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PHARMACOGNOSTIC AND *IN-VITRO* ANTIOXIDANT, ANTI-INFLAMMATORY & ANTIDIABETIC ACTIVITY OF THE FLOWER OF THE HIMALAYAN *RHODODENDRON ARBOREUM*

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Antidiabetic, Antioxidant, Alpha-amylase, *Rhododendron arboreum*, DPPH

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ABSTRACT: Objective: To investigate the Pharmacognostic and *in-vitro* Antioxidant, Anti-inflammatory & Anti-diabetic Activity of the flower of the Himalayan *Rhododendron Arboreum*. **Methods:** The collected plant flowers of *Rhododendron Arboreum* were cleaned with distilled water and air-dried at room temperature under shade (25-27 °C) and reduced to the appropriate size of the powder. The powdered plant materials are further extracted by cold maceration with hydroalcoholic as well as the aqueous solvent. The filtrates obtained from the successive maceration were concentrated under reduced pressure using a rotary evaporator followed by hot air set at 40 °C. The dry extract obtained was harvested, and the dried extract was transferred into airtight bottles and stored in a refrigerator at -4 °C. The powdered flowers of *Rhododendron Arboreum* and hydroalcoholic and aqueous extract were standardized via pharmacognostic parameters. Phytochemical screening of Hydro-alcoholic and aqueous extract of *Rhododendron Arboreum* revealed the presence of tannins, flavonoids, phenol, triterpenes, Steroids & carbohydrates. Different flowers extract of *Rhododendron Arboreum* accessed for in vitro antioxidant potential by using the DPPH assay and NO Scavenging assay by using ascorbic acid as a standard drug. Further, the Hydro-alcoholic and aqueous extract of *Rhododendron Arboreum* flower are to be subjected for the assessment of its anti-inflammatory potential by use of Heat-induced hemolysis as well as Hypotonicity-induced hemolysis approach by the use of the aspirin or diclofenac sodium as a standard drug. Further, the Different flowers extract of *Rhododendron Arboreum* is accessed for its antidiabetic potential by using the *in-vitro* alpha-amylase inhibition and alpha-glucosidase inhibition approach by using the Acrabose as standard drug. **Results:** Phytochemical screening of Hydro-alcoholic and aqueous extract of *Rhododendron Arboreum* flower revealed the presence of tannins, flavonoids, phenol, triterpenes, Steroids & carbohydrates. *In-vitro* study shows that the Hydro-alcoholic and aqueous extract of *Rhododendron Arboreum* flower has excellent antioxidant, anti-inflammatory, and antidiabetic potential in a dose-dependent manner compared to the result standard drugs. **Conclusion:** From this investigation, it is to be suggested that the Hydro-alcoholic and aqueous extract of Rhododendron Arboreum flower is rich in the phenolic & tannin compound. The experimentation study shows the drug is to possesses good antioxidant anti-inflammatory as well as antidiabetic properties.

INTRODUCTION: *Rhododendron Arboreum* is an evergreen widely growing plant associated with red and pink color flowers [1]. The plant's name 'rhododendron' is to be formed from the combination of the two parts one is 'Rhodo', which means rose and the part is 'Dendron', which means tree derived from the Greek word.

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This plant is throughout the Himalayan region from Jammu Kashmir to Nagaland in the cold region mainly [2]. The species is widely distributed between the latitudes 80 °N and 20 S with high

socioeconomic reverence. In the Hindu religion flower of this plant is used as a holy religious botanical. The hilly people used the plant as a folk medicine because it can cure various ailments like diarrhea, headache, inflammation, bacterial and fungal infections [3]. This plant is also having a high nutritive value and is used to make pickles, juice, jam, syrup, honey, squash and *etc.* [4]. The present review describes the therapeutical and food value of Rhododendron by making value-added products to improve the livelihood for sustainable development of the rural tribal population with more job opportunities [5]. This research aims to explore the antioxidant, anti-inflammatory, and antidiabetic potential of the hydroalcoholic extract of the rhododendron flower extract by using the various *in-vitro* estimation approaches.

MATERIAL & METHODS:

Plant Materials: The Fresh flower of *Rhododendron Arboreum* was collected from Seraj valley, Himachal Pradesh in February 2021.

Preparation of Plant Crude Extract: The collected plant flowers were cleaned with distilled water and air-dried at room temperature under shade 25-27 °C and reduced to appropriate size [6]. The powdered plant materials were packed in a plastic bag and kept until extraction [7].

The powdered plant materials were weighed by sensitive digital weighing balance; powdered flowers were macerated with hydroalcoholic solution (30:70) (250 g in 1500 mL) in an Erlenmeyer flask for 72 hrs at room temperature 25-27 °C [8]. The extraction process was facilitated by occasional shaking. After 72 h, the extract was separated from the marc using gauze and further filtered by Whatman filter paper No. 1.

The residue was re- macerated for another 72 h three times using the same volume of hydroalcoholic solution (30:70) to exhaustively extract the plant material.

The filtrates obtained from the successive maceration were concentrated under reduced pressure using a rotary evaporator (Hamato, Japan) followed by hot air set at 40 °C. The extract was further concentrated to dryness by freeze-drying using lyophilizer [9]. After drying, the amount of dry extract obtained was harvested and the dried extract was transferred into airtight bottles and stored in a refrigerator at -4 °C until used. The weight of the dry extract was expressed as a percentage of the total mass of dry plant matter to determine the percentage yield [10].

Pharmacognostical Evaluation: This study is to be the main concern to organoleptic features and Physiochemical Property of the *Rhododendron Arboreum* as shown below in **Fig. 1.**

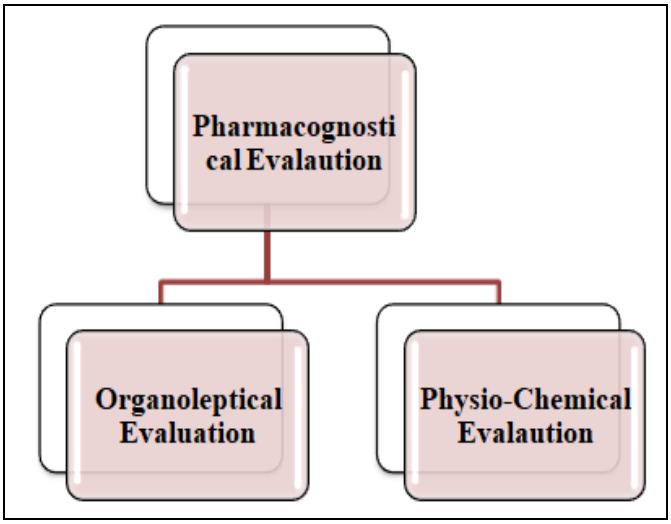

FIG. 1: SHOWN THE PHARMACOGNOSTICAL EVALUATION OF FLOWER *RHODODENDRON ARBOREUM*

Organoleptic Evaluation: The organoleptic characters are the various sensory parameters of the flower of *Rhododendron Arboreum* like shape, size, color, odor, taste and fracture of *Rhododendron Arboreum* were resolution [11]. It encompasses inferences drench from examination ensued due to impressions on organs of senses.

