: himeshsoni@rediffmail.com

Received 8 July 2012, Accepted 26 July 2012

Please cite this article in press as: Soni H *et al.*, Evaluation of Leaves of Aqueous Extract of *Coleus Aromaticus* and Methanolic Extract of *Annona Squamosa* Extracts On Cell Viability. American Journal of PharmTech Research 2012.

INTRODUCTION

Since last many years, plants have beneficial activity in different type of diseases producing in human beings. As per WHO calculate that about 80% of the world's inhabitants problem should treated by medicinal herbal drug for their primary health care [1,2].Cell viability, cell proliferation and many important live-cell functions including apoptosis, cell adhesion, chemotaxis, multidrug resistance, endocytosis, secretion and signal transduction can be stimulated or monitored with various chemical and biological reagents. Many of these processes lead to changes in intracellular radicals free-ion concentrations or membrane potential that can be followed with appropriately responsive fluorescent indicators[3]. The quantification of cellular growth, including proliferation and viability, has become an essential tool in any laboratory working on cell-based studies. Such techniques enable not only the optimization of cell culture conditions, but also the determination of growth factor and cytokine activity. Even more importantly, the efficacy of therapeutic agents in drug screening, the cytostatic potential of anticancer compounds in toxicology testing, and cell-mediated toxicity can be assessed when quantifying cell growth[4]. Cell viability may be judged by morphological changes or by changes in membrane permeability and/or physiological state inferred from the exclusion of certain dyes or the uptake and retention of others[5]. *Coleus aromaticus Benth. syn. C. amboinicus Lour, Plectranthus amboinicus (Lour.) Spreng.* English: Country borage, Indian borage; Sanskrit: Karpuravalli, Sugandhavalakam; Hindi: Patharchur; Bengali: Paterchur; Malyali: Panikkurkka, kannikkurkka; Tamil: Karpuravalli.

Figure 1: *Coleus aromaticus*

It is found throughout the tropics and cultivated in homestead gardens. It is a large succulent aromatic perennial herb with hispidly villous or tomentose fleshy stem. Leaves are simple, opposite, broadly ovate, crenate and fleshy. Flowers are pale purplish in dense whorls at distant intervals in a long slender raceme. Fruits are orbicular or ovoid nutlets. The leaves are useful in

cephalagia, otalgia, anorexia, dyspepsia, flatulence, colic, diarrhoea, cholera, halitosis, convulsions, epilepsy, cough, asthma, hiccough, bronchitis, strangury, hepatopathy and malarial fever[6]. *Annona squamosa* commonly known as custard apple is cultivated throughout India, mainly edible fruit. *Annona squamosa syn.* Arabic (gishta); Bengali (ata);German (Rahm Annone,Rahmapfel,Zimtapfel,Süßsack); Hindi (sitaphal,ata,sharifa); Lao (Sino-Tibetan) (khièb); Malay (nona sri kaya,sri kaya,buah nona); Mandarin (fan-li-chi); Portuguese (atta,fructa do conde); Sanskrit (sitaphal); Spanish (candongo,chirimoya,fructo do conde,anón,anona blanca,pinha,saramuya,anona).

Figure 2: *Annona squamosa*

The plant is traditionally used for the treatment of epilepsy, dysentery, cardiac problem, worm infection, constipation, hemorrhage, antibacterial infection, dysuria, fever, and ulcer. It also has anti fertility, anti tumor and abortifacient properties[7].The present study deals with effect various extracts of both plants leaves material on the dermal cell viability. Further phytochemicals screening of the extracts of both the plants and quantification of secondary metabolites were also carried out to illustrated the efficacy of the extracts.

MATERIAL AND METHOD

Plant material

The leaves were collected from botanical garden L.N.C.P. Bhopal (M.P.) and authenticated. The voucher specimen 004/bot/LNCP/10 & 004/bot/LNCP/11) is preserved in laboratory for reference.

Preparation of extracts

The powdered plant material was extracted successively with redistilled, analytical grade petroleum ether (40-60^{O}C), chloroform, ethanol, methanol and water.

