BioGecko

Vol 12 Issue 03 2023

ISSN NO: 2230-5807

ASSESSMENT OF ANTIOXIDANT POTENCY OF INHOUSE DEVELOPED HERBAL FORMULATION

Ms. Asmita Dashrath Ahir
Research Scholar, Department of Quality Assurance, Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar, Maharashtra, India.

Mr. Hariram Ramashray Yadav
Research Scholar, Department of Quality Assurance, Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar, Maharashtra, India.

Dr. Harshal Ashok Pawar*
Research Guide, Department of Quality Assurance, Dr. L. H. Hiranandani College of Pharmacy, Ulhasnagar, Maharashtra, India.

***Corresponding Author: Dr. Harshal Ashok Pawar**
Email ID: harshal.pawar@dlhhcop.org

ABSTRACT:
Present research work was aimed to investigate the Antioxidant potential of the in-house developed polyherbal formulation. Polyherbal tablets were prepared using a dry extract of Curcuminoids (*Curcuma longa*), Ashwagandha (*Withania somnifera*), and Giloy (*Tinospora cordifolia*). Phenolic content was estimated as Gallic acid equivalent by the Folin–Ciocalteu method, whereas flavonoid content was estimated as Rutin equivalent by the aluminum chloride colorimetric method.tablet formulation was investigated for its antioxidant potential using 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) assay, Phosphomolybdate Assay, or Total Antioxidant Capacity and ferric reducing antioxidant power (FRAP) assay. IC50 value (18.39µg/mL) of the tablet formulation by DPPH method was 18.39µg/mL), whereas standard ascorbic acid (2.89µg/mL). The results of all three assays show that polyherbal formulation possesses significant free radical scavenging and reducing power properties in a concentration-dependent manner. Hence, it can be concluded that in-house developed formulations possess pronounced antioxidant activity.

KEYWORDS: Antioxidant, Phenolic Content, Flavonoid Content, Polyherbal Formulation, DPPH activity.

1. INTRODUCTION:

Since ancient times herbs have been the first choice for the management of a wide range of diseases. The polyherbal formulation is the concept from Ayurveda where a combination of more than one herb or plant is used to achieve a significant level of therapeutic activity. Herbal formulations have played an important role in world health and have made an essential contribution to health care despite the great advances observed in modern medicine. In recent decades an increasing interest in herbal remedies has been observed in several parts of the world. According to an estimate by the World Health Organization (WHO), about 80 % of the population of the developing country uses herbs and other traditional medicines. [1]

Plant-based Antioxidants have the potential to protect cells from oxidative stress via various pathways. Oxidative stress due to Reactive oxygen species (ROS) and other free radicals leads to various degenerative diseases such as cancer, atherosclerosis, cardiovascular diseases, aging, and inflammatory diseases. Reactive free radicals show their action by attacking proteins, lipids, and nucleic acid. Antioxidants fight against free radicals; they exert their action by scavenging, neutralizing, and quenching the free radicals. Increasing evidence suggests that oxidative stress also

impacts the immune system of humans. It is essential to counteract this oxidative stress and thereby enhance the immunity of the human body system. Few modern medicines are available to treat oxidative stress which is costly and has side effects. On the contrary, natural antioxidants are safer and more effective hence there is an increasing interest in antioxidant. [2, 3]

Based on the evidence from the previous literature Curcuminoids (*Curcuma longa*) [4, 5] Ashwagandha (*Withania somnifera*), [6, 7] and Giloy (*Tinospora cordifolia*) [8, 9] are reported to have potent antioxidant activity. In the current work in housed polyherbal formulation was prepared and using a foresaid herbal extract and was assessed for its antioxidant potential.

2. MATERIALS AND METHODS:

Curcuminoids procured from Sahyadri Phytoconstituents Pune, Ashwagandha, Giloy extracts were procured from Himalayan Herberia Noida as a gift sample. Other chemicals such as Gallic acid And Rutin, DPPH, sodium carbonate, aluminum chloride, ascorbic acid, sulphuric acid, ammonium molybdate, sodium phosphate, and potassium ferricyanide used were of analytical grade and were used without any further chemical modification.

2.1 Preparation of Polyherbal Tablet Formulation:

Tablets were prepared usingCurcuminoids (*Curcuma longa*) Ashwagandha (*Withania somnifera*), and Giloy (*Tinospora cordifolia*and suitable excipients by direct compression method. All ingredients were accurately weighed and passed through a sieve of mesh 40 and mixed thoroughly for 20 minutes. Lubricant and Glidants were added and mixed homogeneously and compressed using a capsule shape punch. [10]

2.2 Phytochemical Evaluation:

All three extracts were evaluated for the presence of various phytoconstituents. Phytochemical screening was carried out using a simple chemical test to detect the presence of secondary metabolites such as phenol, alkaloid, tannin, flavonoid, saponin, glycoside, etc. which proved to enhance the antioxidant potential. Using the procedure previously published in the literature. [11, 12]

2.3 Estimation of Phenolic and Flavonoid Content of Curcuminoids, Ashwagandha, and Giloy Extract

2.3.1 Estimation of Total Phenolic Content [13, 14]

Total phenolic content was determined by using the Folin-ciocalteu method. This test is based on the oxidation of phenolic groups with phosphomolybdic/phosphotungstic acids. After oxidation, a green-blue complex formed which was measured at 725 nm.

Preparation of test Solutions: Accurately weighed 10 mg of each of the extracts was dissolved in 100 ml of methanol to get 100µg/ml solution. Further diluted to get 10 µg/ml.