Physicochemical Evaluation: Total moisture content, total ash value obtained, Acid-insoluble ash obtained & extractive values obtained in hydroalcoholic and aqueous flower extract of *Rhododendron Arboreum* were executed as elaborated in Indian Pharmacopoeia [12].

Phytochemical Analysis: The hydroalcoholic flower extract of *Rhododendron Arboreum* was concealed for the presence or absence of the crucial category of active compounds such as tannins, flavonoids, saponins, alkaloids, steroids, triterpenes & glycosides by the standard method [13]. This test on the active moiety is to be performed quantitatively and qualitatively, as shown in **Fig. 2.**

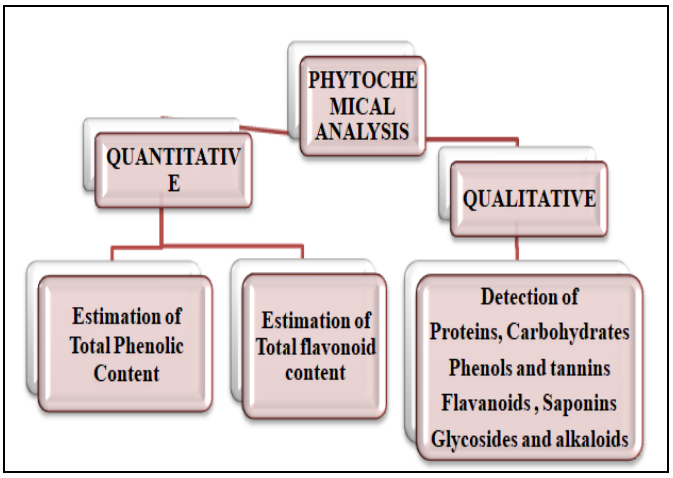

FIG. 2: SHOWN THE PHYTOCHEMICAL ANALYSIS OF FLOWER EXTRACT OF *RHODODENDRON ARBOREUM*

Phytochemical Screening (Quantitative): Quantitative phytochemical screenings of hydro alcoholic and aqueous flower extract of *Rhododendron Arboreum* were performed as per standard protocols to detect the number of total phenols and total flavonoids [14].

Determination of Total Phenolic Content: Folin-Ciocalteu reagent was used to evaluate the total phenolic content of the extract using Gallic acid as standard. The standard curve of Gallic acid was prepared by taking 500, 250, 125, 62.5, 31.25, and 15.625 µg/ml concentrations. The procedure for determining the absorption of various concentrations is the same as follows for in hydroalcoholic flower extract of *Rhododendron Arboreum* powder [15]. All the samples were subjected to a temperature of 60 °C on the water bath for 1h followed by cooling to room temperature. 400 µL of this solution was transferred into the test tube containing 1.6 mL of sodium carbonate (7.5% in deionized water) and 2 mL of Folin Ciocalteu reagent (0.1% in deionized water). Further, all the samples were incubated for 1 h at room temperature. Absorbance was measured at 525 nm using UV Spectrophotometer. All the readings were taken in triplicate. Total phenolic content was expressed in mg Gallic acid equivalent (GAE) per gram of hydroalcoholic and aqueous flower extract of *Rhododendron Arboreum*, using the calibration curve.

Estimation of Flavonoid Content: The most commonly used method to determine total flavonoids contents by taking Quercetin as standard. Different concentrations of in hydroalcoholic and aqueous flower extract of *Rhododendron Arboreum* powder and standard was prepared as above, and 100, 50, 25, 12.5 µg/ml in hydroalcoholic and aqueous flower extract of *Rhododendron Arboreum* powder and standard was added to the test tube containing 75 µL of 5% $NaNO_2$ solution [16]. The mixture was allowed to stand for 10 min. 150 µL of a 10% $AlCl_3.6H_2O$ solution was then added to every sample and were allowed to stand for 5 min. Further 0.5 mL NaOH (1 M) and 2.5 mL of distilled water were added to each sample. Absorbance was measured at 510 nm using UV Spectrophotometer. All the observations were taken in triplicate. Total flavonoid content was calculated as mg Querctine equivalent (QE)/g of hydroalcoholic and aqueous flower extract of *Rhododendron Arboreum* by using the linear regression equation obtained for Quercetin.

Preliminary Phytochemical Screening (Qualitative): Hydro-alcoholic and aqueous flower extract of *Rhododendron Arboreum* was concealed for the presence or absence of the crucial category of active compounds such as tannins, flavonoids, saponins, alkaloids, steroids, triterpenes &glycosides by the standard method [17].

***In-vitro* Antioxidant Activity Assay:**

Antioxidant Activities Assay By DPPH: The DPPH radical scavenging potential assessment elucidated was pursued with slight alterations. The H atom or electron contributing capacity of the hydro alcoholic and aqueous flower extract of *Rhododendron Arboreum* was estimated against the development of the purple-colored methanol solution of DPPH. As a testing agent, this spectrophotometric assay utilized the stable radical, 2, and 2-diphenyl-1-picryl hydrazyl (DPPH), as a testing agent18. The working solutions of the extracts produced in methanol & distinct concentrations of hydroalcoholic and aqueous flower extract of *Rhododendron Arboreum* were utilized. Ascorbic acid was utilized as standard in 0.2-1.0 mg/ml solution. DPPH (0.002%) was processed in methanol & one ml of DPPH solution; 1 ml of sample hydroalcoholic and aqueous flower extract of *Rhododendron Arboreum* in solution was added. The solution mixture was supported in the dark for 30 min and absorbance was estimated at 517 nm. DPPH solution of one ml was utilized as

blank. All procedures were performed in triplicate and the results are expressed as & percentage of DPPH scavenging (mean ± SD) as per the following equation.

DPPH scavenging activity (%) = (The absorbance of the control) - (Absorbance of the sample) / (The absorbance of the control) × 100

Nitric oxide (NO) Scavenging Technique: NO radical scavenging potential of the specimen can be executed by utilizing Griess. 75 µl of different concentrations of hydroalcoholic and aqueous flower extract of *Rhododendron Arboreum* is incubated with 75 µl sodium nitroprusside under visible light polychromatic light for 60 min in a 96 - well microplate. For control, 75 µl 95% methanol is added in place of samples. 150 µl Griess reagent is then appended to the reaction mixture, and absorbance is recorded spectrophotometrically around 550 nm [19]. All procedures were performed in triplicate, and the results are expressed as & Percent NO Scavenging (mean ± SD) as per the following equation.

