

In Vitro Free Radical Scavenging Activity and Antimicrobial Activity of Two Varieties of *Cyamopsis tetragonoloba* L

Ms. Priya Kumari Jain^{1*} & Dr. Shilpi Rijhwani²

Research Scholar, Department of Botany, ICG, The IIS University, Jaipur, Rajasthan

Head Department of Botany, ICG, The IIS University, Jaipur, Rajasthan

ABSTRACT

Cyamopsis tetragonoloba L. (Fabaceae) commonly known as guar is widely used as a traditional medicine in India for curing various disease and also finds use in modern industrial processes as a source of commercially important gum. In the present study, the plant samples were identified and collected from Rajasthan Agricultural Research Institute Durgapura Jaipur, India. The present work focusses on evaluation of antioxidant and antimicrobial activity of different parts of two varieties of *Cyamopsis tetragonoloba* L. (M83 and RGC 1038) The extraction was done by hot soxhlet method using a range of solvents like n-hexane, chloroform, ethyl acetate, methanol and water. The antioxidant properties of all extracts of guar were evaluated by DPPH free radical scavenging assay method. The antimicrobial activity was analysed by agar disc diffusion method against Gram positive, Gram negative bacteria and fungi. The results suggest that none of tested extracts of both variety of guar showed any type of antimicrobial activity against the selected micro-organism so they cannot be explored further as antimicrobial agents. But different extracts, showed different levels of antioxidant activity so they can be explored as a natural source of antioxidants for curing various ailments.

Key words

Cyamopsis tetragonoloba (Guar), M83, RGC 1038, antioxidant activity, antimicrobial activity.

1. INTRODUCTION

India is a rich country in term of medicinal and aromatic plant wealth (Chandra *et al*, 2012). Plants have been connected with human health from time immemorial. They have been an important reservoir of medicine since human civilization came into being (Kajaria *et al*, 2011). In present years, human pathogenic micro-organisms have developed multiple drug resistance due to inappropriate use of these drugs against treatment of infectious disease. Medicines derived from plants play an important role to cover the basic health issues in the developing countries for the treating old and emerging disease because they are better and safer than synthetic drugs that create problems like side effects and carcinogeneity. Therefore, the interest in search for natural products with pharmaceutical properties has increased considerably. This is because of better acceptability, affordability, effectiveness, availability, compatibility and low toxicity with human body (Benmehdi *et al*, 2012). According to few reports, 122 components which have till date significant biological activity have been derived from 94 plant species. About 2,50,000 flowering plants are present on earth but only 6% have been tested for biological activity (Turker and Usta, 2008). Consequently many pharmaceutical companies are involved in research on plant material which have potential medicinal value with fewer side effects as compared to other systems of medicine (Rao and Sansui, 2001). Free radicals are involved in several degenerative diseases such as diabetes, arthritis, cancer and aging. The harmful effects of free radicals on living organisms could be attenuated by antioxidants. The antioxidants are capable of inhibiting the oxidation of biomolecules by removing free radical intermediates and inhibiting other oxidation reactions. Several commercially available synthetic antioxidants are currently in use but they also show toxic effect on human health and environment. Beans are powerhouse of nutrition because they have soluble fiber and carbohydrates. Beans are digested slowly thus, they provide a stabilizing effect on blood sugar and act as an antidiabetic and hypocholesterolemic agents. They also exhibit anticancerous and antimicrobial activity. The medicinal properties of plants are because of their chemical constituents or groups of compounds that have a definite physiological action to protect themselves from continuous attack of naturally occurring pathogens. These non-nutritive plant chemicals have disease preventing property so that they can protect themselves and

humans against disease (Badugu *et al*, 2012). Such chemical substances are known as secondary metabolites. These phytochemicals are flavanoids, alkaloids, terpenoids, steroids, tannins and phenolic compounds. Extraction and characterization of such bioactive compounds from plants have given birth to the some high profile drugs.