Preparation of Standard Solutions: Accurately weighed 10mg of Gallic acid was dissolved in 100 ml methanol to get (100 µg/ml). This Standard solution was diluted with distilled water to get lower concentrations in a range of 2.5 to 20 µg/ml.

Procedure

1 ml of test and standard solutions were added in a 10 ml volumetric Flask separately. 0.5ml of Folin-Ciocalteu reagent and 2.5ml of 7.5 % sodium carbonate solution were added and the volume was made up with distilled water. The mixture was shaken well and kept at room temperature for 30 min. absorbance was measured using a UV visible Spectrophotometer (Shimadzu, UV-1800) at 725 nm against a blank without extract. The standard curve of gallic acid was plotted. With help of the standard curve equation total, phenolic content was calculated and expressed as Gallic acid equivalent (GAEmg/gm).

2.3.2Estimation of Total Flavonoid Content [13, 15]

Total flavonoid content is determined by the aluminum chloride method. The principle of this method is that aluminum chloride forms acid-stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl groups of flavones. In addition, aluminum chloride forms acid labile complexes with the ortho-dihydroxyl groups in the A or B ring of flavonoids. The concentration of these complexes was measured at 415 nm.

Preparation of Test Solutions

Accurately weighed 10 mg each of the extracts was dissolved in 100 ml of methanol to get 100µg/ml solution. Further diluted to get 10 µg/ml.

Preparation of Standard Solutions

Accurately weighed 10 mg Rutin was dissolved in 100 ml methanol to get 100 µg/ml. This Standard solution was diluted with methanol to get lower concentrations in a range of 5 to 30 µg/ml.

Procedure

1 ml of test and standard solutions were addedto 3 ml of methanol separately. To this solution, 0.2 ml of 10% aluminum chloride ($AlCl_3$), and 0.2 ml of 1 M sodium acetate were added and the volume was made up to 10 ml using distilled water. The mixture was incubated at room temperature for 30 min. absorbance was measured using a UV visible Spectrophotometer (Shimadzu, UV-1800) at 415 nm against a blank without extract. The standard curve of Rutin was plotted. With help of the standard curve equation total flavonoid content, the total flavonoid content was expressed as Rutin equivalent (Rutin mcg/mg).

2.4 DPPH Scavenging Activity [16] [17][18]

All three extracts, tablet formulation, and standard ascorbic acid were subjected to radical scavenging activity with the help of the DPPH assay.

The DPPH free radical is reduced to corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in color and upon reaction with a hydrogen donor changes to yellow in color. It is a discoloration assay, which is evaluated by the addition of an antioxidant to a DPPH solution in ethanol or methanol, and the decrease in absorbance was measured at 516 nm**.**

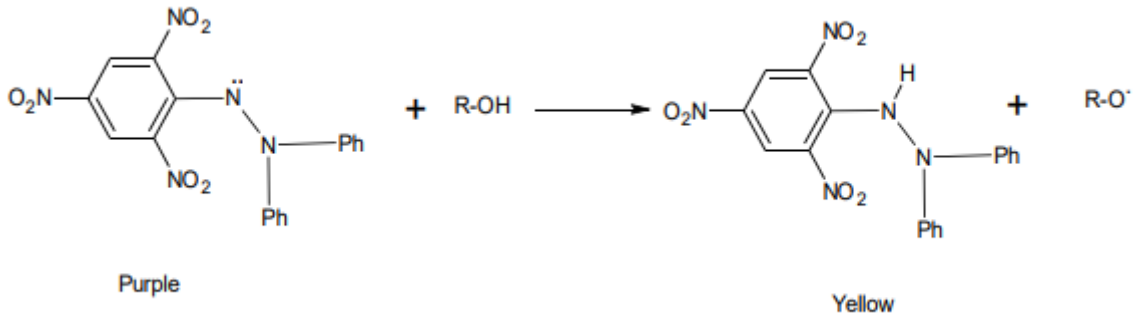

Fig 1: Mechanism of DPPH scavenging assay

Preparation of Test Solutions

Accurately 10 mg of each of the extracts were weighed and dissolved in 10 ml of methanol separately to obtain solutions of 1000µg/ml concentration. These solutions were diluted separately to obtain lower concentrations.

Procedure
Samples were prepared by properly diluting the stock solution and adding 3 ml of methanol and 50 µl of 0.1mM DPPH solution was added and incubated in a dark room at room temperature for 4 minutes. Absorbance was measured at 516 nm using methanol as a blank. A decrease in absorbance in the presence of test samples at different concentrations was noted.
The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (without extract) using the formula.

$$\text{Percentage inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} \times 100$$

2.5Phosphomolybdate Assay [19]
The total antioxidant activity of the optimized formulation was also estimated by the phosphomolybdate method. Ascorbic acid was used as standard. Higher absorbance values indicated a higher total antioxidant potential.

Preparation of the Standard Solution of 10 mg of ascorbic acid dissolved in 100 ml of methanol to get (100µg/ml) this solution was further diluted to lower concentrations in a range of 10-30 µg/ml respectively.

Preparation of the Test Solution equivalent amount of tablet powder was dissolved in 100 ml of methanol to get (100µg/ml) this solution was further diluted to lower concentration in a range of 5- 30 µg/ml respectively.

