IJPRT
Vol.5 No.2 July-December 2013, pp. 97-105

Biological Investigations of the Leaf Extracts of Carissa Carandas

Yakut Bint-e-Sadek[1], Naiyyum Choudhury[1] and Mohammad Shahriar[2]
[1]Department of Mathematics and Natural Sciences, BRAC University, Dhaka, Bangladesh
[2]Department of Pharmacy, University of Asia Pacific, Dhaka, Bangladesh
Email: shahriar_12@yahoo.com, shahriar@uap-bd.edu

Abstract:-*Plant-based medicines play an important role in all cultures, and have been indispensable in maintaining health and combating diseases. Owing to the global trend towards improved quality of life, there is a great demand for medicinal plants. Various parts of this plant have long been used in traditional system of medicine. Hence the present study has been conducted in the light of immense potential of medicinal plants used in various traditional systems. The purpose of the study was to determine the different phytochemical compounds of leaf extracts of Carissa carandas, their antioxidant properties, antimicrobial activities and cytotoxic potentials. Here, ethanolic and n-hexane extracts of the leaves of Carissa carandas were used. Results showed significant antioxidant activities compared to ascorbic acid and BHT in DPPH free radical scavenging with IC_{50} of 1.292 µg/ml and 1.824 µg/ml of ethanolic extract and n-hexane extract. H_2O_2 scavenging activities of the extracts were found to be better than the standard, having IC_{50} values higher than ascorbic acid. Total antioxidant activity and total phenolic content were also determined. Leaf extracts showed no antimicrobial activity through disk diffusion method using extracts ranging from 0.1- 400 µg/disc when compared to Kanamycin disc of 30 µg/disc. Cytotoxic activities of the extractives were comparable to vincristine sulfate, having IC_{50} values of 2.818 and 1.995 of ethanolic extract and n-hexane extract respectively. The present study provided data justifying the use of this plant for medicinal purposes.*

Key words: *Carissa carandas, antioxidant activity, free radical scavenging, antimicrobial activity, cytotoxic activities*

1. INTRODUCTION

For decades, the utilization of herbal plants has drawn avalanche of interest as they could accommodate therapeutic response and are promising candidate to be developed as pharmaceutical products. Presently, complication has arises in severity and extent in combating bacterial and fungal infections in behalf of the development of bacteria and fungi resistant to many current antibiotics [1, 2]. Free radicals have been accused of initiating many serious diseases [3-7]. These free radicals drive oxidative stress and transform the pathophysiological condition of the patient by acting on immune system. It has been known that phenolic and flavonoid compounds of the plant extracts are responsible for antioxidant and antibacterial effects [8-10].

Carissa carandas is a species of flowering shrub in the dogbane family, Apocynaceae. It produces berry-sized fruits that are commonly used as a condiment or additive to Indian pickles and spices. It is a hardy, drought-tolerant plant that thrives well in a wide range of soils. Common names include Koromcha in Bangla and Karanda in English. Its botanical name was in recent years changed to *Carissa congesta* Wight (syn. *C. carandas* Auct formerly widely shown as *C. carandas* L.) [11].

Phytochemical screening of the root extract showed that the crude extract contained small quantities of alkaloids, flavonoids, saponins and large amounts of cardiac glycosides, triterpenoids, phenolic compounds and tannins. [12].

Earlier studies have shown that the extract of the plant possesses cardiotonic, antipyretic and antiviral activity [13-15]. Various cardiac glycosides, a triterpenoidal constituent carissone and β-sitosterol were reported from the root extract of the plant [16]. In Western Ghats region of India, the decoctions and extracts of the roots of this plant are effective remedies in the management and/or control of convulsions and epilepsy. However studies showed that root extract of *C. carandas* may produce its anticonvulsant effects via non-specific mechanisms [12].

In traditional system of medicine the plant is used as an anthelmintic, astringent, appetizer and antipyeretic in stomach disorders, rheumatism, disease of the brain, in biliousness and biliary dysfunction [17].

