ANTICANCER ACTIVITY OF ISOLATED CONSTITUENTS FROM *COCCINIA GRANDIS* BY SULPHORHODAMINE (SRB) ASSAY ON DU-145 AND PC-3 CELL LINES

Shaik Aminabee[*1], V. Deepthi[1], S. Haribabu[1], Shaherbanu[1], S. Karthik[1], M. Tejaswi[1], P. Naga Sai[1], Atmakuri Lakshmana Rao[1]

Department of Pharmacology[*1], V. V. Institute of Pharmaceutical Sciences, Gudlavalleru, Andhra Pradesh.

ABSTRACT:

Aim: To study the anticancer activity of isolated compounds from root of *Coccinia grandis* whole plant by SRB assay method on DU-145 and PC-3 human prostate cancer cell lines. Materials and methods: Anticancer activity of isolated constituents of *Coccinia grandis* was performed on SCC-29B and Ishikawa cancer cell lines by the Advanced Centre for Treatment Research and Education in Cancer (ACTREC) Mumbai, India. Cell line had been developed within RPMI 1640 medium that contains 10% fetal bovine serum and 2 mM L-glutamine with the help of SRB assay along with the absorbance had been recorded on an Elisa plate reader at a wavelength of 540 nm with 690 nm. Results: Isolated constituents particularly kampferol showed LC50, TGI and GI50 activity at >80, 69.7 and <10µg/ ml on DU-145 and >80µg/ ml of GI50 activity on PC-3 cell lines. Conclusion: Kampferol from *Coccinia grandis* has showed potent anticancer activity on DU-145 and PC-3 human prostate cancer cell lines.

Keywords: *Coccinia grandis*, Kampferol, Anticancer activity and Human prostate cancer.

INTRODUCTION:

Plants, the most wonderful gift from nature have been used as an origin of drugs. Various types of drugs are obtained from them. These types of plants are known as medicinal plants[1]. We use one or more of its organ for therapeutic purpose as a precursor of synthesizing of many useful drugs[2]. According to some generous estimates, almost 80% of the present day medicines are directly or indirectly obtained from plants[3].

Coccinia grandis is a type plant belonging to the Cucurbitaceae (commonly known as gourd). It is commonly known as Telachucha, Tindora, Scarlet-fruited gourd and Ivy-gourd. It is natively found in India, Asia and Central Africa[4]. It is a climbing perennial herb which spread vegetatively or by seed. Seeds may be the valuable sources for oils and proteins which can cover both industrial and edible demand[5]. The stem is an herbaceous climber or perennial slender climber with occasional adventitious roots forming where the stem runs

along the ground. The tendrils are long, elastic with coil-like springy character that can wrap around the host to the entire length[6]. The stem and root are the best used in skin disease, asthma, bronchitis, remove joint pains and many other. The most useful organ of these plants is leaves which are classified as palmately simple with five lobes while the shape varies from the heart to pentagon form[7]. The leaves show anti-diabetic, anti-inflammatory, antipyretic, analgesic, antispasmodic, antimicrobial[8], and cathartic, expectorant activities. The leaf constrain also found as hypoglycemic, hypolipidemic and antioxidant activity[9]. The fruit of this plant is ovoid in shape berry type which changes green to red color when become ripen[10]. This part has also medicinal value in curing eczema, tongue sores and cerebral oxidative stress[11]. *Coccinia grandis* contain important raw material for drug production like bioactive compounds such as secondary metabolite like alkaloids, glycoside and saponin, b-amyrine, lupeol, cucubbitacin, cephalandrol, cephalandrine and flavonoids. By considering the significant of this plant, our work aimed at conducting phytochemical screening of this plant to identify the types of compounds present and anticancer activity against DU-145 and PC-3 human prostate cancer cell lines using adriamycin as a reference standard.

MATERIALS AND METHODS:

Procurement and authentication of crude drug

The plant *Coccinia grandis* was collected from Tirupathi during the month of January, 2016. The plants were identified and authenticated by Dr K. Madhava chetty; plant taxonomist, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh. The plant materials were dried under shade for 15 days, coarsely powdered and stored in air tight containers protected from humidity and sunlight for further study.

Preparation of methanolic extracts

250 g of powdered crude drug of *Coccinia grandis* was extracted by cold maceration with 1000 mL of methanol for 18 h. The extracts acquired were concentrated to dryness in vacuum at 40°C and stored at 4°C within the refrigerator until further used. The extracts were subjected to phytochemical and pharmacological assessment[12].

Phytochemical screening

The various extracts of *Coccinia grandis* was subjected to qualitative chemical analysis by using standard procedures as follows. The phytochemical screening of carbohydrates was detected by Molisch's test[13]; proteins were detected by using two tests namely Biuret test and Millon's test and amino acids by Ninhyrdin's test[14]; Steroids was detected by Salkowski, Liebermann-Bur chard's and Liebermann's test[15]; alkaloids were identified with

freshly prepared Dragendroff's Mayer's, Hager's and Wagner's reagents and observed for the presence of turbidity or precipitation[16]. The flavonoids were detected using four tests namely Shinoda, sulfuric acid, aluminum chloride, lead acetate, and sodium hydroxides[17]. Tannins were detected with four tests namely gelatin, lead acetate, potassium dichromate and ferric chloride. The froth, emulsion, and lead acetate tests were applied for the detection of saponins[18]. The steroids were detected by (acetic anhydride with sulfuric acid) and (acetic chloride with sulfuric acid) tests. Sample extracted with chloroform was treated with sulfuric acid to test for the presence of terpenoids[19]. Ammonia solution and ferric chloride solutions were used for the presence of anthraquionones[20].

