|| ISSN(online): 2589-8698 || ISSN(print): 2589-868X ||
International Journal of Medical and Biomedical Studies
Available Online at www.ijmbs.info
NLM (National Library of Medicine ID: 101738825)
Index Copernicus Value 2021: 70.61
Research Article Volume 6, Issue 11; November: 2022; Page No. 22-29

In-Vitro Antioxidant and Anti-Neoplastic Activity of Leaves Extract of Adina Cardifolia

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Article Info: Received 05 August 2022; Accepted 23 October 2022
DOI: https://doi.org/10.32553/ijmbs.v6i11.2622
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Conflict of interest: No conflict of interest.

Abstract
This study of the antioxidant properties of Adina cardifolia ware successfully investigated using DPPH radical scavenging, nitric oxide radical scavenging, Superoxide anion radical scavenging, total antioxidant activity by ABTS*+ radical scavenging and lipid peroxidation inhibitor assays and compared with the standards. Also the anti-neoplastic activity was study by MTT assay using MCF7 Cell line. Percentage growth inhibition by MTT assay using MCF-7 cell line ware found to be 65.54±1.05 by PEAC extract respectively compared to 83.03±1.0% by standard drug Methotrexate.
Keywords: Breast carcinoma, Adina cardifolia, anticancer activity, antioxidant

Introduction

Those chemicals that attach with free radicals and their impact from causing harm to biological molecules. Endogenous antioxidants are generated by our body which is utilized to different free radicals. These days, numerous oral supplements are accessible in the market named as dietary antioxidants[1]. The alternative medicine deals with a wide assorts of herbal plants, which may fill in as key to open the numerous puzzles behind human pathologies. The developing countries relies upon conventional plants for wellbeing necessities, dietary supplements, nutraceuticals, herbal plant extracts and phytoconstituents find wide application in rewarding afflictions going from normal to uncommon infectious and non-infectious diseases[2,3].

Macdonald,J.S.(1999) Oncology, 13 (7 Suppl 3), 33-34.

Rexroth, Gand Scotland, V. (1994) Med.Klin., 89(12), 680-688.

Adina cardifolia is medicinal plants possess various therapeutic characteristics belonging to the family Rubiaceae. Large deciduous tree grows up to 20 meters in height [4]. It has various properties like antioxidant effect[5], anti-neoplastic effect[6] and anti-fertility properties[7], anti-amoebic effect[8].

I. Material and Methods

Collection and Authentication of the leaf

Adinacardifolia leaves were collected from garden of Ashtang Ayurvedic College, Indore and authentication by Dr. Shakun Mishra, Head of Department, S.S.N Govt. P.G. College, Khandwa and under guidance of Dr. Manmeet Singh Saluja, Professor, SunRise University, Alwar, Rajasthan, India.

Preparation of Crude Drug for Extract

Adinacardifolia leaves were keep and dried under shade and grounded. The grounded plant leaves were sieved through sieve no.40 and stowed in a

sealed vessel for extraction[9].

Preparation of extracts of Adinacardifolia

The grounded and sieved plant leaves about 1000gm were sequentially extracted using petroleum ether, ethanol, hydroalcohalic (1:1) and distilled water in soxhlet apparatus material were concentrated After about forty siphons of each solvent extraction step[10, 11].

Drugs and Chemicals

DPPH, sodium nitroprusside, sulphanilamide, naphthylethylene diamine dihydrochloride, Curcumin, nitroblue tetrazolium, NADH, phenazine methosulphate, Catechin, ferric chloride, L-ascorbic acid, trichloroacetic acid, thiobarbituric acid, butylated hydroxy anisole, TROLOX, ABTS, potassium persulfate obtained from Sigma Chemical Co. Ltd, USA. All chemicals and solvents were of reagent grade.

Preliminary Phytochemical Screening

Adinacordifolia extracts were qualitative tests of various active constituents' viz. carbohydrate, glycoside, alkaloid, amino acids, flavonoids, fixed oil, tannins, gum and mucilage, phytosterols etc.