It is used by tribal healers of Western Ghats region of Karnataka to treat liver diseases. And scientific study showed that crude extract of of the roots of *Carissa carandas* showed hepatoprotective activity against CCl_4 and paracetamol induced hepatic oxidative stress in Wistar albino rats [18].

Recent studies indicated that the ethanol and aqueous extracts from roots of *C. carandas* possess significant analgesic, anti-inflammatory and antipyretic activities in rodent models. [19].

Experimental data also indicated that the uripe *Carissa carandas* Linn fruit extract of has significantly lowered the elevated blood glucose levels comparable to diabetic control. [20].

Leaf and unripe fruit extracts of the plant has shown good anticancer activities according to recent studies. [21]. No studies have carried out to determine the cytotoxic activity of *Carissa carandas* extracts. The present study includes data on cytotoxicity of leaf extracts of *Carissa carandas.*

Antioxidant activities of unripe fruit of *Carissa carandas* has been reported [21], but no antioxidant activities of leaf extracts of *Carissa carandas* has been reported. The current study also includes antioxidant activities of *Carissa carandas* leaf extracts.

2. MATERIALS AND METHODS

Plant Materials

Plant sample (leaves) *Carissa carandas* was collected from Norshingdi in July 2012 and submitted to the National Herbarium, Mirpur for identification with an Accession Number: DACB 37533**.** Leaves were sundried for 7 days and later dried in drier at 40°C for about an hour. The dried leaves were then ground into powder using high capacity grinding machine and stored in airtight plastic container with necessary markings for identification and kept in cool, dark and dry place for the investigation.

The sun dried and powdered plant parts (500 gm) of *Carissa carandas* was successively extracted in a Soxhlet extractor at 50°C-60°C temperature using 250 ml of ethanol followed by n-Hexane. All extracts were filtered individually through filter paper and poured on Petri dishes to evaporate the liquid solvents from the extract to get dry extracts. The dry crude extracts were weighed and stored in air-tight container with necessary markings for identification and kept in refrigerator (0-4)°C for future investigation.

Preliminary Phytochemical Screening

One gram of the methanol extract of *Carissa carandas* was dissolved in 100 ml of methanol and was subjected to preliminary phytochemical screenings for determining nature of phytoconstituents [22,23].

IJPRT
Vol.5 No.2 July-December 2013, pp. 97-105

Antimicrobial Activity

The antimicrobial screening, which is the first stage of antimicrobial drug discovery, was performed by the disc diffusion method [24] against some gram positive and gram negative bacteria (Table 2) collected as pure cultures from the department of microbiology, University of Dhaka, Bangladesh. Standard disc of Kanamycin (30 µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control, respectively. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in mm [25].

Free Radical Scavenging Activity

The free radical scavenging activity of the leaf extract of *Carissa carandas* was evaluated by the published method [26,27]. DPPH was used to evaluate the free radical scavenging activity (antioxidant potential).

H_2O_2 Scavenging Activity

Scavenging activity of extract and its sub-fractions were evaluated by hydrogen peroxide [28]. % inhibition of the H_2O_2 scavenging was measured by using the following equation:

$$\% \ inhibition = \left(\frac{Absorbance\ of\ Control - Absobance\ of\ Extract}{Absorbance\ of\ Control}\right) \text{ x } 100$$

Determination of Total Antioxidant Capacity

The total antioxidant capacity was evaluated by the phosphomolybdenum method [29]. 0.3 ml of extract and sub-fraction in ethanol, ascorbic acid used as standard (5-200 µg/ml) and blank (ethanol) were combined with 3 ml of reagent mixture separately and incubated at 95°C for 90 minutes. After cooling to room temperature, the absorbance of each sample was measured at 695 nm against the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid and was calculated by the following equation:

$$A=(c\times V)/m$$

Where, A = total content of Antioxidant compounds, mg/gm plant extract, in Ascorbic acid Equivalent c = the concentration of Ascorbic acid established from the calibration curve, mg/ml, V = the volume of extract in ml, m = the weight of crude plant extract, gm.

Total Phenolics Analysis

Total phenolic content of the leaf extract of *Carissa carandas* was measured employing the method described by Skerget *et al*, (2005) [30] involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard.