Isolation of constituents from *Coccinia grandis*

Petroleum ether extract (PEE) was subjected to silica-gel column chromatography (elution rate of 2 ml min^{-1} flow having a total elution of 200 ml) and eluted with Petroleum ether and ethyl acetate in various ratios. The resulting fractions were obtained and spotted over precoated silica gel F254 plates (20×20 cm, Merck, Germany). The best resolution had been attained using ethanol: chloroform (10:5 v/v) solvent system as well as the plates was dribbled using formaldehyde-sulphuric acid reagent to visualize the spots. The chloroform portion was subjected to chromatography on silica gel (80-140 mesh, Merck) elided with ethanol: chloroform (10:5) solvent system. Repeated chromatography to obtain flavonoid i.e., kampferol[21]. Soon after extraction, the aqueous portion was attained and leftover to stand in a cool place for 72 hours; a yellow coloured product separated from the solution. The precipitate was filtered and washed with a combination of chloroform: ethyl acetate: ethanol (60:30:30). The undissolved portion of the precipitate was mixed in hot methanol strained, the filtrate was evaporated to dryness to provide 115 mg yellow powder i.e., Rutin, and its melting point had been determined. The ethyl acetate fraction was chromatographed using Diaion HP eluted from water–methanol step gradient (Starting from 100:0 to 0:100). The water–methanol fraction (50:50) had been chromatographed on sephadex LH–20 column eluted with methanol: water (7:3) to give Caffeic acid[22].

Anticancer activity on DU-145 and PC-3 cell lines

The anticancer activity of isolated constituents of *Coccinia grandis* was performed on DU-145 and PC-3 cancer cell lines by the Advanced Centre for Treatment Research and Education in Cancer (ACTREC) Mumbai, India. The cell viability was measured using SRB assay. All the environmental conditions were maintained throughout the experiment for all

the groups. The assay was performed in triplicate for each of the extracts. The growth curve was plotted against molar drug concentration of isolated constituents and % control growth.

Experimental procedure or SRB assay

The cell lines were grown in RPMI 1640 medium containing 10 % fetal bovine serum and 2 mM L-glutamine. For present screening experiment, cells were inoculated into 96 well microtiter plates in 100 µL at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs. Experimental drugs were initially solubilized in dimethyl sulfoxide at 100 mg/ml and diluted to 1 mg/ml using water and stored frozen prior to use. At the time of drug addition, an aliquote of frozen concentrate (1mg/ml) was thawed and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium containing test article. Aliquots of 10 µl of these different drug dilutions were added to the appropriate microtiter wells already containing 90 µl of medium, resulting in the required final drug concentrations *i.e.*10 µg/ ml, 20 µg/ml, 40 µg/ml, 80µg/ml.

After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA[23]. Cells were fixed in situ by the gentle addition of 50 µl of cold 30 % (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells, and plates were incubated for 20 minutes at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540nm with 690nm reference wavelength. Percent growth was calculated on a plate–by–plate basis for test wells relative to control wells. Percent Growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells *100. Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)]; the percentage growth was calculated at each of the drug concentration levels.

Percentage growth inhibition = For concentrations for which Ti >/ = Tz (Ti- Tz) positive or zero = [(Ti –Tz) / (C- Tz)] x 100

For concentrations for which

Ti > / = Tz (Ti - Tz) positive or zero [(Ti - Tz) / (C - Tz) × 100

For concentrations for which

Ti < Tz (Ti - Tz) negative = [(Ti - Tz) / (C - Tz)] × 100

Growth inhibition of 50%

G150 = [(Ti - Tz) / (C - Tz) × 100

GI50 is that value of the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) was calculated from Ti=Tz. The LC_{50} is the drug concentration resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning[24]. During this there is a net loss of 50% cells following treatment is calculated from

[(Ti - Tz) / Tz] × 100 = -50

RESULTS AND DISCUSSION:

Phytochemical screening

The phytochemical screening for various extracts viz., petroleum ether, chloroform, ethyl acetate, methanol and water was carried out and results were displayed in (Table 1).

