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SYNERGISTIC CONTRIBUTION OF QUERCETIN TOWARDS ANTIOXIDANT ACTIVITY OF *STEVIA REBAUDIANA* LEAVES

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ABSTRACT

Host toxicity and unfavourable side effects can frequently be reduced by synergistic interactions. It can also slow the development of drug related other problems. The current study's objective was to shed insight on the relationship between quercetin's antioxidant potential with that on the antioxidant potential of *Stevia rebaudiana* leaves. By using a 2,2-diphenyl-1-picrylhydrazyl inhibition assay; quercetin, extract and their blends (quercetin and stevia extract solutions) were evaluated for *in vitro* antioxidant screening. To build a Loewe additivity model, the drug's dose response relationship was determined. With the exception of the ratios 10:0.0120 and 10:0.0140, combinations have negative synergistism to variable degrees. The largest synergistic effects were seen at a ratio of extract: quercetin (10:0.0120). On the basis of the data, it is conceivable to use changes in the profiles of the extracts or in the ratios between different constituent groups to explain the broken relationship between combination indices and the activity of the extracts. Due to the extensive existence of polyphenols, the study may be applied to many plant components, notably those polyphenols.

Keywords: Quercetin, Antioxidant, Stevia, Synergism, DPPH

INTRODUCTION:

Stevia rebaudiana's polyphenol rich leaves are cherished for their antioxidant, anti-inflammatory, anti-atherogenic and anti-diabetic characteristics **[1]** and have been majorly used as natural sweetener. A polyphenol known as quercetin have positive effects on human health by preventing the detrimental effects of oxidative stress **[2, 3]**. The latter is a complicated procedure with a number of variables. However, an excess of reactive oxygen/nitrogen species (ROS/RNS) are the primary causes because

they triggers uncontrolled oxidation of biological molecules, inflammation and lead to metabolic inefficiency **[4]**. Plant extracts are anticipated to have a wide range of reactivity and remove a large number of ROS/RNS owing to their complex composition **[5]**. The probability of synergistic effects is further increased by the presence of numerous elements **[6]**.

The goal of the current study is to provide light on the antioxidant potential of quercetin, a component of *Stevia rebaudiana* leaves. One of natural remedies key advantages over synthetic medications is their complex makeup, potential synergistic effects of elements that reduce the amount of medication needed to achieve the intended result and the likelihood of unfavourable outcomes. Significant synergy has been reported between compounds having a catechol moiety and antioxidant activity **[7, 8]**. Thus, similar effects between quercetin and the other components of *Stevia rebaudiana* leaves may be predicted.

Based on how much observed combination effects deviate from the predicted response in the absence of interaction, the interaction of biologically or chemically active substances is frequently divided into three categories: synergy, additively (no interaction) and antagonism **[9, 10]**. Measures of medication response are gathered during dosage response tests at various dosing points. Such an experimental layout offers a reference model for the null hypothesis of initial non-interaction, which postulates that pharmacological effects simply build up and do not interact with one another (additivity) **[11]**. Depending on the directions of departure, any variation from the reference models will be seen as either synergy or antagonism. A pharmacological combination is considered to be synergistic if it delivers the same response level with a lower dose than the additive case (the reference model). Given that pharmacological combinations often require lower dosages than single treatments to achieve desired efficacy, such synergistic interactions can frequently minimize host toxicity and undesirable side effects. Additionally, it can lessen the emergence of medication resistance as well as other issues **[12, 13]**.

MATERIALS AND METHODS:

Stevia rebaudiana Bertoni. leaves (family: Asteraceae) were collected from the herbal garden of the Faculty of Pharmacy, Integral University, Lucknow. It was further authenticated (IU/PHAR/HRB/22/03) at Faculty of Pharmacy, Integral University, Lucknow, India. GraphPad Prism 8.4.3.686 was used to determine the statistical variance across groups. Readings were presented as the mean standard error of the mean for three/five observations per set. Comparing the test group to the reference/control group, statistical differences were determined using

a nonparametric paired t-test. Differences were deemed as non significant for $p > 0.05$, significant (*) for $p \leq 0.05$, very significant (**) for $p \leq 0.01$ and highly significant (***) for $p \leq 0.001$.