Procedure
About 0.1 ml of standard and test solution was added to 1 ml of reagent solution (0.6 M sulphuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate). The tubes containing the mixture were covered with silver foil and incubated for 90 minutes in a water bath at 95°C, before allowing the mixture was allowed to cool at room temperature. The absorbance of the solution was determined at 765 nm against a blank. Ascorbic acid was used as standard. Higher absorbance values indicated a higher total antioxidant potential.

2.6 Reducing Power Assay /ferric Reducing Antioxidant Power (FRAP) Assay [67]
The reducing power of optimizedformulation was determined by the method of "Oyaizu" The reduction of ferric to ferrous ion is directly proportional to the absorbance.

Preparation of Test Sample
The equivalent amount of tablet powder was dissolved in 100 ml of methanol to get (100µg/ml) this solution was further diluted to lower concentrations in a range of 1-10 µg/ml respectively.

Procedure
1 ml of the sample solution in methanol was added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide ($K_3Fe(CN)_6$) solution. The mixture was incubated at 50 °C for 20 min which was followed by the addition of 2.5 mL 10% (w/v) trichloroacetic acid and centrifugation at 3000 rpm for 10 min. Finally, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) ferric chloride, and absorbance was measured at 700 nm against a blank. Increased absorbance of the reaction mixture indicated greater reducing power.

3. RESULTSAND DISCUSSION

3.1 Phytochemical Evaluation

The preliminary phytochemical study revealed the presence of various secondary metabolites such as carbohydrates, glycosides, flavonoids, alkaloids, saponins, and tannins, etc. in all three extract results are depicted in Table 1phytochemical Evaluation All three extracts show the presence of secondary metabolites such as phenol, tannin, and flavonoid which contribute to antioxidant activity. All three extracts show the presence of s phenol, tannin, and flavonoid which contribute to antioxidant activity.

Table No.1:Phytochemical Evaluation

Test		Curcuminoids	Ashwagandha	Giloy
Carbohydrates	Molish	+	+	+
	Felhinges	+	-	+
Protein	Millions	+	+	+
Steroids	Salkowski	+	+	+
	Liebermann Burchard	-	+	+
Glycoside	Bontragers	-	+	+
Saponin	Foam	+	+	+
Flavonoid	Shinoda	+	+	+
	Lead acetate	+	+	+
Tannin	Fecl3 test	+	+	+
Alkaloid	Dragendorff	+	+	+
	Mayers And wagners	+	+	-

3.2 Estimation of Phenolic and Flavonoid Content and Curcuminoids, Ashwagandha, and Giloy Extract

3.2.1 Total Phenolic Content

Phenols are one of the phytoconstituents present in the plant with redox properties which are responsible for antioxidant activity. Phenolic content was measured using the Folin–Ciocalteu reagent method. Folin - ciocalteu reagent contains phosphomolybdic/ phosphotungstic acid complexes. The method relies on the transfer of electrons in an alkaline medium from a phenolic compound to form a blue-colored complex where the maxim absorption depends upon the concentration of the phenolic compound. The reduced folin- ciocalteu reagent is detectable with a 690-700 nm spectrometer. The results were derived from a calibration curve ($y = 0.174x - 0.0673$, $R2 = 0.9685$) of gallic acid and expressed as gallic acid equivalent (mg of GAE/g). Phenolic content determined in the extracts by the above method is depicted in Table 2 indicating that phenol content in curcuminoids extract is highest followed by Ashwagandha extract and Giloy extract.

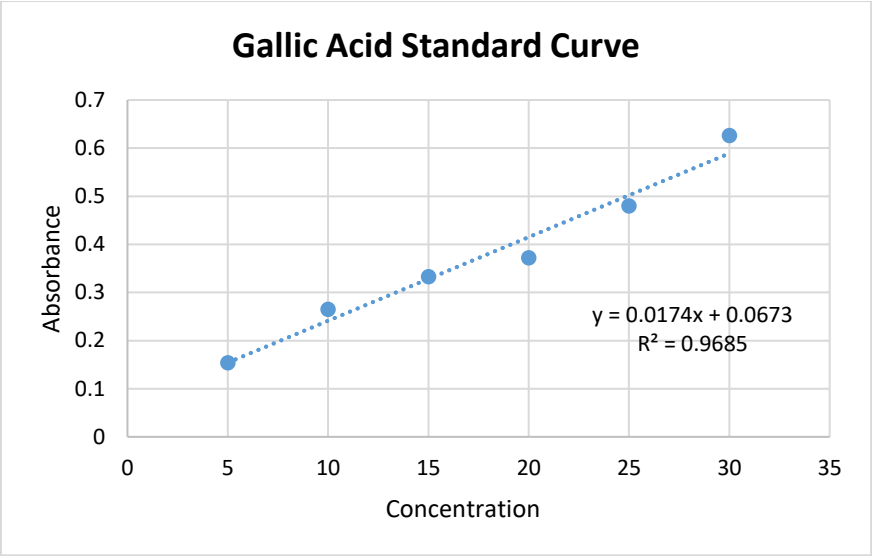

Fig No.2: Standard Curve for Gallic acid

Table No.2: Total Phenol content for selected extracts at 415nm

Extracts	**Total phenolic content (mg of GAE/g).**
Curcuminoids	26.23
Ashwagandha	11.254
Giloy	7.748

3.2.2 Total Flavonoid Content

Total Flavonoids are secondary metabolites with antioxidant activity, the potency of which depends on the number and position of free OH groups. Flavonoid contents in selected plant extracts were determined using the aluminum chloride colorimetric method. The principle of the $AlCl_3$ method is that it forms acid stable yellow colored complexes with the flavones. Which can be measured spetromatically at 415 nm. The results were derived from the calibration curve (y = 0.0362+ 0.0683, R2 = 0.976). Total flavonoid content was expressed as Rutin equivalent (mg of Rutin/g). Flavonoid content was determined in the extracts by the above method as depicted in Table 3 Results indicate that curcuminoids contain the highest amount of flavonoid as compared to ashwagandha and giloy extract.