NO scavenging activity (%) = (The absorbance of the control) ¬ (Absorbance of the sample) / (The absorbance of the control) × 100

***In -vitro* Anti-Inflammatory Activity Assay:** The blood was possessed from normal human volunteers who have not confiscated any NSAIDs which are stand (Non-steroidal and Anti-inflammatory medicine) for 2 weeks preceding the investigation & transmitted to the centrifuge tubes. The tubes were separated at very high-speed rotation at 3000 rpm for 10 min & were cleaned three times with equal volume of normal saline. The volume of blood was estimated and regenerated as 10% v/v suspension with normal saline [20].

Heat-induced Haemolysis: Reaction mixture two ml was appraised of one ml-test hydroalcoholic, and aqueous flower extract of *Rhododendron Arboreum* (250 - 1000 µg/ml) & one ml of 10% RBCs suspension; alternatively, the test sample at most saline was attached to the control test tube. Aspirin was utilized as a standard drug for the comparison of anti-inflammatory activity [21]. Entire centrifuge tubes accommodated reaction mixture were incubated interior the water bath at 56 ºC for 30 min. Finally, of the incubation, the tubes were cooled below the water tap. The reaction mixture was separated by rotating at high speed of 2500 rpm for a time of five min & the absorbance of the supernatants was confiscate at 560 nm. All procedures were performed in triplicate, and the results are expressed as percentage hemolysis (mean ± SD) as per the following equation.

Percentage of inhibition (%) = (The absorbance of the control) ¬ (Absorbance of the sample) / (The absorbance of the control) × 100

Hypotonicity-induced Hemolysis: Hydroalcoholic and aqueous flower extract of *Rhododendron Arboreum* (250 - 1000 µg/ml), reference & control sample were individually mixed among one ml of PBS, two ml of hypo-saline & 0.5 ml of HRBC suspension. Diclofenac sodium concentration of (250-1000 µg/ml), was utilized as a basic reference medicine [22]. Entire assay mixtures were put interior the incubator at about 37 °C for 30 min of time & rotated at high speed around 3000 rpm. The supernatant liquid was poured off or a spectrophotometer predicted the hemoglobin content at 560 nm. All procedures were performed in triplicate, and the results are expressed as percentage hemolysis (mean ±SD) as per the following equation.

Percentage of inhibition (%) = (The absorbance of the control) ¬ (Absorbance of the sample) / (The absorbance of the control) × 100

***In-vitro* Hypoglycemic Activity Assay:** Enzymes inhibition assays are potential of hydroalcoholic and aqueous flower extract of *Rhododendron Arboreum* to inhibit the alpha-amylase alpha-glucosidase enzymes activity was performed following the previously described approach along with the slight modification. The assay was performed in the 96-well plate [23]. For this purpose, 30 µL of the test extract at the different concentration (250-1000 µg) were incubated with the 60 µL enzyme (1U/ml prepared in the phosphate buffer saline (PBS) PH 6.9) for 10 minutes at 37 °C. An equal volume of the distilled water was incubated with the enzymes to serve as a control reaction. The reaction was terminated by adding 30 µL HCL (1M) and 120 µL KI (5% W/V), and the absorbance of each well was recorded at 630 nm by using a UV-Spectrophotometer. All procedures were performed

in the triplicate, and the results are expressed AS % alpha-amylase and alpha-glucosidase enzymes inhibition (mean ± SD) as per the following equation.

% of enzymes inhibition (%) = (The absorbance of the control) ¬ (Absorbance of the sample) / (The absorbance of the control) × 100

RESULTS

Organoleptic Evaluation: The flowers of *R. Arboreum* range in color from a deep scarlet, to red with white markings, pink to white. Bearing up to twenty blossoms in a single truss, this rhododendron is a spectacular sight when in full bloom. It is reported that the bright red forms of this rhododendron are generally found at the lower elevations. Flowers are showy, red in dense globose cymes. Calyx- fine cleft, Corolla-tube spotted funnel-shaped, Stamens-hypogynous declining, Filaments filiform, Anthers-ovate, Style-capitate as shown in **Fig. 3.**

FIG. 3: SHOWING THE MORPHOLOGICAL APPEARANCE OF *RHODENDRON ARBOREUM*

Physicochemical Evaluation: The amount of loss on drying (LOD) at 110 °C revealed the moisture content coeval in the sample, which extended from 78% w/w. The entire ash and acid insoluble ash were accomplished to be 2.04% w/w and 1.23% w/w reciprocally. The ash contents demonstrated the amounts of inorganic matter that exist in the sample and acid insoluble ash virtually within 1%, which revealed low siliceous matter exists in the sample. Successive extractive values of Hydro-alcoholic and aqueous extract of *Rhododendron Arboreum* flower were 5.8%, 4.5% (w/w) reciprocally as appearing in **Table 1.**

TABLE 1: PHYSICOCHEMICAL PARAMETERS OF FLOWER & FLOWER DIFFERENT EXTRACT

Physiochemical constants (%)	Total ash	Acid-insoluble ash	Moisture content
	2.04%	1.23%	78%
Successive extractive values (%)	Aqueous extract of flower 4.5%w/w	Hydroalcoholic extract of flower 5.8%w/w	

Phytochemical Analysis

Phytochemical Screening (Quantitative)

Determination of Total Amount of Phenolic Content: The standard curve is to be constructed by utilizing the standard drug Gallic acid at different concentrations. The total amount of phenolic content of the extract fractions was detected in terms of mg GAE / gram of extracts. Total amount phenolic contents were detected utilizing linear regression equation which is

expressed as y = 0.003 × + 0.033 at R^2 = 0.998 as shown in the **Table 2.**

TABLE 2: STANDARD CALIBRATION CURVE ABSORBANCE/CONCENTRATION OF GALLIC ACID

S. no	Concentration (µg/ml)	Absorbance
1	500	1.98
2	250	1.07
3	125	0.52
4.	62.5	0.266
5.	31.25	0.156
6	15.625	0.084

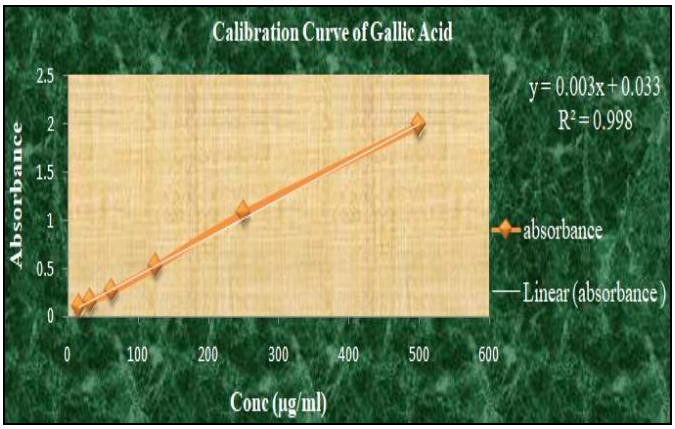

FIG. 4: STANDARD CURVE OF GALLIC ACID FOR DETERMINING TOTAL AMOUNT OF PHENOLIC CONTENT

The total amount of phenolic content in Hydro-alcoholic and aqueous extract of *Rhododendron Arboreum* flower were accounted to be 86.5 & 78.5 mg GAE/gram of extract fractions respectively.