Plant material *(Stevia rebaudiana* leaves) have been authenticated and subjected to aqueous extraction by percolation method to yield to dark brown extract (unpublished data, communicated in RJPT).

Preparation of assortments of stevia extract and quercetin solutions

a. Stevia extract solution (10 µg/mL): Stevia extract (100 mg) was dissolved and volume made upto 100 mL using methanol to obtain a solution having a concentration of 1 mg/mL. It was further serially diluted with methanol to obtain a concentration of 10 µg/mL.

b. Quercetin solution (10 µg/mL): Quercetin (100 mg) was dissolved and volume made upto 100 mL using methanol to obtain a solution having a concentration of 1 mg/mL. It was further serially diluted with methanol to obtain a concentrations of 0.002-0.030 µg/mL.

Blending the solutions of quercetin with stevia extract: Quercetin and stevia extract solutions were mixed in (w/w) ratios of 0.00**:**1.0 (B_0), 0.01**:**1.0 (B_1), 0.02**:**1.0 (B_2), 0.03**:**1.0 (B_3), 0.04**:**1.0 (B_4), 0.05**:**1.0 (B_5), 0.06**:**1.0 (B_6), 0.07**:**1.0 (B_7), 0.08**:**1.0 (B_8), 0.09**:**1.0 (B_9), 0.10**:**1.0 (B_{10}), 0.11**:**1.0 (B_{11}), 0.12**:** 1.0 (B_{12}), 0.13**:**1.0 (B_{13}), 0.14**:**1.0 (B_{14}) and 0.15**:**1.0 (B_{15}).

***In vitro* antioxidant assay**

Quercetin, extract and the blends (quercetin and stevia extract solutions: B_0-B_{15}) were analyzed *in vitro* antioxidant screening by 2,2-diphenyl-1-picrylhydrazyl inhibition assay.

Sample stock solution (100 ng/mL) was prepared in methanol and serially diluted to obtain the test solution (10, 20, 30, 40, 50 ng/mL). Three mL of methanolic 2,2-diphenyl-1-picrylhydrazyl (50 ng/mL) was added in a quartz cuvette and the absorbance measured at 517 nm. After mixing 1 mL of test with 2,2-diphenyl-1-picrylhydrazyl solution, the mixture was incubated at 22 ± 2 °C for 60 minutes in the dark before the absorbance at 517 nm was measured using a UV-Visible spectrophotometer. Ten/Fifteen calibration points curve was used to obtain the dose response curve.

Prediction of Synergism

Loewe Additivity Model postulates that medicines operate on the same route in comparable ways. Drug's dose response relationship was obtained to create a Loewe additivity model. Let medication doses 1 and 2 have the values y^1 and y^2, respectively. The following equation[14] was used to represent the Loewe additivity model: $(y^1/Y_1) + (y^2/Y_2) = 1$. Here Y_1 and Y_2 are the drug doses that produce the same level of response as the drug combination. *Combination Indices* (CI) were determined

using equation: CI = $(C_1/C_{x1}) + (C_2/C_{x2})$, where C_1 and C_2 represented the actual concentrations of the chemicals used in the experiment. C_{x1} and C_{x2} represented the theoretical values necessary (determined from the dose-response curves) to induce the observed effect x. The CI equal to 1 indicated additivity, when it was less than 1 or greater than 1, synergy or antagonism got indicated.