Cyamopsis tetragonoloba commonly known as guar has been grown in India since ancient time for vegetable and fodder purposes. At present, it is grown more for mucilage gum production. In India it is grown in Rajasthan, Gujarat, Haryana and Punjab, Uttar-Pradesh, Madhya Pradesh and Orissa. It belongs to family Fabaceae. It works as an appetizer, cooling agent, digestive aid, laxative, and is useful in dyspepsia and anorexia and has anti-ulcer, anti-secretory, cytoprotective, hypoglycemic, hypolipidemic and anticancerous properties. The pod contains condensed tannins, p-coumaric, caffeic, gallic and gentisic acids, quercetin, kaempferol, 3-arabinoside, p-hydroxycinnamyl, coniferyl and coniferyl alcohol. It also shows antibacterial and antihelminthic activity and is used in diabetes, asthma, inflammation, obesity (Mukhtar *et al*, 2004).

In the present work, antimicrobial and antioxidant activity was carried out on different parts of *Cyamopsis tetragonoloba* L. variety RGC 1038 and M83.

2. MATERIAL AND METHOD

2.1 Plant Material

Fresh guar seeds and leaves of two certified varieties (RGC1038 & M83) were procured from Rajasthan Agriculture Research Institute Durgapura Jaipur. These fresh seeds and leaves were washed in tap water, air dried and then homogenized to fine powder and stored for further use in airtight container.

2.2 Plant Extract

The powder of both varieties of *Cyamopsis tetragonoloba* seeds, leaves and fruit was extracted successively in the ratio of 1:10 (20 g of dry powder: 200ml of solvent) in each of the solvents viz. n-hexane, chloroform, ethyl acetate, methanol and water by Soxhlet extraction for relevant period of time. All the extracts were dried in the oven and stored in a container for further use.

2.3 Quantitative Estimation:

2.3.1 Determination of Total Alkaloids (Harborne, 1973)

In a 250 ml beaker, 5 g of the plant sample and 200 ml of 10% acetic acid in ethanol was added. The reaction mixture was covered and allowed to stand for 4 h. This was later filtered and the extract was concentrated on a water bath to one-fourth of the original volume. Concentrated ammonium hydroxide was added and a white precipitate appeared. Continue adding ammonium hydroxide to the extract, drop-by-drop until the precipitation is complete. The whole solution was then allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue obtained is the alkaloid component, which was dried and weighed to a constant mass.

2.3.2 Determination of Total Flavonoids (Bohm and Kocipal-Abyazan, 1994)

About 10 g of the plant material was extracted repeatedly with 100 ml of 80% aqueous methanol and allowed to stand it for 1 hour. After that extract was filtered by using Whatman filter paper No 42. The filtrate was later transferred into a crucible. Then it was evaporated over a water bath for obtaining the residue. After that it was dried and weighed to a constant weight.

2.3.3 Determination of Total Saponins (Obadoni and Ochulo, 2001)

20 g of plant samples was put into a conical flask and 100 ml of 20% ethanol was added in it. Then the mixture was shaken for 30 min. on an orbital shaker and subjected to continuous stirring when it was heating in a hot water bath at 55° C for 4 h. After that mixture was filtered and residue was obtained. This residue was re-extracted with a 200 ml of 20% ethanol. Then the combined extracts were put over a water bath at 90°C to reduce their volume at 40 ml. Later it was transferred into a separating funnel and 20 ml of diethyl ether was added in it and then shaken vigorously. After completing the process two distinct layers were appeared. When the ether layer was discarded, the aqueous layer was recovered. The entire process was repeated three times.

Then 60 ml of n-butanol was added into it and washed it twice with 10 ml of 5% aqueous sodium chloride. The persisting solution was heated on a water bath to reduce their volume. After evaporation, the samples were dried in the oven and weighed it to achieve a constant weight.

2.3.4 Determination of Total Tannins (Harborne, 1998)

About 28g of the plant material was weighed into a 100ml of capped conical flask and to it 35ml of acetone: water (70:30) was added. The mixture was shaken and stirred overnight at a specific temperature on a rotary shaker. This was then filtered using Whatman filter paper No. 1. After filtration, the acetone was removed from the extract by using dry heat. 10ml of dichloromethane and 20ml of diethyl ether were now added to the mixture. An organic layer was formed which was discarded. In the end, sodium sulphate was added and solution filtered. The mixture was concentrated to dryness. Solid material was obtained in term of tannins; it was calculated as percentage of starting material.

2.4 Antioxidant Activity

The antioxidant activity of the all extract (n-hexane, ethyl acetate, chloroform, methanol and water) of M83 variety of guar fruit was evaluated by DPPH free radical scavenging method. 1mg of each extract was dissolved in 1ml of methanol/water for making the stock solution.