Free Radical Scavenging and Antioxidant Activity

The antiradical and antioxidant activities of the different extracts in five in-vitro models, including DPPH radical scavenging, Nitric oxide radical scavenging, Superoxide anion radical scavenging, total antioxidant activity by ABTS●+ radical scavenging and lipid peroxidation inhibitor assays were investigated.

DPPH radical Scavenging Activity

DPPH i.e. 2,2-diphenyl-1-picrylhydrazyl, an organic chemical compound. This free radical, stable at room temperature, and get reduced in the presence of an antioxidant molecule, that give rise to colorless solution DPPH, were read at 517 nm (Blois MS 1958). To a 0.1mM solution of DPPH in methanol add 3ml methanolic solution of different extract in concentration of 10, 20, 30, 40, 50 and 100 µg and incubate for 30 min at 517nm and check the absorbance. The percentage reduction initial and final absorbance of each solution.

Percentage scavenging of DPPH radical was calculated using the formula

% Scavenging of DPPH = [Abs Control-AbsTest]/ [AbsControl] ×100

Nitric oxide radical Scavenging Activity

Sodium nitroprusside in aqueous solution generate Nitric oxide that reacts with oxygen to yield nitrite ions which were measured by the Griess reaction. Nitric oxide with oxygen leading to reduced production of nitric oxide[12, 13]. Reaction mixture (3ml) was prepared by mixing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and the extracts in different concentrations (10, 20, 30, 40, 50 and 100 µg). These mixtures were incubated at 25^0C for 150 min. At an interval of every 30 min, 0.5 mL of the incubated extracts was removed and 0.5 mL of Griess reagent (1% sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H3PO4) was added. The chromophore formed was measured at an absorbance of 546 nm. All the analyses were performed in triplicate and the results were averaged. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test. Curcumin ware used as a reference compound.

Superoxide anion radical Scavenging Activity

A reaction mixture was prepared by adding 1 mL of nitroblue tetrazolium (NBT) solution (156 µM NBT in 100 mM phosphate buffer, pH 7.4), 1 mL of NADH solution (reduced form of β-nicotinamide adenine dinucleotide) (468 µM in 100 mM phosphate buffer, pH 7.4) and 0.1 mL of extracts in different concentration (10, 20, 30, 40, 50 and 100 µg) in distilled water. The reaction was started by addition of 100 µl of phenazine methosulphate (PMS) solution (60 µM PMS in 100 mM phosphate buffer, pH 7.4). This reaction

mixture ware incubated at 250C for 5 min and measured the absorbance at 560 nm against blank samples. An increased in superoxide anion scavenging activity ware indicated by decreased absorbance of the reaction mixture. Catechin used as reference compound. All the experiments performed in triplicate and the results averaged. The percentage of inhibition was determined by comparing the results of control and test samples[14].

Lipid Peroxidation Inhibitory Activity

For this activity, proper formation of liposome ware ensured by sonicating 300mg of egg lecithin with 30ml of phosphate buffer, pH 7.4 in an ultrasonic sonicator for 30 min. This sonicated solution, plants extracts and standard (ascorbic acid) of different concentrations (10, 20, 30, 40 50, and 100 µg/mL) were added and incubate for 10min. The induction of Lipid peroxidation was done by adding ferric chloride (0.5ml, 400 mM) and L-ascorbic acid (0.5ml, 400 mM) and incubated for 1 h at 370C. The reaction was stopped by adding hydrochloric acid (2 mL, 0.25 N) containing trichloroacetic acid (TCA, 150 mg/ mL), thiobarbituric acid (TBA, 3.75 mg/mL) and butylated hydroxy anisole (BHA, 0.50 mg/mL). Heat the reaction mixture at 800C for 20 min, cooled, centrifuged for 10 min and the absorbance of the supernatant liquid was measured at 532 nm[15]. Experiments were performed in triplicate and averaged; the % inhibition at varying concentration was calculated by the following formula-

$$\% \text{ Inhibition} = [1-(Vt/Vc)] \times 100$$

Where, Vt = mean absorption of test, Vc= mean absorption of control

The IC50 value was derived from the % inhibition at different concentration.

