How to Cite:
Khairnar, A. S., Majaz, Q., Khan, G. J., & Pawar, S. P. (2022). Phytochemical analysis and evaluation of antioxidant activity of the leaves of Begonia picta smith. *International Journal of Health Sciences*, *6*(S4), 9462–9482. https://doi.org/10.53730/ijhs.v6nS4.10791

Phytochemical analysis and evaluation of antioxidant activity of the leaves of Begonia picta smith

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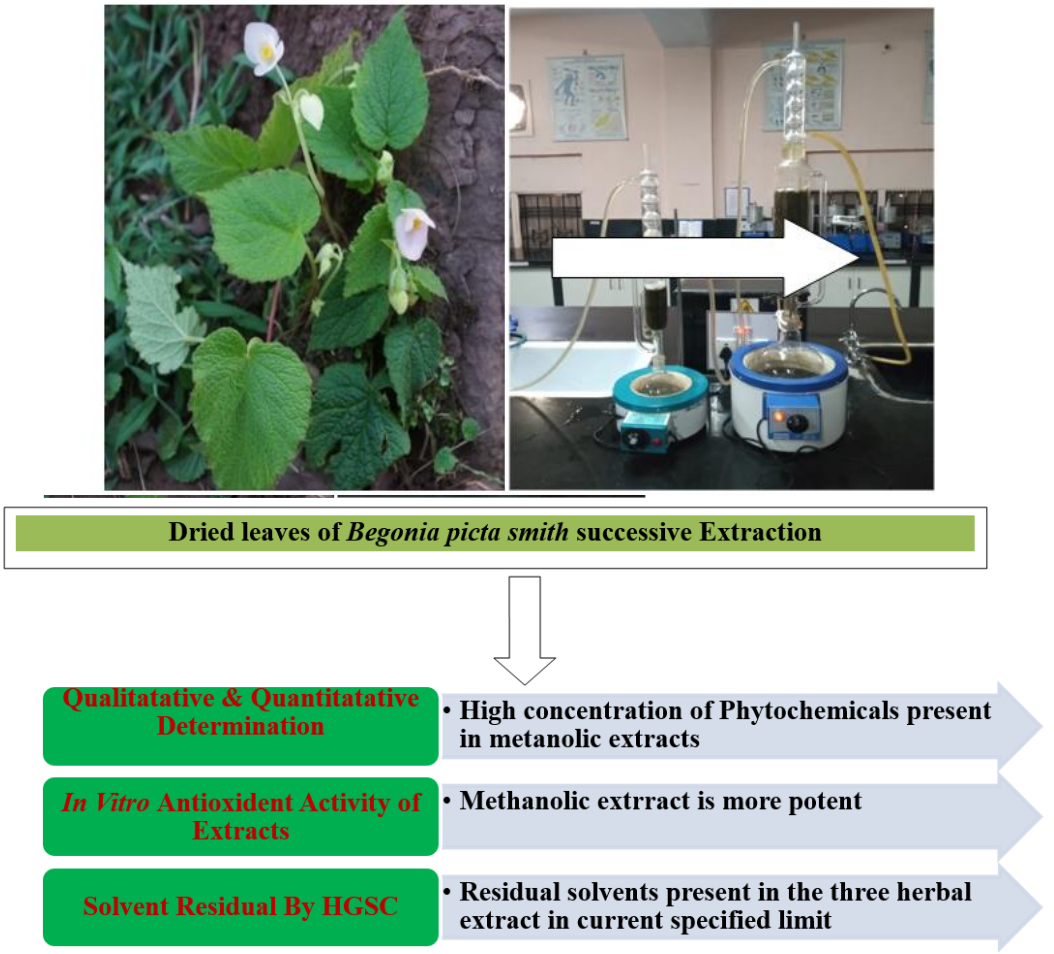
***Abstract*---**Like most other plants *begonia picta smith* contains various secondary metabolites with great potentials. The aim of this paper is to evaluate the phytochemicals by using quantitative and qualitative analysis of Petroleum ether , Chloroform, Methanol extracts ,with the help of standard methods as well solvent residual analysis byGCHS The findings from quantification and phytochemical screening showed the presence of alkaloids, flavonoids, reducing sugars, Phenols, proteins, amino acids, saponins, tannins, terpenoids,Antioxidant activity was determined by 1, 1-diphenyl-2-picryl hydrazyl (DPPH)scavenging method, nitric oxide scavenging method, reducing power assay method and Inhibitory concentrations (IC50) were calculated. the residual solvents present in the three herbal extract also in current specified limits. Evaluation of In Vitro Anti-Oxidant activity reveals that methanolic extract is most potent as compare to other extracts by taking Ascorbic acid as standard. This information clearly represent that the plant *Begonia picta smith* is the good source of dietary antioxidant.

Keywords---quantitative, phytochemical, leaves, begonia, antioxidant.