Characterization of isolated phytoconstituents from *Coccinia grandis*

Kaempferol

Physical form, yellow powder; the results suggested that a flavonoid is present without any glycoside functionality. The NMR results, suggested that the molecular formula was $C_{15}H_{10}O_6$. The IR spectrum contained peaks at 3318 and 1250-1050 cm^{-1}, which were consistent with the existence of hydroxyl groups. A peak was also observed at 1661 cm^{-1}, which was attributed to the stretching vibration of a carbonyl group. The vibrational absorption peaks observed at 1613, 1569, 1508 and 1438 cm^{-1} were consistent with the presence of a phenyl ring skeleton, and the peaks at 1611, 1522 and 1408 cm^{-1} indicated the presence of an oxygen-containing heterocycle. ^{1}H NMR (300 MHz, DMSO-d6) δ: 12.50 (1H, s, 5-OH), 10.83 (1H, s, 7-OH-7), 9.38 (3H, s, -OH), 7.67 (1H, d, *J* = 2.1 Hz, H-2⊠), 7.53 (1H, dd, *J* = 7.2, 1.9 Hz, H- 6⊠), 6.88 (1H, d, *J* = 8.5 Hz, H-5⊠), 6.40 (1H, d, *J* = 1.7 Hz, H-8), 6.18 (1H, d, *J* = 1.7 Hz, H-6); ^{13}C NMR (75 MHz, MeOD) δ: 175.9 (C-4), 164.2 (C-7), 161.1 (C-9), 156.8 (C- 5), 147.4 (C-2), 146.6 (C-4⊠), 144.8 (C-3⊠), 135.7 (C-3), 122.7 (C-1⊠), 120.3 (C-6⊠), 114.8 (C-5⊠), 114.5 (C-2⊠), 103.1 (C-10), 97.8 (C-6), 93.0 (C-8).

These data were found to be consistent with those reported in the literature for kaempferol[25-28] and so the compound was identified as kaempferol.

Rutin

Light yellow powder. The NMR results, suggested that the molecular formula was C_{27} H_{30} O_{16}. IR (KBr) V_{max}cm-1:3408, 3321(OH-stretching), 2483 (CH_2-stretching), 2714 (C-H bonding), 1462 (C=O groups) and 1383 (C-OH vibrations). ^{1}H NMR(300 MHz, CD3OD) δppm = 6.18 (*d, J=2.0* HZ, 1H,H-6), 6.36 (*d, J*=2.0Hz,1H,H-8), 7.66 (*d,J*=2.05Hz,1H,H-2'), 6.88 (*d, J*=8Hz,1H,H-5'), 7.62 (*dd, J*=8.5 & 2.2Hz, 1H, H-6'), 5.12 (*d, J*=7.5Hz, 1H,H-1"), 4.50 (*d,J*=1.5Hz,1H,H-1"'), 1.12 (*d,J*=6.3Hz,3H,H-6"'), 3.32-3.86(m). ^{13}C NMR (75.5 MHz,CD3OD) δppm=158.6 (C-2), 135.8 (C-3), 179.5 (C-4), 162.6 (C-5,) 100.1 (C-6), 166.1 (C-7), 94.9 (C-8), 159.3 (C-9,) 105.7 (C-10,) 123.2 (C-1'), 117.7 (C-2'), 145.8 (C-3'), 149.8 (C-4') 116.1 (C-5'), 123.4 (C-6'), 104.8 (C-1'') 75.7 (C-2''), 77.3 (C-3''), 71.5 (C-4''), 78.1 (C-5'') 68.7 (C-6''), 102.5 (C-1'''), 72.1 (C-2'''), 72.2 (C-3''') 73.9 (C-4'''), 69.8 (C-5'''), 18.0 (C-6'''). These data were found to be consistent with those reported in the literature for rutin[29-30] and so the compound was identified as rutin.

Ellagic acid

It is a highly crystalline material with needle like structures of nanometre sizes. The NMR results, suggested that the molecular formula was $C_{14}H_5O_8$. The IR spectrum of the compound exhibit broad band in the range, 2800-3700 cm^{-1} which is attributed to the -OH stretching while the band observed at 1725 cm^{-1} corresponds to C=O stretching. The bands observed in the range, 1669-1500 cm^{-1} are due to aromatic ring vibrations while the ones at 1190 and 1052 cm^{-1} are due to ester linkage. The band at 751 cm^{-1} is assigned to aromatic C-H bending vibration. ^{1}H NMR (DMSO-d6) ppm: 7.45 (s, 2H, ArH), 10.67 (s, 4H, -OH), ^{13}C NMR (DMSO-d6) ppm: 159.08 (C7), 148.08 (C4), 139.55 (C3), 136.35 (C2), 112.27 (C1), 110.21 (C5), 107.59 (C6). These data were found to be consistent with those reported in the literature for ellagic acid[31-32] and so the compound was identified as ellagic acid.

Betulinic acid

It was crystallized from water as white powder. The NMR results, suggested that the molecular formula was $C_{30}H_{48}O_3$. IR (KBr): 3473, 3063, 2953, 2887, 2712, 1682, 1643, 1457, 1375, 1221, 1194, 1106, 1035, 980, 876, 871, 789 cm^{-1}; ^{1}H NMR ($CDCl_3$): 0.65, 0.77, 0.98, 1.14 and 1.34 (5s, 15H, all tertiary –CH_3), 1.37 (m, 2H, H-21), 1.38 (m, 2H, H-16), 1.45 (m, 2H, H-20), 1.51 (m, 4H, H-18, H-19 and H-15), 2.09 (m, 3H, H-1 and H-9), 2.13 (m, 2H, H-