Brine Shrimp Lethality Bioassay

Brine shrimp lethality bioassay [31,32] technique was applied for the determination of general toxic properties of the plant extractives. DMSO solutions of the samples were applied against *Artemia salina* in a 1-day *in vivo* assay. For the experiment, 4 mg of each of the ethanol and n-hexane soluble fractions were

dissolved in DMSO and solutions of varying concentrations (100, 50, 25, 12.50, 6.25, 3.125, 1.563 µg/ml) were obtained by serial dilution technique using DMSO. Vincristine sulphate was used as positive control.

3. RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

In preliminary phytochemical screening, the methanol extract of leaf of *Carissa carandas* demonstrated the presence of alkaloids, carbohydrate, unsaturated sterols, phenolics and saponins (Table 1).

Table 1: Qualitative analysis of Alkaloid, Carbohydrate and Glycoside of the leaf extracts of *Carissa carandas*

Tests	**Extracts**	
	Ethanol	**n-Hexane**
Test for alkaloids		
Hager's Test	**+**	**+**
Test for carbohydrates		
Molish's Test	**+**	**+**
Fehling's Test	**+**	**+**
Glycosides	**+**	**N/A**
Test for unsaturated sterols		
Leiberman Burchard's Test	**+**	**+**
Test for Phenolic Compounds		
Ferric Chloride Test	**-**	**-**
Alkaline Reagent Test (flavonoids)	**+**	**-**
Lead Acetate	**+**	**-**
Gelatin Test	**-**	**-**
Test for Saponins		
Saponins	**+**	**-**

[+: presence; - : Absence.]

IJPRT
Vol.5 No.2 July-December 2013, pp. 97-105

Antimicrobial Activity

The leaf extracts of *Carissa carandas* when subjected to antimicrobial screening at 0.1 µg/disc to 400 µg/disc the ethanol extract (EE) and n-hexane soluble fraction (nE) revealed antimicrobial activity against the tested microorganisms (Table 2).

Table 2: Results of antimicrobial activity of extracts of leaves of *Carissa carandas*

Name of Organisms	Concentrations													
	Standard Kanamycin (30 µg/disc) (mm)	0.1 µg/disc		0.5 µg/disc		1 µg/disc		30 µg/disc		50 µg/disc		100 µg/disc		400 µg/disc	
		EE	nE	EE	nE	EE	nE	EE	nE	EE	nE	EE	nE	EE	nE
S. aureus	26.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B. subtilis	28	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B. cereus	26.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. coli	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V. cholerae	29	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Free Radical Scavenging Activity

All the extractives of *Carissa carandas* were subjected to free radical scavenging activity using DPPH by using ascorbic acid (ASA) and *tert*-butyl-1-hydroxytoluene (BHT) as reference standards (Table 3). In this investigation, the ethanol and n-hexane extract showed significant free radical scavenging activity with IC_{50} value of 1.292 µg/ml and 1.824 µg/ml respectively.

Table 3: Table to show the IC_{50} values of ethanol and n-Hexane extracts and standards of DPPH free radical activity test

Name of Standard and Sample	IC_{50} (µg/ml)
Ethanol	1.292
n-hexane	1.824
Ascorbic Acid (ASA)	0.733
Butylated Hydroxy Toluene (BHT)	0.783

H_2O_2 Scavenging activity

All the extractives of *Carissa carandas* were subjected to free radical scavenging activity using DPPH by using ascorbic acid (ASA) as reference standards (Table 4). In this investigation, the ethanol showed significant free radical scavenging activity with IC_{50} value of 2.038 µg/ml.

Table 4: Table to show the IC_{50} values of ethanol and n-Hexane extracts and standards of H_2O_2 scavenging test

Name of Standard and Sample	IC_{50} (µg/ml)
Ethanol	2.038
n-hexane	1.802
Ascorbic acid	1.78

Total Antioxidant Capacity

Total antioxidant capacity of the different extracts of *Carissa carandas* was evaluated by the phosphomolybdenum method and was expressed as ascorbic acid equivalents (AAE) per gram of plant extract. Total antioxidant capacity of the test samples was calculated using the standard curve of ascorbic acid (y = 0.002x + 0.001; R2 = 0.997). n-hexane extract of *Carissa carandas* was found to possess the highest total antioxidant capacity (Table 5).

