Section A-Research paper

IN-VITRO ANTIOXIDANT EVALUATION OF DIOSMIN LOADED PHYTOSOME BY DPPH ASSAY (2,2-DIPHENYL-1-PICRYLHYDRAZYL ASSAY)

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Abstract:

Objective: The current research work was at attempt to investigate the *in-vitro* antioxidant activity of diosmin loaded phytosomal preparation by using DPPH Assay. **Method**: Phytosome was prepared with Diosmin and Phospholipid by using solvent evaporation method. The *in-vitro* antioxidant activity was measured using DPPH method for evaluation of FRSA. **Results:** the outcome of the research work clearly indicated that Diosmin loaded Phytosmal formulation replicated better free radical scavenging activity(FRSA) and results showed 78.21±0.82 % with Diosmin and 85.11±0.24% with phytosome at Highest concentration (80µg/ml)and IC50 of Diosmin was 43.15 µg/ml and IC50 for Diosmin Loaded Phytosome was 39.01 µg/ml. **Conclusion**: Experimental results showed that Diosmin Phytosome has significant Antioxidant Activity.

Keywords: Diosmine-Phytosome, Antioxidant activity, DPPH (2,2-Diphenyl-1-picrylhydrazyl assay)

Introduction:

Medicinal plants are the richest source, which provide new opportunities for the treatment of various diseases due to presence of pharmacologically active constituents. Which makes them s a more suitable candidate having low dose with minimal side effect. Different plants having active constituents are used as therapeutic active agent as anticancer, anti-inflammatory, hepato-protective, antioxidant activity, anti-inflammatory action. In the pharmaceutical industry, medicinal plants are valued for their active constituents such as polyphenols and flavonoids, glycosides, alkaloids, and tannins, which may be used as therapeutic agents in the synthesis of drugs. Besides medicinal purposes, they also used as nutritional purpose or used to remove disease symptoms. Oxidative stress is one of the primary causes of progression of different diseases like cancer, hyper-lipidemia, gastric ulcer and diabetes. Antioxidant protects the body from damage caused by free radical initiative reaction. Free radicals and different reactive oxygen species (ROS) are developed constantly in the human body and excess species are removed by the different enzyme and non–enzyme antioxidant defense mechanism. Reactive oxygen species such as free radicals, superoxide anion radicals, Hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-) and non–free radicals and singlet oxygen (O_2)

along with various forms of active oxygen are actively involved in various physicochemical and biochemical processes in the body and aging derivatives of oxygen attributed as reactive oxygen species, are continuously generated inside the human body during normal life. The generated ROS are detoxified by the antioxidants present in the human body. Excess production of ROS and/or inadequate antioxidant defense can easily affect and persuade oxidative damage to various bimolecular such as proteins, lipids, lipoproteins and Nucleic acid[12].Antioxidants behave as micronutrients substance having ability to neutralize free radicals or their action. Different compound such as Anthocyanin, Coumarines, Flvanoids ,lignans, catechins and isocatechins used as antioxidant effect.

Active Constituents: Diosmin is a natural occurring flavonoid used for medicinal purpose to treat different diseases. Disomin act as vascular protecting therapeutic agent used to treat chronic venous insufficiency, antioxidant activity, Hepatoprotective action but possess practically low solubility in water, low bioavailability, poor drug dissolution and higher dose [4].it is also a nutrient used to improve metabolic disorders like glucose metabolism. Diosmin is worked as vascular protecting agents and used to improve chronic hemorrhoids, lymphedema, chronic venous insufficiency and varicose veins.[6]

Diosmin has molecular formula $C_{28}H_{32}O_{15}$.Basic and clinical research studied that the diosmin drug (IUPAC name 5-hydroxy-2- (3-hydroxy-4-methoxyphenyl) -7- [(2S,3R,4S,5S,6R) -3,4,5-trihydroxy-6- [[(2R,3R,4R,5R,6S) -3,4,5- trihydroxy -6- methyloxan -2- yl] oxymethyl] oxan-2-yl] oxychromen -4- one.) [6]. the diosmin loaded phytosome is the one approach to increase the physicochemical properties, Therapeutic activity and bioavailability of drug in comparison of pure drug. [7]