14), 3.17 (t, 2H, *J* = 7 Hz, H-2), 3.38 (s, 2H, H-7), 4.56 (s, 2H, H-11), 4.59 (s, 2H, H-12). ^{13}C NMR (pyridine-*d5*): δ 39.3 (C-1), 28.3 (C-2); 78.2 (C-3); 39.6 (C-4); 56.0 (C-5); 18.8 (C-6); 34.9 (C-7); 41.2 (C-8); 51.0 (C-9); 37.6 (C-10); 21.3 (C-11); 26.2 (C-12); 38.7 (C-13); 42.9 (C-14); 31.3 (C-15); 32.9 (C-16); 56.7 (C-17); 47.8 (C-18); 49.8 (C-19); 152.5 (C-20); 30.3 (C-21); 37.6 (C-22); 28.7 (C-23); 16.4 (C-24); 16.5 (C-25); 16.5 (C-26); 15.0 (C-27); 178.9 (C-28); 110.0 (C-29); 19.5 (C-30). These data were found to be consistent with those reported in the literature for betulinic acid[33-34] and so the compound was identified as betulinic acid.

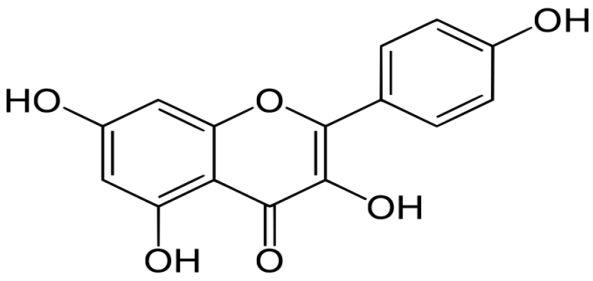
Caffeic acid

White powder. The NMR results, suggested that the molecular formula was $C_9H_8O_4$. IR (KBr) νmax 960.11, 1118, 1156, 1217, 1278, 1295, 1326, 1353, 1449, 1566, 1602, 2350 and 3424 cm^{-1}; ESMS m/z (%): 177, 178, 179 (M-1), 180, 135.2; ^{1}H NMR (400 MHz, DMSO) δppm: 3.41 (1H, s), 6.15-6.19 (1H, d), 6.75-6.77 (1H, s), 6.95-6.98 (1H, q), 7.031-7.035 (1H, d), 7.4-7.44 (1H, d), 9.12 (1H, s), 9.51 (1H, s), 12.1 (1H, s); ^{13}C NMR (400 MHz, DMSO) δppm: 114.63 (C-6), 115.12 (C-8), 115.74 (C-3), 121.08 (C-4), 125.71 (C-5), 144.52 (C-7), 145.53 (C-1), 148.08 (C-2), 167.81 (C-9). These data were found to be consistent with those reported in the literature for caffeic acid[35-36] and so the compound was identified as caffeic acid.

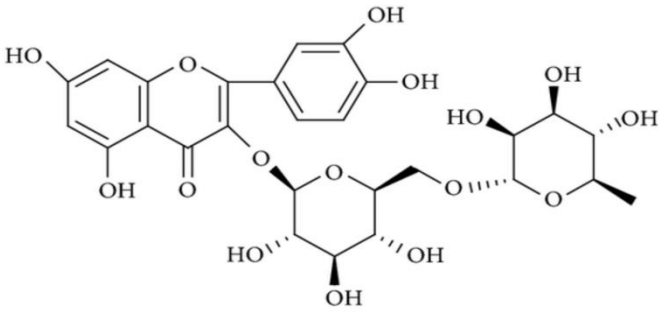
Table 1: Phytochemical screeninbg of successive solvent extraction of *Coccinia grandis*

Phytoconstituents	Method	Aqueous extract	Methanolic extract	Ethyl acetate extract	Chloroform extract	Pet. Ether extract
Flavonoids	Shinoda Test	+	+	+	+	-
	Zn. Hydrocholride Test	+	+	+	+	-
	Lead acetate Test	+	+	+	+	-
Volatile Oil	Stain Test	-	+	-	-	-
Alkaloids	Wagner Test	-	-	-	-	-
	Hager's Test	-	-	-	-	-
Tannins & Phenols	Fecl3 Test	+	+	-	+	-
	Pot. Dichromate Test	+	+	-	+	-
Saponins	Foaming Test	+	+	-	-	-

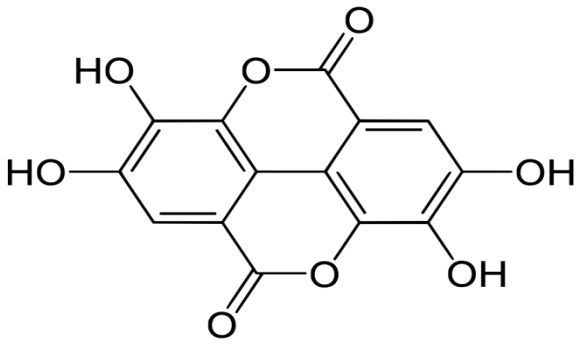
Steroids	Salkowski Test	+	+	-	-	+
Carbohydrates	Molish Test	-	-	-	-	-
Acid Compounds	Litmus Test	-	-	-	-	-
Glycoside	Keller-Killani Test	+	+	-	-	-
Amino Acids	Ninhydrin Test	-	-	-	-	-
Proteins	Biuret	-	-	-	-	-



