AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Canvassing Pharmacological Activities Expressed by *Hibiscus Sabdariffa* Calyx Extracts Using *In Vitro* Models

Lana Yousif Mutalib[1]*
1. Department, Pharmacy College, Hawler Medical University, Erbil, Kurdistan Region, Iraq.

ABSTRACT

Efforts have been spend for discovering drugs from medicinal plants. Hibiscus sabdariffa plant a well known plant with many traditional uses for treating different aliments have been selected for canvassing pharmacological activities expressed by the different polarity extracts. Results showed significant antidiabetic activity at different concentrations of glucose solution (5, 10 and 20 mM) (p value < 0.001). Significant moderate anti-inflammatory activity comprising in membrane stabilizing activity were expressed by ethanolic extract ranging (29.573±0.709 - 55.040 ±1.659 %) (p value < 0.001) while mild activity were expressed by petroleum ether extract ranging (10.273±0.413 - 22.347± 1.049 %). Approximately similar thrombolytic activity was exhibited by both extracts ranging (56.663±1.3139-21.533±0.656) consider as significant activity in comparison to the control negative (p value < 0.0001). Results conclude canvass the pharmacological activities expressed by different polarity extracts which were showed significant activity in some aspects which may open new venue for production of new drugs derived from nature with less side effects.

Keywords: *Hibiscus sabdariffa*, Membrane stabilizing, Thrombolytic, In-vitro anti-diabetic

*Corresponding Author Email: lanayousif82@yahoo.com
Received 06 October 2015, Accepted 17 October 2015

Please cite this article as: Mutalib LY *et al.*, Canvassing Pharmacological Activities Expressed by *Hibiscus Sabdariffa* Calyx Extracts Using *In Vitro* Models. American Journal of PharmTech Research 2015.

INTRODUCTION

An important role have been played by medicinal plants in developing of phytotherapeutic agents used in treatment of different aliments specially diseases without complete cure using conventional drug therapy such as, liver disease, cancer and arthritis. Plant phytochemicals exert variant physiological activity may serve as anti-diabetes, anti-inflammatory, thrombolytic, antiarthritic agents. Generally medicinal plants used as dietary supplement and in curing of various diseases without knowledge of their physiological action. In spite of the fact that few medicinal plants have been examined scientifically for their physiological effects, they continued play an important role in physiotherapy in several countries[1,2]. Previous epidemiological researches expressed that natural antioxidant intakes decreased risk of many diseases such as diabetes, thrombus and inflammatory diseases. Recently there was a great demand for discovering drugs from natural sources, since treatment of diseases with conventional drugs were costly[3,4,5]. Diabetes is a chronic disease characterized by elevated level of blood sugar either fasting or post prandial, diabetes is a disease with high prevalence worldwide about 4% in 1995 with predication of increasing percentage to about 5.4% at 2025 allocated specially in developing countries[6]. Inflammation is defined as disturbed physiological condition characterized by swelling, redness, heat caused by injury, or infection of the body tissue[7]. Allopathic drugs used in treatment of inflammation were non-steroidal anti-inflammatory drugs[8]. Recently there were an elevated rate of cardiovascular diseases caused by the blot clot formation (thrombus) which was considered as most series diseases of cardiovascular system[9]. Conventional allopathic drugs used in thrombus lysis conditions are streptokinase, tissue plasminogen activitor (t-PA) and urokinase, but their usage were combined by series side effects such as heamorrhage, anaphylactic shock[9-13]. Researchers were always looking for drugs derived from nature with minimum adverse effects specially plants are the most common target for developing modern drugs[14]. *Hibiscus sabdariffa* belong to Malvaceae plant family, were assigned by various pharmacological activity such antihypertensive[15], liver diseases[16], antibacterial antibiofilm[17] and an anti-inflammatory[18]. Hibiscus sabdariffa plant locally known by "Kujarat" used by the local community for different purposes either culinary or medicinally. In the present study *Hibiscus sabdariffa* was selected to be assessed in different pharmacological aspects in various extract forms, pharmacological points include anti-diabetic, anti-inflammatory and thrombolytic activity using *In-vitro* models.

MATERIAL AND METHOD

Plant Collection

Dried calyx of Hibiscus sabdariffa plant purchased by local herbalists in Erbil city were collected and authenticated by Pharmacognosy Department, Pharmacy College, Hawler Medical University.

