



“*Taraxacum officinale* Herb as an Anti-inflammatory Medicine”

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Date of Receipt- 31/01/2015
Date of Revision- 12/02/2015
Date of Acceptance- 21/02/2015

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ABSTRACT

Taraxacum officinale is a very well known medicinal herb in Ayurvedic medicine since times immemorial. The presence of various phytochemicals in the concerned plant in the form of alkaloids, flavonoids, and terpenoids made it efficient anti inflammatory drug. The inhibition of hypotonicity induced HRBC membrane lysis was taken as a measure of the anti inflammatory activity. The percentage of membrane stabilisation for dichloromethane, ethyl acetate, methanol and water extracts of the plant (Root, Stem and Flower) and Diclofenac sodium were done at different concentrations. The percentage stabilization of stem water extract was found to be highest, followed by methanol extract of stem. The root and flower extracts also follow the same trend as the stem extracts as per their percentage stabilization against inflammation. The percentage of stabilization was found concentration dependent in all the plant extracts, i.e., percentage of stabilization increases with the increase in the concentration of plant extracts. The polar solvents potentially show more stabilization potential against inflammation as compared to the non-polar solvents.

Keywords: Phytochemicals, *Taraxacum officinale*, Anti-inflammatory, *In-vitro*, Hypotonicity.

INTRODUCTION

The only kingdom upon this planet is the plant kingdom which furnishes food to whole organisms which get exists over this planet. Not only the food but they provide furniture, fodder, etc and most important phytomedicines which are help humans from time to time to remove all types of diseases of any origins. The plants helped in

improving health problems due to the chemical constituents of plants, and the probable therapeutic applications of the plant extracted medicines help in improving health problems. Plants act as chemical constituent vessels that possess pharmacological potential to overcome all diseases¹.

Taraxacum being the largest genus of *Asteraceae* family, is a herbaceous perennial herb commonly called dandelion, found especially in lawns and along roadsides, and it is used as a medicinal herb and in food preparations. Dandelion has been extensively used as traditional folk medicine, and as diuretic in modern phytotherapy. It is used to treat a variety of diseases including cancer^{2,3} in China, Arab and Native America. This plant has anti-angiogenic, and through its inhibition of NO production and cyclooxygenase-2 (COX-2) acts as anti-inflammatory and anti-nociceptive⁴.

MATERIALS AND METHODS

Plant materials

The plant material was collected from Kupwara region of Kashmir and was authenticated from FRI Dehradun. The collection process was preferably done in the dry condition. Plant was weighed before and after the removal of unwanted material kept under shade at room temperature for the removal of extra moisture. The plant samples were air dried and grounded into uniform powder with a grinder. All the plants parts i.e. stem, flowers and roots were collected separately and were subjected to different operations individually.

Experimental

All the chemicals used in this investigation were of analytical reagent (AR) grade and were purchased from Sigma Merck. De-ionized water was used for the complete study. All the glass wares and equipments used for handling were stabilized properly prior to use.

Preparation of plant samples

The separated and segregated plant parts (viz, Roots, Stem and Flowers) were collected. The shade dried dirt free plant parts were powdered in the grinder and

stored in the air tight container in the dark until further use.

Preparation of plant extracts

The extraction procedure was carried out in series of organic solvents based upon their polarity index. Extracts were prepared in 1000ml of different solvents, using Soxhlet Extraction Procedure. The various solvents used for extraction purpose involve Dichloromethane, Ethylacetate, Methanol and finally Water in order of increasing polarity. From the extraction results it was found that methanol and water have better extracting power in terms of yield so for obtained. Water having the highest polarity index (9.0) was found to be the best solvent for extraction purpose as per the percentage yield of the extracts in all the three parts of the plants extracted separately (Root, Stem, and Flower). The physical properties, percentage yield of the extracts are mentioned in the table 1-3.

Preparation of human red blood cells (HRBC) suspension

Fresh whole human blood was collected and mixed with equal volume of sterilized Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.05% citric acid and 0.42 % sodium chloride in water). The blood was centrifuged at 3000 rpm for 10 min and packed cells were washed three times with isosaline (0.85%, pH 7.2). The volume of the blood was measured and reconstituted as 10% v/v suspension with isosaline^{5,6}.

Heat induced hemolysis

The principle involved here is stabilization of human red blood cell membrane by hypo tonicity induced membrane lysis. The assay mixture contains 1ml phosphate buffer [pH 7.4, 0.15 M], 2 ml hypo saline [0.36 %], 0.5 ml HRBC suspension [10 % v/v] with various concentrations of plant extracts and standard

drug diclofenac sodium of various concentrations (50, 100, 250, 500, 1000, 2000 µg/ml) and control (distilled water instead of hypo saline to produce 100 % hemolysis) were incubated at 37°C for 30 min and centrifuged respectively. The hemoglobin content in the suspension was estimated using spectrophotometer at 560 nm⁷.

The percentage of hemolysis of HRBC membrane can be calculated as follows,

% Hemolysis = (Absorbance of Test sample / Absorbance of Control) X 100.

The percentage of HRBC membrane stabilization can be calculated as follows,

% Protection = 100 – [(Absorbance of Test sample / Absorbance of Control) X 100]. (See table 4-15 and figure 1-24.)

RESULTS AND DISCUSSION

Taraxacum officinale plant have been analysed for the in vitro analysis of anti-inflammatory effect of various plant extracts (T1-T12) (Table 4-15). The diclofenac sodium has been used as a reference drug. All the plant extracts have been analysed spectrophotometrically and percentage of stabilisation against hypotonicity induced membrane lysis. The various plant extract follow the order as per their percentage of stabilisation (T4 > T3 > T11 > T8 > T7 > T12 > T6 > T10 > T5 > T2 > T1 > T9). The percentage of stabilisation of all the plant extracts was found to be concentration dependent, as the stabilisation percentage increases correspondingly with the increase in the concentration of plant extracts. The graph was plotted between the plant extract concentration and the percentage of stabilization, and straight line was obtained which gets ended with the line of saturation.

Among the all plant extracts the stem extracts were found be more effective as per their anti-inflammatory effect, (T1–T4) followed by plant root extracts (T5–T8). The

flower extracts have been found to posses the less anti-inflammatory effect than the stem and root extracts. As per the polarity of solvent, the polar solvent extracts (T3, T4, T7, T8, T11, and T12) showed marginally higher response towards the anti-inflammatory activity than the non polar solvents (T1, T2, T5, T6, T9, and T10). The percentage of stabilisation of all the plant extracts was found to less than the standard reference compound (Diclofenac Sodium).

The anti inflammatory activity of the plant was measured on the basis of stabilization of HRBC membrane in which the lysis was carried out by hypotonicity. The membrane lysis was carried out by hypotonicity and its stabilization was determined against the plant extracts and compared with known medicine Diclofenac Sodium. The results obtained from the plant extracts were interesting and when compared with the known anti inflammatory drug, showed that the concerned plant has a high potential to act as a good anti inflammatory medicine.

The anti-inflammatory activity showed by different plant extracts are due to the different ionic potential of various solvents used so far. Among the four solvents used so far, the more ionic solvents (Methanol and Water) showed highest activity against inflammation as compared to less polar solvents (Dichloromethane and Ethyl acetate). The ionic solvent extracts marginally have more anti inflammatory activity than the non ionic (Kaleab Asres⁸).

CONCLUSION

The hypotonicity induced membrane lysis and its membrane stabilisation by plant derived products was developed to establish a mechanism of anti- inflammatory activity of the *Taraxacum officinale*. So the present study of in vitro activity anti inflammatory activity was carried out and results demonstrated the depression of inflammation.

The concerned activity of the plant extracts was expected due to presence of biological important components such as alkaloids, flavonoids, terpenoids, and phenolic compounds. Hence the *Taraxacum officinale* can be used as potential anti-inflammatory medicine.