Estimation of the Total Amount of Flavonoid Content: The standard curve of Quercetin was represented at different concentrations. Linear regression was an appeal to the acquired curve. The total amount of flavonoid content was then detected from the equation, which is expressed as follows y = 0.009 × -0.014 at R^2 = 0.999 in terms of mg QE/g of extract fraction as shown in **Table 3.**

TABLE 3: STANDARD CALIBRATION CURVE OF ABSORBANCE/CONCENTRATION QUERCETIN

S. no	Concentration (µg/ml)	Absorbance
1	100	0
2	50	0.054
3	25	0.112
4	12.5	0.218
5	6.75	0.051

The total amount of flavonoid content in Hydro-alcoholic and aqueous extract of Rhododendron Arboreum flower was 53.78 & 63.89 mg QE/g of extract fractions, respectively.

Phytochemical Screening (Qualitative): Hydro-alcoholic and aqueous extract of *Rhododendron Arboreum* revealed the presence of tannins, flavonoids, phenol, triterpenes, Steroids & carbohydrates. Glycosides, protein and alkaloids were accomplished to be absent, as shown in **Table 4.** Flavonoids & tannins are recognized for their antioxidant, astringent, anti-inflammatory & hepatoprotective potential. The plant occupies a high percentage of tannins, flavonoids, and triterpene. This may be one of the rationale behind the antioxidant, astringent, anti-inflammatory & hepatoprotective potential of this botanical.

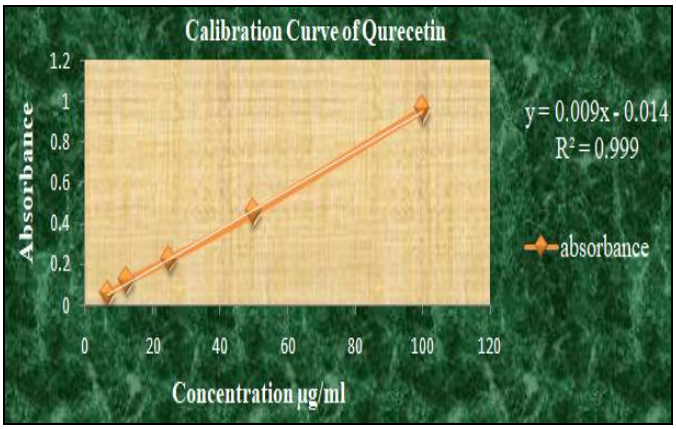

FIG. 5: STANDARD CURVE OF QUERCETIN FOR DETERMINING TOTAL AMOUNT OF FLAVONOID CONTENT

TABLE 4: PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF AQUEOUS AND HYDROALCOHOLIC EXTRACT OF *RHODODENDRON ARBOREUM*

Test for active constituent	Hydroalcoholic extract	Aqueous extract
Tannins	+++	+++
Flavonoids	++	+
Phenol	+++	++
Triterpenes	+	–
Glycosides	-	-
Saponins	-	-
Protein	+	–
Steroids	+++	++
Alkaloids	-	-
Carbohydrates	+	+
Coumarins	–	–
Xanthoprotein	–	–
Number of the compound in the extract	07	05

(-) Absent ; (+) Low; (++) Average; (+++) High;

***In-vitro* Anti-Oxidant Activity Assay:**

Antioxidant Activities Assay (Spectrophotometric Analysis) by DPPH: The more frequent basis utilization of DPPH assay is

straightforward and extremely precise. DPPH is depreciated in the radical form through its strength. The present radical appears a secure absorption maximum at a wavelength of 517 nm (purple). In the existence of antioxidants, the color turns from purple to yellow. Consequently, the sole apparatus essential for the assay is a UV- Vis Spectrophotometer. The DPPH free radical scavenging capabilities of aqueous and hydroalcoholic extract of *Rhododendron Arboreum* at distinct concentrations were estimated and contrasted with that of the standard ascorbic acid **Table 3.** Five distinct working solutions of aqueous and hydroalcoholic extract of *Rhododendron Arboreum* were utilized having varying concentrations (250, 500, 750 & 1000 µg/ml). Decolouration due to reaction of antioxidant in samples with the stable DPPH free radical detected by spectrophotometrically. It was perceived that as the concentration of samples enhances, the free radical scavenging potential will also be enhanced. The antioxidant consequence of botanical products is primarily due to the radical scavenging potential of phenolic compounds like flavonoids, polyphenols, tannins, and phenolic compounds. When these compounds enhanced in dose, the antioxidant potential enhanced correspondingly in all the samples as shown in **Table 5.**

TABLE 5: DPPH FREE RADICAL SCAVENGING POTENTIAL IN (%) OF AQUEOUS AND HYDROALCOHOLIC EXTRACT OF *RHODODENDRON ARBOREUM* & ASCORBIC ACID (N = 3; MEAN ± SD).