RESULT AND DISCUSSION:

The analytes examined in this investigation included dried aqueous extracts of *Stevia rebaudiana* leaves and Quercetin **(Table 1-3)**. Total activity ratings for the extract ranged considerably from 10.936 to 57.967 demonstrating a concentration dependent capacity to scavenge the 2,2-diphenyl-1-picrylhydrazyl at lower doses **(Table 2)**. The examination of blends of quercetin and *Stevia rebaudiana* leaves revealed their impact on the extracts' antioxidant capacity. With a total activity range of 12.8716 to 62.4702, the evaluated blends scavenged 2,2-diphenyl-1-picrylhydrazyl in a dose dependent manner at lower doses **(Table 3)**. The dose response curves were reported against 2,2-diphenyl-1-picrylhydrazyl. For each of the examined analytes, the experimental data was first fitted into sigmoid functions **(Figure 1-3)**. The quercetin-extract blends examined in 15 concentration ratios as synergy depended on both the characteristics and precise dosage of each element[15]. Combination indices (CI) were generated **(Table 4)** to assess the effects that were seen. The Loewe additivity model, which is the foundation for the CIs, compared the actual concentrations of the analytes utilized in the assay to the theoretical concentrations needed to have the same impact (derived from the individual dose response curves) **[16]**.

As seen in **Table 4** and **Figure 4**, combinations had negative synergistism, albeit to varying degrees, with the exception of the ratios 10:0.0120 and 10:0.0140. The proportion 10:0.0120 showed the highest synergistic effects. Based on the data, it is possible that variations in the extracts' profiles, or in the ratios between various constituent groups, can be used to explain the disrupted link between combination indices and the extracts' activity. For instance, phenolic-rich extracts displayed behaviours resembling those of proportions, which are relatively low in polyphenols but stand out for their well balanced composition **[17, 18]**. The health promotion involves investigations **[19]** and methods **[20]** to achieve optimal health[21]. The thiazolidione may be also development as novel pharmaceuticals **[22, 23, 24]**.

Table 1: 2,2-diphenyl-1-picrylhydrazyl assay of Quercetin IC_{50} (µg/mL)

Concentration (µg/mL)	Percentage Inhibition	p value	Level of significance
0.003	13.7389 ± 0.1513	0.0022	Very significant
0.006	24.3674 ± 0.8812	0.0080	Very Significant
0.009	33.7735 ± 1.2119	0.0023	Very significant
0.012	42.0253 ± 1.2434	0.0270	Significant
0.015	51.7113 ± 1.2758	0.0304	Significant
0.018	56.2932 ± 1.3089	0.0013	Very significant
0.021	61.5664 ± 1.3430	0.0226	Significant
0.024	68.0824 ± 1.3779	0.0043	Very significant
0.027	68.1246 ± 1.4137	0.0034	Very significant
0.030	68.2682 ± 1.4505	0.0269	Significant

Table 2: 2,2-diphenyl-1-picrylhydrazyl assay of the extract IC_{50} (µg/mL)

Concentration (µg/mL)	Percentage Inhibition	p value	Level of significance
6	10.936 ± 0.5815	0.0007	Highly significant
12	19.396 ± 1.1226	0.0001	Highly significant
18	26.883 ± 1.3648	0.0159	Significant
24	33.451 ± 1.7081	0.0051	Very significant
30	41.161 ± 1.7525	0.0048	Very significant
36	51.176 ± 1.7981	0.0030	Very significant
42	56.965 ± 1.8448	0.0005	Highly significant
48	57.376 ± 1.8928	0.0006	Highly significant
54	57.665 ± 1.9420	0.0005	Highly significant
60	57.967 ± 1.9925	0.0133	Significant

Table 3: 2,2-diphenyl-1-picrylhydrazyl assay of the blends IC_{50} (µg/mL)