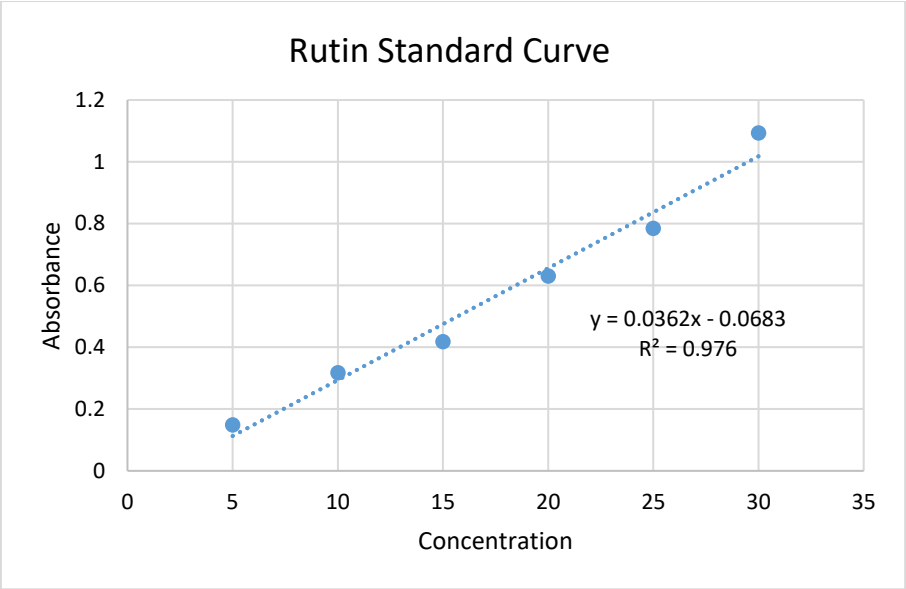

Fig No.3: Standard Curve for Rutin

Table No.3: Total flavonoid content for selected extracts at 415nm

Extracts	**Total flavonoid content (mg of Rutin /g)**
Curcuminoids	24.17
Ashwagandha	1.67
Giloy	1.1

2.4 DPPH Scavenging Activity

DPPH is a free radical that reacts with hydrogen donors. Initially DPPH radical is purple in color but upon reaction with a hydrogen donor, it becomes colorless and leads to the formation of a non-radical form of DPPH All the test Samples showed concentration-dependent increases in radical scavenging capacity. In the case of extracts, The greatest DPPH radical scavenging potency with a minimum IC50 value was recorded for curcuminoids extract (25.35µg/mL), followed by Ashwagandha Extract(72.5 µg/mL), and Giloy Extract with (205.5 µg/mL).% inhibition for each extract is summarized in Table 4

The IC 50 value of the optimized tablet formulation was recoated with (18.38 µg/mL). While IC50 value of standard ascorbic acid (2.89 µg/mL), as presented in Tablet 5.

Results of IC 50 value of individual extract and formulation indicate. The formulation has a lower IC50 value indicating higher antioxidant potential. As compared to individual extract. From this, it can be concluded that a mixture of extracts increases antioxidant activity more than individual extracts.

Table No.4: % Inhibition by Curcuminoids, Ashwagandha extract, Giloy extract.

Curcuminoids		**Ashwagandha**		**Giloy**	
Conc. (µg/ml)	**% Inhibition**	**Conc. (µg/ml)**	**% Inhibition**	**Conc. (µg/ml)**	**% Inhibition**
1	6.14	1	1.85	75	24
10	27.19	25	8.88	100	34.12
20	47.36	50	25	125	42.06

30	54.38	75	58.3	150	45.23
40	78.94	100	67.59	175	50.00
50	84.21	125	75	200	52.38
				300	57.93
IC_{50}	**25.35**	**IC_{50}**	**72.5**	**IC_{50}**	**205.5**

Table No.5: % Inhibition by Standard Ascorbic Acid and Optimized formulation

Ascorbic acid		**Formulation**	
Conc. (µg/ml)	**% Inhibition**	**Conc. (µg/ml)**	**% Inhibition**
1.5	34.72	5	18.72
2.5	48.63	10	28.31
3.5	58.83	15	46.11
4.5	61.11	20	63.47
5.5	74.3	25	65.29
6.5	87.5	30	73.51
		35	77.62
IC_{50}	**2.89**	**IC_{50}**	**18.39**