Plant Extract Preparation

Dried calyx of *Hibiscus sabdariffa* plant introduced for successive extraction using different polarity solvents according to standard procedure protocols of ordinary reflux method. One hundred gram of dried calyx extracted using low polarity solvent petroleum ether (PE), the marc has been collected, dried and introduced for second extraction step using higher polarity solvent ethanol (70%) (ETH). The extracts were concentrated and dried. The dried extracts were reconstituted using distilled water forming a stock solution with concentration of (1mg\ml). Further serial dilution of the extracts were prepared ranging between (200-1000 mcg\ml) introduced in different assessments.

Estimation of *In-vitro* Anti-diabetic Activity

In vitro anti-diabetic activity of *Hibiscus sabdariffa* calyx have been estimated using glucose uptake by yeast cells method as described by Gupta et al, 2013[19]. Briefly, commercially available baker's yeast washed repeatedly using distilled water (3000xg: 5min.) until supernatant fluid were clear, the yeast cells were reconstituted using distilled water to make a suspension (10 % (v\v)). A mixture of (1ml) of glucose solution with concentrations (5mM, 10mM and 25mM) with different concentration (200, 400, 600, 800, 1000 mcg\ml) of both extracts were incubated for 10 min at 37^0C. The reaction mixture was started by the addition of (0.1 ml) of yeast suspension and further incubation for 60 min. at 37^0C. After incubation period the tubes were centrifuged (2500gx 5 min). Metformin drug incorporated in the study as a standard positive control at similar concentration range of the extracts. The percentage of glucose uptake by yeast cells were estimated from recording the optical density of the supernatant using spectrophotometer at (630 nm) from following equation:

% Increase glucose uptake = (OD_s - OD_c \ OD_s) x 100

OD_s is the optical density of sample (extract or standard drug), OD_c is the optical density of control.

Estimation of *In-vitro* Anti-inflammatory Activity

Anti-inflammatory activity of plant extracts have been estimated from heat induced red blood cell membrane stabilizing activity following standard procedure protocol:

1. Preparation of Red Blood Cell (RBC) Suspension

Sample with volume (10ml) of fresh whole human blood from volunteers (with history of not taking non-steroidal anti-inflammatory drugs at least 7 days period) was collected in clean sterile

centrifuge tubes, then centrifuged for 10 min at 3000 rpm and washed with equal volume of normal saline three times. The volume of blood cells were measured and dilute with normal saline in percentage of 10% (v\v) [20, 21].

2. Heat Induced Haemolytic Test

A volume of (1ml) blood cell suspension and (1ml) of extracts at concentration range (200, 400, 600, 800 and 1000 mcg\ml) were mixed forming reaction mixture in separate tubes. The reaction mixture in centrifuge tubes were incubated at 56 ^{0}C water bath for 30 min, the tubes were cooled and centrifuged at 2500 rpm for 5 min. Aspirin drug and normal saline samples were incorporated in the study as standard control positive (at similar concentration range of extracts). The percentage of haemolysis inhibition of extracts were estimated from measuring the optical density of the supernatant of the reaction mixture after incubation period using spectrophotometer at 560 nm, which calculated from following equation[22]:

% of inhibition of haemolysis = 100 x (OD1-OD2 \OD1)

OD1 is the optical density of the control (the reaction mixture contains normal salin instead sample to be tested), OD2 is the optical density of the tested sample (extract or control positive).

Estimation of *In-vitro* Thrombolytic Activity

Fresh human blood sample (10ml) were taken from healthy human volunteers (n=10) without a history of taking anti-inflammatory and contraceptive for a minimum of 7 days period. A volume of (0.5 ml) blood samples were transferred to ten pre-weighed properly labelled eppendorf tubes in a sterile aseptic condition. Each filled properly labelled eppendorf tube were incubated at 37 ^{0} C for 45 min for clot formation process. After incubation period of clot formation the serum (yellow liquid) was removed without disturbing the clot using syringe and the tubes were reweighed. The clot weight was estimated from the following equation:

Weight of clot= weight of clot filled tube - weight of empty tube

A volume of (0.1ml) of each concentration of different extracts of *H. sabdariffa* at concentration range (200, 400, 600, 800 and 1000 mcg\ml) have been added to each eppendorf tube, and incubated further more for 90 min at 37^{0}C. Commercially available Streptokinase (SK) of 1500000 I.U (Abott) drug and distilled water were incorporated in the study as control positive and negative respectively. The supernatant fluid released from the clot lysis after incubation period were removed (yellow liquid) using syringe without disturbing the clot and the tubes were again re-weighed The percentage of clot lysis (thrombolytic activity) were calculated from the deviation in the clot weight during the two periods of incubation by the following equation[23, 24].