Concentration (µg/ml)	Absorbance at 560 nm		
	Ascorbic acid	HA Extract	Aqueous Extract
1000	0.028 ± 0.009	0.098 ± 0.016	0.176 ± 0.019
750	0.119 ± 0.011	0.115 ± 0.024	0.357 ± 0.014
500	0.194 ± 0.031	0.235 ± 0.018	0.576 ± 0.029
250	0.295 ± 0.021	0.437 ± 0.016	0.781 ± 0.033
Concentration (µg/ml)	**% DPPH free radical scavenging**		
1000	96.96	89.37	80.91
750	87.09	87.52	61.27
500	78.95	74.71	37.52
250	68.00	52.60	15.29

The control showed absorbance at 560 nm (0.922) used to compare the % DPPH free radical scavenging

Around the entire aqueous and hydroalcoholic extract of *Rhododendron Arboreum* samples investigations, the aqueous and hydroalcoholic extract of *Rhododendron Arboreum* appearing in the concentration of 1.0 mg/ml exhibited the optimum free radical scavenging potential of 80.91 & 89.37%. Correspondingly, 0.75 mg/ml aqueous and hydroalcoholic extract of *Rhododendron Arboreum* exhibited the optimum free radical scavenging potential of 61.27 & 87.52%. Aqueous and hydroalcoholic extract of *Rhododendron Arboreum* at 0.50 mg/ml had the highest free radical scavenging potential 37.52 & 74.71%. 0.25 mg/ml of aqueous and hydroalcoholic extract of *Rhododendron Arboreum* had optimum free radical scavenging potential of 15.29 & 52.60%. It was additionally recognized that the entire tested samples appeared lower DPPH radical scavenging potential when collating with the Ascorbic acid as a standard. The optimum free radical scavenging potential was acquired for the ascorbic acid at 1mg/ml was raise to be 96.96%. Additionally, **Fig. 6.** demonstrates that the scavenging percentage of aqueous and hydroalcoholic extract of *Rhododendron Arboreum* was in increasing sequence with the increase in concentration.

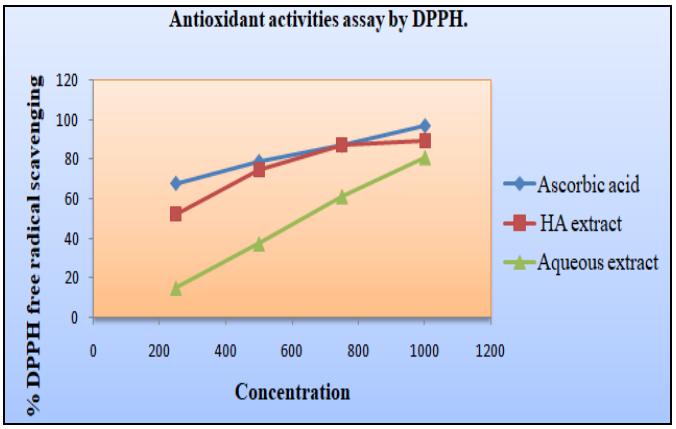

FIG. 6: DEMONSTRATE THAT THE DPPH SCAVENGING PERCENTAGE OF AQUEOUS AND HYDROALCOHOLIC EXTRACT OF *RHODODENDRON ARBOREUM* & ASCORBIC ACID

Nitric oxide (NO) Scavenging Technique: Nitric oxide radical accused from sodium nitroprusside in aqueous solution at physiological pH connect among the oxygen to generate nitrite ions which

were deliberated by Griess reaction. Nitric oxide radical accused from nitroprusside at physiological pH was accomplished to be inhibited by the hydroalcoholic and aqueous flower extract of *Rhododendron Arboreum* and ascorbic acid as manifested in **Table 4.** Four diverse working solutions of hydroalcoholic and aqueous flower extract of *Rhododendron Arboreum* and ascorbic acid were utilized having diverse concentrations (0, 250, 500, 750, and 1000 µg/ml) were utilized. Decolouration due to the reaction of antioxidants in samples with the nitric oxide free radical was deliberated spectrophotometrically. It was perceived that when the concentration of samples enhanced, the percentage nitric oxide scavenging potential also enhanced, as shown in **Table 6.**

TABLE 6: PERCENTAGE NITRIC OXIDE SCAVENGING ACTIVITY OF AQUEOUS AND HYDROALCOHOLIC EXTRACT OF *RHODODENDRON ARBOREUM* & ASCORBIC ACID (N = 3; MEAN ± SD).

Concentration (µg/ml)	Absorbance at 560 nm		
	Ascorbic acid	HA Extract	Aqueous Extract
1000	0.029 ± 0.017	0.128 ± 0.021	0.215 ± 0.011
750	0.128 ± 0.028	0.146 ± 0.031	0.476 ± 0.027
500	0.185 ± 0.008	0.209 ± 0.021	0.545 ± 0.024
250	0.327 ± 0.017	0.378 ± 0.017	0.676 ± 0.019
Concentration (µg/ml)	**% Nitric oxide scavenging**		
1000	96.28	83.61	72.47
750	83.61	81.30	39.05
500	76.31	73.23	30.21
250	58.31	51.60	13.44
The control showed absorbance at 560 nm (0.781) used to compare the % Nitric oxide scavenging			

Around the entire aqueous and hydroalcoholic extract of *Rhododendron Arboreum* samples investigations, the aqueous and hydroalcoholic extract of *Rhododendron Arboreum* appearing in the concentration of 1.0 mg/ml exhibited the optimum percent nitric oxide scavenging potential of 72.47 & 83.61%. Correspondingly, in 0.75 mg/ml aqueous and hydroalcoholic extract of *Rhododendron Arboreum* exhibited the optimum percent nitric oxide scavenging potential 39.05& 81.30 %. Aqueous and hydroalcoholic extract of *Rhododendron Arboreum* at 0.50 mg/ml had the highest percent nitric oxide scavenging potential 30.21 & 73.23%. 0.25 mg/ml of aqueous and hydroalcoholic extract of *Rhododendron Arboreum* had optimum percent nitric oxide scavenging potential 13.44 & 51.60%.

It was also recognized that the entire tested samples appeared to have percent nitric oxide scavenging potential when collating with the standards. The optimum percent nitric oxide scavenging potential was acquired for the ascorbic acid at 1mg/ml was raise to be 96.28%. Additionally **Fig. 7.** demonstrates that the NO scavenging percentage of aqueous and hydroalcoholic extract of *Rhododendron Arboreum* was in increasing sequence with the increase in concentration.

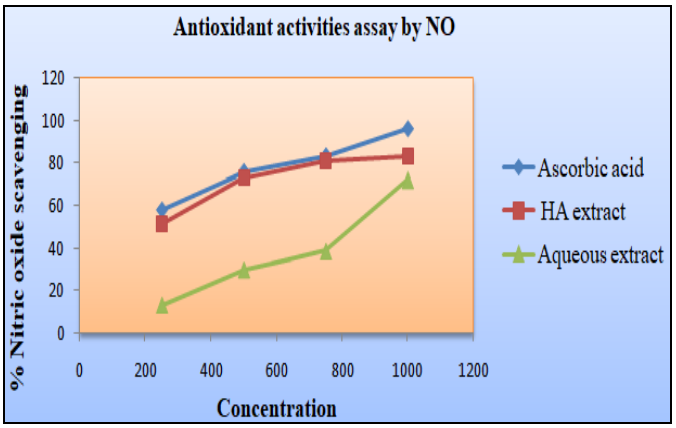

FIG. 7: DEMONSTRATE THAT THE NO SCAVENGING PERCENTAGE OF AQUEOUS AND HYDROALCOHOLIC EXTRACT OF *RHODODENDRON ARBOREUM* & ASCORBIC ACID

In-vitro Anti-Inflammatory Activity Assay:

Heat Induces Hemolysis: Stabilization of the cell membrane of RBCs when asserting with direct controlled heat was investigated to access membrane stabilization potential of diverse drugs concentration in collation to diclofenac.