2.4.1 DPPH Scavenging Assay

1ml of 0.3mM DPPH in methanol was mixed to 2ml of each sample solutions (concentrations as per protocol) and allowed to react at room temperature in the dark for 30 minutes. The blank was prepared with 2ml of sample solution and 1ml methanol. The control was 1ml DPPH solution and 2ml methanol. The absorbance was measured at 517 nm. (Mensor *et al*, 2001)

%AA (antioxidant activity) was calculated using the formula:

$$\%AA = \{100 - [(A_s - A_b) \times 100] \div A_c\}$$

Where,

A_s was the absorbance of sample

A_b was the absorbance of blank

A_c was the absorbance of control

Ascorbic acid (1mg/ml) was used as the standard.

2.4.2 Calculating of 50% Inhibitory Concentration (IC_{50}):

The concentration (mg/ml) of the fractions that was required to scavenge 50% of the radical was calculated by using the percentage scavenging activities at seven different concentrations of the extracts. Percentage inhibition (I %) was calculated using the formula.

$$I\% = [A_c - A_s \div A_c \times 100]$$

Where,

A_c is the absorbance of control

A_s is the absorbance of sample

IC_{50} value was calculated graphically.

2.5 Antimicrobial Activity

Antimicrobial activity was carried out by Agar disc diffusion method against Gram positive, Gram negative bacteria and fungal strain.

2.5.1 Microorganisms Tested

The ATCC bacterial strains were obtained from Dept. of Microbiology, Mahatma Gandhi Medical College and Hospital, Jaipur, Rajasthan, India and MTCC strains from Dept. of Microbiology, The IIS University, Jaipur, Rajasthan, India. Both ATCC and MTCC strains selected for the study were the Gram positive *Staphylococcus aureus*, the Gram negative *Escherichia coli* and *Pseudomonas aeruginosa* and a fungus *Candida tropicalis*.

2.6 Statistical Analysis

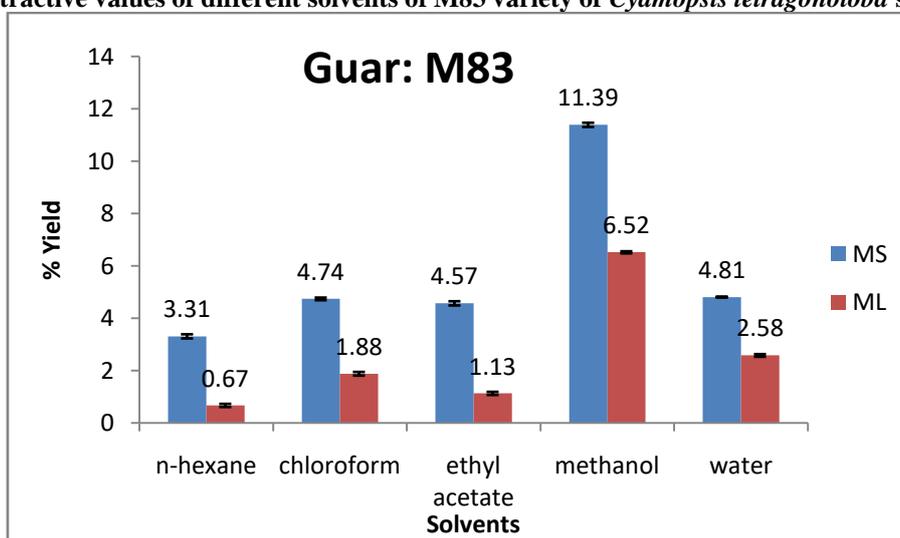
Each sample was evaluated individually in triplicates and the result are expressed as mean value (n=3) ± standard error of the mean.