Table 5: Table to show the values of ethanol and n-hexane extracts and standards of Total Antioxidant Activity test

Name of Extracts	**Total Anti-oxidant capacity (mg/gm), Ascorbic Acid Equivalent**
Ethanol	3.61±1.74
n-hexane	4.88±3.44

Values are the mean of duplicate experiments and represented as mean ± SD

Total phenolics analysis

All the extractives of *Carissa carandas* were tested for total phenolic content. The highest phenolic content was found in ethanolic extracts (8.02±2.24 mg/gm of GAE/gm of extractives) (Table 6).

Table 6: Table to show the values of ethanol and n-Hexane extracts and standards of Total Phenolic Content

Name of Extracts	**Total Phenolic Content (mg/gm), Gallic Acid Equivalent**
Ethanol	8.02±2.24
n-hexane	6.11±1.34

Values are the mean of duplicate experiments and represented as mean ± SD

Brine shrimp lethality bioassay

In the brine shrimp lethality bioassay, the LC50 values of ethanol and n-hexane extracts of *Carissa carandas* were found to be 2.818 and 1.995 respectively (Table 7).

Table 7: LC_{50} values of the five extracts of *Carissa carandas* and Standard

Test Samples	**Regression Line**	**R^2**	**LC_{50}**
Vincristine sulfate	y = 29.79x + 64.62	0.927	0.927
Ethanol extract	y = 35.59x + 33.818	0.961	2.818
n-hexane extract	y = 29.65x + 36.03	0.976	1.995

4. CONCLUSION

Herbal medicine is still the mainstay of maximum world population; mainly in the developing countries for primary healthcare not because they are inexpensive but also for better cultural acceptability, better compatibility with the human body and minimal side effects. The identification of active principles and their molecular targets from traditional medicine provides an enormous opportunity for drug development. The current study takes us a step ahead in the process of drug development as well as new validated treatment of a traditionally used medicinal plant. *Carissa carandas* a widely available medicinal

plant in Bangladesh as well in other parts of Asia has shown remarkable antioxidant activities. Although its leaf extracts did not show any antimicrobial activity through the method used in the present study but other researches showed that it has antimicrobial activities. The leaf extracts also showed some level of cytotoxic activities on *Artemia salina.* Hence, it could be concluded that the ethanolic and n-Hexane extracts of leaves of *Carissa carandas* has antioxidant and cytotoxic activities when extracted through hot extraction process.