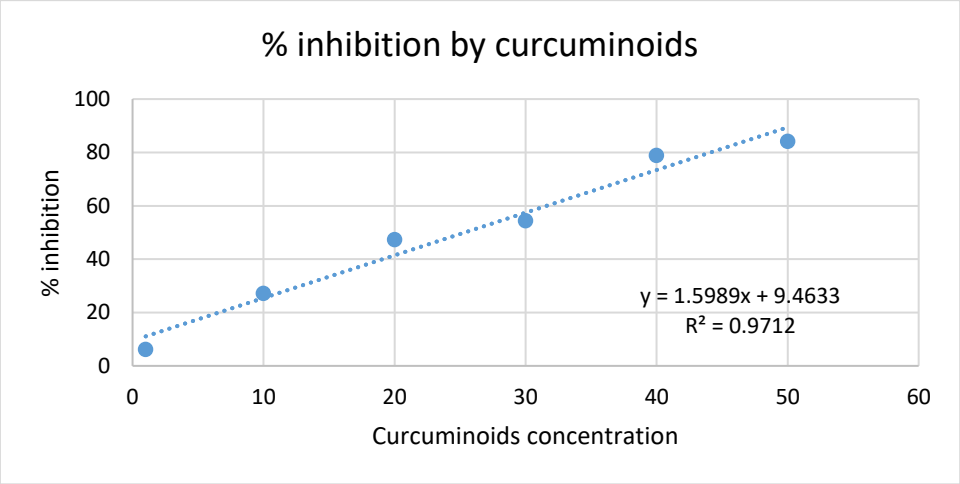

Fig No. 4: Standard Curve for % Inhibition by DPPH Assay (Curcuminoids)

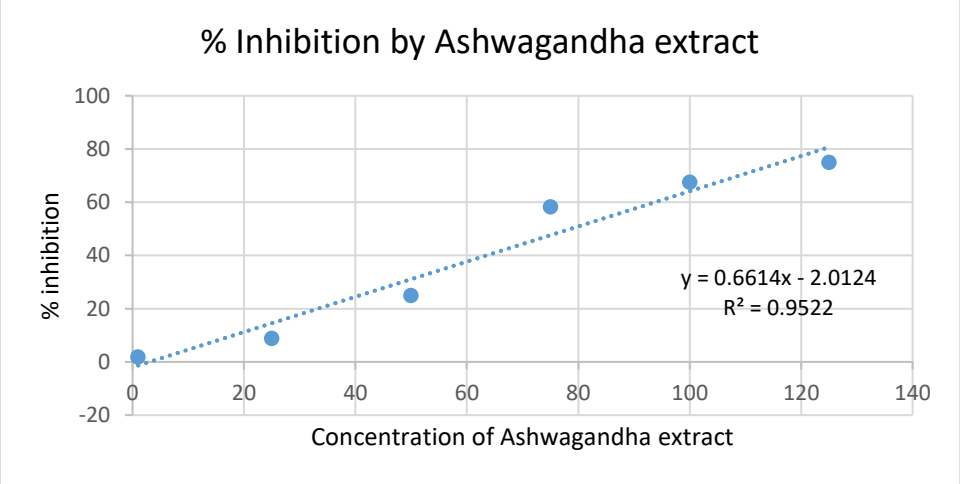

Fig No. 5: Standard Curve for % Inhibition by DPPH Assay (Ashwagandha Extract)

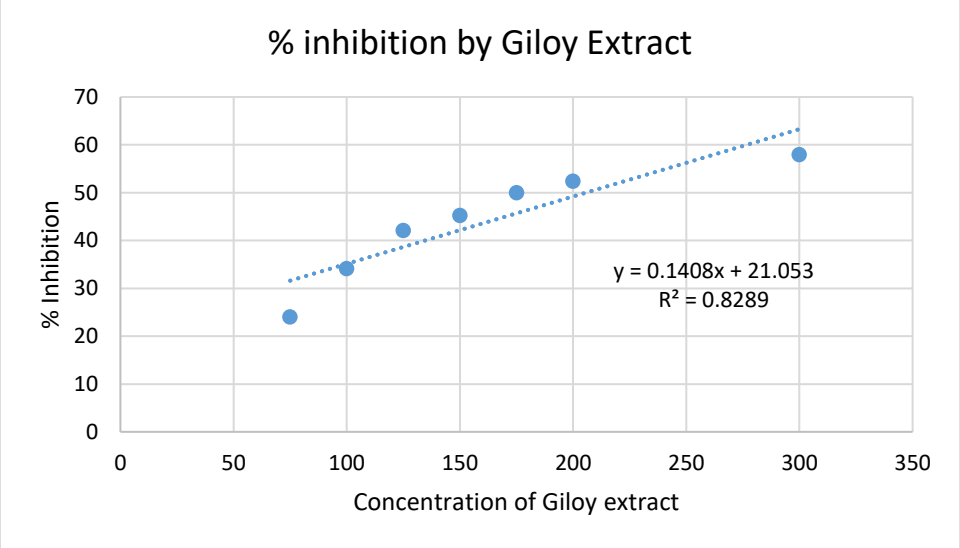

Fig No.6: Standard Curve for % Inhibition by DPPH Assay (Giloy Extract)

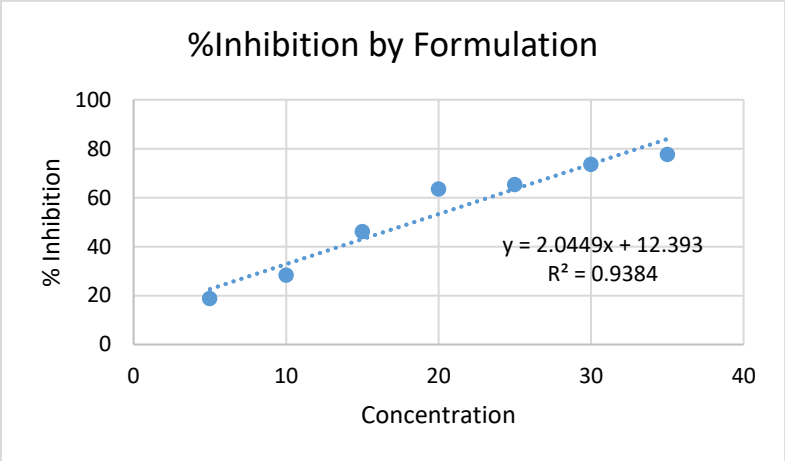

Fig No. 7: Standard Curve of % Inhibition by DPPH Assay (Optimized Formulation)