3. RESULT AND DISCUSSION

3.1 % Yield

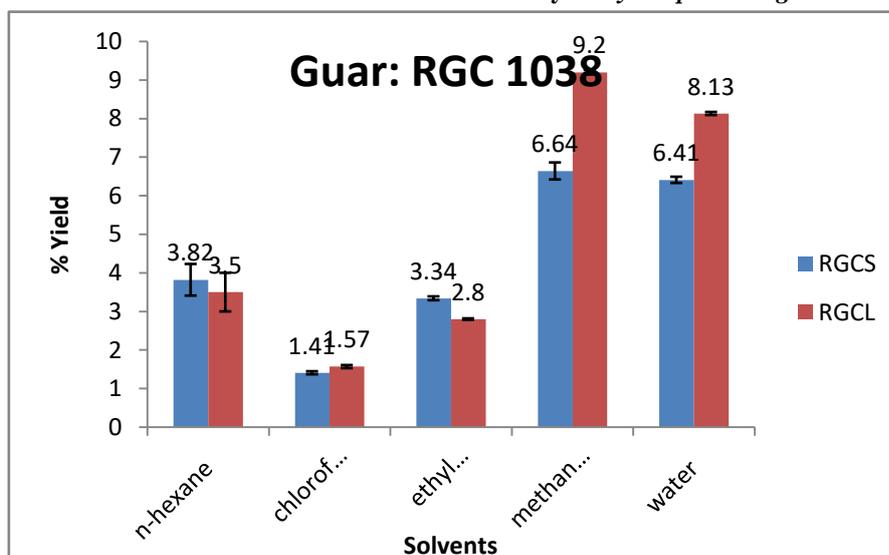
Figure 3.1 (A & B) indicates the % yield of both varieties of Guar seeds and leaves extract. For RGC1038 seeds, maximum yield was obtained in methanolic extract and minimum in chloroform extract, for RGC1038 leaves maximum yield was seen in aqueous extract and minimum in chloroform extract, for M83 seeds and leaves maximum yield was observed in methanolic extracts and minimum in n-hexane extracts. The obtained yield varies in all solvent extracts of different parts because phytoconstituents are presents in plant parts in different amount and extraction yield is related to the polarity of solvents and nature of the components present in the sample.

Fig 3.1 (A) Extractive values of different solvents of M83 variety of *Cyamopsis tetragonoloba* seeds and leaves



MS: M83 Seeds, ML: M83 Leaves

Fig 3.1 (B) Extractive values of different solvents of RGC1038 variety of *Cyamopsis tetragonoloba* seeds and leaves

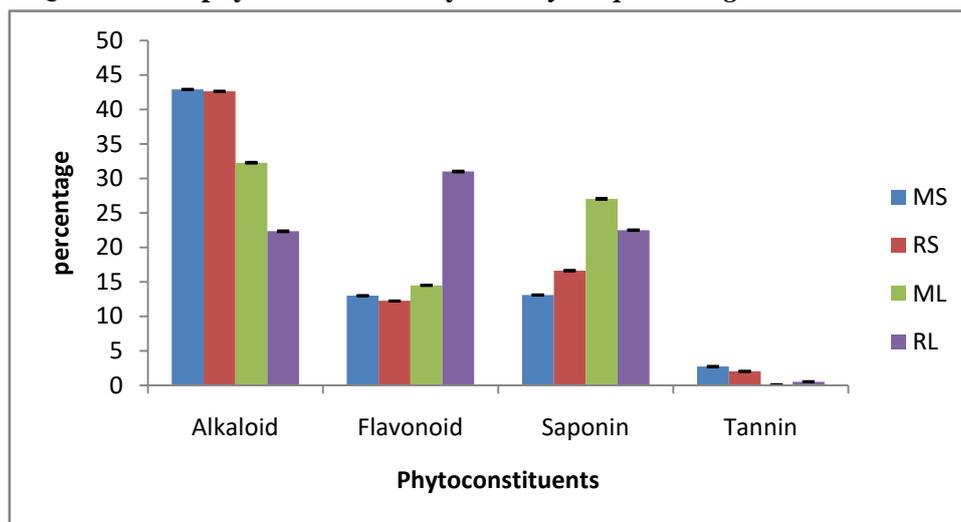


RGCS: RGC1038 Seeds, RGCL: RGC1038 Leaves

3.2 Quantitative Analysis

The results presented in Figure 3.2 revealed that maximum Alkaloid and Tannin contents were found in M83 seeds, Flavonoid contents in RGC1038 leaves and Saponin contents in M83 leaves. Phytochemical estimation of the n-hexane, chloroform, ethyl acetate, methanol and aqueous extracts of both varieties of *Cyamopsis tetragonoloba* seeds and leaves used in this analysis showed that the maximum crude extracts comprised Alkaloids, Flavanoids and Phytosteroids and some extracts comprised Saponins, Glycosides and Tannins & Phenolic compounds.