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Table 1. Represents the physical properties, yield obtained and percentage yield of the Stem of *Taraxacum officinale*

Solvents	Physical Properties	Yield Obtained g/1000ml	Percentage Yield
Dichloromethane (T1)	Greenish Orange-Solid	5.5	11%
Ethyl acetate (T2)	Blackish Green Powder	3.7	7.4%
Methanol (T3)	Honey Brown Powder	9.5	19%
Aqueous (T4)	Honey Brown Powder	14.7	29.4%

Table 2. Represents the physical properties, yield obtained and percentage yield of the Root of *Taraxacum officinale*

Solvents	Physical Properties	Yield Obtained g/1000ml	Percentage Yield
Dichloromethane (T5)	Chocolate Brawn-Powder	7.5	15%
Ethyl acetate (T6)	Brawn Powder	4.8	9.6%
Methanol (T7)	Honey Brown Powder	6.9	13.8%
Aqueous (T8)	Honey Brown Powder	11.7	23.4%

Table 3. Represents the physical properties, yield obtained and percentage yield of the Flower of *Taraxacum officinale*

Solvents	Physical Properties	Yield Obtained g/1000ml	Percentage Yield
Dichloromethane (T9)	Yellowish Green Powder	6.4	12.8%
Ethyl acetate (T10)	Yellow Brawn Powder	5.7	11.4%
Methanol (T11)	Brown Powder	9.8	19.6%
Aqueous (T12)	Blackish Brown Powder	15.4	30.8%

Table 4. Report of anti-inflammatory effect of (T1) extract of *Taraxacum officinale* and standard on HRBC membrane hemolysis and membrane stabilization

Conc. of Standard/ Plant Extract ($\mu\text{g/ml}$)	% Hemolysis of <i>T. officinale</i>	% Stabilisation of <i>T. officinale</i>	% Hemolysis of % Diclofenac	% Stabilisation of Diclofenac
50	24.10	56.70	47.97	50.9
100	18.37	62.21	27.89	64.60
250	16.24	69.85	15.63	78.97
500	14.63	75.18	11.54	84.99
1000	10.15	81.22	6.44	95.52
2000	7.33	88.17	1.10	97.98

Table 5. Report of anti-inflammatory effect of (T2) extract of *Taraxacum officinale* and standard on HRBC membrane hemolysis and membrane stabilization

Conc. of Standard/ Plant Extract (µg/ml)	% Hemolysis of <i>T. officinale</i>	% Stabilisation of <i>T. officinale</i>	% Hemolysis of % Diclofenac sodium	% Stabilisation of Diclofenac sodium
50	65.56	45.23	47.97	50.9
100	51.56	53.34	27.89	64.60
250	44.34	67.78	15.63	78.97
500	33.78	74.98	11.54	84.99
1000	22.45	81.19	6.44	95.52
2000	12.11	89.21	1.10	97.98

Table 6. Report of anti-inflammatory effect of (T3) extract of *Taraxacum officinale* and standard on HRBC membrane hemolysis and membrane stabilization

Conc. of Standard/ Plant Extract (µg/ml)	% Hemolysis of <i>T. officinale</i>	% Stabilisation of <i>T. officinale</i>	% Hemolysis of % Diclofenac sodium	% Stabilisation of Diclofenac sodium
50	50.95	50.89	47.97	50.9
100	42.71	61.39	27.89	64.60
250	33.73	72.19	15.63	78.97
500	22.12	79.17	11.54	84.99
1000	12.91	87.43	6.44	95.52
2000	5.89	93.73	1.10	97.98

Table 7. Report of anti-inflammatory effect of (T4) extract of *Taraxacum officinale* and standard on HRBC membrane hemolysis and membrane stabilization

Conc. of Standard/ Plant Extract (µg/ml)	% Hemolysis of <i>T. officinale</i>	% Stabilisation of <i>T. officinale</i>	% Hemolysis of % Diclofenac sodium	% Stabilisation of Diclofenac sodium
50	31.25	65.73	47.97	50.9
100	18.77	75.21	27.89	64.60
250	15.25	83.12	15.63	78.97
500	11.23	88.58	11.54	84.99
1000	7.45	91.57	6.44	95.52
2000	5.15	93.98	1.10	97.98

Table 8. Report of anti-inflammatory effect of (T5) extract of *Taraxacum officinale* and standard on HRBC membrane hemolysis and membrane stabilization

Conc. of Standard/ Plant Extract (µg/ml)	% Hemolysis of <i>T. officinale</i>	% Stabilisation of <i>T. officinale</i>	% Hemolysis of % Diclofenac sodium	% Stabilisation of % Diclofenac sodium
50	55.92	51.61	47.97	50.9
100	46.16	63.45	27.89	64.60
250	31.8	72.11	15.63	78.97
500	23.45	78.16	11.54	84.99
1000	16.56	85.51	6.44	95.52
2000	5.43	89.33	1.10	97.98

Table 9. Report of anti-inflammatory effect of (T6) extract of *Taraxacum officinale* and standard on HRBC membrane hemolysis and membrane stabilization

Conc. of Standard/ Plant Extract (µg/ml)	% Hemolysis of <i>T. officinale</i>	% Stabilisation of <i>T. officinale</i>	% Hemolysis of % Diclofenac sodium	% Stabilisation of % Diclofenac sodium
50	28.20	61.65	47.97	50.9
100	22.67	70.23	27.89	64.60
250	18.15	79.17	15.63	78.97
500	12.43	81.48	11.54	84.99
1000	9.35	89.67	6.44	95.52
2000	6.78	90.45	1.10	97.98

Table 10. Report of anti-inflammatory effect of (T7) extract of *Taraxacum officinale* and standard on HRBC membrane hemolysis and membrane stabilization

Conc. of Standard/ Plant Extract (µg/ml)	% Hemolysis of <i>T. officinale</i>	% Stabilisation of <i>T. officinale</i>	% Hemolysis of % Diclofenac sodium	% Stabilisation of % Diclofenac sodium
50	45.15	52.51	47.97	50.9
100	38.32	59.23	27.89	64.60
250	31.26	68.32	15.63	78.97
500	24.21	79.45	11.54	84.99
1000	15.32	85.67	6.44	95.52
2000	7.23	91.89	1.10	97.98

Table 11. Report of anti-inflammatory effect of (T8) extract of *Taraxacum officinale* and standard on HRBC membrane hemolysis and membrane stabilization

Conc. of Standard/ Plant Extract (µg/ml)	% Hemolysis of <i>T. officinale</i>	% Stabilisation of <i>T. officinale</i>	% Hemolysis of % Diclofenac sodium	% Stabilisation of Diclofenac sodium
50	55.95	52.81	47.97	50.9
100	47.12	65.25	27.89	64.60
250	32.89	74.01	15.63	78.97
500	25.56	81.56	11.54	84.99
1000	18.34	89.41	6.44	95.52
2000	7.34	92.43	1.10	97.98

Table 12. Report of anti-inflammatory effect of (T9) extract of *Taraxacum officinale* and standard on HRBC membrane hemolysis and membrane stabilization

Conc. of Standard/ Plant Extract (µg/ml)	% Hemolysis of <i>T. officinale</i>	% Stabilisation of <i>T. officinale</i>	% Hemolysis of % Diclofenac sodium	% Stabilisation of Diclofenac sodium
50	39.43	56.12	47.97	50.9
100	31.54	65.34	27.89	64.60
250	22.43	71.78	15.63	78.97
500	15.23	79.76	11.54	84.99
1000	10.43	84.65	6.44	95.52
2000	6.12	89.15	1.10	97.98

Table 13. Report of anti-inflammatory effect of (T10) extract of *Taraxacum officinale* and standard on HRBC membrane hemolysis and membrane stabilization

Conc. of Standard/ Plant Extract (µg/ml)	% Hemolysis of <i>T. officinale</i>	% Stabilisation of <i>T. officinale</i>	% Hemolysis of % Diclofenac sodium	% Stabilisation of Diclofenac sodium
50	40.21	57.52	47.97	50.9
100	35.65	66.21	27.89	64.60
250	26.23	72.43	15.63	78.97
500	16.54	81.10	11.54	84.99
1000	11.46	85.43	6.44	95.52
2000	7.15	89.69	1.10	97.98

Table 14. Report of anti-inflammatory effect of (T11) extract of *Taraxacum officinale* and standard on HRBC membrane hemolysis and membrane stabilization

Conc. of Standard/ Plant Extract ($\mu\text{g/ml}$)	% Hemolysis of <i>T. officinale</i>	% Stabilisation of <i>T. officinale</i>	% Hemolysis of % Diclofenac sodium	% Stabilisation of Diclofenac sodium
50	55.56	56.91	47.97	50.9
100	45.56	61.98	27.89	64.60
250	34.34	70.45	15.63	78.97
500	27.45	79.89	11.54	84.99
1000	15.15	82.56	6.44	95.52
2000	8.13	92.67	1.10	97.98

Table 15. Report of anti-inflammatory effect of (T12) extract of *Taraxacum officinale* and standard on HRBC membrane hemolysis and membrane stabilization