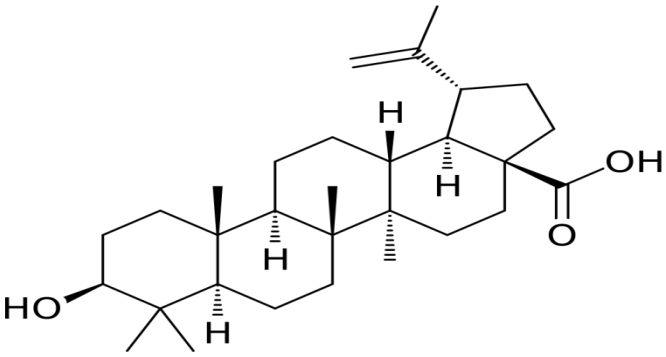
Kaempferol



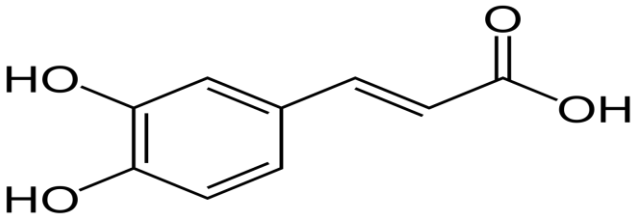
Rutin



Ellagic acid



Betulinic acid



Caffeic acid

Figure 1: Isolated constituents from *Coccinia grandis*

Anticancer activity

In the present investigation the anticancer activity of isolated constituents from *Coccinia grandis* were carried on DU-145 and PC-3 human prostate cancer cell lines by SRB assay. After completion of protocol the absorbance was read on Elisa plate reader at a wavelength of

540 nm. Photography of cell cultures were taken Figure 2, Figure 3 and values were plotted on graph and LC50, TGI and GI50 were then calculated from the graph Figure 4 and 5. Along with adrinamycin and isolated constituents treated cells also showed kariolysis, apoptosis, roundening of cell Figure 2, Figure 4. GI50 means the drug concentration resulting in a 50% reduction in the net protein increase as compared to control cells. TGI is the drug concentration resulting in total growth inhibition. The LC50 is the drug concentration resulting in a 50% reduction in the measured protein at the end as compared to the beginning. Isolated constituents particularly Kaempferol showed LC50, TGI and GI50 activity at >80, 69.7 and <10µg/ml on DU-145 and >80µg/ml of GI50 activity on PC-3 cell lines. Caffeic acid showed TGI and GI50 activity at 37.2 and <10 µg/ml on Ishikawa and 60.2, <10 and <10µg/ml of LC50, TGI and GI50 activity on PC-3 cell lines respectively (Table 2-5). The chemotherapeutic agents extensive utilized in oncologic therapy produce deleterious unwanted effects which enhance the fatality as well as morbidity brought on by malignancy. Safer therapies are thus frantically required, a few of that you can get within natural substances like phytochemicals. Having well known chemopreventive activities and preclinical antitumor effects, phytochemicals give a novel restorative strategy which value additional exploration[37]. Phenols and polyphenols, flavonoids and their derivatives, are ubiquitous in plants and more than 8,000 different compounds are included in this group and many of them are antioxidants. They are associated with the inhibition of vascular disease and malignancy[38]. Flavonoids have drawn a lot of interest with regards to their potential benefits on health[39]. Flavonoids have been shown to possess antimalignant effects[40]. The anticancer effects of methanol extract of *Coccinia grandis* might be related to their content of Flavonoids. Reported by Pradhan, flavonoids might exert their chemopreventive role in malignancy via their results on signal transduction in cell proliferation as well as angiogenesis[41].

Results from present investigation indicate that *Coccinia grandis* has anticancer activity (*in vitro*) on DU-145 and PC-3 cancer cell line. The isolated constituents throughout the studies showed negative activity on cell lines except Kaempferol and Caffeic acid that showed comparable activity to the standard compound Adriamycin for DU-145 and PC-3 human prostate cancer cell lines. The effective concentration of major isolated constituents was observed to be < 80 µg/ml. Our results are in concordance with some of the previous studies on this plant. These previous studies indicate that this plant has some phytochemicals which

can have possible anticancer activity, either singly or in combination. The present results, together with previous studies, suggest that *Coccinia grandis* possess anticancer activity.