The aqueous and hydroalcoholic extract of *Rhododendron Arboreum* was efficacious in inhibiting heat-induced hemolysis at diverse concentrations. This result is demonstrated in graphical form in **Table 7.**

TABLE 7: EFFECT OF AQUEOUS AND HYDROALCOHOLIC EXTRACT OF *RHODODENDRON ARBOREUM ON HEAT-INDUCED HEMOLYSIS OF ERYTHROCYTE (N = 3; MEAN ± SD).*

Concentration (µg/ml)	Absorbance at 560 nm		
	Diclofenac sodium	HA Extract	Aqueous Extract
1000	0.062 ± 0.021	0.071 ± 0.007	0.106 ± 0.014
750	0.073 ± 0.018	0.086 ± 0.014	0.121 ± 0.022
500	0.084 ± 0.011	0.098 ± 0.021	0.135 ± 0.023
250	0.098 ± 0.007	0.123 ± 0.033	0.157 ± 0.032
Concentration (µg/ml)	**% Inhibition of Heat-induced Hemolysis**		
1000	77.12	73.80	60.88
750	73.06	68.26	55.35
500	69.00	63.83	50.18
250	63.83	54.61	42.06

The control showed absorbance at 560 nm (0.271) used to compare the % Inhibition of Heat-induced Hemolysis

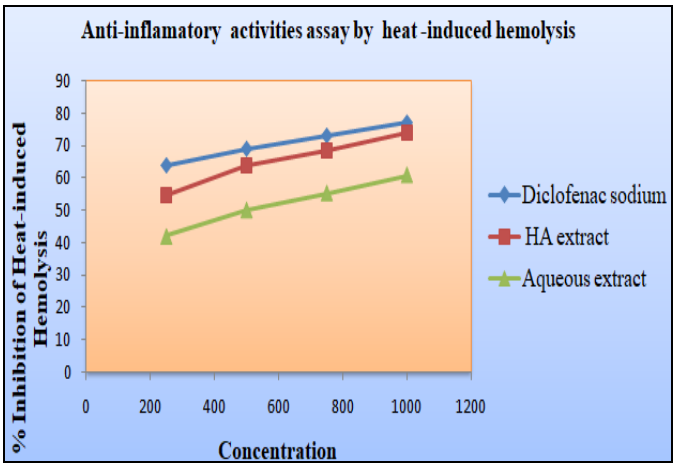

FIG. 8: DEMONSTRATE THE HEAT-INDUCED HEMOLYSIS OF AQUEOUS AND HYDROALCOHOLIC EXTRACT OF *RHODODENDRON ARBOREUM* WITH DICLOFENAC SODIUM AS A STANDARD

Around the entire aqueous and hydroalcoholic extract of *Rhododendron Arboreum* samples investigations, the aqueous and hydroalcoholic extract of *Rhododendron Arboreum* appearing in the concentration of 1.0 mg/ml exhibited the optimum percent Inhibition of Heat-induced Hemolysis potential of 60.88 & 73.80%.

Hydro- Correspondingly, in 0.75 mg/ml aqueous and alcoholic extract of *Rhododendron Arboreum* exhibited the optimum percent of Inhibition of Heat-induced Hemolysis potential 55.35 & 68.26%. Aqueous and hydroalcoholic extract of *Rhododendron Arboreum* at 0.50 mg/ml had the percent of Inhibition of Heat-induced Hemolysis potential 50.18 & 63.83%. 0.25 mg/ml of aqueous and hydroalcoholic extract of *Rhododendron Arboreum* had optimum percent of Inhibition of Heat-induced Hemolysis potential 42.06 & 54.61%.

It was also recognized that the entire tested samples appeared to have a percent Inhibition of Heat-induced Hemolysis potential when collating with the standards. The optimum percent of Inhibition of Heat-induced Hemolysis potential was acquired for the Diclofenac sodium at 1 mg/ml was raise to be 77.12%. Additionally, **Fig. 8.** demonstrates that the percent of Inhibition of Heat-induced Hemolysis of aqueous and hydroalcoholic extract of *Rhododendron Arboreum* was in increasing sequence with the increase in concentration.

TABLE 8: EFFECT OF AQUEOUS AND HYDROALCOHOLIC EXTRACT OF *RHODODENDRON ARBOREUM* ON HYPO-TONICITY INDUCED HEMOLYSIS OF ERYTHROCYTE (N = 3; MEAN ± SD)

Concentration (µg/ml)	Absorbance at 560 nm		
	Diclofenac sodium	HA Extract	Aqueous Extract
1000	0.074 ± 0.004	0.095 ± 0.022	0.112 ± 0.015
750	0.084 ± 0.009	0.113 ± 0.024	0.124 ± 0.014
500	0.092 ± 0.017	0.132 ± 0.018	0.135 ± 0.005
250	0.114 ± 0.035	0.145 ± 0.017	0.168 ± 0.031
Concentration (µg/ml)	**% Hypo-tonicity Induced Haemolysis**		
1000	71.31	63.17	56.58
750	65.50	56.20	51.93
500	64.34	48.83	47.67
250	55.81	43.79	34.88

The control showed absorbance at 560 nm (0.258) used to compare the % Inhibition of Hypo-tonicity Induced Haemolysis

Hypotonicity Induced Haemolysis: The RBC membrane stabilization was repeatedly tested by changing related conditions for hemolysis. The consequences manifested that aqueous and hydroalcoholic extract of *Rhododendron Arboreum* of at concentration range of 250-1000 µg/ml shield, represented below in **Table 8.** Around the entire aqueous and hydroalcoholic extract of *Rhododendron Arboreum* samples investigations, the aqueous and hydroalcoholic extract of *Rhododendron Arboreum* appearing in the concentration of 1.0 mg/ml exhibited the optimum percent Inhibition of Hypo-tonicity Induced Haemolysis potential of 56.58 & 63.17%.