Conc. of Standard/ Plant Extract ($\mu\text{g/ml}$)	% Hemolysis of <i>T. officinale</i>	% Stabilisation of <i>T. officinale</i>	% Hemolysis of % Diclofenac sodium	% Stabilisation of Diclofenac sodium
50	40.10	55.51	47.97	50.9
100	32.43	67.14	27.89	64.60
250	21.27	73.35	15.63	78.97
500	14.14	81.65	11.54	84.99
1000	8.42	88.70	6.44	95.52
2000	5.11	91.10	1.10	97.98

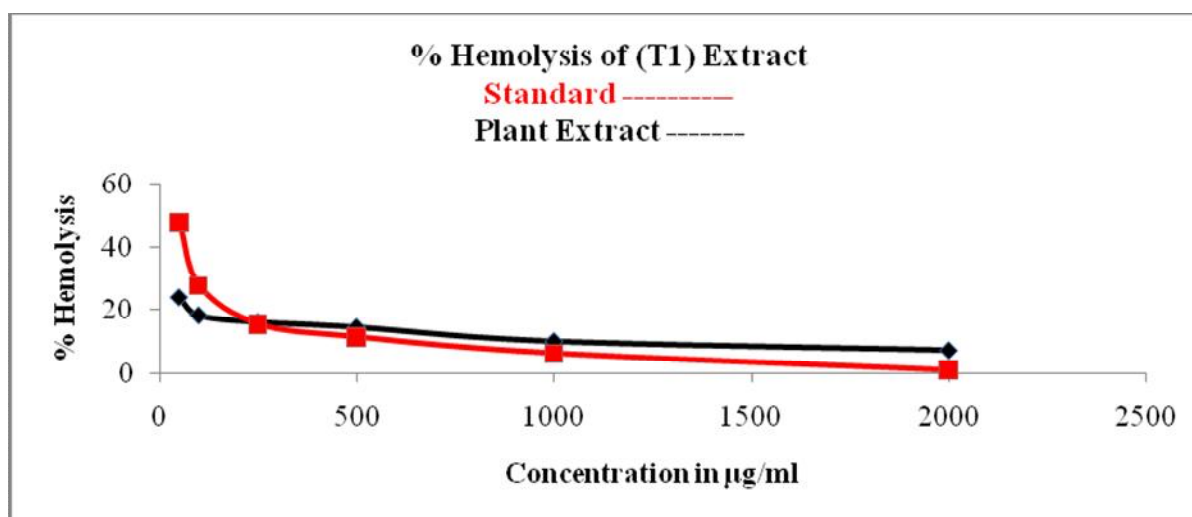


Figure 1. Graph represents HRBC Membrane Hemolysis by (T1) extract and Standard

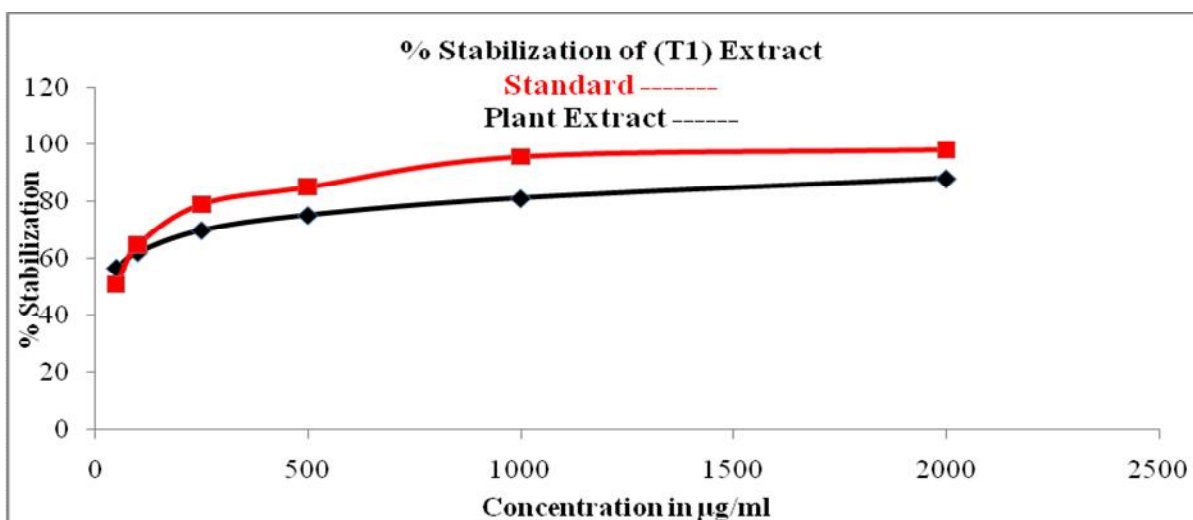


Figure 2. Graph represents HRBC Membrane Stabilization by (T1) extract and Standard

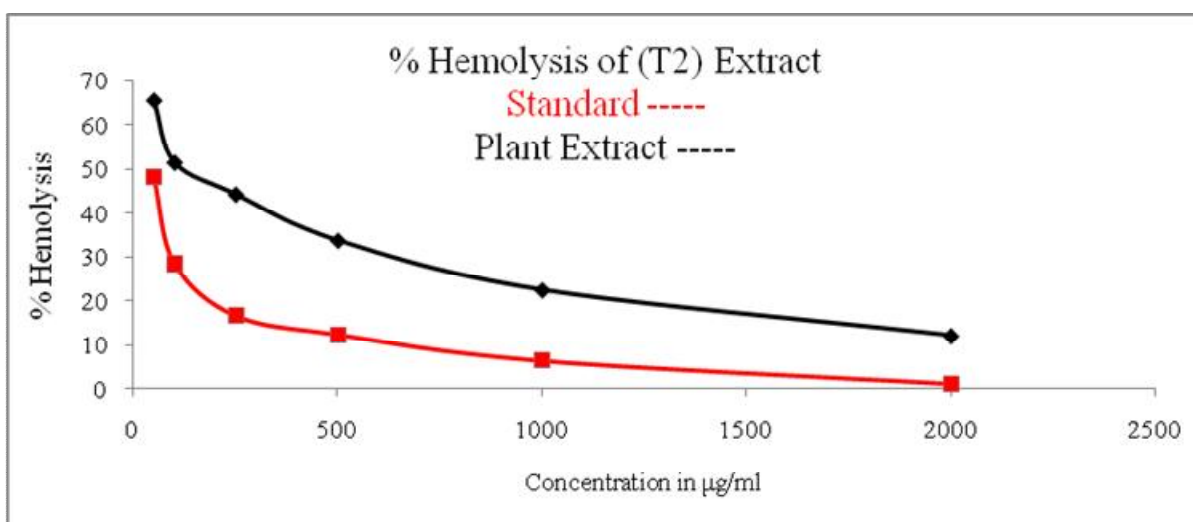


Figure 3. Graph represents HRBC Membrane Hemolysis by (T2) extract and Standard

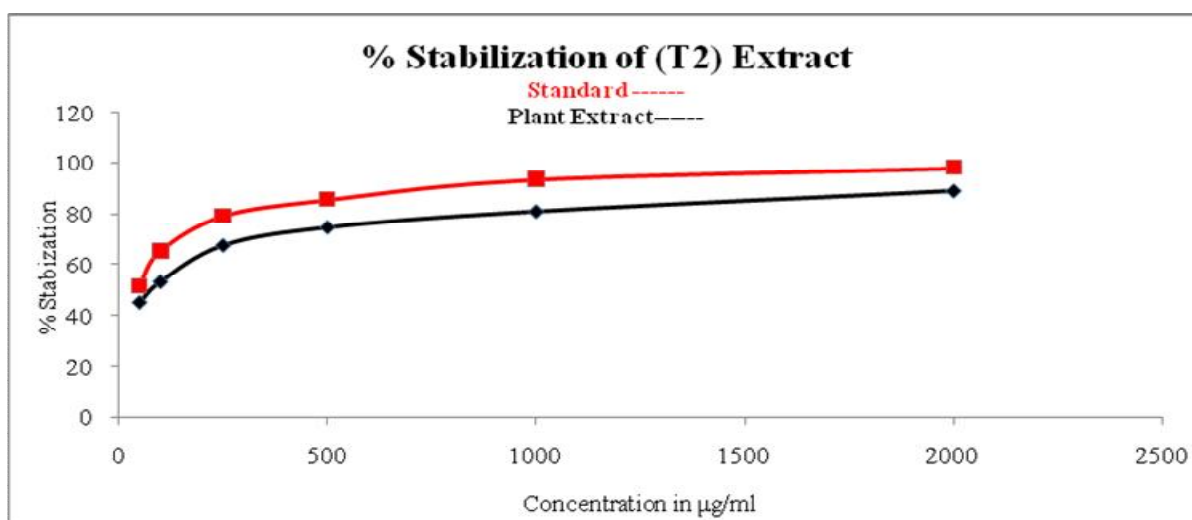


Figure 4. Graph represents HRBC Membrane Stabilization by (T2) extract and Standard