% clot lysis = (weight of released clot \ weight of clot) x 100

Statistical Analysis

All experiments were carried out in triplicate; the results were expressed as mean ± standard deviation (SD). Comparison between means with controls performed using one tail unpaired t-test and two way ANOVA methods using Graph pad prism 6 program considering (p value < 0.001) statistically significant.

RESULTS AND DISCUSSION

1. Estimation of *In-vitro* Anti-diabetic Activity

In-vitro anti-diabetic evaluation of petroleum ether and ethanolic extracts of *H. sabdariffa* showed a dose dependent manner activity for all tested concentration of extracts and different concentrations of glucose solution. Highest activity were exhibited at 25mM for both extracts and standard drug (metformin). Significant activity was exhibited by the ethanolic extract at concentration (1000 mcg\ml) in comparison with petroleum extract at same concentration (p value < 0.001) (Table.1, Table.2 and Table.3). The results showed promoting the glucose transference through yeast cells, consequently control glucose concentration. This activity will aid type II diabetes patients for controlling blood glucose level which characterized by low insulin level and elevated blood glucose. Studies in type II diabetes rats results where compatible with our findings, since *H. sabdariffa* extracts which decreases hyperglycaemia[25].

2. *In-vitro* Anti-Inflammatory Activity

Ethanolic extract of *H. sabdariffa* exhibited significant membrane stabilizing activity in comparison to the petroleum ether (p value<0.001). Highest activity were recorded at greatest concentration of both extracts (Figure 1). There were a linear manner exhibited by both extracts with concentration gradient. Mild activity of membrane stabilizing were expressed by the petroleum ether extract ranging (10.273±0.413 - 22.347± 1.049 %) and moderate activity of ethanolic activity ranging (29.573±0.709 - 55.040 ±1.659 %) in comparison with control positive (aspirin). The anti-inflammatory activity were recorded in many studies[26, 27].

3. *In-vitro* Thrombolytic Activity

There were continuous studies of phytochemical constituents from medicinal plant with cardio protection activities since they were safer and more economic than conventional drugs, as a part of the nature revolution for developing safe drugs. *H. sabdariffa* extracts showed significant activities in comparison to the control negative (distilled water) (p value < 0.0001). Generally the thrombolytic activity expressed by the petroleum ether extract were greater than that expressed by

the ethanolic extract at different tested concentrations. Mild to moderate activity were expressed by the petroleum ether extract in comparison to the control positive streptokinase.

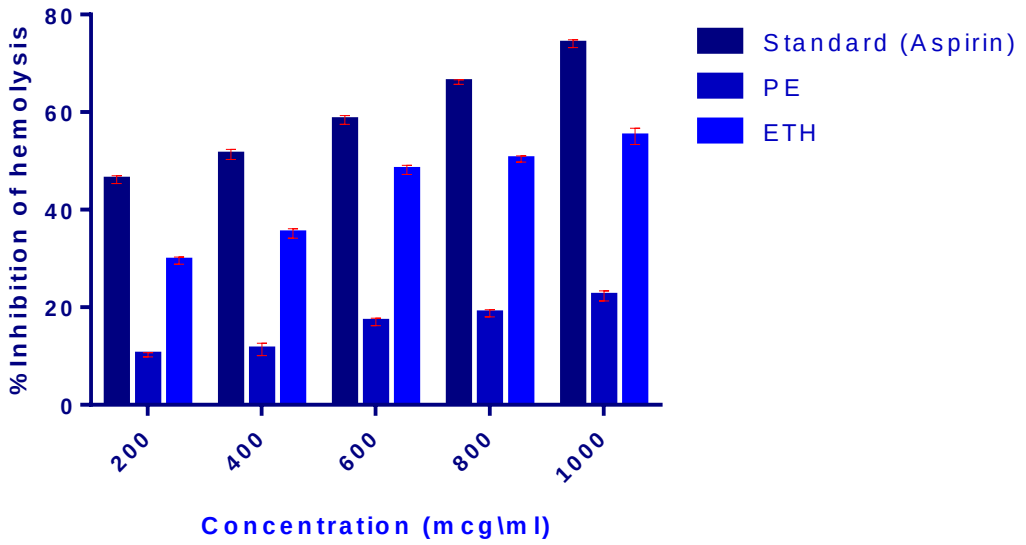

Figure 1: Percentage of Inhibition of Haemolysis by Different Extracts of *Hibiscus Sabdariffa* Plant and Standard Drug (Aspirin) at Different Concentration Range