Correspondingly, 0.75 mg/ml aqueous and hydroalcoholic extract of *Rhododendron Arboreum* exhibited the optimum percent of Inhibition of Hypo-tonicity Induced Haemolysis potential 51.93 & 56.20%. Aqueous and hydroalcoholic extract of *Rhododendron Arboreum* at 0.50 mg/ml had the percent of Inhibition of Hypo-tonicity Induced Haemolysis potential 47.67 & 48.83%. 0.25 mg/ml of aqueous and hydroalcoholic extract of *Rhododendron Arboreum* had optimum percent of Inhibition of Hypo-tonicity Induced Haemolysis potential 34.88 & 43.79%. It was additionally recognized that the entire tested samples appeared percent Inhibition of Hypo-tonicity Induced Haemolysis potential when collating with the standards. The optimum percent of Inhibition of Hypo-tonicity Induced Haemolysis potential was acquired for the Diclofenac sodium at 1mg/ml was raise to be 71.31%. Additionally **Fig. 9.** demonstrates that the percent of Inhibition of Hypo-tonicity Induced Haemolysis of aqueous and hydroalcoholic extract of *Rhododendron Arboreum* was in increasing sequence with the increase in concentration.

***In-vitro* Hypoglycemic Activity Assay:**

% Alpha-Amylase Inhibition: The aqueous and hydroalcoholic extract of *Rhododendron Arboreum* obtained by extraction methods were subjected to *in-vitro* Model of the diabetic assay, which includes the use of digestive enzymes alpha-amylase and alpha-glucosidase. The extent of the inhibition of aqueous and hydroalcoholic extract of *Rhododendron Arboreum* on these enzymes was studied and made to compare with standard drug acarbose, which has been used widely as an enzyme inhibitor as shown in **Table 9.**

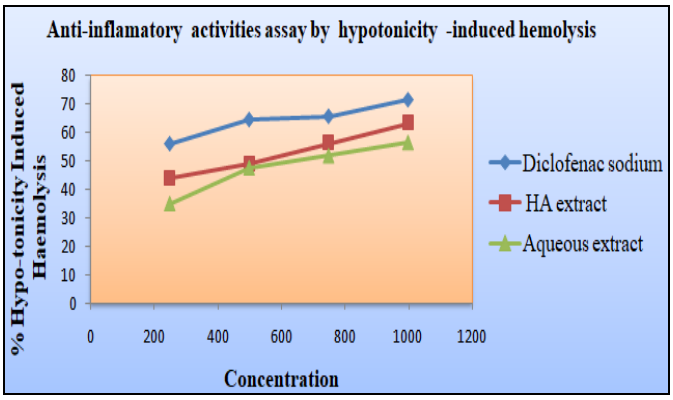

FIG. 9: DEMONSTRATING THE HYPO-TONICITY INDUCED HEMOLYSIS OF AQUEOUS AND HYDROALCOHOLIC EXTRACT OF *RHODODENDRON ARBOREUM* WITH DICLOFENAC SODIUM AS A STANDARD DRUG

TABLE 9: EFFECT OF AQUEOUS AND HYDROALCOHOLIC EXTRACT OF *RHODODENDRON ARBOREUM* ON % ALPHA-AMYLASE INHIBITION (N = 3; MEAN ± SD).

Concentration (µg/ml)	Absorbance at 560 nm		
	Acarbose	HA Extract	Aqueous Extract
1000	0.107 ± 0.019	0.103 ± 0.021	0.138 ± 0.013
750	0.146 ± 0.018	0.198 ± 0.007	0.204 ± 0.030
500	0.187 ± 0.024	0.297 ± 0.031	0.325 ± 0.022
250	0.227 ± 0.027	0.425 ± 0.011	0.486 ± 0.015
Concentration (µg/ml)	**% alpha amylase inhibition**		
1000	81.42	82.11	76.04
750	74.65	65.62	64.58
500	67.53	48.43	43.57
250	60.59	26.21	15.52

The control showed absorbance at 540 nm (0.576) used to compare the % alpha-amylase inhibition

Around the entire aqueous and hydroalcoholic extract of *Rhododendron Arboreum* samples investigations, the aqueous and hydroalcoholic extract of *Rhododendron Arboreum* appearing in the concentration of 1.0 mg/ml exhibited the optimum percent alpha-amylase inhibition potential

of 76.04 & 82.11%. Correspondingly, in 0.75 mg/ml aqueous and hydroalcoholic extract of *Rhododendron Arboreum* exhibited the optimum percent alpha-amylase inhibition potential 64.58 & 65.62%.

Aqueous and hydroalcoholic extract of *Rhododendron Arboreum* at 0.50 mg/ml had the percent alpha-amylase inhibition potential 43.57 & 48.43%. 0.25 mg/ml of aqueous and hydroalcoholic extract of *Rhododendron Arboreum* had optimum percent alpha-amylase inhibition potential 15.52 & 26.21%.

It was also recognized that the entire tested samples showed percent alpha-amylase inhibition potential when collating with the standards. The optimum percent of alpha-Amylase inhibition potential was acquired for the Acarbose at 1mg/ml was raised to be 81.42%. Additionally **Fig. 10.** demonstrates that the percent of alpha-Amylase inhibition of aqueous and hydroalcoholic extract of *Rhododendron Arboreum* was in increasing sequence with the increase in concentration.

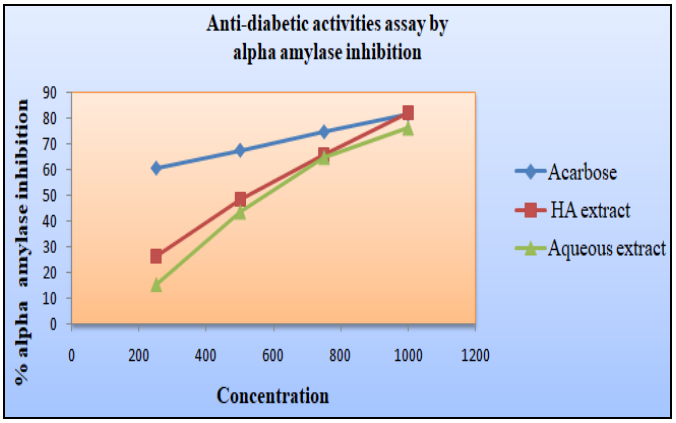

FIG. 10: DEMONSTRATING THE % ALPHA-AMYLASE INHIBITION OF AQUEOUS AND HYDROALCOHOLIC EXTRACT OF *RHODODENDRON ARBOREUM* WITH ACARBOSE AS A STANDARD DRUG

TABLE 10: EFFECT OF AQUEOUS AND HYDROALCOHOLIC EXTRACT OF *RHODODENDRON ARBOREUM* ON % ALPHA-GLUCOSIDASE INHIBITION (N = 3; Mean ± SD).

