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EVALUATION OF ANTIOXIDANT ACTIVITY, TOXICITY STUDY, AND STANDARDIZATION OF RV FORTE CAPSULE

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ABSTRACT:

Background and objective: Oxidative stress is a common factor for various diseases like cancer, diabetes, metabolic disorders, atherosclerosis, and cardiovascular diseases. The incidence of such diseases is growing day by day around the globe and causes various complications. The disease not only affects the quality of life but also the mortality rate with this disease is too high. Considering these factors, it is important to come up with a simple yet potential solution for reducing oxidative stress. Therefore, RV New Visions Health Care Pvt. Ltd has developed an RV forte capsule as a potential treatment for reducing oxidative stress and help to fight against cancer.

Materials and Methods: Antioxidant activity of RV forte capsule was evaluated In-vitro by using DPPH scavenging assay, H2O2 radical scavenging assay, and Reducing power assay. Further, toxicity testing in animal models and the standardization using the HPTLC method was done.

Results: The formulation RV forte capsule showed significant anti-oxidant action when compared with standard ascorbic acid. The percentage scavenging activity of RV forte capsule was found to be 14.2 - 60.05 % at 50 - 500 ug/ml by DPPH scavenging assay,17.57 – 55.27 % by hydrogen radical scavenging assay, and the reducing power was found to be increasing with increased absorbance at 50-500 ug/ml concentrations. In toxicity testing, no toxicity was found at the dose of 100mg/kg.

Conclusion: The study thus proves, RV forte capsule has significant antioxidant activity

Keywords: Oxidative stress; Antioxidant; Cancer; Standardization; Herbal formulation; Toxicity testing

INTRODUCTION:

Oxidative stress is a phenomenon caused by an imbalance between the production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products. [1]

Superoxide radicals ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and singlet oxygen (O_2) are commonly defined reactive oxygen species (ROS); they are generated as metabolic by-products by biological systems. [2,3] Processes, like protein phosphorylation, activation of several transcriptional factors, apoptosis, immunity, and differentiation, are all dependent on proper ROS production and presence inside cells that need to be kept at a low level. [4] When ROS production increases, they start showing harmful effects on important cellular structures like proteins, lipids, and nucleic acids. [5] A large body of evidence shows that oxidative stress can be responsible, with different degrees of importance, in the onset and/or progression of several diseases (i.e., cancer, diabetes, metabolic disorders, atherosclerosis, and cardiovascular diseases). [6]

The use of traditional medicine is widespread and plants still represent a large source of natural antioxidants that might serve as leads for the development of novel drugs. Several examples have revealed the fact that plant which contains antioxidant potential demonstrate the beneficial effects in inflammatory diseases. [7]

Oxidative stress is a severe cause of various diseases, especially cancer, there is a great need to develop a potential solution to reduce oxidative stress.

Considering the benefits of herbal drugs in reducing oxidative stress, RV New Visions Healthcare Pvt. Ltd. has come up with a formulation named RV forte capsule as a potent anti-oxidant. The RV forte capsule is composed of Citrus Bioflavonoids which are proven to support blood circulation, as an antioxidant, an anti-allergic, antiviral and anti-arthritic, Resveratrol which has antioxidant and immunomodulatory activities, Guduchi which is an anti-stress and adaptogenic, catechins, Ashwagandha is an anti-oxidant and immunomodulatory, ginger which possess anti-cancer activity, Liquorice is an anti-viral and anti-HIV drug, as key ingredients out of total 11 ingredients.

The current study aims at the evaluation of the antioxidant activity of RV forte capsule by in-vitro, in-vivo, and standardization by HPTLC.

MATERIAL AND METHODS:

Materials: RV forte capsule was used for the analysis purpose aiming for the evaluation of antioxidant activity, toxicity testing, and standardization of the same.