Extract: Quercetin blends (w:w) ratio	Percentage Inhibition	p value	Level of significance
10:0.002	12.8716 ± 1.1814	0.0010	Highly significant
10:0.004	22.5987 ± 2.2382	0.0012	Very significant
10:0.006	31.0024 ± 2.2964	0.0231	Significant
10:0.008	34.1794 ± 2.3561	0.0001	Highly significant
10:0.010	46.4896 ± 2.4173	0.0152	Significant
10:0.012	57.1926 ± 2.4802	0.0050	Very significant
10:0.014	61.5928 ± 2.5446	0.0076	Very significant
10:0.016	61.3647 ± 2.6108	0.0249	Significant
10:0.018	62.3881 ± 2.6787	0.0081	Very significant
10:0.020	62.3248 ± 2.7483	0.0024	Very significant
10:0.022	62.3448 ± 2.8198	0.0291	Significant
10:0.024	62.3748 ± 2.8931	0.0005	Highly significant
10:0.026	62.4148 ± 2.9683	0.0269	Significant
10:0.028	62.4470 ± 3.0455	0.0003	Highly significant
10:0.030	62.4702 ± 3.2059	0.0029	Very significant

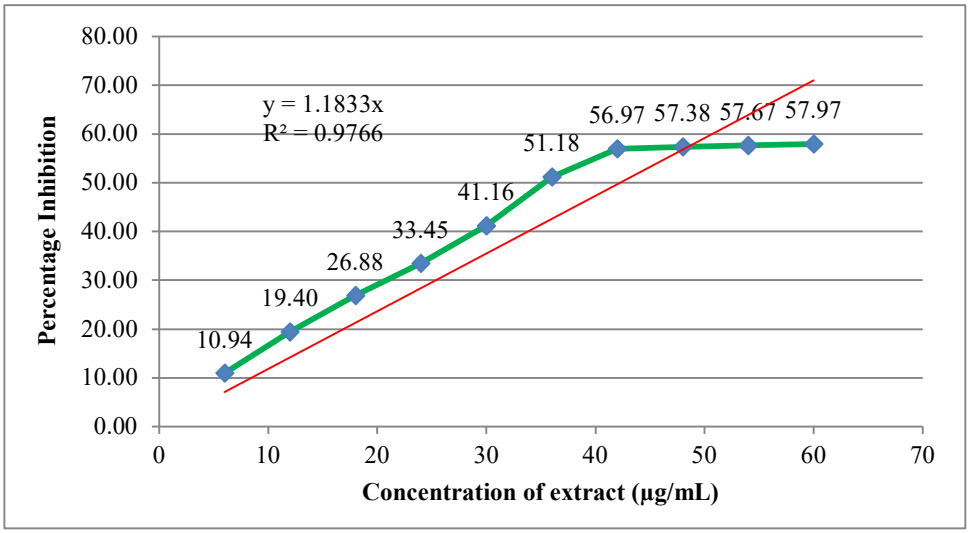

Figure 1: Dose response curve for Extract

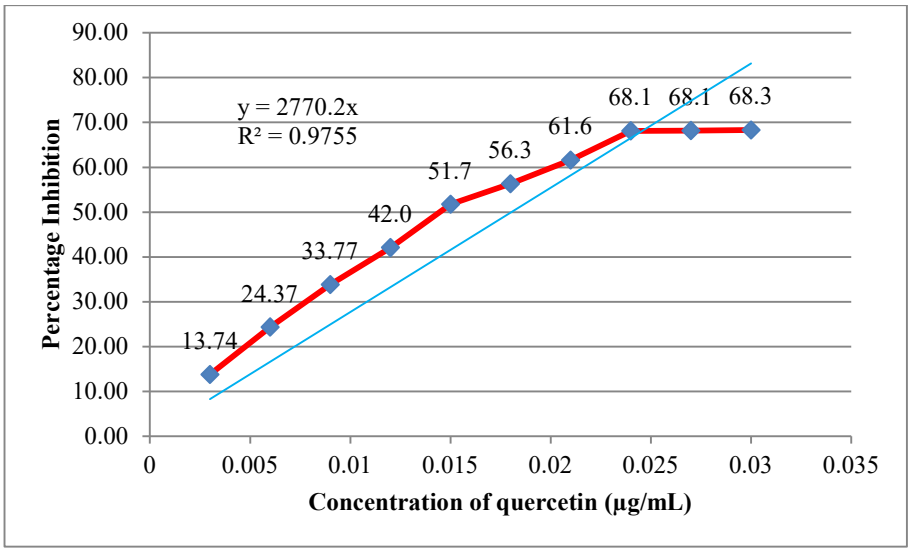

Figure 2: Dose response curve for Quercetin

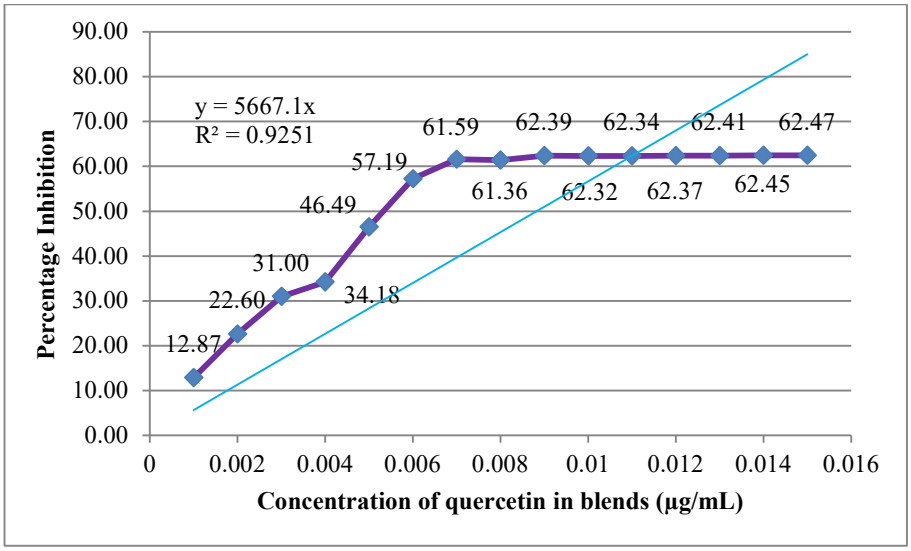

Figure 3: Dose response curve for Quercetin-Extract Blends

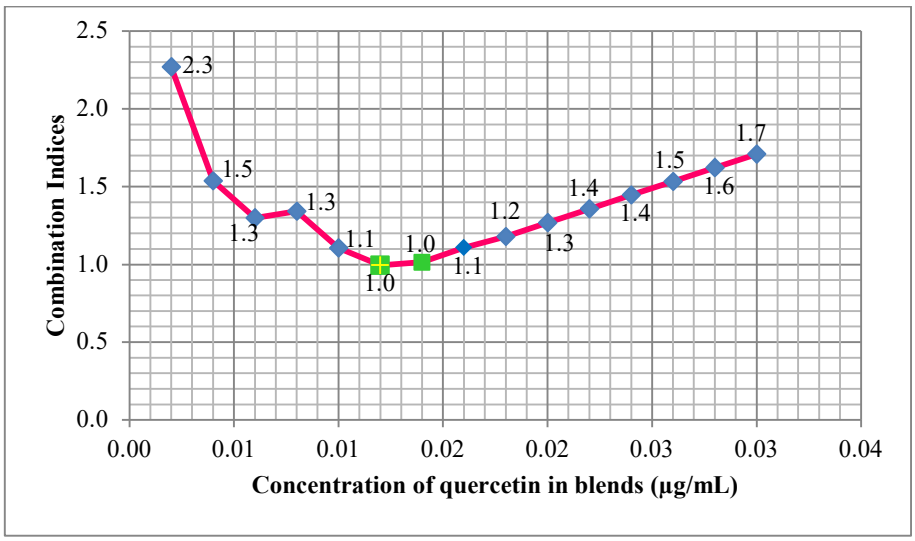

Figure 4: Combination Indices for Quercetin-Extract Blends

Table 4: Synergistic effects between the main extracts' constituents