Total Antioxidant Activity

Antioxidant activity of plants extracts were compared with standard antioxidant TROLOX (6-hydroxy-2, 5, 7, 8- tetramethychroman-2-carboxylic acid) to scavenge ABTS●+ cation radical. ABTS●+ [2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] is a radical cation generated directly in stable form through the reaction between ABTS and potassium per sulfate. When this radical reacts with plant extract containing antioxidants, it reduces, and thus the extent of radical's inhibition is determined and calculated relatively to standard TROLOX.

To perform this assay, dissolve ABTS in water to make a 7 mM stock solution. The ABTS●+ radical cation was formed by reaction between ABTS stock solution with 2.45 mM potassium persulfate. Allow this mixture to stand up to 12–16 h in the dark at room temperature, then check the absorbance, once the absorbance become stable then it was ready to use. The ABTS●+ solution was diluted with water and equilibrated to 0.70 (± 0.02) at 30°C. Add 10 µL of plant extracts and 0-15 µL of standard TROLOX separately to 1 ml ABTS●+ solution and analyze the absorbance at 734 nm after 6 min, perform this experiment at least three times[16.17,18]. Calculate the percentage inhibition of absorbance and was plotted as a function of the concentration of standard and sample to calculate the TROLOX equivalent antioxidant concentration (TEAC).

For calculation of TEAC, the gradient of the plot for the sample ware divided by the gradient of the plot for TROLOX and scavenging activity of the samples calculated by the formula-

$$S\% = [(Acontrol - Asample)/Acontrol)] \times 100$$

Where Acontrol is the absorbance of the blank control (ABTS●+ solution without test sample) and Asample is the absorbance of the test sample.

In-Vitro Cytotoxicity Activity by MTT assay Method

In vitro cytotoxicity activity of plants extracts by MTT assay method using MCF 7 cell line. MTT assay is based on the supposition that dead cells or their products do not reduce tetrazolium. The assay relies on both the mitochondrial activity per cell and number of cells present. The assay is based on the principle that the mitochondrial

enzyme succinate dehydrogenase present in living cells cleaves 3-(4, 5 dimethyl thiazole2yl)-2, 5-diphenyl tetrazolium bromide (MTT) into a blue formazan derivative. The amount of cells was found to be proportional to the extent of formazan production by the cells used[19].

Cells were seeded in a 96-well flat-bottom plate (5000 cells/well) and permitted to adhere for 24h at 370 C with 5% CO2 atmosphere. Different drug concentration was added and incubated further for 48hrs. Before 4h of the completion of incubation, 20µl of MTT (5mg/ml) was added. Dead cell percentage was determined using an ELISA plate reader set to record absorbance at 570nm. The percentage growth inhibition was calculated using the formula given below[20].

% growth inhibition = 100 X (Control OD-Sample OD/Control OD)

Sample OD=Absorbance of treated cells (Plant Extracts and Standard Drug)
Control OD=Absorbance of control (untreated)

III. Results and Discussion

Photochemical Screening

The extraction ware performed by using different solvents ranging from polar to non polar for eg. Distilled water, hydroethanolic (1:1), ethanol, and petroleum ether. The abbreviations used for the extracts along with the extractive values are reported in the Table 1. The phytochemical screening of all extracts showed positive reaction for the flavonoids, phenols, saponin, alkaloids, triterpanoids and tannins. The results of phytochemical analysis depicted in the Table 2.

Table No. 1: Abbreviation for extracts and extractive value of extracts

S.N0.	Plant	Extract	Abbreviation used	Extractive values
1.	Adina cardifolia	Pet. Ether	PEAC	34.26
		Ethanol	ETAC	38.96
		Hydro alcohol	HAAC	42.66
		Distilled Water	DWAC	41.08

Table No. 2: Photochemical analysis of leaves extract of A.cardifolia by using different solvents.

S.N.	Photochemical	PEAC	ETAC	HAAC	DWAC
1.	Steroids	-	-	-	-
2.	Fatty acids	++	+	-	-
3.	Alkaloids	++	+++	++	++
4.	Phenols	+	++	++	+
5.	Flavanoids	+	+++	+++	+++
6.	Saponins	++	+++	+	+++
7.	Tannins	++	++	+	++

Where symbol (+++) indicates presence in high concentration, symbol (++) indicates presence in moderate concentration, symbol (+) indicates presence in trace concentration and symbol (–) indicates absence of the respective phytochemical.