Methods:

1) Evaluation of antioxidant activity:

a) DPPH radical scavenging activity:

The ability of the formulations to scavenge DPPH radicals was determined by using the following method. 50 µl aliquot of each formulation, in 50 mm Tris-HCl buffer (pH 7.4), was mixed with 450 µl of Tris–HCl buffer and 1.0ml of 0.1mM DPPH in methanol. After 30 min incubation in darkness and at ambient temperature, the resultant absorbance was recorded at 517 nm. The percentage inhibition was calculated.

b) Hydroxyl radical scavenging activity

The degradation of Deoxyribose generated by the Fenton reaction was measured spectrophotometrically in the presence and absence of the test compound. The final reaction mixture in each test tube consisted of 0.3 ml each of Deoxyribose (30 mM), ferric chloride (1mM), EDTA (1 mM), H2O2 (20mM), in the phosphate buffer having pH 7.4 and 0.3 ml of test compound at different concentration. the test tubes were incubated for 30 min at 37^{o}C after incubation, trichloroacetic acid (0.5 ml, 5%) and the thiobarbituric acid (0.5 ml, 1%) were added and the reaction mixture was kept in a boiling water bath at 30 min. it was then cooled and the absorbance was measured at 532 nm. The result was expressed as a % of scavenging of hydroxyl radical.

c) Reducing power activity

The reducing power of the formulations was determined. Extracts at different concentrations in 1 ml of distilled water were mixed with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml potassium ferricyanide [K3Fe (CN) 6] (1%), and then the mixture was incubated at 50^{0}C for 30 min. Afterward, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of upper layer solution was mixed with 2.5 ml distilled water and 0.5 ml FeCl3 (0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated an increase in reducing power.

2) Toxicity testing:

Groups of five male and five female mice were administered RV Forte Capsule by oral gavage daily at the dose of 1000 mg/kg body weight for 28 days and were sacrificed on day 28 to evaluate its toxicity. The concurrent control group receiving the vehicle was also maintained. Additionally, satellite groups of five mice per sex receiving test articles at 1000 mg/kg level were further observed for 14 days for assessment of reversibility, persistence, or delayed occurrence of toxicity.

3) Standardization by HPTLC method:

a) Preparation of Mobile Phase:

9 mL n-hexane, 3 mL Ethyl acetate, and 0.3mL Glacial acetic acid was measured individually using a graduated measuring cylinder and transferred into a 50 mL stoppered conical flask. The mobile phase was properly mixed, sonicated, and transferred into a 10x10 cm TLC development chamber and allow equilibrating for 15 minutes.

b) Preparation of Test Solution:

about 200 mg extract was weighed and transferred into a 20 mL volumetric flask; about 15 mL of methanol was added. It was sonicated for 15 minutes in cold water. It was diluted up to the mark with diluent. The solution was filtered through Whatman filter paper no. 41. The resulting solution was used as a test solution**.**

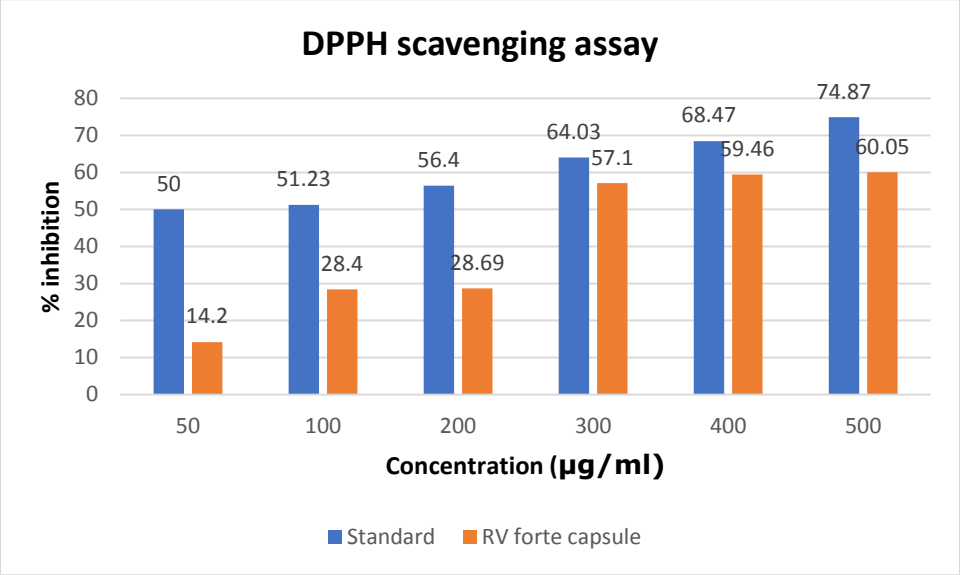
RESULTS:

1) Evaluation of Anti-oxidant activity

a) DPPH radical scavenging activity

All the concentrations were capable of scavenging DPPH radicals at pH 7.4 in a dose-dependent fashion. All the concentrations of the test compound were capable of scavenging the free radicals and had shown anti-oxidant activity. Graph 1 depicts the percentage inhibition of RV forte capsule at different concentrations of test compounds and also the comparison with different concentrations of Standard i.e., ascorbic acid. The % inhibition of test formulation at concentrations of 50 ug/ml, 100 ug/ml, 200 ug/ml, 300 ug/ml, 400 ug/ml, 500 ug/ml were found to be 14.2 %, 28.4 %, 28.69 %, 57.10 %, 59.46 %, 60.05 % respectively. When compared statistically between the groups, the P-value was found to be 0.0081 and the test results were found to be significant.

Graph 1: The graphs show % inhibition of RV forte tablet as compared to standard at different concentrations of test compound and standard.

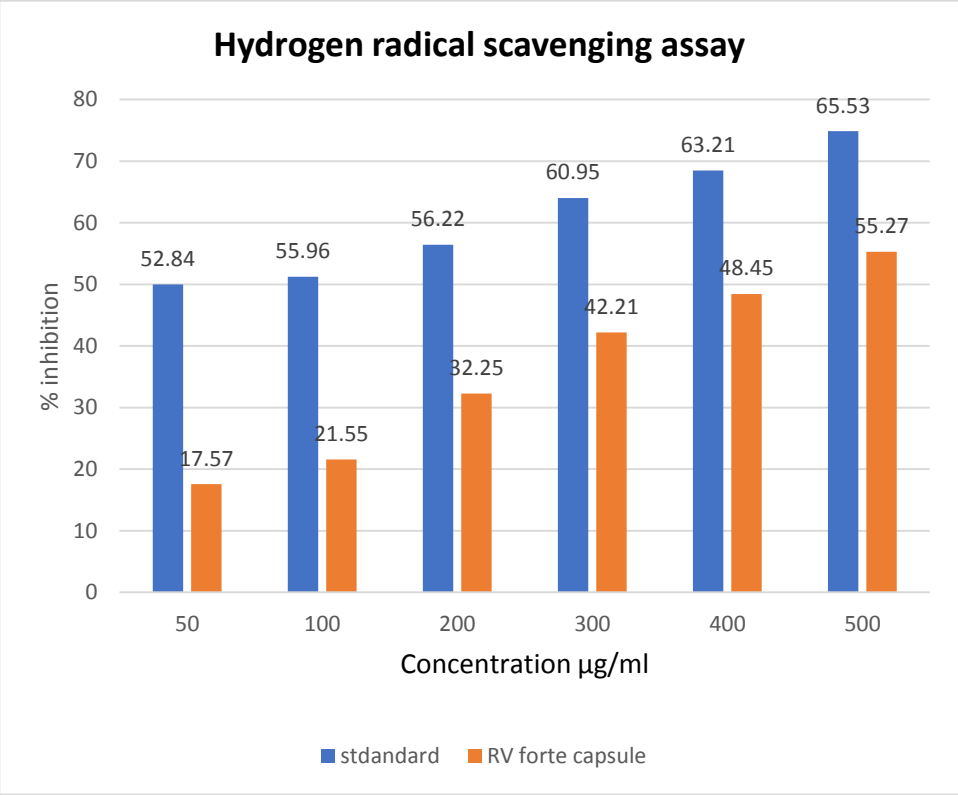


The values are % inhibition calculated from the mean of absorbance of specific concentrations ($P<0.001$)

b) Hydrogen radical scavenging activity:

Hydroxyl radicals are very reactive, can be generated in biological cells to the Fenton reaction. The test compounds exhibited concentration-dependent scavenging activity against hydroxyl radical generated in the Fenton reaction system. RV forte capsule demonstrated scavenging of hydroxyl radicals ranging from17.57% to 55.27 %. The P-value was found to be 0.0363 and the results were found to be significant. The results are depicted in Graph 2

Graph 2: It indicates the % inhibition of RV forte capsule in comparison with Standard

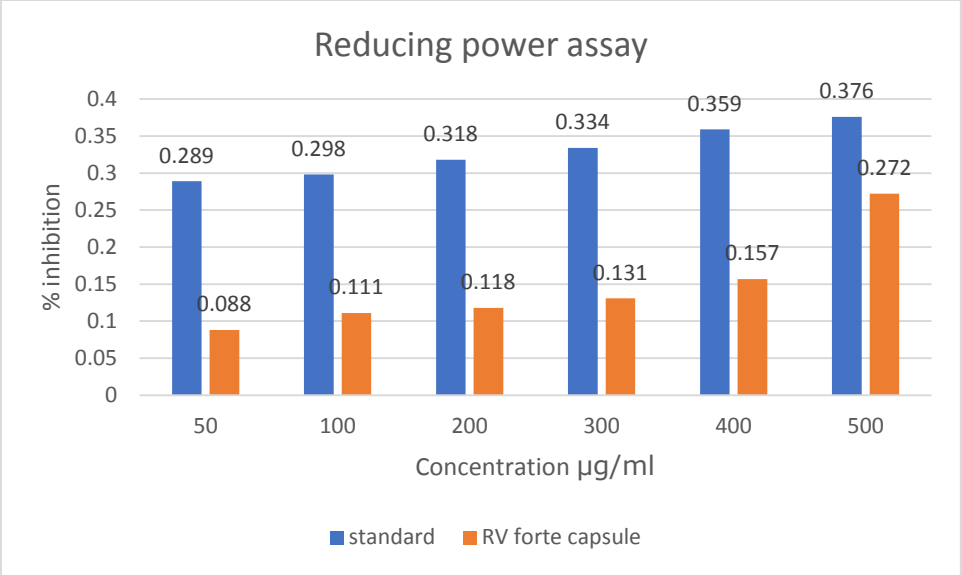


The values are % inhibition calculated from the mean of absorbance of specific concentrations ($P<0.001$)

c) Reducing Power Assay:

For the measurements of the reductive ability of test compounds, the Fe^{3+} – Fe^{2+} transformation was investigated in the presence of test compounds. In this assay, the yellow color of the test solution changes to various shades of green and blue depending on the reducing power of each antioxidant sample. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form[16]. The reducing power of test compounds is enhanced with increasing concentration of samples. The absorbance of the samples increased together with the reducing power. The reducingpower of test compounds was found to be concentration. The results are depicted in Graph 3.

Graph 3: The above graph depicts the absorbance of the RV forte capsule in comparison with the standard.



The values are mean of absorbance of specific concentrations. ($P<0.001$)

2) Toxicity testing:

The results for toxicity testing were found to be prominently significant. At the dose of 1000mg/kg, there were no toxicity signs for the RV forte capsule.

a) Bodyweight:

During the study period of 28 days, the changes in body weight were observed and were wound to be with a weight gain of 14.37 and 12.92% in females and males respectively.

b) Clinical signs and mortality:

Clinical signs and mortality were assessed for 28 days and no abnormal clinical signs and mortality were observed in both male and female rats.

c) Individual animal organ weights:

After 28days the animals were sacrificed and organs were dissected and weighed. The individual animal organs weight was noted and is depicted in table 2

Table no. 1: The table represents the average weight of individual organs.