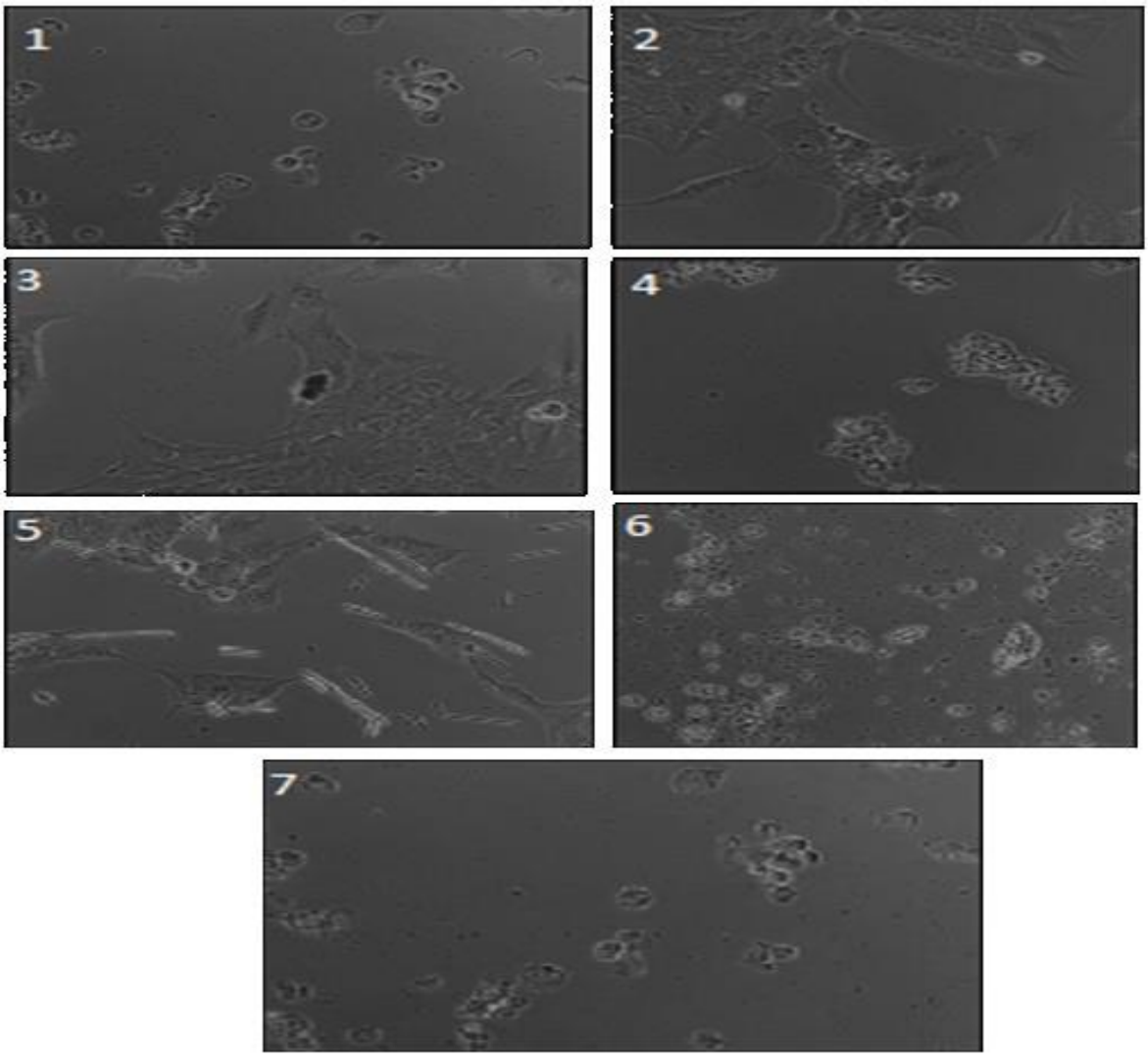


Figure 2: Morphology of the DU-145 human prostate cancer cell lines

1: Phase contrast photography of DU-145 cell line

2: Phase contrast photography of DU-145 cell line with Ellagic acid

3: Phase contrast photography of DU-145 cell line with Rutin

4: Phase contrast photography of DU-145 cell line with Kaempferol

5: Phase contrast photography of DU-145 cell line with Betulinic acid

6: Phase contrast photography of DU-145 cell line with Caffeic acid

7: Phase contrast photography of DU-145 cell line with Adriamycin

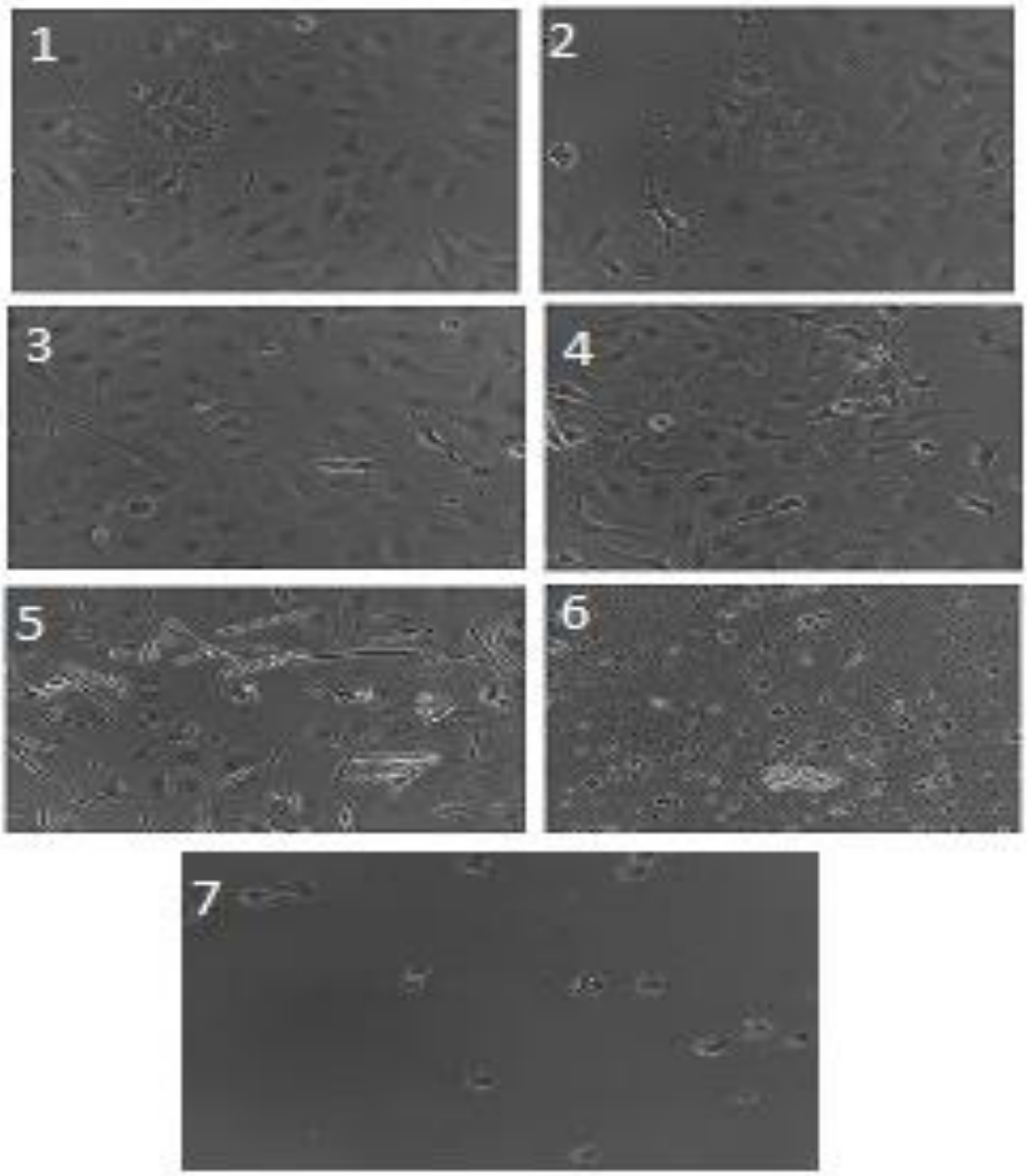


Figure 3: Morphology of the PC-3 human prostate cancer cell lines

1: Phase contrast photography of PC-3 cell line

2: Phase contrast photography of PC-3 cell line with Ellagic acid

3: Phase contrast photography of PC-3 cell line with Rutin

4: Phase contrast photography of PC-3 cell line with Kaempferol

5: Phase contrast photography of PC-3 cell line with Betulinic acid

6: Phase contrast photography of PC-3 cell line with Caffeic acid