DPPH free radical Scavenging assay

To the various extracts HAAC have shown more significant inhibition of DPPH by 43.18±0.03% compared to 67.45 ± 3.467% inhibitions by standard Ascorbic acid. Table No.3. Shows percentage inhibition of DPPH.

Table No. 3: DPPH radical scavenging activity by *A.cardifolia*

Sample	10 µg/mL	20µg/mL	30 µg/mL	40 µg/mL	50 µg/mL	100 µg/mL	IC50
Ascorbic acid	17.64±0.03	30.41±0.02	43.53±0.13	56.80±0.04	68.43±0.02	95.25±0.03	39.29
PEAC	3.46±0.01	6.71±0.27	11.55±0.61	15.74±0.43	19.58±0.44	28.77±0.47	167.09
ETAC	10.21±0.21	18.67±0.26	21.45±0.07	26.46±0.13	29.88±0.44	41.29±0.04	120.42
HAAC	11.32±0.15	16.54±0.13	22.97±0.36	22.83±0.03	32.38±0.30	44.15±0.03	108.98
DWAC	8.40±0.01	11.56±0.04	14.94±0.07	18.70±0.06	21.49±0.14	37.70±0.25	137.4

All readings are mean ± SD, n = 3, IC50 value reported as Conc. ± SEM, P < 0.01 Vs standard

Nitrous oxide radical scavenging activity

In the Table no. 4 demonstrates the percentage inhibition of nitric oxide production by varying concentrations of plant extracts. The potent % inhibition value of extracts was found to be 48.29±0.10% exhibited by HAAC compared to standard curcumin which produce 91.42±0.22% inhibition.

Table No.4: Nitrous oxide radical scavenging activity by *A.cardifolia*

Sample	10 µg/mL	20µg/mL	30 µg/mL	40 µg/mL	50 µg/mL	100 µg/mL	IC50
Curcumin	16.12±0.24	29.86±0.14	38.42±0.02	48.76±0.04	61.35±0.32	91.42±0.22	44.57
PEAC	4.91±0.13	9.27±0.17	11.39±0.38	15.24±0.12	20.38±0.04	34.29±0.02	146.35
ETAC	13.15±0.003	18.33±0.01	23.06±0.11	27.55±0.10	31.66±0.04	44.97±0.01	118.85
HAAC	11.25±0.19	17.15±0.05	25.67±0.24	31.49±0.01	34.04±0.02	48.29±0.10	97.05
DWAC	4.38±0.06	11.68±0.06	15.54±0.01	18.61±0.02	23.09±0.09	38.52±0.21	128.51

All readings are mean ± SD, n = 3, IC50 value reported as Conc. ± SEM, P < 0.01 Vs Standard

Superoxide anion radical scavenging activity

In the Table No.5 shows the superoxide scavenging effect of plant extracts and catechin. The potent % inhibition value of extracts was found to be 53.08±0.9 % exhibited by ETAC compared to standard catechin which produce 93.14+0.34% inhibition.

Table No.5: Superoxide radical scavenging activity by *A.cardifolia*

Sample	10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL	100 µg/mL	IC50
Catechin	31.51± 0.80	49.28±0.52	65.22±0.41	76.40±1.32	89.23±0.28	93.14±0.34	39.53
PEAC	5.59±0.05	9.84±0.28	19.82±0.03	23.85±0.13	25.19±0.33	36.2±0.22	133.27
ETAC	5.38±0.14	9.83±0.12	23.32±0.18	34.1±0.61	38.5±0.43	53.08±0.9	84.19
HAAC	9.44±0.05	20.95±0.32	32.28±0.21	36.84±0.11	44.91±0.17	52.74±0.09	80.21
DWAC	9.64±0.15	20.83±0.09	26.24±0.48	31.37±0.28	33.44±0.35	45.58±0.02	105.95

All readings are mean ± SD, n = 3, IC50 value reported as Conc. ± SEM, P < 0.01 Vs Standard

Lipid Peroxidation inhibitory activity

In the Table 6. shows inhibition of radical were found to be 39.33±0.41% exhibited ETAC compared to standard Ascorbic acid which produce 99.83+0.047% inhibition.