Figure 6: Isolation of mice ear pinna

Figure7: Separation of dermis

Figure 8:Transfer of tissues in RPMI media

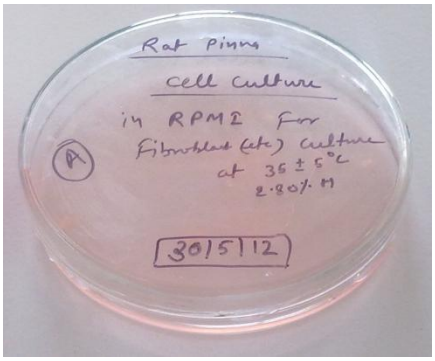

Figure 9: Change in media colour (2 day)

Figure 10: Change in media colour (4 day)

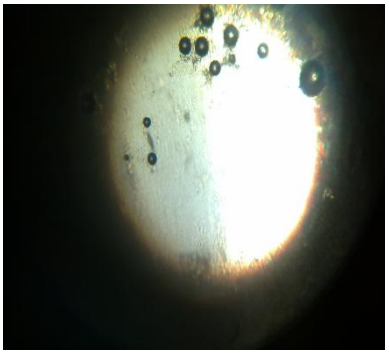

Figure11: Microscopic observation of cell in fibroblast suspension

Fibroblast Cell Suspension

Wash skin samples in PBS by gently shaking or agitating in a 50-ml petri-dish. Incubate with 0.3% trypsin in PBS 7.4 in a 37^0C water bath for 10 minute. (Figure.6) Place the sample on the lid of a 50 ml tissue culture dish with the epidermal side up and scrape off the dermis mechanically using two pairs of forceps. Place the dermal sample on the lid of a 50 ml tissue culture dish and cut it into small (2- to 3-mm) squares. (Figure. 7) Place 5 to 10 skin pieces in the center of a 50 ml tissue culture dish or 6-well plate. Add 20 ml of 4^0C complete growth medium (RPMI) into the petri dish. Place the culture in a humidifier 37^0C, 5% CO_2 incubator. Check the

fibroblast outgrowth every 3 to 4 days under an inverted phase-contrast microscope and change medium every 3 to 4 days.(Figure 11) The cell proliferation curve was prepared by taking absorbance at 545nm.(Figure 5)

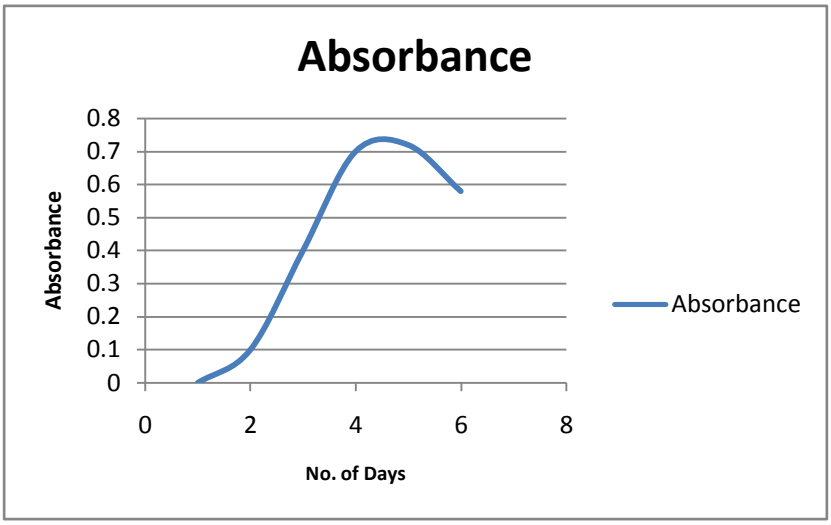

Figure 5: Growth Curve of the Cell

Assessment of Cell Viability

Trypan blue dye exclusion technique

Principle

Trypan Blue is a blue acid dye that has two azo chromophores group. Trypan blue will not enter into the cell wall of plant cells grown in culture. Trypan Blue is an essential dye, use in estimating the number of viable cells present in a population[8].