Graphical Abstract

Introduction

India is leading in biodiversity and well practiced knowledge of traditional herbal medicine so India can a great role of supplier of herbal products not only to meet domestic needs but also advantages of export potential so innovative strategies to improve process of plant collection, bioassay,screening compounds isolation and compound development must be employed in order to get more accurate results should be go for clinical trials to evaluate safety and efficacy.[1]Medicinal plants contain numerous biologically active compounds which are helpful inthe treatment of various diseases and improving the life They are possessed to have various properties likeantioxidant, anti-inflammatory, anti-cancer, anti-diabetic,etc.[2]*Begonia Picta smith* (*Begoniaceae*) is a genus of *Begonia*, a perennial flowering plant, the genus contains 1,795 different plant species.Most of the

Begonia species contain high amount of acids. While visiting the natural habitats of Indian Begonias interviews of tribal inhabitants of the surrounding areas are taken to trace out the ethnomedicinal uses of *Begonia picta* smith and found that used againstindigestion, stomach-pains, diarrhea etc.*B. picta,* taken as *Chatni*withmeat-items. Leaves of *Begonia pictasmith*isboiled and sour curry is prepared with small fish. Incase of dehydration, juice of *Begonia pictasmith*is taken or the stem is taken raw with salt.are used by many tribesof Maharashtra,Arunachal Pradesh, Meghalaya, Manipur, Nagaland and Tripura for thesepurposes.[3]Phytochemicals are naturally occurring substances found in plants which provide healthbenefits. These are known as secondary metabolites and may often be created by modifiedsynthetic pathways from primary metabolite or share substrates of primary metabolite origin[4] Alkaloids, flavonoids, tannins, phenolics, saponin, steroids, glycoside, terpenes and etc. They protect plants from disease and contribute for plant's color, aroma and flavor. Further, they have a role in protection of human health when their dietary intake is significant. This is the rare, endangered and threatened plants of Maharashtra compiled by The Maharashtrastate biodiversity board with information from the various institutions and scientists therefore The present study was to determine the phytoconstituents of *Begonia picta smith* in different solvents by qualitative and quantitative phytochemical screening to provide direction for further research.

Materials and Methods

Chemicals

Petroleum ether, Chloroform, Methanolwere purchased from S.D fine chemicals Ltd.(Mumbai, India).

Plant material

The leaves of Begonia picta smith were collected from the rural area of Dab Village, Nandurbar district and taxonomic Identification was done by comparing morphological features and duly identified as *Begonia picta smith*by Dr. Priyanka A. Ingale, Scientist B, Botanical Survey of India, Pune (Voucher Specimen number-01) and a sample voucher specimen of plant was prepared and deposited at that institute as well taken No Objection certificate from MSSB , Nagpur for access to Biological resources and associated traditional knowledge under Rule 17 of the Maharashtra Biological Diversity), Rules. 2008.

Extracts preparation

Plant leaves were collected dry-cleaned and the foreign organic material (FOM) was separated. They were shade dried and grinded to obtain the powder with the help of grinder. The powder form of sample of 500g was taken and subjected to soxhlet extraction. Three different solvent were chosen to run extraction process as per their polarity i.e. Petroleumether,chloroform, methanol.Different liquid extract obtained were dried separately usingRotavapour drier below 40^{o}C and solid extracts were preserved in refrigerator at 4^{o}c.Extractive value of each extract were determined.

Phytochemical Analysis

Freshly prepared each extract was subjected to standard methods of phytochemical analyses to detected by their color reactions with different reagents and the presence of phytoconstituents i.e.flavonoids, carbohydrates, glycosides, saponins,tannins, proteins and alkaloids .These tests are carried out by standardMethods[5,6].

Residual Solvent Analysis By Head Space Gas Chromatography

Residual Solvent analysis of Petroleum ether, Chloroform and Methanol herbal extract with sample ID NO: 3265/20-21was carried out at Sophisticated Instrumentation Centre for Applied Research and Testing (SICART), Anand, by Head Space Gas Chromatography with Perkin Elmer (Auto system XL)

Quantification of phytochemicals

Determination of total carbohydrate content, Determination of total protein, Determination of total sponin, Determination of total steroids, Determination of total alkaloids , Determination of total flavanoid, Determination of total tannin Content, Determination of total phenolic content, and Determination of total anthraquinone glycoside content were analyzed.[7,8,9]

Determination of total carbohydrate content

Phenol–Sulfuric acid method is based on simple colorimetric measurement. The basic principle of this method is that sulfuric acid dehydrated carbohydratesproduce furfural derivatives which develop detectable color with phenol. For determinationof total carbohydrate content 2 ml of a test solution was mixed with 1 ml of 5% aqueous solution of phenol and 5 ml of concentrated sulfuric acid in a test tube. The resulting mixture allowed to stand for 10 min and vortexed for 30 second and then placed for 20 min in a water bath at room temperature for color development. Then the intensity of absorption of developed color was recorded at 490 nm by using spectrophotometer. Glucose was used as standard solution and calibration curve was prepared in range of 3 µg/ml to 30 µg/ml.[10,11]