Standard $C_{quercetin}$	Measured $C_{quercetin}$	Standard $C_{quercetin}$ / Measured $C_{quercetin}$	Standard $C_{extract}$	Measured $C_{extract}$	Standard $C_{extract}$ / Measured $C_{extract}$	Combination Indices [Approximation]	Predicted Effect
0.0020	0.0046	0.4304	20.0000	10.8777	1.8386	2.2691 [2.3]	Antagonism
0.0040	0.0082	0.4903	20.0000	19.0980	1.0472	1.5376 [1.5]	Antagonism
0.0060	0.0112	0.5361	20.0000	26.1999	0.7634	1.2995 [1.3]	Antagonism
0.0080	0.0123	0.6484	20.0000	28.8848	0.6924	1.3408 [1.3]	Antagonism
0.0100	0.0168	0.5959	20.0000	39.2881	0.5091	1.1049 [1.1]	Antagonism
0.0120	0.0206	0.5812	20.0000	48.3331	0.4138	0.9950 [1.0]	Synergism
0.0140	0.0222	0.6297	20.0000	52.0517	0.3842	1.0139 [1.0]	Synergism
0.0160	0.0222	0.7223	20.0000	51.8590	0.3857	1.1080 [1.1]	Antagonism
0.0180	0.0225	0.7992	20.0000	52.7238	0.3793	1.1786 [1.2]	Antagonism
0.0200	0.0225	0.8890	20.0000	52.6703	0.3797	1.2687 [1.3]	Antagonism
0.0220	0.0225	0.9775	20.0000	52.6872	0.3796	1.3571 [1.4]	Antagonism
0.0240	0.0225	1.0659	20.0000	52.7126	0.3794	1.4453 [1.4]	Antagonism
0.0260	0.0225	1.1540	20.0000	52.7464	0.3792	1.5331 [1.5]	Antagonism
0.0280	0.0225	1.2421	20.0000	52.7736	0.3790	1.6211 [1.6]	Antagonism
0.0300	0.0226	1.3303	20.0000	52.7932	0.3788	1.7092 [1.7]	Antagonism

CONCLUSIONS:

The synergistic contribution of Quercetin towards antioxidant activity of *Stevia rebaudiana* leaves has evaluated. The 2,2-diphenyl-1-picrylhydrazyl scavenging test showed a positive trait for synergistic output and act as a springboard for more research on comparable outcomes in more intricate biological models. The found relationships between the phenolic compound and the extract under investigation may also be utilized to assess the antioxidant properties of auxiliary plant components with comparable chemical make up. The study may be applied to different plant components, particularly the polyphenols, due to the widespread presence of polyphenols.

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DECLARATION OF CONFLICT OF INTEREST:

None

REFERENCES:

[1] Kultur S. Medicinal Plants Used in Kirklareli Province (Turkey). J. Ethnopharmacol. 2007 111: 341-364.

[2] Pandey KB, Rizvi SI. Plant Polyphenols as Dietary Antioxidants in Human Health and Disease. Oxid. Med. Cell. Longev. 2009 2: 270-78.

[3] Zhang H, Tsao R. Dietary Polyphenols, Oxidative Stress and Antioxidant and Anti-inflammatory

Effects. Curr. Opin. Food Sci. 2016 8: 33-42.

[4] Droge W. Free Radicals in the Physiological Control of Cell Functions. Physiol. Rev. 2002 82: 47-95.

[5] Efferth T, Koch E. Complex Interactions between Phytochemicals. The multi-target Therapeutic Concept of Phytotherapy. Curr. Drug Targets. 2011 12: 122-32.

[6] Sonam KS, Guleria S. Synergistic Antioxidant Activity of Natural Products. Annal. Pharmacol. Pharm. 2017 2: 1-6.