2,2-Diphenyl-1-picrylhydrazyl Assay(DPPH Assay): Several methods have been used for evaluation of total antioxidant activity of Drug. The 2,2-Diphenyl-1-picrylhydrazyl assay or DPPH assay is one of the widely used method .DPPH is a dark colored crystalline powder , which can accommodate a large number of samples in a short period of time and is enough sensitive to identify for quantitative analysis of the natural compounds at low concentrations so it was used in this study for the primary screening of antioxidants for phytosomal activity [15]. From this present study evaluate the free radical scavenging activity (FRSA) (DPPH activity) of Pure drug Diosmin, Ascorbic acid and Optimized diosmin loaded phytosome to compare their highest antioxidant potential.[16]

Materials and Methods:

Diosmin (purchased from Sigma-Aldrich, Mumbai, India), Phospholipid and DPPH(2,2-Diphenyl-1-picrylhydrazyl), Ascorbic acid and chemical reagents are of analytical grade.

Method for Phytosome Development:

Phytosome is a complex system prepared by using Solvent evaporation method. Diosmin phytosome was prepared by using round bottom flask. Firstly Diosmin were dissolve in Dimethylsulphoxide (DMSO) and taken with different molar ratio of phospholipid in round bottom flask (RBF). Mixture was refluxed using water bath for Three hours at 45^0 C and after three hour, the mixture was cooled and then pour to wide mouth petridish. The petridish was kept open overnight at room temperature for evaporation of solvent and after this dried under vacuum at 60^0 C for Three hour. Diosmin loaded phytosomes residue were collected and stored in desiccators for overnight[7].Optimized Formulation having Diosmin to phospholipid molar ratio (1:1.75) was used for *in-vitro* antioxidant evaluation.

In-Vitro Antioxidant Activity:

Antioxidant Assay: Antioxidant activity of Diosmin and Diosmin Loaded Phytosome were determined by in-vitro DPPH free radical scavenging assay.

Method for *In-Vitro* Antioxidant Activity:

2,2-Diphenyl-1-picrylhydrazyl assay (DPPH assay)

For the study of antioxidant activity of active constituents Diosmin and Diosmin Loaded Phytosomes were analyzed as per the Williams *et al.* method by using DPPH method. For evaluation a stock solution of DPPH (0.1mM solution)was prepared in analytical methanol and kept in ambered colored glass container for 30 min at room temperature[8].For activity analysis used different dilution from 10 to 80 µg/ml (10,20,30,40,50,60,70 and 80 µg/ml) of 2 ml sample were prepared and added to 1 ml of 0.1 mM DPPH solution. The mixture was incubated for 30 min at 37 ^{0}c and check the absorbance in *UV-VIS* spectrophotometer (1800 model, Shimadzu) at 517 nm[9]. IC_{50} was calculated from percentage inhibition. For standard solution activity ascorbic acid used for this purpose and experiment was performed in triplicate manner (n=3) and All the

Results are prepared as mean ± standard deviation and the percentage of DPPH radical scavenging activity was evaluated by following equation [14].

$$AA\% = (1\text{-}Abs_{SAMPLE} / Abs_{CONTROL}) \times 100$$

AA% = percentage antioxidant activity (AA) or DPPH radical scavenging percentage
ABS_{SAMPLE} = Absorbance of Sample
$ABS_{CONTROL}$ =Absorbance of Control

Result and Discussion:

***In-Vitro* Antioxidant Activity by DPPH Method:** the present study was evaluated for the antioxidant activity by DPPH a comparable antioxidant with that of standard ascorbic acid (for different concentration 10 to 80 µg/ml). There was observed that diosmin showed a concentration dependent activity at all used concentration range (for various concentration range 10 to 80 µg/ml) .In the study table-1 clearly reflects and found to be that diosmin phytosome was better active antioxidant comparison to pure diosmin drug .At highest concentration 80 µg/ml the Ascorbic acid showed 95.75±0.56% Percentage free radical scavenging activity and with Diosmin at Higher concentration showed 78.21±0.82 % and with Highest concentration of Diosmin loaded Phytosome showed 85.11±0.24 %.The IC50 Value was observed for Ascorbic acid was 32.09 µg/ml,IC50 for Diosmin 43.15 µg/ml and IC50 For diosmin Loaded Phytosome showed 39.01 µg/ml .Therfore the result showed that the Percentage FRSA by DPPH increased with Phytosme formulation against Pure drug diosmin and showing the Betterment of Phytosme therapeutic activity for antioxidant property[13] [17].