7: Phase contrast photography of PC-3 cell line with Adriamycin

Table 2: Drug concentration (µg/ml) and percentage of growth inhibition on DU-145 human prostate cancer cell lines

	Experiment 1				Experiment 2				Experiment 3				Average values			
	10	**20**	**40**	**80**	**10**	**20**	**40**	**80**	**10**	**20**	**40**	**80**	**10**	**20**	**40**	**80**
Ellagic acid	135.6	185.6	194.5	148.1	107.9	111.7	117	127.8	105.8	106.8	117.9	131.2	116.4	134.7	143.1	135.7
Rutin	151.7	169.8	171.1	146.5	106.9	111.9	112.7	122.6	103.5	107.5	109.9	130.3	120.7	129.7	131.2	133.1
Kaempferol	123.2	149.5	108.3	-27.6	106.8	110.3	72.7	-36.7	103.5	111.9	67.8	-31.5	111.2	123.9	82.9	-31.9
Betulinic acid	122.5	173.2	165.2	134.5	105.5	115.4	115.8	117.9	88.6	106.9	104.6	125.9	105.5	131.8	128.5	126.1
Caffeic acid	119.1	21.3	7.8	-9.8	60	-31.5	-51.6	-21.9	39.9	-46.9	-59.6	-28.7	73.0	-19.0	-34.5	-20.1
Adriamycin	4.9	-2.1	-25.8	-35.5	9.4	1.3	-18.4	-36.2	1.9	-7.2	-27.9	-37.9	5.4	-2.7	-24.0	-36.5

Table 3: Drug concentration (µg/ml) and percentage of growth inhibition on PC-3 human prostate cancer cell lines