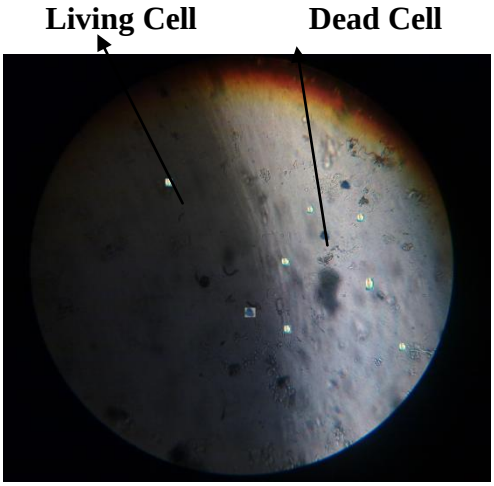

Figure12: Microscopic observation of Viable and dead cell in cell culture + extract + Trypan dye

Procedure:

Collect fibroblast suspensions into a 15-ml polypropylene centrifuge tube and centrifuge 10 min

at 10000 rpm on 4^0C. Aspirate the supernatant, tap the pellet to dissociate the cells, and resuspend them in 100 to 200 µl of fresh 4^0C complete growth medium. Mix a 10 to 20µl sample of the cell suspension with an equal volume of 0.4% trypan Blue and then add 200 µl plant extracts then count total and viable cells under a phase contrast microscope using a hemacytometer (Figure 12)Plate 3-10, 10^4viable cells in 5 ml of fresh complete growth medium in a 25-cm^2 tissue culture flask[9]. Calculate percent viability by using formula:

% viability = (live cell count/total cell count)*100

Phytochemicals Screening

Extracts were tested for the presence of active principles such as phytosterols, tannins, flavonoids, saponins, alkaloids, glycoside, triterpenoids and proteins. Following standard procedures were used [10].

Total Flavonoids contents

The content of total flavonoids was determined by aluminum chloride colorimetric method. The content of flavonoids was determined as quercetin equivalent. 10 mg/ml of plant extract in respective solvent (stock solution SS) was mixed with 2 ml $AlCl_3$ (2% w/v) in methanol and the solution was made up to 25ml with methanolic solution of acetic acid (0.5% v/v) (Probe solution PS). 1ml of SS was made up to 25ml with methanolic solution of acetic acid (contrast solution CS).The absorbance of PS and CS was measured at 420nm after 30 minutes. The result expressed as % of total Flavonoids content[11].

%TFC = Absorbance at 420 x dilution x 100 / $E^{1\%}_{1\,cm}$ x wt. of extract in gms

RESULT AND DISCUSSION

Percentage of viable cell can be obtained by performing trypan blue dye exclusion technique. Percentage cell viability of fibroblast cell suspension were tabulated in table 1 &Graphical representation were showed in figure 3 and 4.

Table 1: Cell Viability assay of different extracts of *C.aromaticus* and *A. squamosa*

Cell Line	% Cell Viability						Total cell Count						pH
Dermal Fibroblast	*C.aromaticus*			*A.squamosa*			*C.aromaticus*			*A.squamosa*			
	Aq.	MeOH	ETOH	Aq.	MeOH	ETOH	Aq.	MeOH	ETOH	Aq.	MeOH	ETOH	7.4
	65.2	62.1	47.3	70.6	71.2	50.7	1.3x 10^4	1.24 x10^4	0.94x 10^4	1.41x 10^4	1.42x 10^4	1.014 x10^4	