Table 1: Concentration Vs Percentage Free radical Scavenging Activity by DPPH

S.No.	Concentration(µg/ml)	Percentage Free radical Scavenging Activity by DPPH		
		Ascorbic acid	**Diosmin**	**Phytosome**
1.	10	28.61±0.11	23.21±0.84	27.03±0.74
2.	20	37.12±0.23	28.74±0.65	33.47±0.21
3.	30	47.32±0.51	36.16±0.11	41.60±0.23
4.	40	54.71±0.41	51.51±0.79	51.45±0.45
5.	50	66.08±0.70	54.36±0.68	57.12±0.85
6.	60	72.89±0.32	68.25±0.57	70.21±0.12
7.	70	86.24±0.87	72.11±0.75	74.24±0.91
8.	80	95.75±0.56	78.21±0.82	85.11±0.24
IC50 Value (µg/ml)		32.09	43.15	39.01

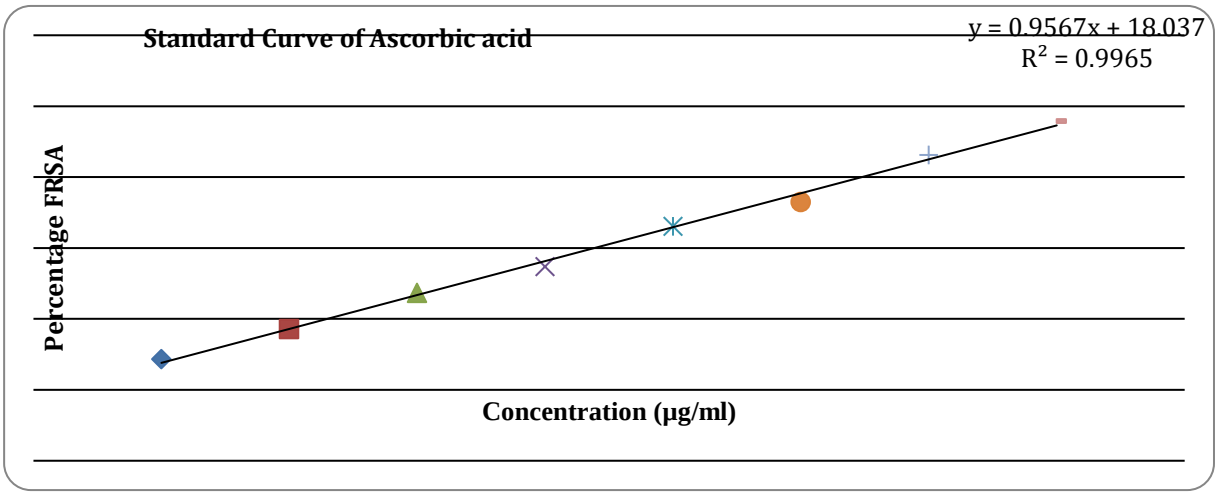

Figure 1: Standard Curve of Ascorbic acid

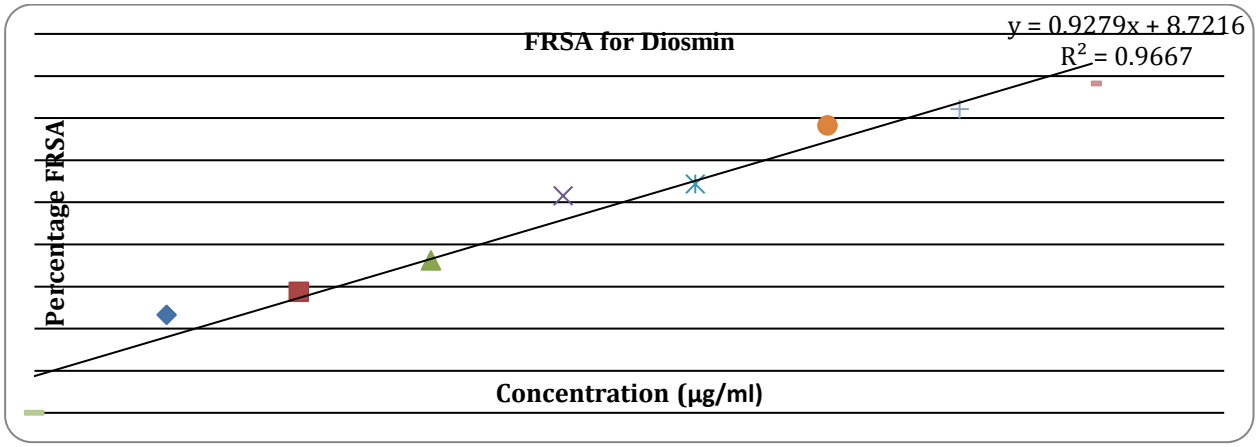

Figure 2:FRSA study for DIOSMIN

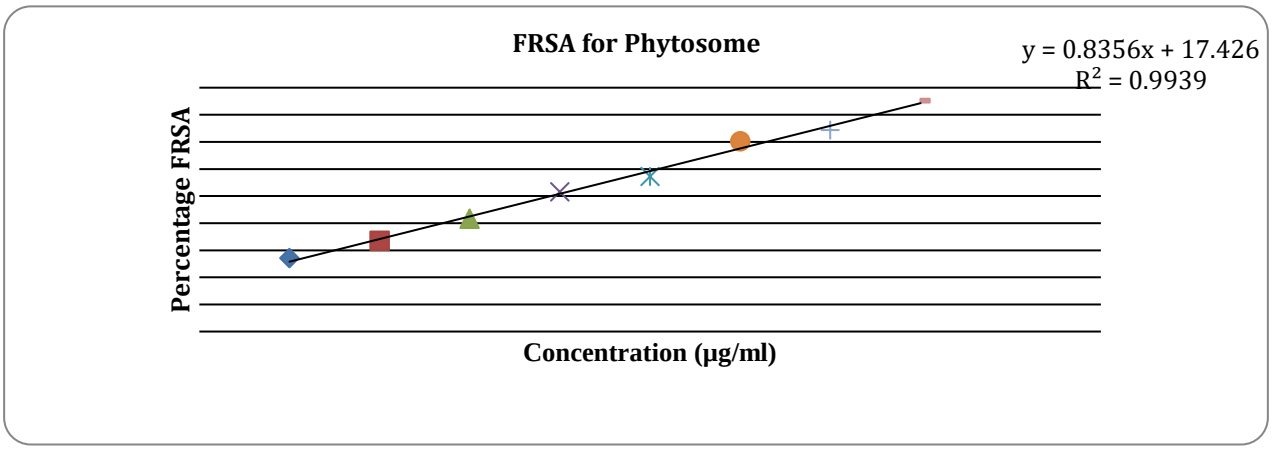

Figure 2:FRSA study for Phytosome

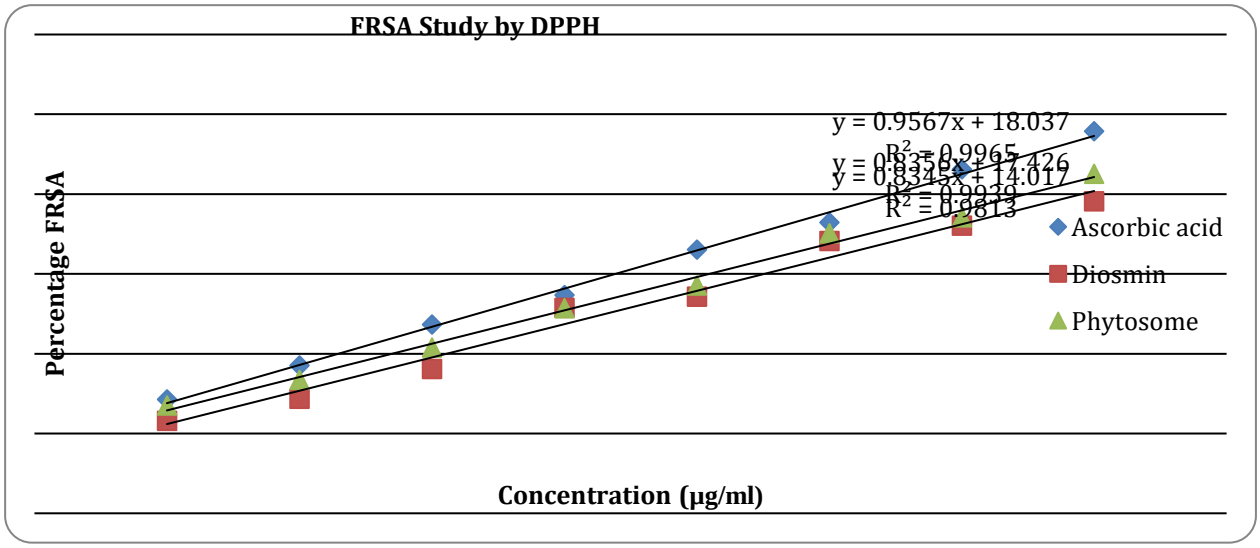

Figure 1: Comparison of DPPH free radical scavenging activity of Ascorbic Acid , Diosmin acid and Diosmin Loaded phytosome.