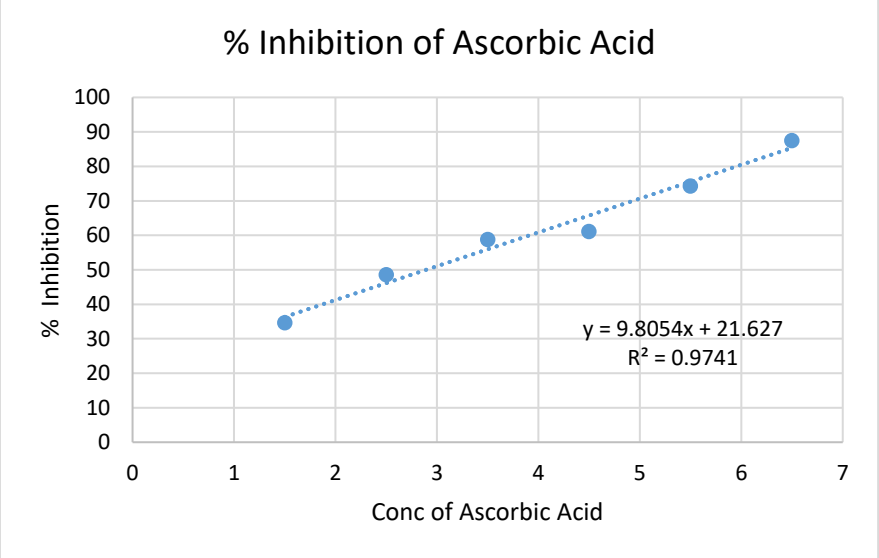

Fig No.8:Standard Curve of % Inhibition by DPPH Assay (Standard ascorbic acid)

3.5 Phosphomolybdate Assay or Total Antioxidant Capacity:
The phosphomolybdate method is quantitative, as the total antioxidant capacity (TAC) is expressed as equivalent to ascorbic acid. This assay is based on the reduction of phosphomolybdate ion in the presence of an antioxidant resulting in the formation of a green phosphate/MoV complex which is measured spectrophotometrically at 695 An increase in absorbance indicates an increase in antioxidant activity. Ascorbic acid equivalent was found to be (9.34mg of ascorbic acid /g). The overlay graph of the Phosphomolybdate assay of Ascorbic Acid And Formulation is given in, figNo 10, Table 6. Indicates absorbance increase in a concentration-dependent manner which is an indicatorof antioxidant activity**.**