Fig. 3.2 Quantitative phytochemical analysis of *Cyamopsis tetragonoloba* seeds and leaves



MS: M83 Seeds, ML: M83 Leaves, RS: RGC1038 Seeds, RL: RGC1038 Leaves

3.3 Antioxidant Activity

3.3.1 DPPH Free Radical Scavenging Activity

This method is more acceptable because it is simple as well as independent from polarity of sample extract and also effective when evaluating multiple samples. Antioxidants react with the stable free radical DPPH (deep violet color) and by decoloration convert it into 1, 1- diphenyl-2 picryl hydrazine. All extracts significantly scavenged DPPH. The scavenging effect of extracts were expressed as percentage activity (%AA) given in Figure 3.3 and Table 3.3.1 Aqueous extract showed highest scavenging activity (IC_{50} 0.63 mg/ml) followed by methanolic extract (IC_{50} 0.71 mg/ml), ethyl acetate extract (IC_{50} 0.84 mg/ml), chloroform extract (IC_{50} 1.59 mg/ml). N-hexane did not show any scavenging activity. Table 3.3.2 Showed that results were compared with ascorbic acid (IC_{50} 18.08 μ g/ml). Polar solvent extracts showed good scavenging potential so it can be stated that fruit of guar is the good source of antioxidants.

Table 3.3.1 Antioxidant activity of all extracts of *Cyamopsis tetragonoloba* fruit of M83 variety

Conc. (mg/ml)	n-hexane	Ethyl acetate	Chloroform	Methanol	Water
0.10	8.48 \pm 0.50	7.93 \pm 1.25	12.89 \pm 0.71	28.53 \pm 0.45	21.01 \pm 0.60
0.25	16.25 \pm 0.44	21.95 \pm 0.75	20.96 \pm 0.60	37.17 \pm 0.39	33.48 \pm 0.58
0.50	19.47 \pm 0.56	41.60 \pm 1.20	28.76 \pm 0.46	43.02 \pm 0.15	44.03 \pm 0.46
0.75	23.03 \pm 0.55	45.46 \pm 0.79	32.86 \pm 0.35	51.06 \pm 0.45	54.80 \pm 0.70
1.00	38.51 \pm 0.57	58.08 \pm 0.74	41.62 \pm 0.61	57.74 \pm 0.62	60.54 \pm 0.46
1.50	45.29 \pm 0.57	70.44 \pm 0.90	47.69 \pm 0.64	74.87 \pm 0.56	66.08 \pm 0.57
2.00	48.69 \pm 0.74	79.32 \pm 0.73	60.90 \pm 0.35	86.19 \pm 0.56	70.66 \pm 0.41
IC ₅₀	-	0.84 mg/ml	1.59 mg/ml	0.71 mg/ml	0.63 mg/ml

Table 3.3.2 Antioxidant activity by Ascorbic acid assay

Conc.(µg/ml)	% Antioxidant activity
5	0.488
10	20.07
15	36.07
20	56.92
25	71.79
IC50	18.08 µg/ml

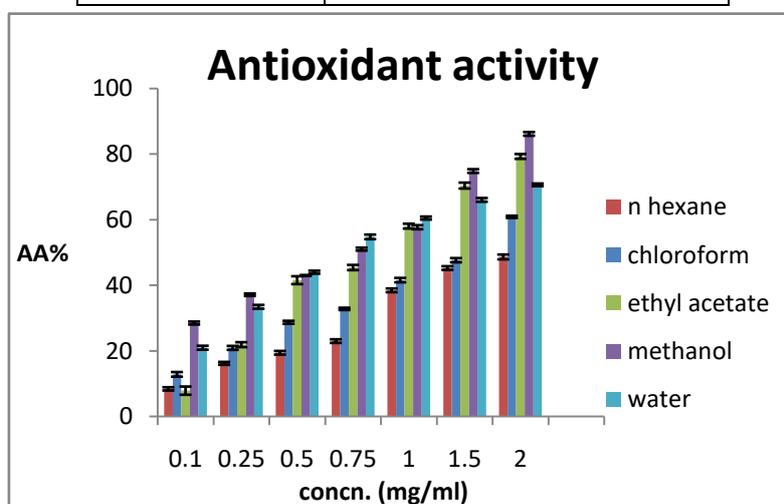


Fig. 3.3 Antioxidant activity of different extracts of M83 variety of *Cyamopsis tetragonoloba* fruit

3.4 Antimicrobial Activity

Table 3.4 reveals that antimicrobial activity of both variety of guar seeds and leaves are tested in Gram positive, Gram negative bacteria and the fungus strain. All five types of solvent extracts with three dilutions (10, 10⁻¹ and 10⁻² µl) showed negligible antimicrobial activity.