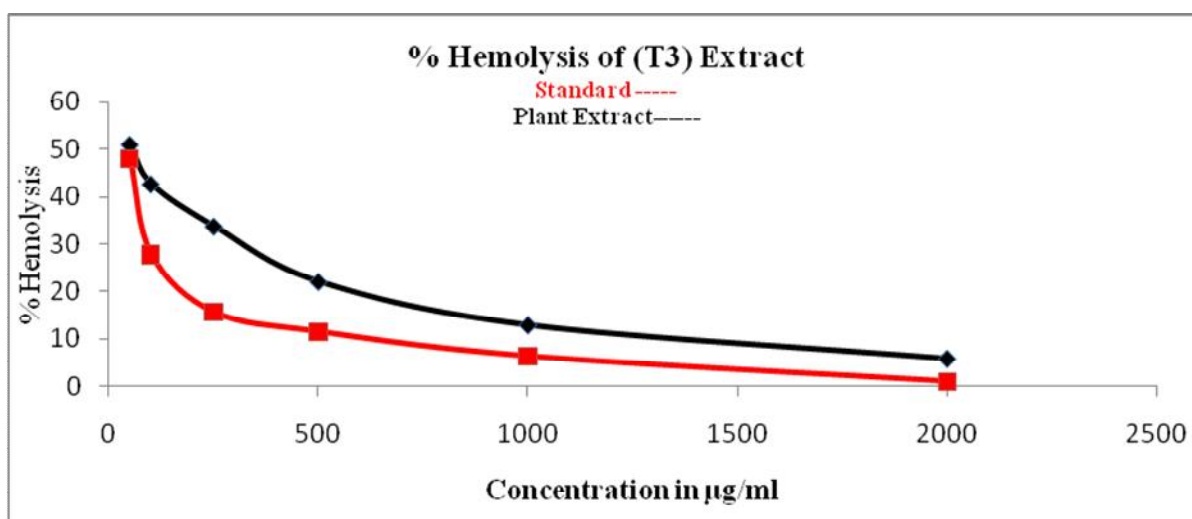


Figure 5. Graph represents HRBC Membrane Hemolysis by (T3) extract and Standard

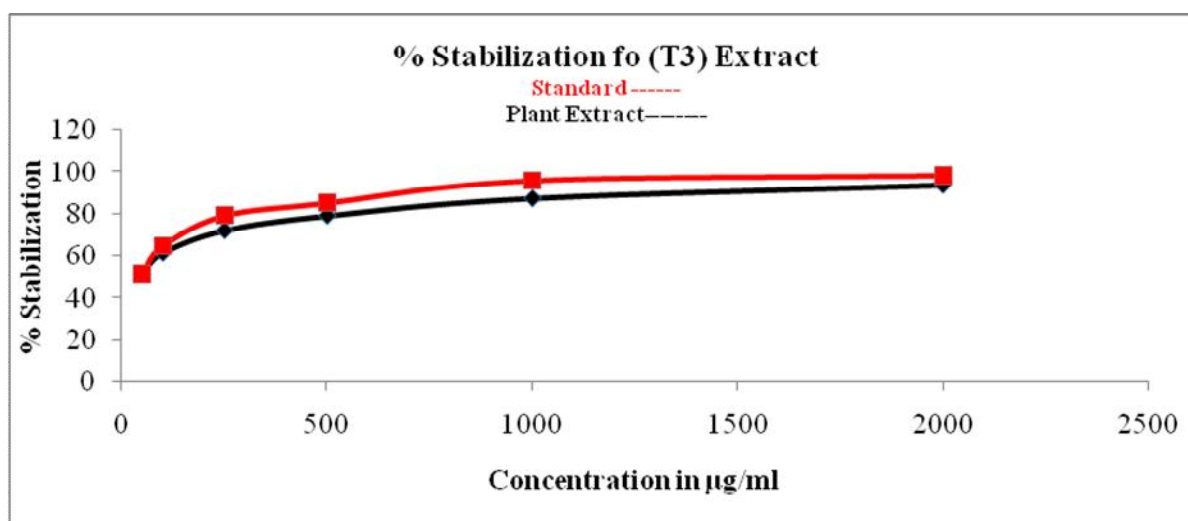


Figure 6. Graph represents HRBC Membrane Stabilization by (T3) extract and Standard

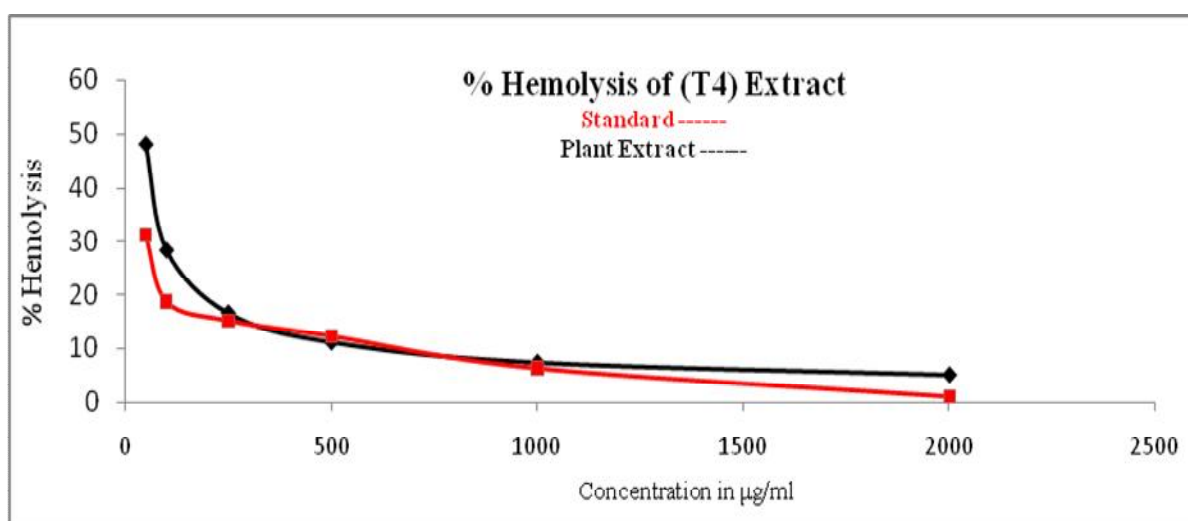


Figure 7. Graph represents HRBC Membrane Hemolysis by (T4) extract and Standard

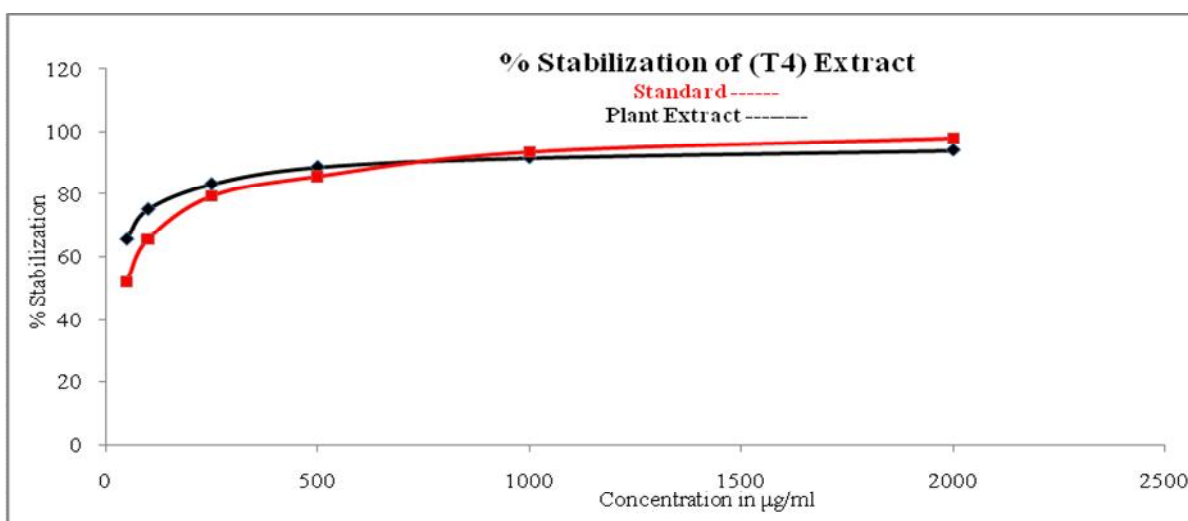


Figure 8. Graph represents HRBC Membrane Stabilization by (T4) extract and Standard