Table No. 6: Lipid peroxidation inhibitory activity by *A.cardifolia*

Sample	10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL	100 µg/mL	IC50
Ascorbic Acid	9.04± 0.30	21.47±0.03	31.64±0.43	40.60±0.36	53.26±0.36	99.83±0.047	49.03
PEAC	3.60±0.18	6.86±0.40	11.24±0.09	15.15±0.17	19.29±0.41	26.75±0.21	182.95
ETAC	6.79±0.21	6.86±0.40	13.62±0.09	23.96±0.56	28.48±0.26	39.33±0.41	123
HAAC	8.61±0.21	16.36±0.12	23.05±0.76	26.33±0.42	30.19±0.44	37.02±0.17	132.9
DWAC	6.22±0.18	8.57±0.49	11.93±0.23	18.15±0.33	22.75±0.17	30.00±0.42	165.27

All readings are mean ± SD, n = 3, IC50 value reported as Conc. ± SEM, P < 0.01 Vs Standard

Total antioxidant activity

In the Table.7. Shown the comparative analysis of outcome. Inhibition of radical were found to be 39.60±0.06% exhibited by HAAC compared to standard TROLOX which produce 97.09±0.29 % inhibition.

Table No.7: Total antioxidant activity by *A.cardifolia*

Sample	10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL	100 µg/mL	IC_{50}
TROLOX	25.51±0.54	34.73±0.43	44.57±0.71	53.87±0.43	62.76±0.79	97.09±0.29	37.76
PEAC	4.14±3.65	7.90±0.28	13.41±0.46	17.18±0.21	21.71±0.22	30.80±0.33	158.63
ETAC	5.80±0.30	9.10±0.15	14.69±0.41	17.53±0.35	23.86±0.27	32.33±0.47	152.51
HAAC	6.47±0.41	12.06±0.36	17.50±0.56	25.86±0.54	32.30±0.54	39.60±0.06	117.66
DWAC	9.08±0.34	13.26±0.17	17.71±0.53	22.55±0.10	28.49±0.19	36.27±0.19	136.99

All readings are mean ± SD, n = 3, IC50 value reported as Conc. ± SEM, P < 0.01 Vs Standard

In-Vitro Antineoplastic activity by MTT assay Method on MCF7 Human Breast Cancer Cell line

The effect of different plant extract on the growth of MCF-7 cell line ware investigated and compared with standard drug Methotrexate by the MTT assay.
The highest percentage inhibition value obtained at 500µg concentration ware 65.54±1.05% by PEAC compared with 83.03±1.0% by standard drug.Table.8 shows results of MTT assay.

Table No. 8: In-vitro cytotoxic activity ofextract of A. cordifolia by MTT assay

Concentration (µg/ml)	% growth Inhibition				
	PEAC	ETAC	HAAC	DWAC	MXT
500	65.54±1.05	62.13±1.08	60.85±1.58	40.45±0.70	83.03±1.0
166.66	52.66±1.32	50.56±1.21	50.09±0.67	25.31±0.16	69.26±1.04
55.55	50.30±1.66	48.80±0.28	48.01±0.95	23.54±0.76	65.64±0.62
18.52	47.05±2.87	46.90±1.18	45.95±0.97	22.30±1.07	63.08±0.99
6.17	44.51±2.42	43.71±0.83	43.64±1.1	20.40±1.29	59.82±0.37
2.06	41.91±2.75	41.52±0.44	41.45±0.90	18.39±0.55	57.57±0.99
0.68	39.60±2.76	39.22±0.62	39.27±1.00	16.20±0.74	55.02±0.90
0.23	37.54±2.65	37.21±0.64	37.43±0.50	14.25±0.74	52.70±1.06
0.076	35.11±3.14	35.26±0.82	35.36±0.47	12.60±0.90	50.45±1.72
0.025	33.10±3.10	33.07±0.46	33.53±0.84	10.41±0.97	48.43±1.51

Values are expressed as mean ± SEM, n=3, P<0.01Vs Standard

Conclusion

The hydroalcohalic extracts specify the more affirmative results for its antioxidant activity in-vitro models while petroleum ether extract shows major in-vitro anticancer activity by MTT assay. This research reveals that the plant base medicine have excellent antioxidant activity and encouraging anticancer activity and also in further drugs in pharmaceutical area, different biologically active constituents and secondary metabolites are precious for further analysis.