[7] Sak K. A Supposed Mechanism of Synergistic Action of Catechol Containing Natural Polyphenols. Int. J. Phytomed. 2017 9: 207-12.

[8] Hajimehdipoor H, Shahrestani R, Shekarchi M. Investigating the Synergistic Antioxidant Effects of Some Flavonoid and Phenolic Compounds. Res. J. Pharmacogn. 2014 1: 35-40.

[9] Berthoud HR. Synergy: A Concept in Search of a Definition. Endocrinology. 2013 154(11): 3974-77.

[10] Geary N. Understanding synergy, American Journal of Physiology-Endocrinology and Metabolism. 2013 304(3): E237-53.

[11] Greco WR, Bravo G, Parsons JC. The Search for Synergy: A Critical Review from a Response Surface Perspective, Pharmacol Rev. 1995 47(2): 331-85.

[12] Greco WR, Faessel H, Levasseur L. The Search for Cytotoxic Synergy between Anticancer Agents: A Case of Dorothy and the Ruby Slippers? J Natl Cancer Inst, 1996 88(11): 699-700.

[13] Worthington RJ, Melander C. Combination Approaches to Combat Multidrug-resistant Bacteria. Trends Biotechnol. 2013 31(3): 177-84.

[14] Greco WR, Bravo G, Parsons JC. The Search for Synergy: A Critical Review from a Response Surface perspective. Pharmacol Rev. 1995 47(2): 331-85.

[15] Tallarida RJ. Quantitative Methods for Assessing Drug Synergism. Genes Cancer. 2011 2, 1003-8.

[16] Zhao L, Au JLS, Wientjes MG. Comparison of Methods for Evaluating Drug-Drug Interaction. Front Biosci. 2010 2, 241-49.

[17] Matczak M, Marchelak A, Michel P, Owczarek A, Piszczan A, Kolodziejczyk-czepas J, Nowak P, Olszewska MA. *Sorbus domestica* L. Leaf Extracts as Functional Products: Phytochemical Profiling,

Cellular Safety, Pro-inflammatory Enzymes Enhibition and Protective Effects Against Oxidative Stress *in vitro*. J Funct. Foods. 2018 40, 207-18.

[18] Kushwaha SP, Kumar P, Rawat SK, A preface noesis regarding general health awareness among the rural mass. Asian Journal of Pharmacy and Technology, 2012 2(1), 12-14.

[19] Ansari J, Kushwaha SP, Ansari VA, Singh K, Hasan SM. Agar well diffusion: A prominent method for in vitro screening of antimicrobials. International Journal of Botany Studies, 2021; 6(5):836-839.

[20] Kushwaha SP, Gupta SK, Kumar S. Phytochemical screening of aqueous extract derived from Stevia rebaudiana leaves, Musa acuminata fruits and Solanum lycopersicum fruits. International Journal of Botany Studies, 2021; 6(2):510-512.

[21] Kushwaha SP, Singh K, Kumar A, Hasan SM. Compassionate role of synergism in herbal formulations. International Journal of Biology, Pharmacy and Allied Sciences. 2022; 11(12):5689-5895.

[22] Kapoor A, Hafeez A, Kushwaha P. Nanocarrier Mediated Intranasal Drug Delivery Systems for the Management of Parkinsonism: A Review. Curr Drug Deliv. 2023 Jun 16. doi:10.2174/1567201820666230523114259.

[23] Izhar, M.P., Hafeez, A., Kushwaha, P. et al. Drug Delivery Through Niosomes: A Comprehensive Review with Therapeutic Applications. J Clust Sci 2023; 34, 2257–2273.

[24] Gupta P, Hafeez A, Kushwaha P. Development and Evaluation of Topical Ethosomal Gel for Fungal Infections. Drug Res (Stuttg). 2023 Jan; 73(1):46-53.