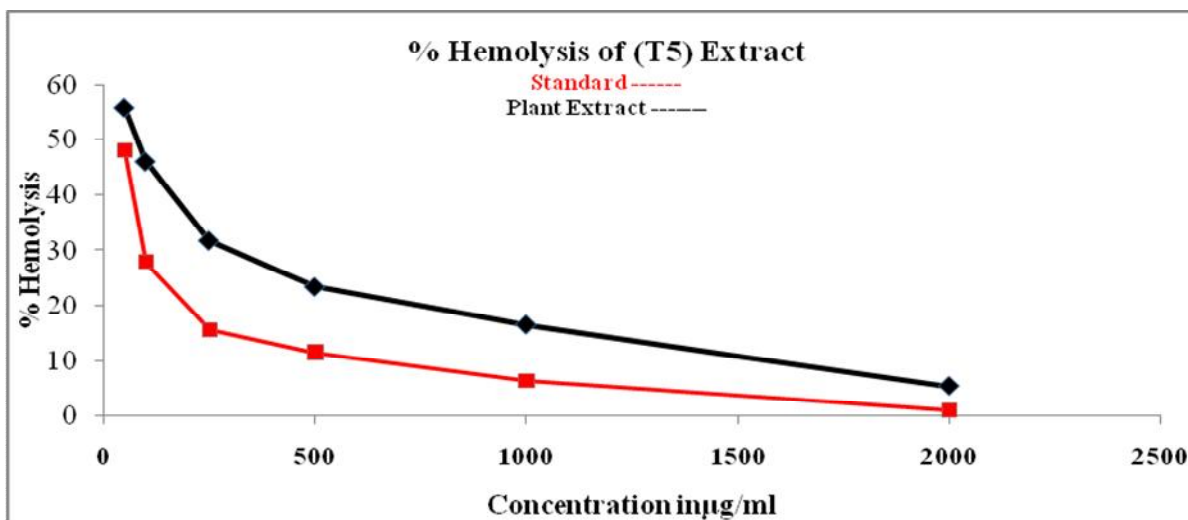


Figure 9. Graph represents HRBC Membrane Hemolysis by (T5) extract and Standard

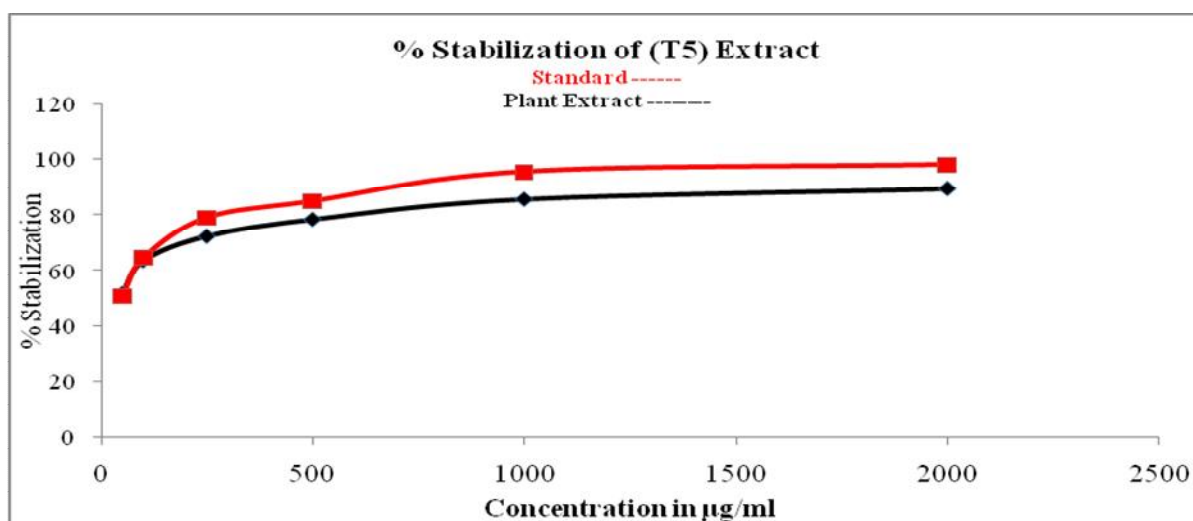


Figure 10. Graph represents HRBC Membrane Stabilization by (T5) extract and Standard

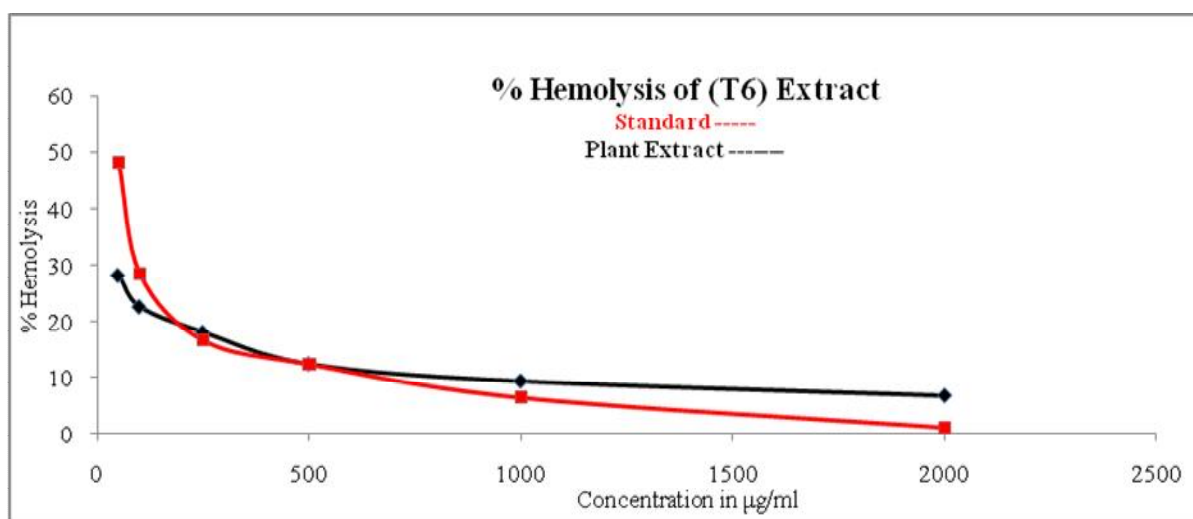


Figure 11. Graph represents HRBC Membrane Hemolysis by (T6) extract and Standard

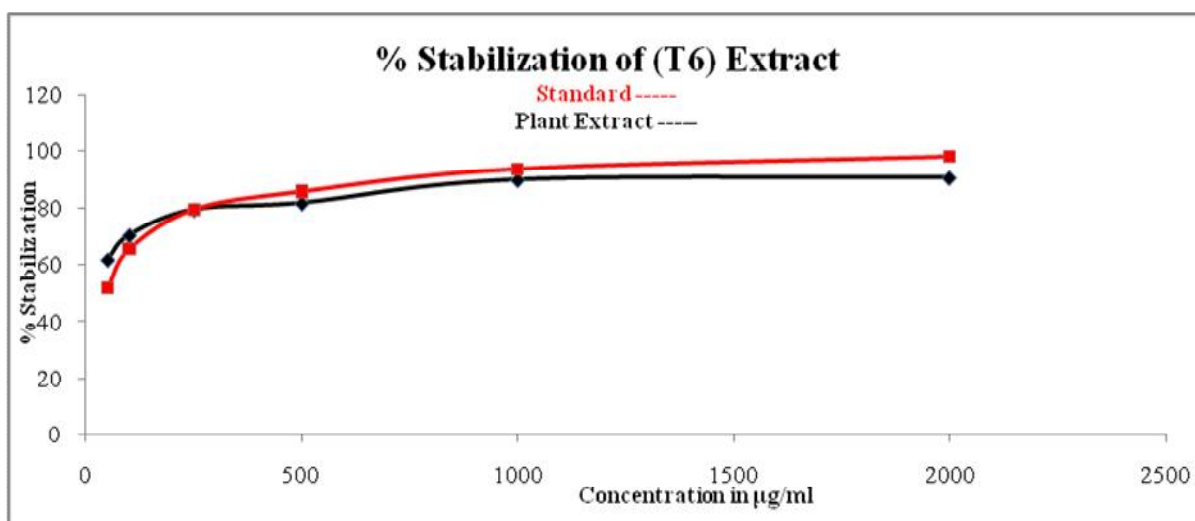


Figure 12. Graph represents HRBC Membrane Stabilization by (T6) extract and Standard