Table 3.4 Antimicrobial activity of RGC 1038 and M83 variety of *Cyamopsis tetragonoloba* Seeds and leaves extract:

Extract (µl)	N-hexane			Ethyl acetate			Chlorofom			Methanol			Water		
	10	10 ⁻¹	10 ⁻²	10	10 ⁻¹	10 ⁻²	10	10 ⁻¹	10 ⁻²	10	10 ⁻¹	10 ⁻²	10	10 ⁻¹	10 ⁻²
<i>E.coli</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Proteus vulgaris</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

4. CONCLUSION

The dietary intake of vegetables and fruits can be useful in the management of oxidative stress and age related disease because in the present day stressful life style trending over the world its becomes necessary to focus on medicinally important fruits and vegetables to minimize the risk of life. By the use of these green components the diseases can be cured without any side effects unlike the modern drugs. The present study was carried out with the cluster bean in search for effective, nontoxic natural compounds with antioxidative and antimicrobial activity and it can be concluded that although guar could not show any antimicrobial activity. It is a potentially important source of phyto-constituents and dietary antioxidants. They may help in combating oxidative stress.

References

- Badugu, L.R. 2012. Phytochemical screening, quantitative estimation, total phenolics and total flavonoids, antimicrobial evaluation of *Cyamopsis tetragonoloba*. *Int J Res Pharm Biol Sci* 3: 1339-1142.
- Benmehdi, H., Hasnaoui, O., Benali, O., Sahli, F. 2012. Phytochemical investigation of leaves and fruits extract of *Chamaerops humilis* L. *J Mater Environ* 3: 320-327.
- Boham, B.A., Kocipai-Abyazan, R. 1974. Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium vaticulatum* and *V. calycinium*. *Pac Sci* 48: 458-463.
- Chandra, S., Saklani, S., Mishra, A.P., Bamrara, A. 2012. Nutritional profile and phytochemical screening of Garhwal Himalaya medicinal plant *Dioscorea bulbifera*. *Int Res J Pharm* 3: 289-294.
- Harborne J.B. 1973. *Phytochemical methods: A guide to modern techniques of plants analysis*. 1st edn. London. Chapman and Hall, Ltd. 49-188.
- Harborne, J.B. 1998. *Phytochemical methods: A guide to modern techniques of plants analysis*. 3rd edn. Chapman and Hall, London, ISBN: 0-12-57260-5 pp: 1-302.
- Kajaria, D.K., Gangwar, M., Sharma, A. K., Nath, G., Tripathi, Y. B., Tripathi, J. S., Tiwari, S. K. 2011. Comparative evaluation of phenol and flavonoid content of poly herbal drugs. *Pharmacology online* 3: 1365-1373.
- Mensor LL, Menezes FS, Leitao GG, Reis AS, Santos TC, Coube CS, Leitao SG. 2001. Screening of Brazilian plants extracts for antioxidant activity by the use of DPPH free radical method. *Phytother. Res* 15: 127-130.
- Mukhtar, H.M., Ansari, S.H., Ali, M., Bhat, Z.A., Naved, T. 2004. Effect of aqueous extract of *Cyamopsis tetragonoloba* Linn. Beans on blood glucose level in normal and alloxan- induced diabetic rats. *IJEB* 42: 1212-1215.
- Obdoni, B O., Ochuko, P O. 2001. Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta States of Nigeria. *Global J Pure Appl Sci* 8: 203-208.
- Rabo, E. T., Sanusi, S. S. 2001. *An Inventory of Medicinal Plants of the Nigeria Savannah*. Levianthan Books, Lagos, Nigeria pp: 21-24.
- Turker, A. U., Usta, C. 2008. Biological screening of some Turkish medicinal plant extracts for antimicrobial and toxicity activities. *Nat Prod Res* 22(2): 136-146.