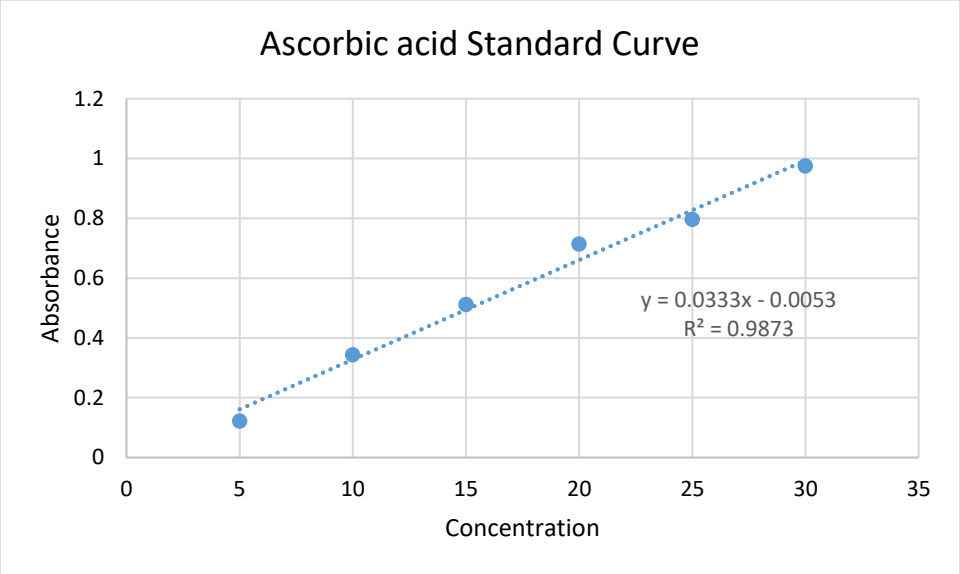

Fig No. 9: Standard Curve for Ascorbic acid at 695 nm for Phosphomolybdate assay

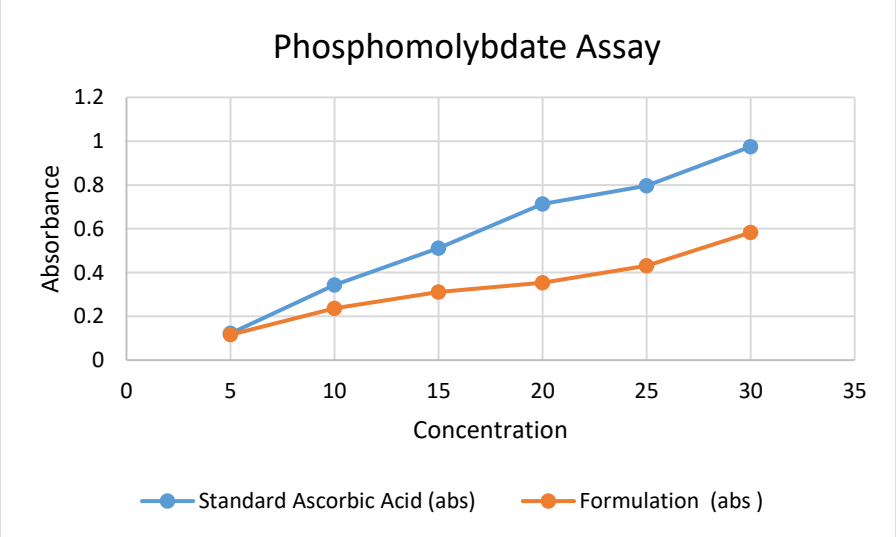

Fig No.10: Overlay Phosphomolybdate assay of Ascorbic Acid and Formulation

Table No.6: Absorbance of Standard Ascorbic Acid Formulation at 698 nm

Concentration (µg/ml)	Standard Ascorbic Acid (abs)	Formulation (abs)
5	0.122	0.116
10	0.344	0.236
15	0.512	0.311
20	0.714	0.353
25	0.797	0.431
30	0.975	0.583

3.6 Ferric Reducing Antioxidant Power (FRAP) Assay:
The transformation ability of compounds from Fe3+/ferricyanide complex to Fe2+/ferrous form acts as a potential indicator for antioxidant activity. In the FRAP assay, the yellow color test solution changes to green and blue depending on the reduction capacity of extracts or compounds. The presence of reductants in the test solution reduces Fe3+ to Fe2+, which can be monitored by measurement of Perl's Prussian blue color at 700 nm. Fig no 11 shows a graph showing an increase in absorbance with increased concentration which indicates a reducing power property.

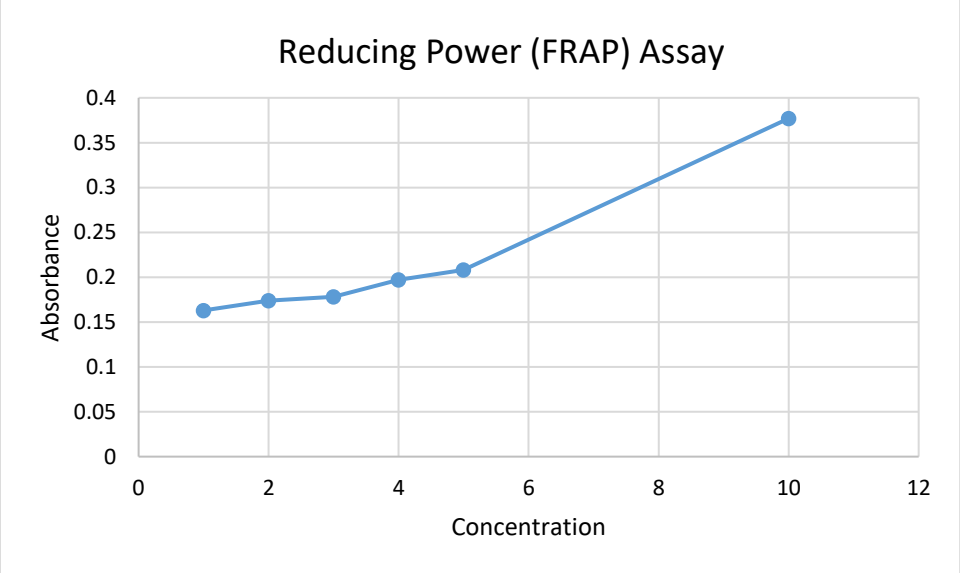

Fig No. 11: Reducing Power Assay of Formulation

Table No. 7: Absorbance of Formulation at 700 nm

Concentration (µg/ml)	Absorbance (nm)
1	0.163
2	0.174
3	0.178
4	0.197
5	0.208
10	0.377

4. CONCLUSION:
Replacement of synthetic antioxidants with natural antioxidants may be advantageous in various ways such as being safer with lesser side effects, cost-effective, and also widely accepted. In the present research work, well-established antioxidant plant extracts were selected for the development of formulation. Results of the phytochemical evaluation indicate the presence of various

phytoconstituents in selected herbal. Flavonoid and phenol content estimated quantitatively are responsible for antioxidant activity. Results of the DPPH assay indicate that Formulation possesses higher antioxidant potential as compared to individual extract. Results of the phosphomolybdate assay and ferric-reducing antioxidant power assay also prove the antioxidant potential of the formulation. Polyphenolic curcuminoids in Curcuminoids extract, Withanoilds and other alkaloids in Ashwagandha extract and other various phytoconstituents in Giloy extract are contributes to the antioxidant property of the formulation.Although the parameters used in this study were not disease-specific, the quantification of antioxidant properties can serve as a guide for the use of these plants for reactive oxygen species related diseases. Further investigation into the identification of responsible antioxidant components and their mechanism of action to better understand their ability to control diseases that have a significant impact on quality of life.

5. CONFLICT OF INTREST:

The authors declare that there is no conflict of interest regarding the publication of this paper.