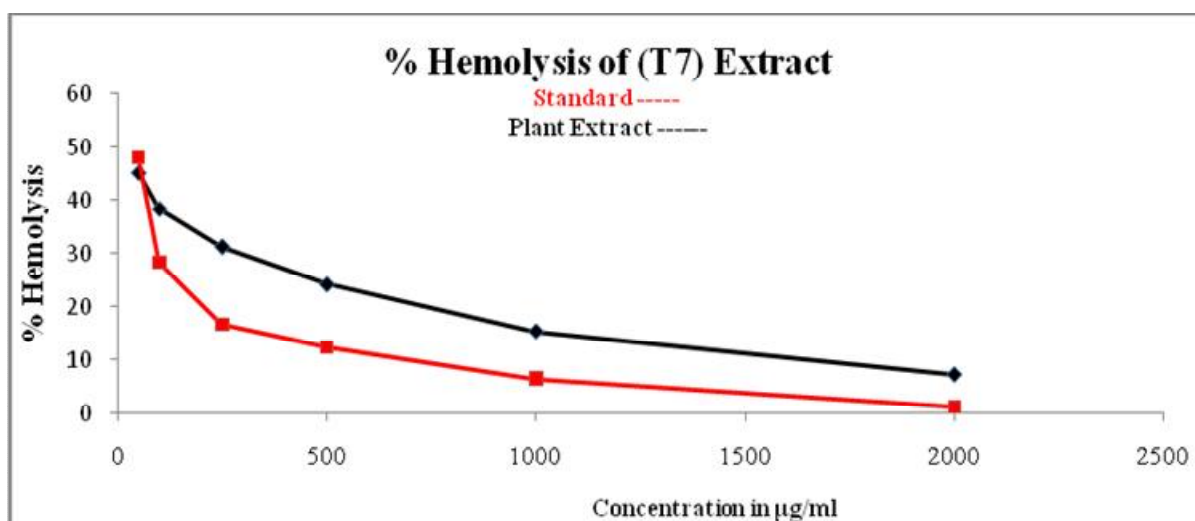


Figure 13. Graph represents HRBC Membrane Hemolysis by (T7) extract and Standard

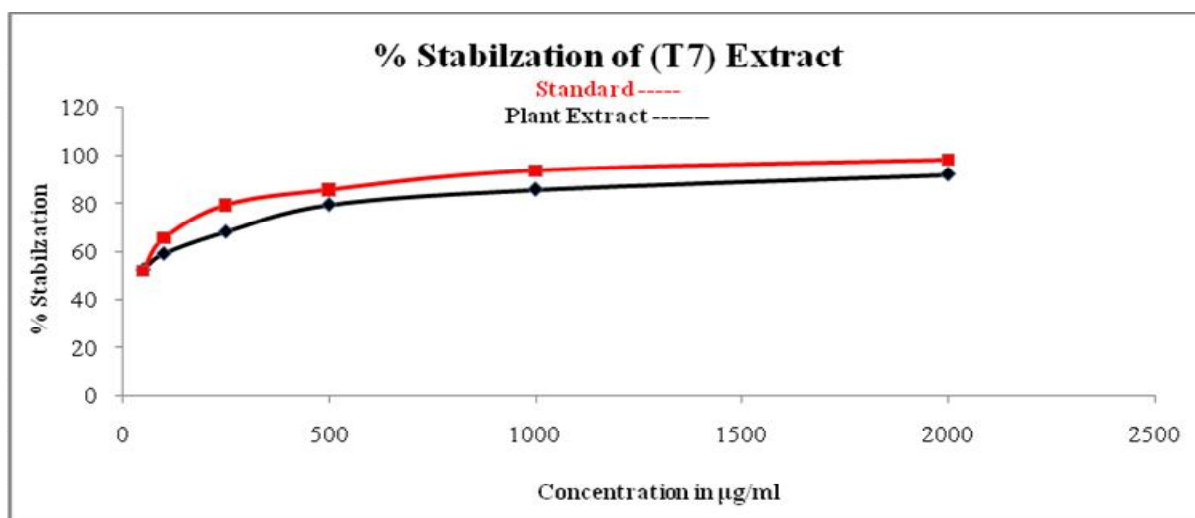


Figure 14. Graph represents HRBC Membrane Stabilization by (T7) extract and Standard

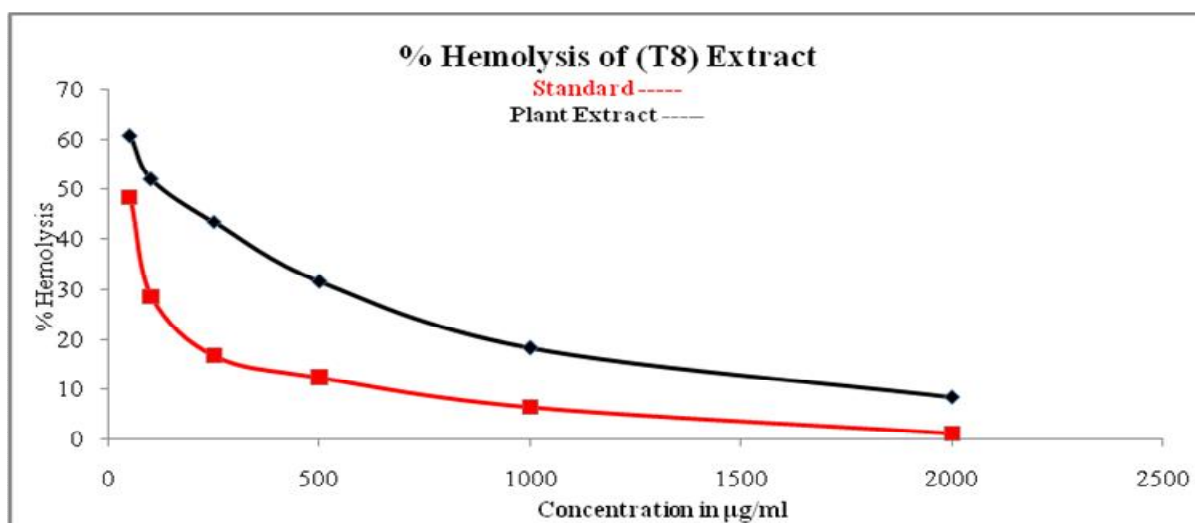


Figure 15. Graph represents HRBC Membrane Hemolysis by (T8) extract and Standard

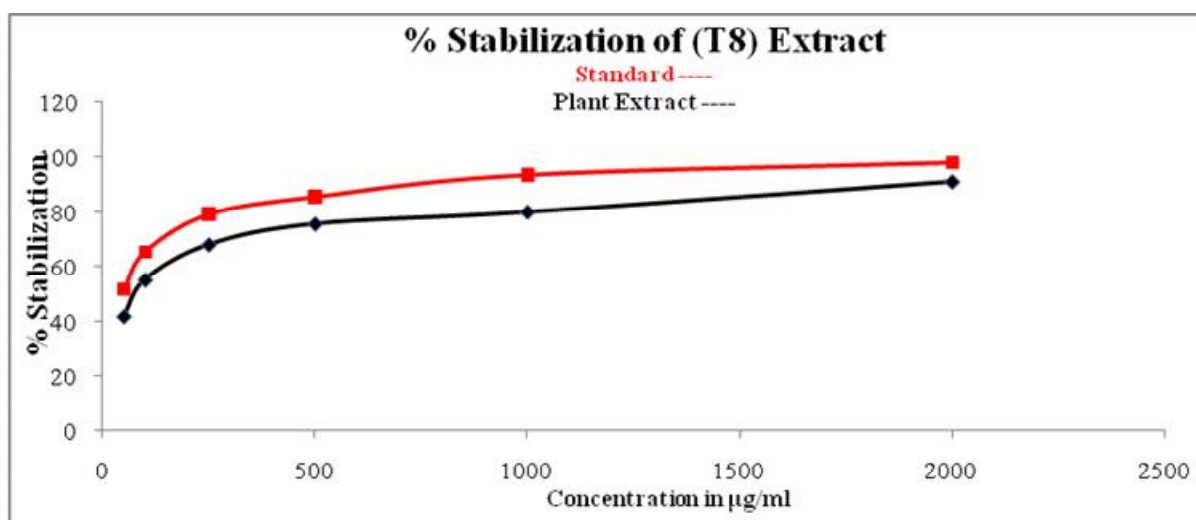


Figure 16. Graph represents HRBC Membrane Stabilization by (T8) extract and Standard