Aq.= Aqueous extract, MeOH= Methanolic extract & ETOH= Ethanolic extract

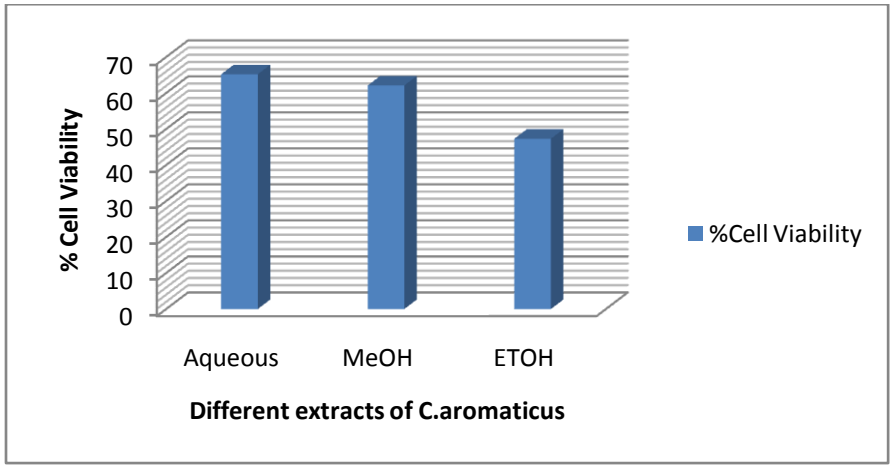

Figure:3 Comparision of % Cell Viability of different extract of *C.aromaticus*

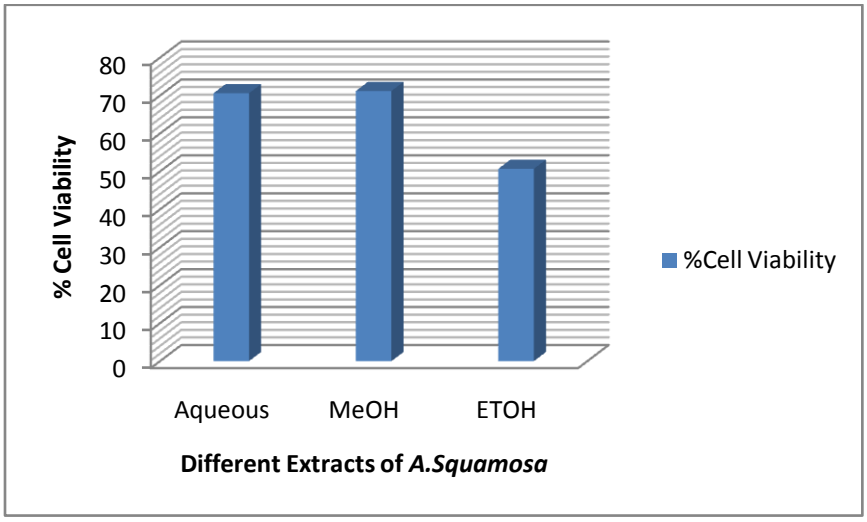

Figure:4 Comparision of % Cell Viability of different extract of *A. squamosa*

The % cell viability and total cell count of aqueous extract of *C.aromaticus* and methanolic extract of *A.squamosa* were found to be 65.2, $1.3x10^4$ and 71.2, $1.42x10^4$respectively.Cell proliferation curve was showed in Figure: 5. Further phytochemicals screening of the extracts of both the plants revealed presence of Flavonoids on the above extracts(table 2 & 3). The total flavonoids content were determined by aluminum chloride colorimetric method. The total flavonoids contents of aqueous extract *Coleus aromaticus* & methanolic extracts of *Annona squamosa* leaves were found to be 2.60% and 2.4% respectively.

Table 2: Phytochemical Screening of *Annona* leaves

Test	Pet.ether	Chloroform	Ethanolic	Methanolic	Aqueous
Carbohydrate					
Molish	(+)ve	(-)ve	(-)ve	(-)ve	(-)ve
Benedict	(+)ve	(-)ve	(-)ve	(+)ve	(-)ve
Starch	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
Hexose sugar	(+)ve	(+)ve	(-)ve	(+)ve	(-)ve

Tannin					
$FeCl_3$	(+)ve	(+)ve	(-)ve	(+)ve	(+)ve
Protein					
Biuret	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
Xanthoprotein	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Amino acid					
Ninhydrin	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Alkaloids					
Dragnodroff	(-)ve	(+)ve	(+)ve	(+)ve	(-)ve
Mayer	(-)ve	(+)ve	(-)ve	(-)ve	(+)ve
Steroid					
Salkowski	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve
Libermann –Bucher	(-)ve	(+)v	(+)ve	(+)ve	(+)ve
Flavonoids					
Shinoda	(-)ve	(+)ve	(-)ve	(+)ve	(+)ve
NaOH	(-)ve	(+)ve	(-)ve	(+)ve	(-)ve
Lead acetate	(-)ve	(+)ve	(+)ve	(+)ve	(+)ve
Coumarin	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
Glycosides					
Baljet	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Legal	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Killer-Killani	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve

(+)ve = Present (-)ve Absent

Table 3:Phytochemical Screening of *Coleus aromaticus* leaves

Test	Pet.ether	Chloroform	Ethanolic	Methanolic	Aqueous
Carbohydrate					
Molish	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Benedict	(-)ve	(+)ve	(-)ve	(+)ve	(-)ve
Starch	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
Hexose sugar	(-)ve	(-)ve	(-)ve	(+)ve	(-)ve
Tannin					
$FeCl_3$	(-)ve	(-)ve	(+)ve	(-)ve	(-)ve
Protein					
Biuret	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Xanthoprotein	(-)ve	(-)ve	(-)ve	(-)ve	(-)v
Amino acid					
Ninhydrin	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve
Alkaloids					
Dragnodroff	(-)ve	(+)ve	(-)ve	(-)ve	(+)ve
Mayer	(-)ve	(+)ve	(-)ve	(-)ve	(+)ve
Steroid					
Salkowski	(-)ve	(+)ve	(+)ve	(-)ve	(+)ve
Libermann – Bucher	(-)ve	(+)ve	(+)ve	(-)ve	(+)ve
Flavonoids					
Shinoda	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
NaOH	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve

Lead acetate	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
Coumarin	(-)ve	(-)ve	(-)ve	(-)ve	(+)ve
Glycosides					
Baljet	(-)ve	(+)ve	(-)ve	(-)ve	(+)ve
Legal	(-)ve	(+)ve	(-)ve	(-)ve	(+)ve
Killer-Killani	(-)ve	(-)ve	(-)ve	(-)ve	(-)ve

(+)ve = Present (-)ve Absent

CONCLUSION

Assessment of cell viability may be accomplished with a microscope, using dyes that mark nonviable cells by dye exclusion. The most commonly used dye is trypan blue, but others may be used as well. Viable cells have intact membranes and exclude the dye; nonviable cells are labeled with the dye and are visible with bright field optics. As well as being useful as a means of assessing functional integrity, trypan blue exclusion is widely used as an objective method of determining viable cell count prior to using cells; a simple protocol for this application is presented, along with other basic cell culture techniques. The effect of various extracts of the both the plants revealed that aqueous extract *Coleus aromaticus* & methanolic extracts of *Annona squamosa* leaves showed significant result due to present of the flavonoids. Biochemical assays and morphological observations showed that they could improve cell viability. The protective effects of the flavonoid in a fibroblast culture system showed maximum viability of the cells and promote cell proliferation. Flavonoids are polyphenolic compounds that occur naturally in various plant species. They are utilized mainly as a source of starting material in the pharmaceutical and food industries and show numerous biological activities of interest, for example, antioxidant capacity, anti-inflammatory action, wound healing property and stimulation of the immune system [12].

ACKNOWLEDGEMENT

The authors are thankful to LNCT College of Pharmacy, Bhopal, MP, India for providing necessary facilities to carry out this research work.

REFERENCES

1. Who diet, nutrition and the prevention of chronic diseases, [world health organization] technical report series, 916, who, geneva, 2003:451-1493.
2. Etkin nl. A hausa herbal pharmacopoeia: biomedical evaluation of commonly used plant medicine. J Ethnopharmacolo, 1981; 4:75-98.
3. Hayes AW. Principles and Methods of Toxicology, Third Edition, Raven Press 1994; 1231–1258.

4. Ionela Zevedei-Oancea and Stefan Schuster Topological analysis of metabolic networks based on Petri net theory *In Silico* Biology 2003; 3:29.
5. Becker B, Clapper J, Harkins KR, Olson JA. In situ screening assay for cell viability using a dimeric cyanine nucleic acid stain. Anal. Biochem 1994; 221:78-84.
6. Soni Himesh, Singhai AK, Sharma Sarvesh. Quantitative estimation of DNA isolated from leaves and stems of *coleus aromaticus.* Int J Pharm 2012; 2(1):84-89.
7. Soni Himesh, Singhai AK, Sharma Sarvesh. Quantitative estimation of DNA isolated from various parts *annona squamosa.* Int Res J Pharm 2011; 2(12):169-171.
8. Phillips HJ, Terryberry JE. Counting actively metabolizing tissue cultured cells. Cell research 1957; 13: 341-347.
9. Masters rw. Animal cell culture, trypan blue assay sop. 3rd ed. 2000, 1 – 3.
10. Kokate ck. Practical pharmacognosy. Pune:vallabh prakashan ; 2003:210-211
11. Chen HY, Lin YC , Hsieh CL. Evaluation of antioxidant activity of aqueous extract of some selected neutraceuticals herbs. Food chemistry 2007; 104: 1418-20.
12. Harborne JB, Williams CA. Advances in flavonoid research since 1992. photochemistry 2000; 55: 481–504.