Concentration (µg/ml)	Absorbance at 560 nm		
	Acarbose	HA Extract	Aqueous Extract
1000	0.145 ± 0.012	0.098 ± 0.024	0.126 ± 0.007
750	0.179 ± 0.004	0.145 ± 0.008	0.135 ± 0.021
500	0.208 ± 0.014	0.198 ± 0.015	0.218 ± 0.031
250	0.325 ± 0.023	0.276 ± 0.024	0.348 ± 0.024
Concentration (µg/ml)	**% alpha - glucosidase inhibition**		
1000	72.84	81.64	76.40
750	66.47	72.84	74.71
500	61.04	62.92	59.17
250	39.13	48.31	48.31
The control showed absorbance at 560 nm (0.534) used to compare the % alpha-glucosidase inhibition			

% Alpha-glucosidase Inhibition: The aqueous and hydroalcoholic extract of *Rhododendron Arboreum* obtained by extraction methods were subjected to in vitro model of the diabetic assay, which includes the use of digestive enzymes alpha-glucosidase. The extent of the inhibition of aqueous and hydroalcoholic extract of *Rhododendron Arboreum* on these enzymes was studied and made to compare with standard drug acarbose, which has been used widely as an enzyme inhibitor.

Around the entire aqueous and hydroalcoholic extract of *Rhododendron Arboreum* samples investigations, the aqueous and hydroalcoholic extract of *Rhododendron Arboreum* appearing in the concentration of 1.0 mg/ml exhibited the optimum percent alpha-glucosidase inhibition potential of 76.40 & 81.64%. Correspondingly, in 0.75 mg/ml aqueous and hydroalcoholic extract of *Rhododendron Arboreum* exhibited the optimum percent alpha-glucosidase inhibition potential 74.71 & 72.84%. Aqueous and hydroalcoholic extract of *Rhododendron Arboreum* at 0.50 mg/ml had the percent alpha-glucosidase inhibition potential 59.17 & 62.92%. 0.25 mg/ml of aqueous and hydroalcoholic extract of *Rhododendron Arboreum* had optimum percent alpha-glucosidase inhibition potential 48.31 & 48.31%. It was also recognized that the entire tested samples appeared to have percent alpha-glucosidase inhibition potential when collating with the standards. The optimum percent of alpha-glucosidase inhibition potential was acquired for the Acarbose at 1mg/ml was raise to

be 72.84%. Additionally, **Fig. 10** demonstrates that the percent of alpha-glucosidase inhibition of aqueous and hydroalcoholic extract of *Rhododendron Arboreum* was in increasing sequence with the increase in concentration.

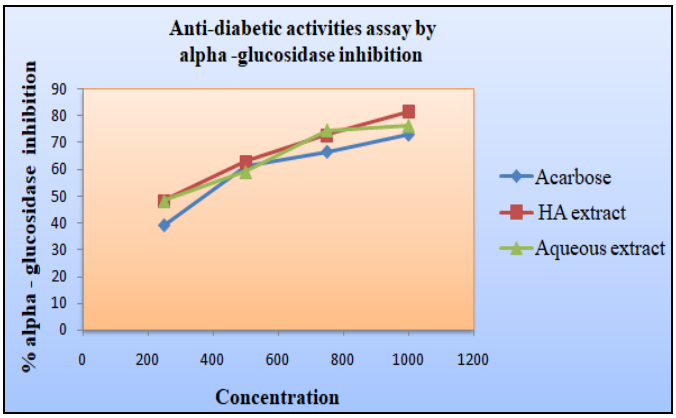

FIG. 11: DEMONSTRATING THE % ALPHA-GLUCOSIDASE INHIBITION OF AQUEOUS AND HYDROALCOHOLIC EXTRACT OF *RHODODENDRON ARBOREUM* WITH ACARBOSE AS A STANDARD DRUG

The outcomes of the study demonstrate that the inhibition of alpha-glucosidase enzymes by the extract was seen more when comparing with the alpha-Amylase enzyme.

DISCUSSION: Aqueous and hydroalcoholic extract of *Rhododendron Arboreum* obtained by cold maceration of the flower of yield value up to 5% w/w. The phytochemical screening of Aqueous and hydroalcoholic extract of *Rhododendron Arboreum* is to be showing the presence of the good content of the tannin and flavonoid in them. This compound possesses good antioxidant, anti-inflammatory & antidiabetic potential.

Further, these properties of the Aqueous and hydroalcoholic extract of *Rhododendron Arboreum* were investigated by using in vitro estimation approach. When these compounds enhanced in dose, the antioxidant potential enhanced correspondingly in all the samples. It is to be observed that the Aqueous and hydroalcoholic extract of *Rhododendron Arboreum* is to show comparable antioxidant potential in the comparison of the ascorbic acid. This study is to be suggested that the drug is to possesses excellent antioxidant potential. Further, the Aqueous and hydroalcoholic extract of *Rhododendron Arboreum in-vitro* anti-inflammatory study suggests the Aqueous and hydroalcoholic extract of *Rhododendron Arboreum* is to possess good action against the inflammatory disorder in the body. The antidiabetic activity of the Aqueous and hydroalcoholic extract of *Rhododendron Arboreum* by using the enzymes inhibition approach of alp[ha amylase and alpha-glucosidase etc. *Rhododendron Arboreum* in vitro anti-inflammatory study suggests that the Aqueous and hydroalcoholic extract of Rhododendron Arboreum possesses good enzyme inhibitory action and possesses good antidiabetic potential. This study is to be recommended that the Aqueous and hydroalcoholic extract of *Rhododendron Arboreum* is to be good antioxidant and anti-inflammatory & antidiabetic action to cure the various body disorders.

CONCLUSION: This study is to be presented us that the drug yield value can be enhanced by using the extraction of such type of the modified method. Since the yield value with both extract aqueous and hydroalcoholic extract, *Rhododendron Arboreum*, with the value of the high yield up to 5%. It also has shown the high content of the tannin, flavonoids & phenolic compounds.

DPPH and NO radical scavenging approaches show the good antioxidant potential of the aqueous and hydroalcoholic extract *Rhododendron Arboreum*., The heat-induced, as well as hypo-tonicity induced approach, demonstrates that the drug is having a good anti-inflammatory activity of the aqueous and hydroalcoholic extract *Rhododendron Arboreum* in the human body.

Further, the antidiabetic activity is accessible by using the pancreatic alpha-amylase and intestinal alpha-glucosidase enzymes that possess excellent antidiabetic potential. The result of the investigation is to be suggested that the aqueous and hydroalcoholic extract *Rhododendron Arboreum* is rich in the phenolic compound and the experimentation study shows the drug is to possesses good antioxidants, anti-inflammatory, and antidiabetic properties.

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Human and Animal Rights: No Animals/Humans were used for studies that are base on this research.

Consent for Publication: Not applicable

CONFLICTS OF INTEREST: The authors declare no conflict of interest, financial or otherwise.

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