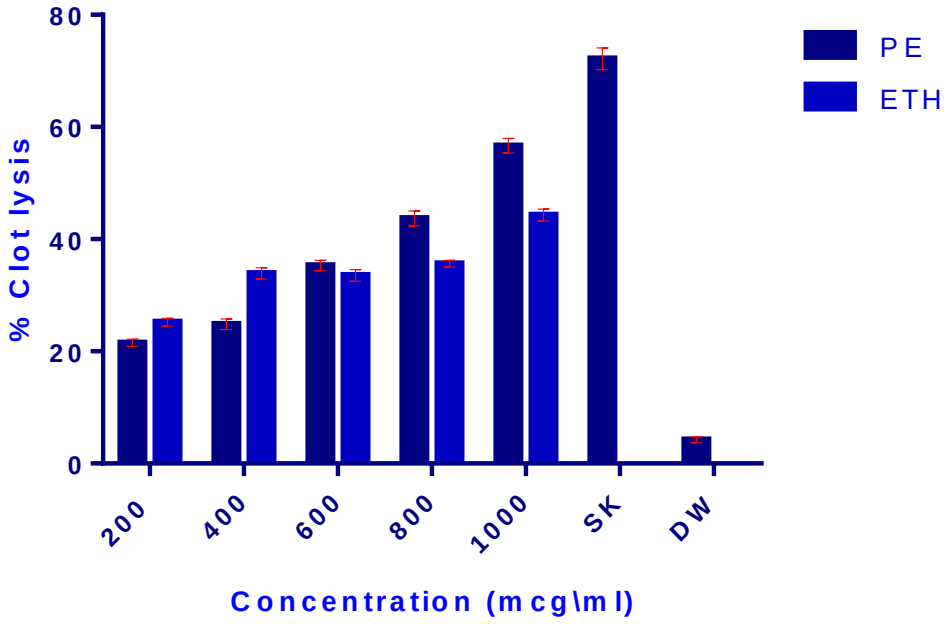

Figure.2: Percentage of Clot Lysis Exhibited by Ethanol and Petroleum Ether (PE) and Ethanol (ETH) Extract of *H. Sabdariffa* Plant along with Control Positive (SK) and Control Negative (DW)

Table 1: % Increase Glucose Uptake of Petroleum Ether and Ethanolic Extracts of *Hibiscus Sabdariffa* at 5m M Glucose Solution

Concentration (mcg\ml)	% Increase Glucose Uptake (Mean ± SD)		
	Standard (Metformin)	PE[1]	ETH[2]
1000	74.200 ± 1.873	41.639 ± 1.109	44.720 ± 1.050
800	61.213 ± 0.737	28.513 ± 2.501	42.587 ± 1.125
600	57.347 ± 1.035	22.060 ± 1.184	38.570 ± 0.816
400	55.427 ± 2.250	21.593 ± 0.970	35.247 ± 1.130
200	53.213 ± 0.990	19.517 ± 1.117	34.797 ± 0.940

1 Petroleum ether extract, 2 Ethanol extract, *n= 3*

Table 2: % Increase Glucose Uptake of Petroleum Ether and Ethanolic Extracts of *Hibiscus Sabdariffa* at 10m M Glucose Solution

Concentration (mcg\ml)	% Increase Glucose Uptake (Mean ±SD)		
	Standard (Metformin)	PE[1]	ETH[2]
1000	87.980 ± 0.766	68.380 ± 1.021	77.273 ± 0.547
800	87.420 ± 1.030	63.257 ± 0.681	74.837 ± 1.075
600	86.483 ± 0.931	61.177 ± 0.837	72.317 ± 0.799
400	84.240 ± 0.963	58.907 ± 0.721	65.897 ± 0.509
200	83.357 ± 1.070	57.730 ± 0.684	53.933 ± 0.351

1 Petroleum ether extract, 2 Ethanol extract, *n= 3*

Table 3: % Increase Glucose Uptake of Petroleum Ether and Ethanolic Extracts of *Hibiscus Sabdariffa* at 25mM Glucose Solution