6. ACKNOWLEDGMENT:

The authors are very much thankful to Dr. P. S. Gide principal of Hyderabad Sindh National Boards (HSNCB's) Dr. L.H. Hiranandani College of Pharmacy, Ulhasnagar, for his continuous support, guidance, and encouragement

5. REFERENCES:

1. Pan, J., He, S., Zheng, J. et al. The development of an herbal material quality control strategy considering the effects of manufacturing processes. Chin Med 14, 38 (2019). https://doi.org/10.1186/s13020-019-0262-9.
2. Alok S, Jain SK, Verma A, Kumar M, Mahor A, Sabharwal M. Herbal antioxidant in clinical practice: a review. Asian Pac J Trop Biomed. 2014 Jan; 4(1):78-84. doi: 10.1016/S2221-1691(14)60213-6.
3. Ali, R., Sajjad, N., Qureshi, S., Hassan, S., Ahmad, M., Ganie, S. A., And Hamid, R. Evaluation of Antioxidant and Immunomodulatory Potential of Methanolic Extract of Juniperus squamata Buch.-Ham. Ex D.Don. Free Radicals and Antioxidants, (2018). 8(2), 111–119.
4. Ak, T., And Gülçin, İ. Antioxidant and radical scavenging properties of curcumin. Chemicobiological interactions, (2008)174(1), 27-37.
5. Menon, V. P., And Sudheer, A. R. Antioxidant and anti-inflammatory properties of curcumin. The molecular targets and therapeutic uses of curcumin in health and disease, (2007). 105-125.
6. Davis, L., And Kuttan, G. Immunomodulatory activity of Withania somnifera. Journal of ethnopharmacology, 71(1-2), (2000)193-200.
7. Agarwal, R., Diwanay, S., Patki, P., And Patwardhan, B**. (1999).** Studies on immunomodulatory activity of Withania somnifera (Ashwagandha) extracts in experimental immune inflammation. *Journal of Ethnopharmacology*, *67*(1), 27-35.
8. Kapil, A., And Sharma, S. (1997). Immunopotentiating compounds from Tinospora cordifolia. Journal of ethnopharmacology, 58(2), 89-95.
9. Mathew, S., And Kuttan, G. Immunomodulatory and antitumour activities of Tinospora cordifolia. Fitoterapia, (1999) 70(1), 35-43.
10. GAONKAR, V. And MANNUR, V. And MASTIHOLIMATH, V.S. And Hullatti, K. K... Development and Evaluation of Herbal Supplement: A Quality by Design Approach. Indian Journal of Pharmaceutical Sciences. (2020) 82. 10.36468/pharmaceutical-sciences.690.
11. Kokate, C.K.; Purohit, A.P.; Gokhale, S.B. Pharmacognosy; Ed.55th; Nirali Publication. India, 2018 pp.14.138. SYNOPSIS Page | 9
12. Pawar, H. Phytochemical Evaluation and Curcumin Content Determination of Turmeric Rhizomes Collected From Bhandara District of Maharashtra (India). Medicinal Chemistry, 2014,

13. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. Plants (Basel). 2019 Apr 11;8(4):96. doi: 10.3390/plants8040096. PMID: 30978964; PMCID: PMC6524357
14. Soobrattee, M.A.; Neergheen, V.S.; Luximon-Ramma, A.; Aruoma, O.I.; Bahorun, T. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutat. Res.-Fund. Mol. Mutagen. 2005, 579, 200–213.
15. Panche, A.N.; Diwan, A.D.; Chandra, S.R. Flavonoids: An overview. J. Nutr. Sci. 2016, 5, e4 Umamaheswari, M., And Chatterjee, T. K. (2008). In vitro antioxidant activities of the fractions of Coccinia grandis L. leaf extract. *African Journal of Traditional, Complementary and Alternative Medicines*, *5*(1), 61-73.
16. Rahman, M.M., Islam, M.B., Biswas, M. et al. In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. BMC Res Notes 8, 621 (2015). https://doi.org/10.1186/s13104-015-1618-6.
17. Umamaheswari M, Chatterjee TK. In vitro antioxidant activities of the fractions of Coccinia grandis L. leaf extract. Afr J Tradit Complement Altern Med. 2007 Oct 27;5(1):61-73. PMID: 20162057; PMCID: PMC2816591.
18. 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. Anal Biochem. 1999 May 1;269(2):337-41. doi: 10.1006/abio.1999.4019. PMID: 10222007.
19. khatoon, M., Islam, E., Islam, R. et al. Estimation of total phenol and in vitro antioxidant activity of Albizia procera leaves. BMC Res Notes 6, 121 (2013). https://doi.org/10.1186/1756-0500-6-121
20. Vijayalakshmi, M., and K. Ruckmani. "Ferric Reducing Anti-Oxidant Power Assay in Plant Extract". Bangladesh Journal of Pharmacology, vol. 11, no. 3, June 2016, pp. 570-2, doi:10.3329/bjp.v11i3.27663.
21. Binay Kumar Mahto, Ajay Kumar Shukla. Development and Assessment of Antioxidant, Anticancer Activity of Poly-Herbal Combination Research And Reviews: Journal of Agriculture and Allied SciencesRRJAAS| Volume 11 | Issue 4 |April, 2022
22. Bhalodia NR, Nariya PB, Acharya RN, Shukla VJ. In vitro antioxidant activity of hydro alcoholic extract from the fruit pulp of Cassia fistula Linn. Ayu. 2013 Apr;34(2):209-14. doi: 10.4103/0974-8520.119684. PMID: 24250133; PMCID: PMC3821253.
23. Soobrattee M.A., Neergheen V.S., Luximon-Ramma A., Aruoma O.I., Bahorun T. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutat. Res.-Fund. Mol. Mutagen. 2005;579:200–213. doi: 10.1016/j.mrfmmm.2005.03.023.