Animal ID	Adrenals	Ovary	Brain	Kidney	Liver	Heart	Spleen	Epididymis	Thymus	Uterus
AVG Wt.	0.004	1.004	0.497	0.356	1.369	0.225	0.089	NA	0.000	0.163
SD	0.001	0.381	0.036	0.046	0.070	0.047	0.013	NA	0.018	0.026

d) Average hematological parameters:

The hematological parameters like total leukocyte count, RBC count, Hemoglobin, Hematocrit, MCV, MCH, MCHC, platelet count, etc. were done for all the groups were checked and results are depicted in table no 3

Table 2: The table represents the average hematological parameters

TEST	Total Leukocyte Count (X 10^3/µL)	RBC Count (X 10^6/µL)	Hemoglobin (Hb) (g/dL)	Hematocrit (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Platelet Count (X 10^3/µL)
AVG	1.36	5.96	11.56	33.62	53.34	21.61	34.43	493.80
SD	0.40	0.86	0.72	1.88	6.24	2.84	2.11	156.12

e) Histopathology:

Histopathology of one animal in each group was performed at the end of the studies and the results were found as depicted in the following figures. The histopathology of the brain, heart Prostrate, adrenal, kidney, large intestine, liver, lung, spleen, urinary bladder, stomach, thyroid was performed. The histopathology of the brain, kidney, liver, and lungs are presented in the images as follows:

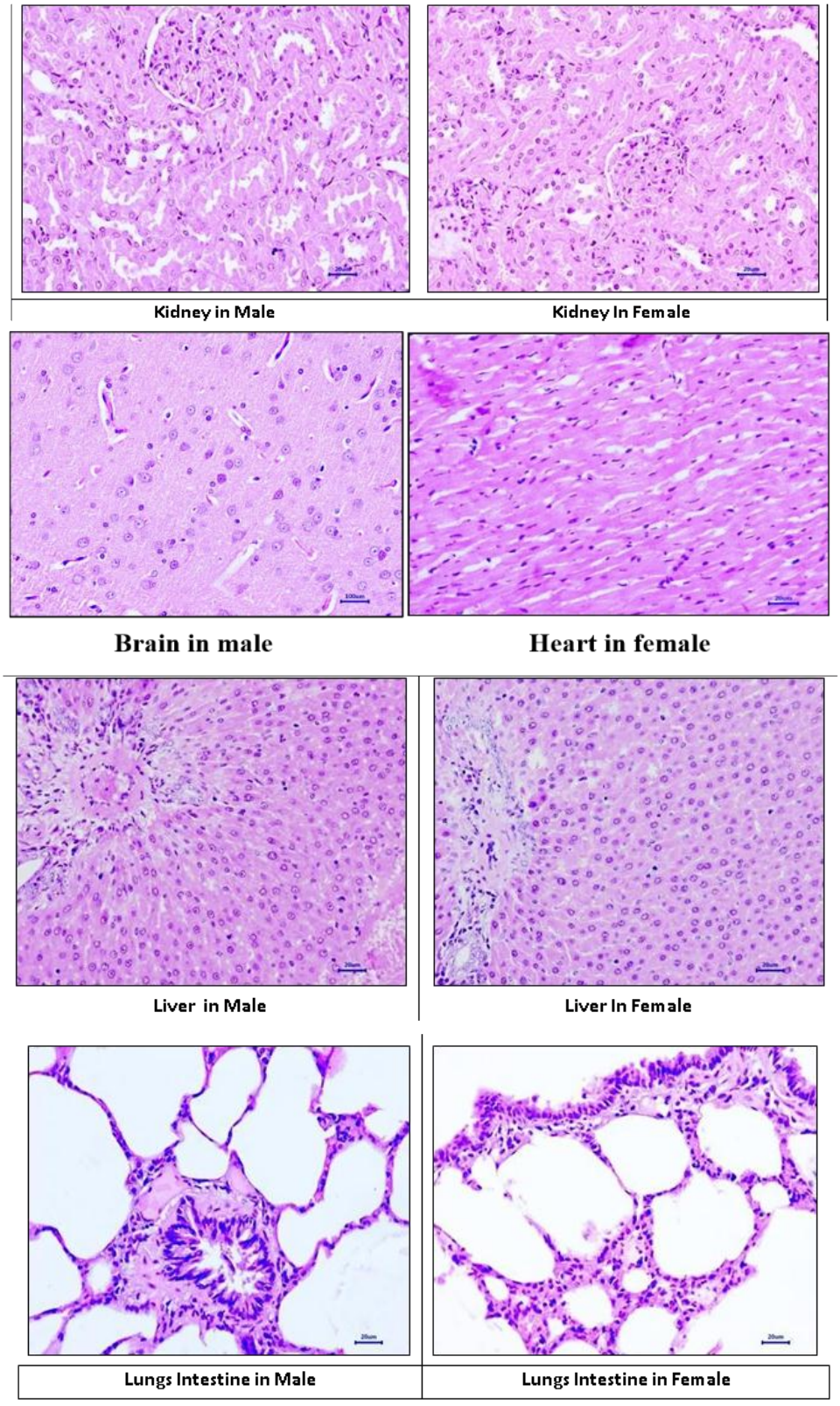


Figure 1: The figures above represent the histopathological images of the individual organ isolated form the animal after dissection at termination of the study.

3) Standardization by HPTLC:

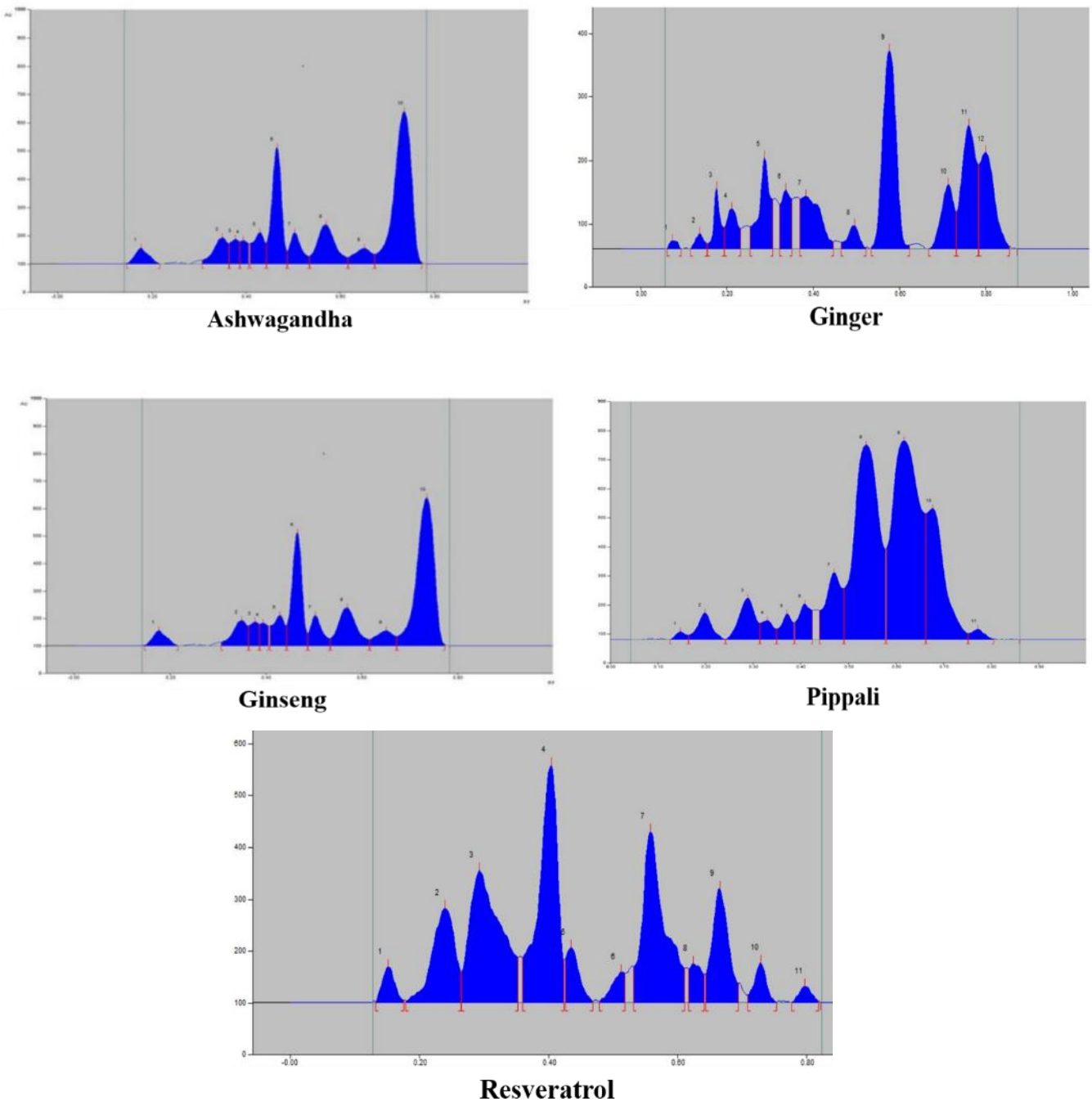


Figure 2: The above images represent the chromatograms of Ashwagandha, ginger, ginseng, Pippali, resveratrol obtained by performing the standardization by HPTLC.

DISCUSSION AND CONCLUSION:

Oxidative stress is the result of an imbalance in the body between the oxidizing system, consisting mainly of free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS), and antioxidant systems. Oxidative stress is likely to be involved in the age-related development of cancer. The reactive species produced in oxidative stress can cause direct damage to the DNA and are therefore mutagenic, and it may also suppress apoptosis and promote proliferation, invasiveness, and metastasis.[8] Reactive oxygen species are a common type of free radical and cause oxidative stress. The imbalance of ROS decreases the antioxidant activity of molecules. The ROS has a beneficial and deleterious role. [9] At low concentrations of ROS, it produces beneficial effects and when there is overproduction it can damage or inhibit the normal function of lipids proteins and DNA. Due to the intracellular reduction of O2 to ROS, it becomes toxic to cells and tissue. [10] The ROS is produced from an endogenous and exogenous substance. The endogenous production of ROS is from mitochondria, Cytochrome P450, peroxisomes, inflammatory cell activation. The mitochondria generate hydrogen peroxide and use 90% of the cellular oxygen. [11] During the process of reducing oxygen for the production of water, superoxide (O2), hydrogen peroxide (h2O2), and hydrogen radical (OH) are produced. [12] All these free radicals are toxic to the cells and destroy cells. The cell's destruction in turn causes free radical generation. The other endogenous sources of free radicals are neutrophils, eosinophils, macrophages. [13] The activated macrophages increase the oxygen] uptake and give rise to a variety of Reactive oxygen species. The liver macrophages participate in free radical-induced hepatoxicity and liver cancer. Similarly, other macrophages participate in the development of several types of cancers. [14]

Considering the impact of oxidative stress and its correlation with cancer, there is a great need to develop a potential solution to the same. To the needful required to tackle the increasing prevalence and considering its impact on the life of people, RV NEW VISIONS HEALTH CARE has developed an RV forte capsule for reducing oxidative stress. The current study aims to evaluate the antioxidant activity of the RV forte capsule by in-vitro antioxidant activity. Also, it aims at the toxicity testing of RV capsules in animal models and furthers the standardization of the same.

The possible mechanism through which the RV forte capsule gives its antioxidant and immunomodulatory actions is via its key ingredient which is resveratrol that enhances immunity by inhibiting Camp and downregulating the signaling cascade. It upregulates the NAD+ and AMPK. Thus, improves mitochondrial function and physical stamina, and energy creation. [15] It shows antioxidant activity by decreasing the reactive oxygen generation, directly scavenging the free radical, improving endogenous antioxidant enzymes like Catalase, Superoxide dismutase, etc., and improving mitochondrial energy biogenesis. [16]

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CONFLICTS OF INTEREST:

There are no conflicts of interest.

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