Concentration (mcg\ml)	% Increase Glucose Uptake (Mean ±SD)		
	Standard (Metformin)	PE[1]	ETH[2]
1000	95.203 ± 0.136	75.120 ± 0.737	77.050 ± 0.330
800	94.870 ± 0.704	73.370 ± 1.005	75.410 ± 1.145
600	94.923 ± 0.612	69.647 ± 0.405	74.836 ± 0.430
400	94.203 ± 0.895	66.910 ± 0.442	72.953 ± 0.178
200	93.613 ± 1.188	65.053 ± 0.705	65.183 ± 1.002

1 Petroleum ether extract, 2 Ethanol extract, *n= 3*

Table 4: Percentage of Clot Lysis Exhibited by Petroleum Ether Extract of *Hibiscus Sabdariffa*

Concentration (mcg\ml)	% Clot lysis	t-value	P value (In comparison to the control negative)
1000	44.293 ± 0.703	57.685	<0.0001
800	35.634 ± 1.015	69.024	<0.0001
600	33.910 ± 0.586	44.027	<0.0001
400	33.550 ± 1.026	44.932	<0.0001
200	25.203 ± 1.081	41.366	<0.0001

1 mean ± SD, *n=3*

Table 5: Percentage of clot lysis exhibited by ethanol extract of *Hibiscus sabdariffa*

Concentration (mcg\ml)	% Clot lysis	t- value	P value (In comparison to the control negative)
1000	56.663 ± 1.314	64.148	<0.0001
800	43.683 ± 1.333	47.661	<0.0001
600	35.314 ± 0.935	50.013	<0.0001
400	24.850 ± 0.935	33.256	<0.0001
200	21.533 ± 0.656	35.605	<0.0001

1 mean ± SD, *n=3*

Table.6: Percentage of clot lysis of control positive (Streptokinase (SK)) and control negative (Distilled water (DW))

Control	% Clot lysis[1]
Streptokinase	72.164 ± 1.945
Distilled water	4.245 ± 0.526

1 mean ± SD, *n=3*

CONCLUSION

In an attempt to canvass the pharmacological activities attributed by the different polarity extracts of Hibiscus sabdariffa calyx, the extracts showed a linear manner of activity with concentration gradient in tested aspects of the pharmacological parameters.

ACKNOWLEDGEMENTS

Research author was very gratefully thanks members from Ministry of Health for their cooperation for preceding the research works, and Pharmacognosy Department for encouraging me to perform the research.

CONFLICT OF INTEREST

There was no any conflict of interest to be declared by the author.

REFERENCES

1. Dinesh K, Mitra A, Manjunatha M. In vitro and in vivo studies of antidiabetic Indian plants: A review. Journal of Herbal Medicine and Toxicology 2009; 3(2): 0973-4643.
2. Yamamoto J, Yamada K, Naemura A, Yamashita T, Arai R. Testing various herbs for antithrombotic effect. Nutrition 2005; 2: 580–587.
3. Sushil KM, Talambedu U, Pranav T, Kirti YM, Tarun J, Bhargavi B, Yogini PM, Veena P. An In vitro studies on indigenous ayurvedic plants, having hypoglycemic activity. Asian Pacific Journal of Tropical Disease 2012; 46-49.
4. Marles RJ, Farnsworth NR. Plants as sources of antidiabetic agents. Economic and medicinal plant research 1994; 6: 149-187.

5. Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Braz J Med Biol Res 2000; 33(2).
6. Mohamed B, Abderrahim Z, Hassane M, Abdelhafid T, Abdelkhaleq L. Medicinal plants with potential antidiabetic activity - A review of ten years of herbal medicine research (1990-2000). Int J Diabetes & Metabolism 2006; 14: 1-25.
7. HerbalGram. Reliable herbal medicine information. The American Botanical Council 1997; 40:21.
8. Sangita C, Priyanka C, Protapaditya D, Sanjib B. Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. Asian Pacific J Tropical Biomedicine 2012; 178- 180
9. Sai SY, Mamata P, Divya GC, Beena DB. Evaluation of in vitro thrombolytic activity of phytochemicals in *Bacopa monnieri* Linn. J Pharm Res 2012; 5(1):100-101.
10. Apurba SA, Fariha AC, Farjana K, Jamaluddin ATM, Atiqul HPAP. Phytochemical Screening and In vitro Evaluation of Pharmacological Activities of *Aphanamixis polystachya* (Wall) Parker Fruit Extracts. Tropical Journal of Pharmaceutical Research 2013; 12 (1): 111-116.
11. Mucklow JC. Thrombolytic treatment – streptokinase is more economical than Alteplase. British Medical J 1995; 311: 1506.
12. Sweta P, Rajpal SK, Jayant YD, Hemant JP, Girdhar MT, Hatim FD. Effect of *Fagonia Arabica* (Dhamasa) on in vitro thrombolysis. BMC Complementary and Alternative Medicine 2007; 7: 36.
13. Rouf SA, Moo-Young M, Chisti Y. Tissue-type plasminogen activator: characteristics, applications and production technology. Biotechnology Advances 1996; 14: 239-226.
14. Galib BS, Behera K, Kar AC. Lithiotripic activity of swadamstradi kwatha seadamstradi chan Vati-an experimental evaluation. JDRAS 2006; 27: 8-18.
15. Odigie IP, Ettarh RR, Adigun SA. Chronic administration of aqueous extract of *Hibiscus sabdariffa* attenuates hypertension and reverses cardiac hypertrophy in 2K-1C hypertensive rats. J Ethnopharmacol 2003; 86:181-185.
16. Wang CJ, Wang JM, Lin WL, Chu CY, Chou FP, Tseng TH. Protective effect of Hibiscus anthocyanins against tert-butyl hydroperoxide–induced hepatic toxicity in rats. Food Chem Toxicol 2000; 38: 411-416.
17. Lana YM, Sayran HH, Saffa TH. Estimation of bacterial growth inhibition and antibiofilm activity of Hibiscus *sabdariffa* plant extracts against standard pathogenic bacteria. World J Pharm Sci 2015; 3(8): 1525-1530.

18. Daffallah AA, Al-Mustafa Z. Investigation of the anti-inflammatory activity of the *Acacia nilotica* and *Hibiscus sabdariffa* . Am J Chin Med 1996; 24(3-4): 263-269.
19. Gupta D, Kondongala SC, Girish P et al. In vitro antidiabetic activity of pentacyclic tritrprnoida and fatty acid ester from bauhinia purpurea. Int J Pharmacol Pharma Technol 2013; 2(1).
20. Sakat S, Juvekar AR, Gambhire MN, et al. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. I. J. Pharm. Pharm. Sci. 2010; 2(1): 146-155.
21. Govindappa M, Naga Sravya S, Poojashri MN, Sadananda TS, Chandrappa CP. Antimicrobial, antioxidant and *in vitro* anti-inflammatory activity of ethanol extract and active phytochemical screening of *Wedelia trilobata* (L.) Hitchc. J Pharmacognosy and Phytotherapy 2011; 3(3): 43-51.
22 Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on anti-diabetic medicinal plants having insulin mimetic Property. Asian Pacific J Tropical Biomedicine 2012; 320-333.
23 Sweta P, Rajpal SK, Jayant YD, Hemant JP, Girdhar MT, Hatim FD. Effect of *Fagonia arabica* (Dhamasa) on in vitro thrombolysis. BMC Complement Altern Med 2007; 7: 36.
24 Daginawala HF, Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM. Development of an *In vitro* model to study clot lysis activity of thrombolytic drugs. Thromb J 2006; 4: 14.
25 Peng CH, Chyau CC, Chan KC, Chan TH, Wang CJ, Huang CN. *Hibiscus sabdariffa* polyphenolic extract inhibits hyperglycemia, hyperlipidemia, and glycation-oxidative stress while improving insulin resistance. J Agricultural and Food Chemistry 2011; 59(18): 9901–9909.
26 Ogundipe OO, Moody JO, Oluwole FS, Fakeye TO. Anti-inflammatory and antimicrobial activities of selected Nigerian Plant foods. In Proceedings of the 1st International Workshop on Herbal Medicinal Products. University of Ibadan, Ibadan 1998; 138–146.
27 Beltran-Debon R, Alonso-Villaverde C, Aragones J, Rodriguez-Medina I, Rull A, Micol V *et al*. The aqueous extract of *hibiscus sabdariffa* calices modulates the production of monocyte chemo attractant protein-1 in humans. J Phytomedicine 2010; 17(3-4): 186-191.

AJPTR is

- **Peer-reviewed**
- **bimonthly**
- **Rapid publication**

Submit your manuscript at: editor@ajptr.com