Conclusion:

The experimental work revealed that diosmin loaded phytosomes showed improved antioxidant activity by inhibiting DPPH.The experimental data of the study proves its antioxidant property. Whereas diosmin loaded phytosomes are comparatively more significant than diosmin alone. As formulation of phytosome improved the bioavailability, better release and significantly low dose is required. Thus improvement of antioxidant activity with minimal side effects makes this formulation more promising .It definitely needs more research to use clinically.

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Conflict of interest: Author has no conflict.

Reference:

[1] Kumar, Shashank, and Abhay K. Pandey. "Chemistry and biological activities of flavonoids: an overview." *The scientific world journal* 2013 (2013).

[2] Agrawal, A. D. "Pharmacological activities of flavonoids: a review." *Int. J. Pharm. Sci. Nanotechnol* 4.2 (2011): 1394-1398.

[3] Burda, Stanislaw, and Wieslaw Oleszek. "Antioxidant and antiradical activities of flavonoids." *Journal of agricultural and food chemistry* 49.6 (2001): 2774-2779.

[4] Bogucka–Kocka, Anna, et al. "Diosmin–isolation techniques, determination in plant material and pharmaceutical formulations, and clinical use." *Natural Product Communications* 8.4 (2013): 1934578X1300800435.

[5] H. Shariare, Mohammad, et al. "Development and optimization of epigallocatechin-3-gallate (EGCG) nano phytosome using design of experiment (DoE) and their in vivo anti-inflammatory studies." *Molecules* 25.22 (2020): 5453.

[6] Mustafa, Saad, et al. "Plant metabolite diosmin as therapeutic agent in human diseases." *Current Research in Pharmacology and Drug Discovery* (2022): 100122.

[7] Freag, May S., Yosra SR Elnaggar, and Ossama Y. Abdallah. "Lyophilized phytosomal nanocarriers as platforms for enhanced diosmin delivery: optimization and ex vivo permeation." *International journal of nanomedicine* (2013): 2385-2397.

[8] Karthivashan, Govindarajan, et al. "Optimization, formulation, and characterization of multiflavonoids-loaded flavanosome by bulk or sequential technique." *International Journal of Nanomedicine* 11 (2016): 3417.

[9] Nariya, Pankaj B., et al. "In vitro evaluation of antioxidant activity of Cordia dichotoma (Forst f.) bark." *Ayu* 34.1 (2013): 124.

[10] H. Shariare, Mohammad, et al. "Development and optimization of epigallocatechin-3-gallate (EGCG) nano phytosome using design of experiment (DoE) and their in vivo anti-inflammatory studies." *Molecules* 25.22 (2020): 5453.

[11] Mutee, A. F., et al. "In vivo anti-inflammatory and in vitro antioxidant activities of Peperomia pellucida." *Int J Pharmacol* 6.5 (2010): 686-90.

[12] Bondet, V., W. Brand-Williams, and C. L. W. T. Berset. "Kinetics and mechanisms of antioxidant activity using the DPPH. free radical method." *LWT-Food Science and Technology* 30.6 (1997): 609-615.

[13] Murugesan, Manikkampatti Palanisamy, et al. "Evaluation of anti-cancer activity of phytosomes formulated from Aloe vera extract." *Materials Today: Proceedings* 42 (2021): 631-636.

[14] Nariya, Pankaj B., et al. "In vitro evaluation of antioxidant activity of Cordia dichotoma (Forst f.) bark." *Ayu* 34.1 (2013): 124.

[15] Benzie, Iris FF, and John J. Strain. "The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay." *Analytical biochemistry* 239.1 (1996): 70-76.

[16] Ahmad, Nisar, et al. "Free radical scavenging (DPPH) potential in nine Mentha species." *Toxicology and Industrial Health* 28.1 (2012): 83-89.

[17] Singh, Anita, and Manoj Bisht. "Evaluation of in-vitro antioxidant potential and in-vivo hepatoprotective activity of root extract of Quercus oblongata D. DON." *Journal of Drug Delivery and Therapeutics* 8.5-s (2018): 152-161.