REFERENCES

1. Aderogba MA, Okoh EK, Idowu TO. Evaluation of antioxidant activity of the secondary metabolites from *Poliostigma reticulatum* (DC) hochst. Journal of Biological Sciences 2005; 5:239-242.
2. Rabaud C, Tronel H, Fremont S, May T, Canton P, Nicolas JP. Free radicals and HIV infection. Annales de Biologie Clinique 1997; 55: 565-571.
3. Malorni W, Rivabene R, Lucia BM, Ferrara R, Mazzone AM, Cauda R, Paganelli R. The role of oxidative imbalance in progression to AIDS Effect of the thiol supplier *N-acetylcysteine*. AIDS Research and Human Retroviruses 1998; 14(17): 1589-1596.
4. Robert A and Meunier B. Is Alkylation the Main Mechanism of Action of the Antimalarial Drug Artemisinin? Chemical Society Review Articles 1998; 27(4): 273-274.
5. Pauli GF, Case RJ, Inui T, Wang Y, Cho S, Fischer HH, Franzblau SG. New perspectives on natural products in TB drug research. Life Sciences 2005; 78(5): 485-494.
6. Shah PM. The need for new therapeutic agents: What is in the pipeline? Clinical Microbiology and Infection 2005; 11(3): 36-42.
7. Rios JL, Recio MC. Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology 2005; 100(1&2): 80-84.
8. Da-Silva JFM, De-Souza MC, Matta SR, De-Andrade MR, Vidal FVN. Correlation analysis between phenolic levels of *brazilian propolis* extracts and their antimicrobial and antioxidant activities. Food Chemistry 2006; 99: 431-435
9. Majhenic L, Skerget M, Knez Z. Antioxidant and antimicrobial activity of *guarana* seed extracts. Food Chemistry 2007; 104(3): 1258-1268.
10. Pereira JA, Oliveira I, Sousa A, Valentao P, Andrade PB. Walnut (*Juglans regia* L.) leaves: Phenolic com-pounds, antibacterial activity and antioxidant potential of different cultivars. Food and Chemical Toxicology 2007; 45: 2287-2295.
11. Morton, J. *"Fruits of Warm Climates"*. Miami, FL: Julia F. Morton, 1987.
12. Hegde K, Thakker SP, Joshi AB, Shastry CS, Chandrashekhar KS. Anticonvulsant activity of *Carissa carandas Linn.* root extract in experimental mice. Tropical Journal of Pharmaceutical Research 2009; 8(2): 117-125
13. Dhawan BN, Patnaik GK. Investigations on some new cardio-active glycosides. Indian Drugs 1985; 22(6): 285-290.
14. Rajasekaran A, Jeyasudha V, Kalpana B, Jayakar B. Preliminary phytochemical and antipyretic evaluation of *Carissa carandas*. Indian J Nat Prod 1999; 15(1): 27-29.
15. Taylor RSL, Hudson JB, Manandhar NP, Tower GHN. Antiviral activities of medicinal plant of Southern Nepal. J Ethnopharmacol 1996; 53(2): 97-104.
16. Rastogi RC, Vohra MM, Rastogi RP, Dhar ML. Studies on *Carissa carandas* Linn. Part I. Isolation of the cardiac active principles. Indian J Chem 1966; 4: 132.
17. Kirtikar KR, Basu BD. Indian medicinal plant. Volume II, Lalit Mohan Basu, Allahabad, 2003, pp 1546.

18. Hegde K, Joshi AB. Hepatoprotective effect of *Carissa carandas* Linn. Root Extract against CCL_4 and Paracetamol induced Hepatic Oxidative Stress. Indian Journal of Experimental Biology 2009; 47: 660-667.
19. Balakrishnan N, Bhashkar VH. Analgesic, anti-inflammatory and antipyretic activities of *Pergularia daemia and Carissa carandas*. DARU 2009; 17(3): 168-174.
20. Itankar PR, Lokhande SJ, Verma PR, Arora SK, Sahu RA, Patil AT. Antidiabetic potential of unripe *Carissa Carandas* Linn. Fruit extract. Journal of Ethnopharmacology 2001; 135(2): 430-433.
21. Sulaiman SF, Wong ST, Ooi KL, Yusof SR, Tengku M, Tengku S. Anticancer Study of *Carissa carandas* extracts. Project Report. USM, 2008.
22. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. 3rd edn. Chapman and Hall Int Ed., New York, 1998.
23. Kokate CK. Pharmacognosy. 16th edn. Nirali prakashani, Mumbai, India, 2001. Ayafor JF. Limonoids and phytol derivatives from *Cedrela sinensis.* Tetrahedron 1972; 28: 9343.
24. Bauer AW, Kirby WMM, Sherries JC and Tuck M. Antibiotic susceptibility testing by a standardized disc diffusion method. Am J Clin Pathol 1966; 45: 493-496.
25. Choi HY, Jhun EJ, Lim BO, Chung IM, Kyung SH, Park DK. Application of flow injection chemilumineacence to the study of radical scavenging activity in plants. Phytother Res 2000; 14: 250-253.
26. Desmarchelier C, Repetto M, Coussio J, Liesuy S, Ciccia G. Antioxidant and pro-oxidant activities in aqueous extracts of Argentine plants. Int J Pharmacog 1997; 35: 116-120.
27. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical Biochemistry 1999; 269(2): 337-341.
28. Jayaprakasha GK, Jaganmohan RL, Sakariah KK. Antioxidant activities of flavidin in different *in vitro* model systems. Bioorg Med Chem 2004; 12: 5141-5146.
29. Skerget M, Kotnik P, Hadolin M, Hras A, Simonic M, Knez Z. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chem 2005; 89: 191-198.
30. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen JB, Nicholsand DE, Mclaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med 1982; 45: 31-34.
31. Maclaughlin JL, Anderson JE, Rogers LL. The use of biological assays to evaluate botanicals. Drug Info Jour1998; 32: 513-524.