Acknowledgment

Authors are thankful to Dr Anil K Gupta for their valuable guidance.

References

1. Villines Z (2017). Antioxidants and free radicals.
2. Rates SM (2001) Plants as source of drugs. Toxicon. 39(5):603–613.
3. Chaturvedi M, Patel R, Saraswat R (2019) Preliminary Phytochemical Screening of Various Extracts of Leaves and Tubers of Sauromatum guttatum (Wall.) Schott. Pramana Research 9(1): 383-87.
4. Roy GP, Bhaskar BK,Flora of Madhya Pradesh, Ashish Publication House, 1992, 212.
5. Baral P, Dubey A, Tewari S, Vasmatkar P, Verma AK. Total Polyphenolic Contents and Antioxidant Activity of Leaf, Bark and Root of Adina cordifolia Benth. & Hook. Journal of Pharmaceutical, Chemical and Biological. 2016; 4(3):394-401.
6. Saluja MS, Sangameswaran B, Sharma A. In-vitro Cytotoxic activity of leaves of Adina Cordifolia against Ehrlich Ascites Carcinoma (EAC) cell lines, Elixir Pharmacy. 2012; 53: 12157-12159.
7. Sabir, M. and Razdan, M.K. (1970). Antifertility study with leaf extracts of Adina cordifolia (karam ki gaach). Indian Journal of Physiology and Pharmacology14:209-210.
8. Iqbal PF, Bhat AR, Azam A (2009) Antiamoebic coumarins from the root bark of Adina cordifolia and their new thiosemicarbazone derivatives. European Journal of Medicinal Chemistry 44:2252-2259.Jakaria M, Parvez M, Zaman R, Arifujjaman, Hasan MI, Sayeed MA, Ali MH. Investigations of Analgesic Activity of the Methanol Extract of Haldina cordifolia (Roxb.) Bark by using in vivo Animal Model Studies. Research Journal of Botany. 2015; 10: 98-103.
9. Khandelwal KR (2001) Practical Pharmacognosy technique and experiments, Nirali Prakashan, Pune, 2001, 2nd edition, 149-56.
10. Trease GE and Evans WC, Text book of Pharmocognosy. 13th (eds). Alden Press, Oxford, London, 2003; 512-513.
11. Green LC, Wagner DA, Glogowski J et al (1982) Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Analytical Biochemistry 126: 131–36.
12. Marcocci L, Maguire JJ, Droy-Lefaix MT (1994) The nitric oxide scavenging property of Ginkgo biloba extract EGb 761. Biochemical and Biophysical Research Communications 201: 748-55.
13. Nishikimi M, Rao NA, Yagi K (1972) The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and Biophysical Research Communications 46: 849-854.
14. Joo-Shin Kim (2018) Evaluation of In-Vitro Antioxidant Activity of the Water Extract Obtained from Dried Pine Needle (Pinus densiflora) Preventive Nutrition and Food Science. 23(2):134-143.
15. Chang HY, Ho YL, Sheu MJ et al (2007) Antioxidant and free radical scavenging activities of Phellinus merrillii extracts. Botanical Studies 48:407–417.
16. Erel O (2004) A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clinical Biochemistry 37(4):277–285.

17. Robert Re, Pellegrini N, Proteggente A et al (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology Medicine 26(9/10):1231–1237
18. Ramnath V, Rekha PS, Kuttan G et al (2009). Regulation of Caspase-3 and Bcl-2 Expression in Dalton's Lymphoma Ascites Cells by Abrin. Evid. Based Complement. Alternative Medicine 6: 233-238.
19. Hajighasemi F, Mirshafiey A (2010). Propranolol effect on proliferation and vascular endothelial growth factor secretion in human immunocompetent cells. Journal of Clinical Immunology and Immunopathology Research 2: 22-27.