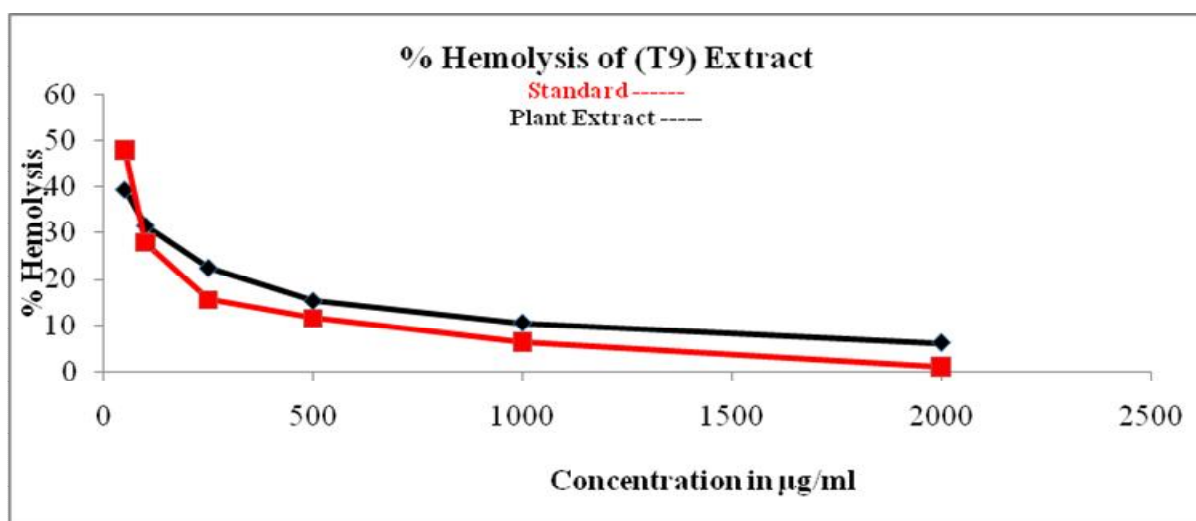


Figure 17. Graph represents HRBC Membrane Hemolysis by (T9) extract and Standard

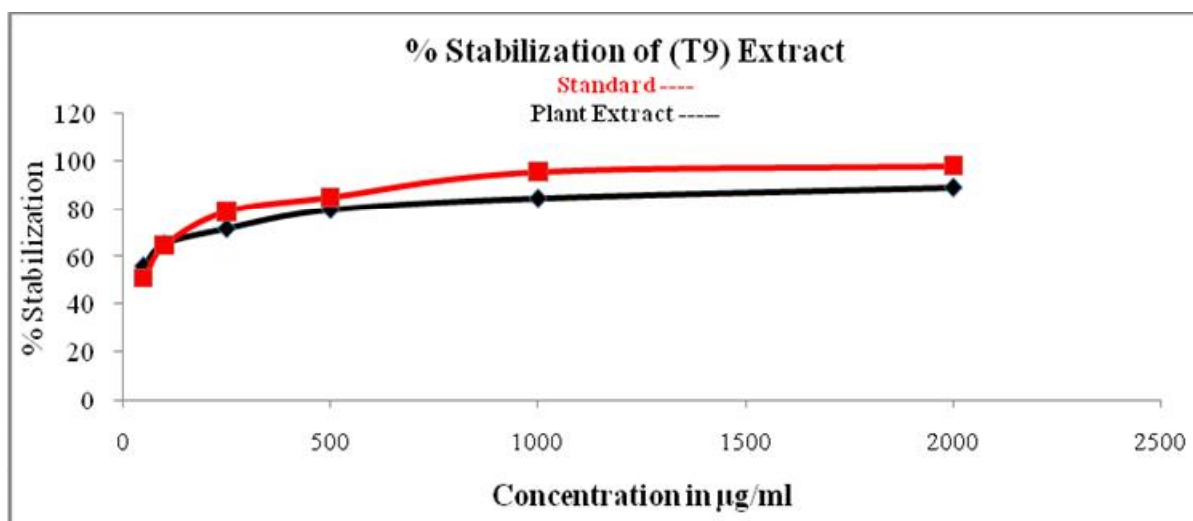


Figure 18. Graph represents HRBC Membrane Stabilization by (T9) extract and Standard

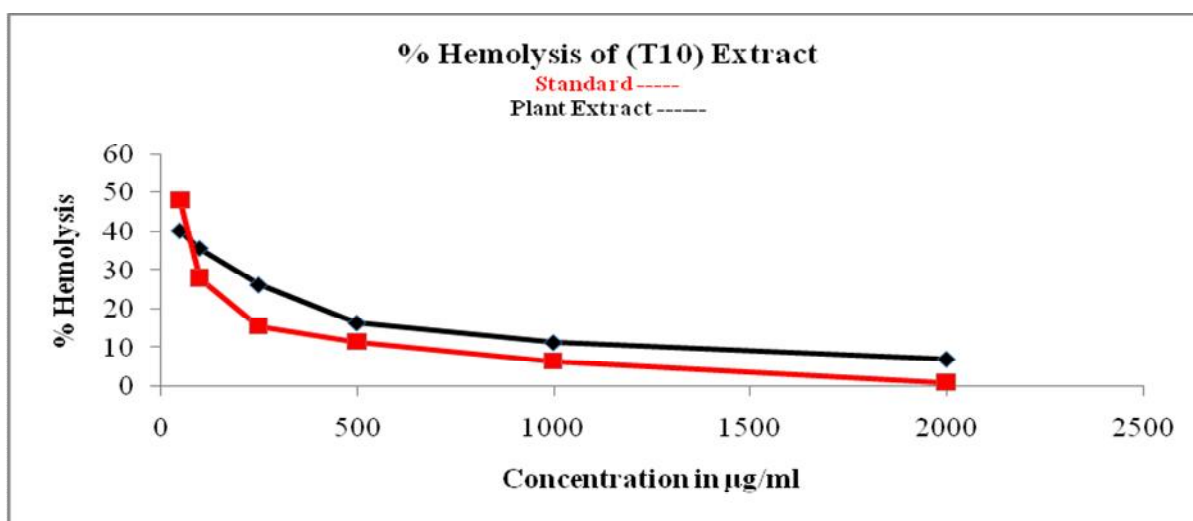


Figure 19. Graph represents HRBC Membrane Hemolysis by (T10) extract and Standard

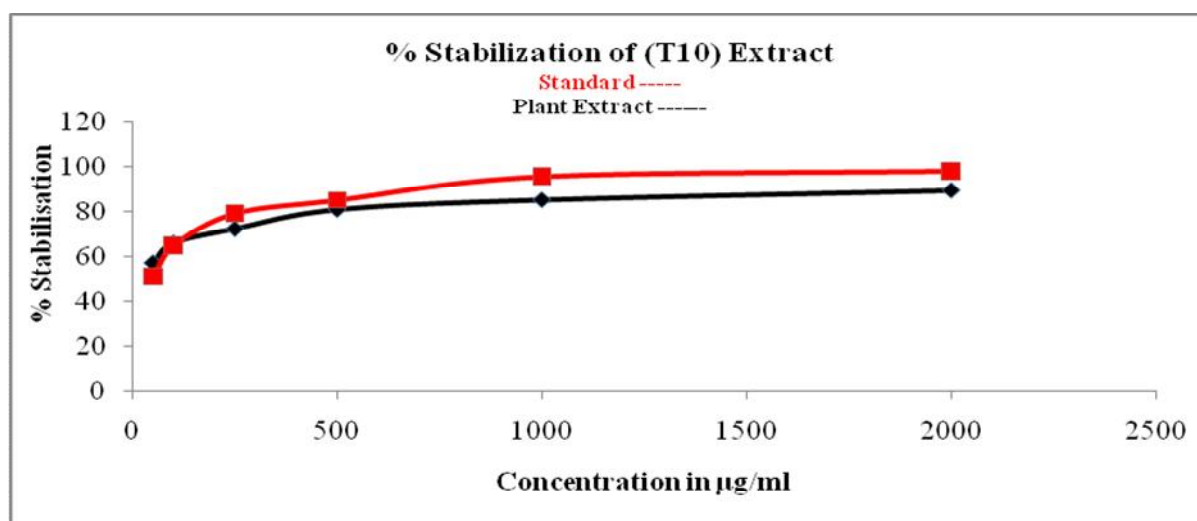


Figure 20. Graph represents HRBC Membrane Stabilization by (T10) extract and Standard