IJPRT
Vol.5 No.2 July-December 2013, pp. 97-105

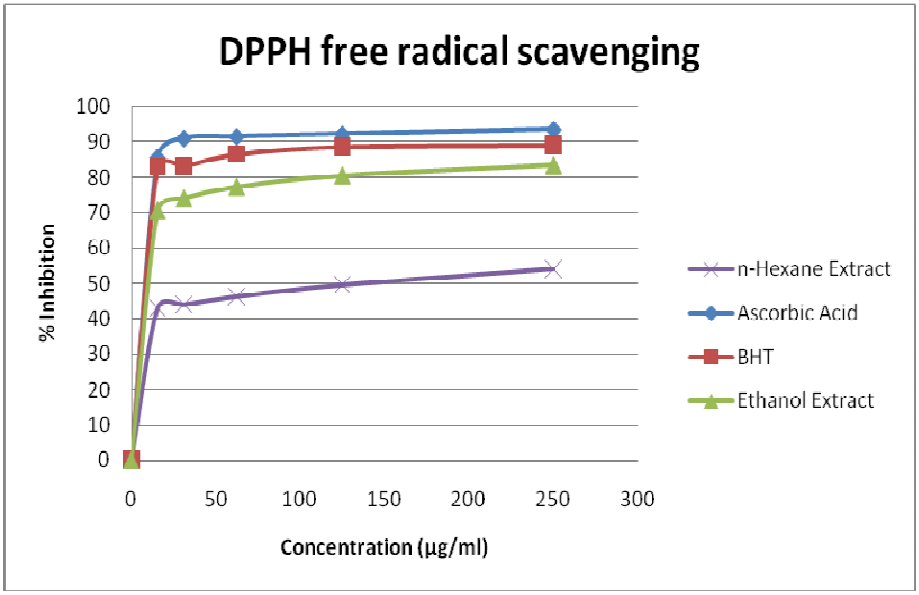

Figure 1: DPPH free radical Scavenging

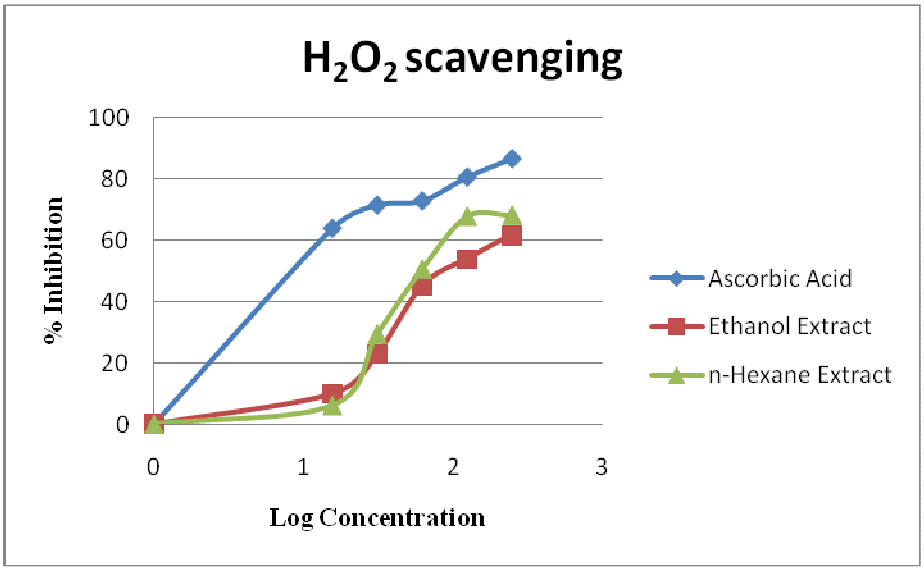

Figure 2: H_2O_2 scavenging