	Experiment 1				Experiment 2				Experiment 3				Average values			
	10	**20**	**40**	**80**	**10**	**20**	**40**	**80**	**10**	**20**	**40**	**80**	**10**	**20**	**40**	**80**
Ellagic acid	89.9	88.8	95.9	110.7	93.8	99.1	106.2	115.2	93.1	106.2	139.9	115.2	92.3	98.0	114.0	113.7
Rutin	97.1	84.3	93.2	107.3	86.2	83.6	95.2	102.9	96.7	101.6	104.9	109.8	93.3	89.8	97.8	106.7
Kaempferol	85.6	91.6	62.1	57.4	84.4	82.9	96.4	34.2	95.6	89.6	93.2	52.1	88.5	88.0	83.9	47.9
Betulinic acid	98.1	97.1	99.8	113.2	87.2	95.1	96.9	108.1	98.2	101.5	103.4	113.4	94.5	97.9	100.0	111.6
Caffeic acid	1.8	-39.1	-74.5	-66.2	-3.5	-36.1	-59.1	-46.2	-23.5	-30.9	-42.9	-42.9	-8.4	-35.4	-58.8	-51.8

Adriamycin	-74.1	-76.9	-78.5	-67.1	-72.9	-77.5	-79.2	-64.5	-75.4	-76.8	-77.8	-63.1	-74.1	-77.1	-78.5	-64.9

Table 4: Drug concentration (µg/ml) calculated from graph on DU-145 cell line

DU-145	LC50	TGI	GI50*
Ellagic acid	NE	NE	> 80
Rutin	NE	NE	> 80
Kaempferol	> 80	69.7	47
Betulinic acid	NE	NE	> 80
Caffeic acid	NE	37.2	< 10
Adriamycin	NE	12.7	< 10

LC50, Concentration of drug causing 50% cell kill; GI50, Concentration of drug causing 50% inhibition of cell growth; TGI, Concentration of drug causing total inhibition of cell growth; NE, Non-evaluable data. Experiment needs to be repeated using different set of drug concentrations

Table 5: Drug concentration (µg/ml) calculated from graph on PC-3 cell line

PC-3	LC50	TGI	GI50*
Ellagic acid	NE	NE	NE
Rutin	NE	NE	NE
Kaempferol	NE	NE	> 80
Betulinic acid	NE	NE	NE
Caffeic acid	60.17	< 10	< 10
Adriamycin	NE	< 10	< 10

LC50, Concentration of drug causing 50% cell kill; GI50, Concentration of drug causing 50% inhibition of cell growth; TGI, Concentration of drug causing total inhibition of cell growth; NE, Non-evaluable data. Experiment needs to be repeated using different set of drug concentrations

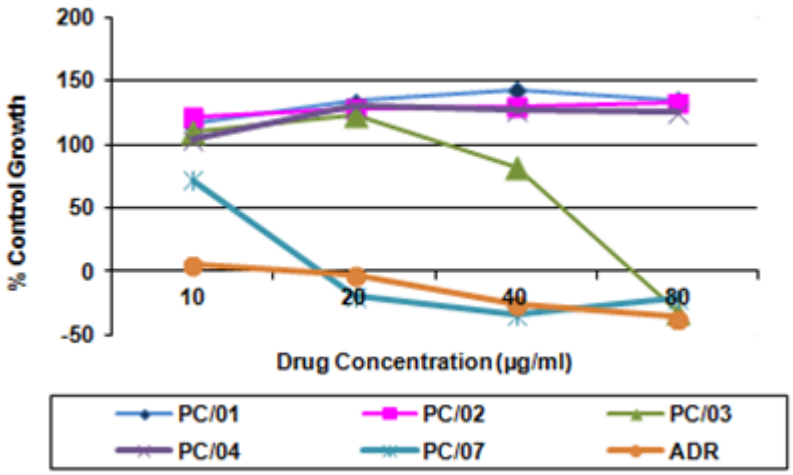


Figure 4: DU-145 cell line growth curve

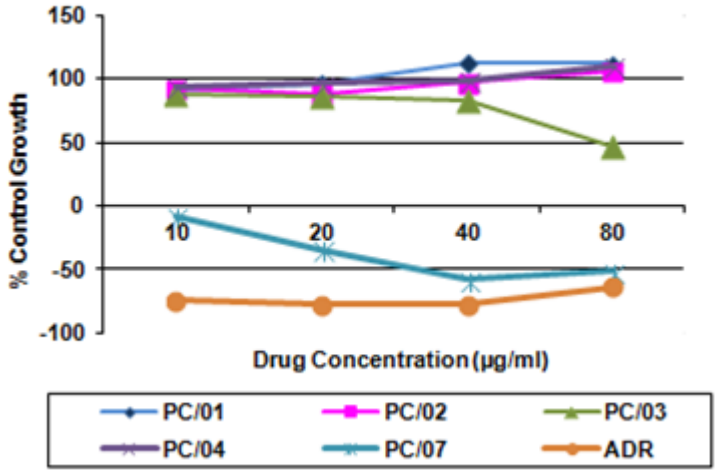


Figure 5: PC-3 cell line growth curve

Conclusion: The outcome attained from the current investigation signifies that the plant owned a considerable anticancer activity may be attributed to the presence of isolated constituents i.e., Kaempferol and Caffeic acid from *Coccinia grandis*, indicating the traditional relevance of the plant, which were non toxic to normal cells. The results of the study will also need to be confirmed using in vivo models and to determine the other active chemical constituents accountable for the anticancer activity.

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