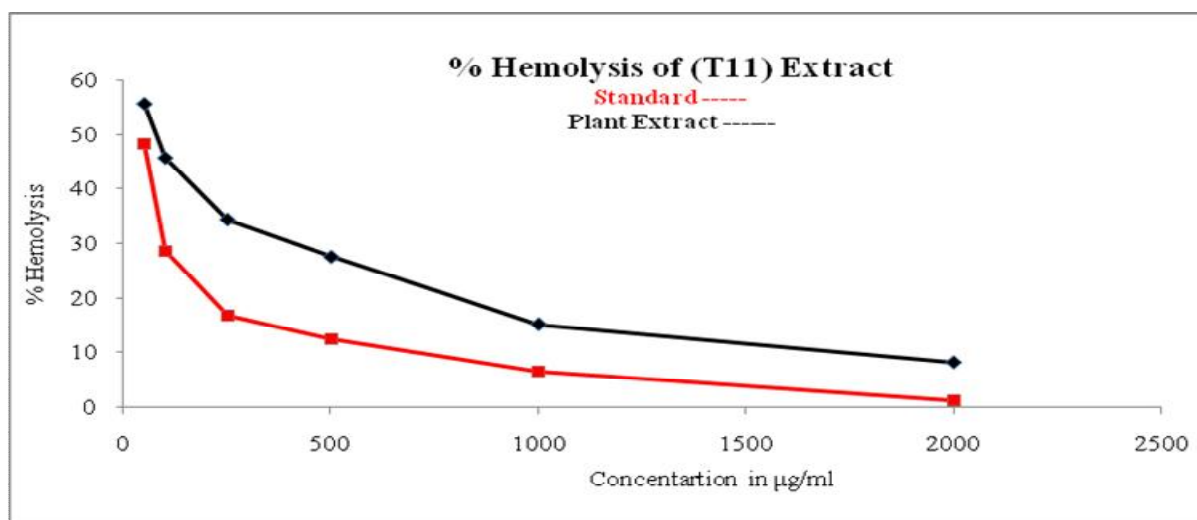


Figure 21. Graph represents HRBC Membrane Hemolysis by (T11) extract and Standard

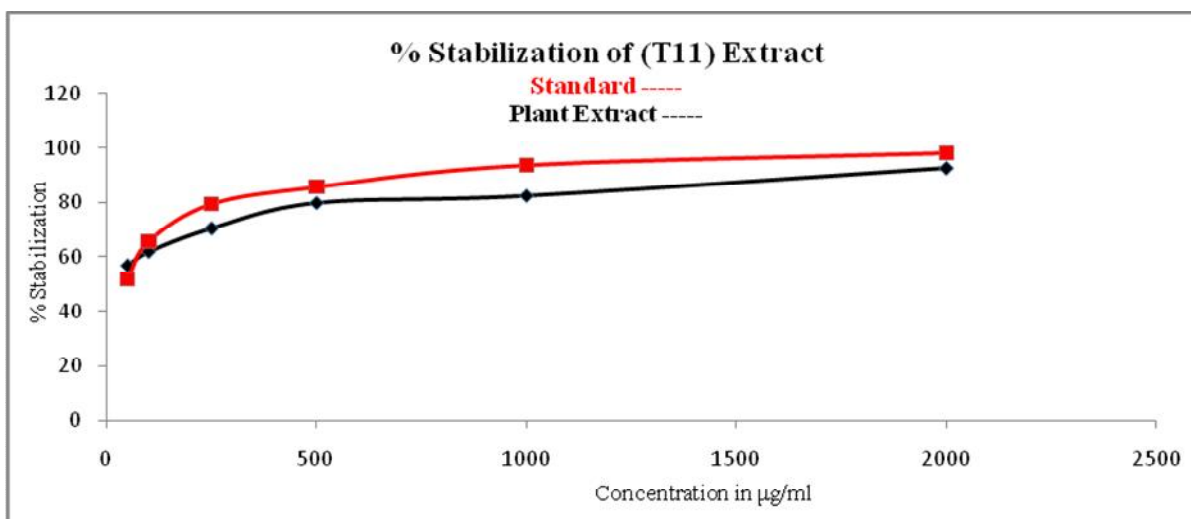


Figure 22. Graph represents HRBC Membrane Stabilization by (T11) extract and Standard

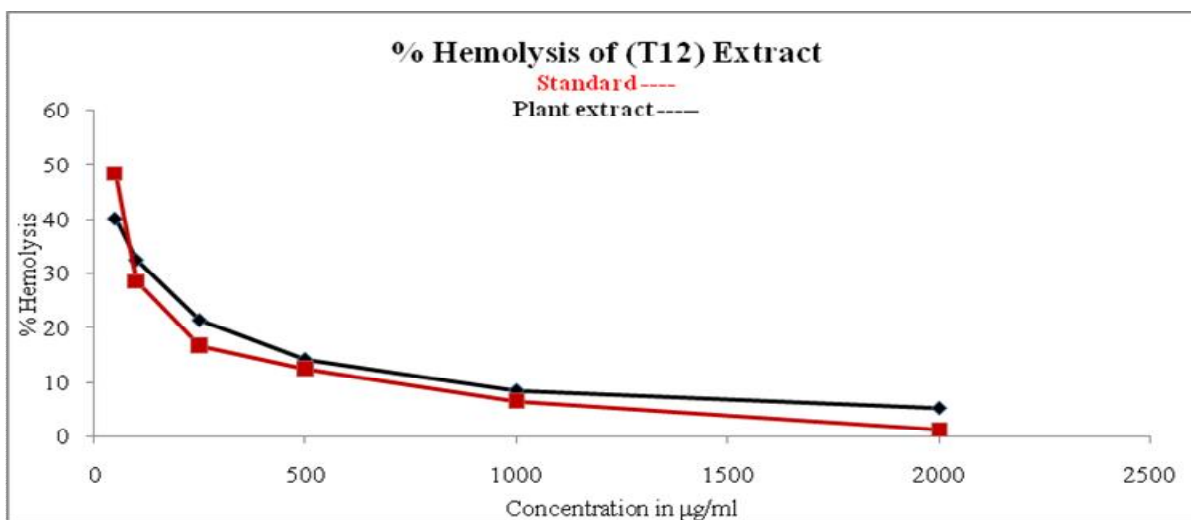


Figure 23. Graph represents HRBC Membrane Hemolysis by (T12) extract and Standard

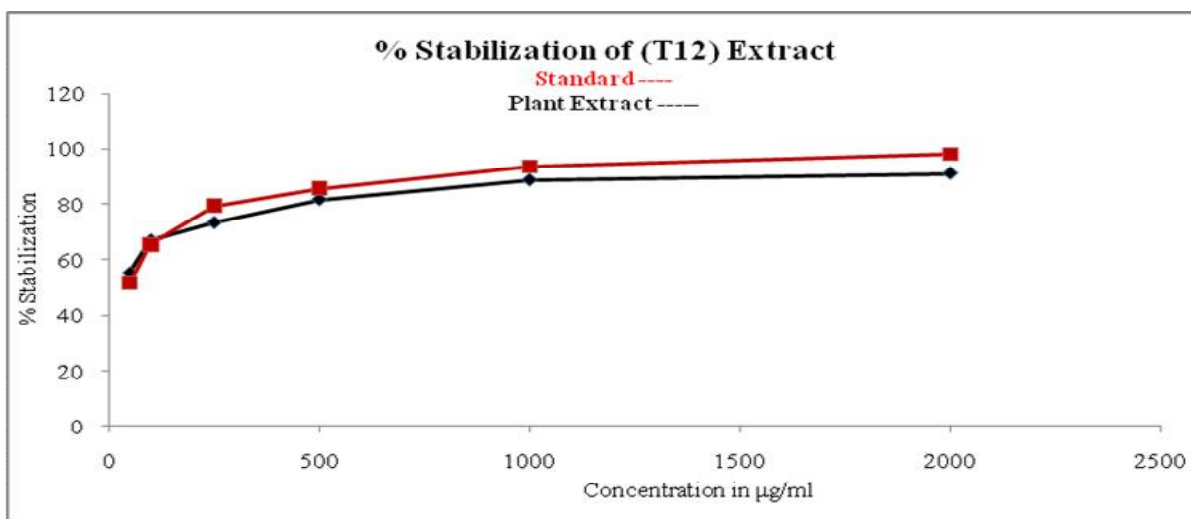


Figure 24. Graph represents HRBC Membrane Stabilization by (T12) extract and Standard