Determination of total protein

The Bradford assay is a based on simple colorimetric measurement. Bradford reagent (light brown) binds with protein results in change in color. For determinationof total protein content the equal volume of test solution was treated with Bradford reagent and incubated for 5 min. Then the intensity of absorption of developed color was recorded at 595 nm by using spectrophotometer. Albumin was used as standard solution and calibration curve was prepared in range of 2 µg/ml to 20 µg/ml.[10]

Determination of total sponin

10 mg crude saponin extracts were dissolved in 5 ml of 50% aqueous methanol. 250 µl of aliquot was transferred to test tubes into which an equal volume of vanillin reagent (8 %) was added followed by 72% (v/v) sulphuric acid. The mixture was mixed and placed in a water bath adjusted at 60°C for 10 min. The tubes were cooled on an ice-cold water bath for 3 to 4 min and absorbance of yellow color reaction mixture was measured at 544 nm using a UV–Vis spectrophotometer (UV-1800 Simadzu) against a blank containing 50% aqueous methanol instead of sample extract. The saponin concentrations were calculated from standard curve and expressed as mg diosgenin equivalents (DE) per g crude extract.[12]

Determination of total steroids

Liberman-Burchard reaction involves oxidation of steroids which results pentaenyliccation with one or more double bond as compare to original compound. The oxidation product SO_3 reduces to SO_2 which form characteristic blue-green color. For determination equal volume of test solution wastreated Liberman-Burchard reagent in test tube and covered by black paper then allowed to stand for 15 minutes. Then the intensity of absorption of green color complex was recorded at 640 nm by using spectrophotometer. Diosgenin was used as standard solution and calibration curve was prepared in range of 2 µg/ml to 20 µg/ml.[13]

Determination of total alkaloids

Bromocresol green reacts with alkaloids and form yellow coloured complex due to nitrogen atom presence in basic ring which easily measured by colorimetrically. Bromocresol green does not react with nitrogen available in side chain so this method is not suitable for determination of amine or amide alkaloids.[8]For determination 1 ml of test solution was treated with 5 ml of pH 4.7 phosphate buffer and 5 ml of BCG solution in separating funnel then extracted with 5 ml of chloroform. Then chloroform extract was transferred in to 10 ml volumetric flask and volume adjusted with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. Atropine was used as standard solution and calibration curve was prepared in range of 1 µg/ml to 10 µg/ml.[14]

Determination of total flavonoid

The principle of determination of total flavonoid content by aluminum chloride method is that aluminum chloride forms acid stable complex with C-4 keto group and either C-3 or C-5 hydroxyl groups of flavonoids. In addition it forms acid liable complexes with orthodihydroxyl groups of flavonoid which can be detected by UV spectroscopy.[8]For determination 5 ml of test solution was treated with 0.3 ml of 5 % sodium nitrite and 3 ml 10% aluminum chloride in 10 ml volumetric flask and after 6 minute 2 ml of sodium hydroxide solution was added then volume adjusted up to 10 ml with distilled water. The solution was mixed well again and the absorbance was measured against a blank at 510 nm with by

UV spectrophotometer. Quercetin was used as standard solution and calibration curve was prepared in range of 2 µg/ml to 20 µg/ml.[15]

Determination of total tannin Content

Tannins reduce phosphotungustomolybdic acid in alkaline media and produce blue colored solution which is proportional to concentration of tannin.[8]For determination 1 ml of test solution was treated with 0.5ml of Folin-Denis reagent and 1 ml of sodium carbonate solution in 10 ml volumetric flask. Final volume was made up to 10 ml with distilled water. The solution was mixed well and kept for about 30 minutes then absorbance was measured against a blank at 760 nm by UV spectrophotometer. Tannic acid was used as standard solution and calibration curve was prepared in range of 2 µg/ml to 20 µg/ml.[16]

Determination of total phenolic content

Folin-Ciocalteu method is useful for determination of phenolic and polyphenolic compounds it work by measuring the amount of the substance being tested needed to inhibit the oxidation of reagent. However, this reagent will reactswith any other reducing substance therefore reagent measure total reducing capacity of substance not just the level of phenolic compounds.[8] For determination 1 ml of test solution was treated with 0.5ml of Folin-Ciocalteaureagent and 1 ml of 2 % sodium carbonate solution in 10 ml volumetric flask. Final volume was made up to 10 ml with distilled water. The solution was mixed well again and kept for about 3 hourwith intermittent shaking then the absorbance was measured against a blank at 760 nm by UV spectrophotometer. Gallic acid was used as standard solution and calibration curve was prepared in range of 2 µg/ml to 20 µg/ml.[17]

Determination of total anthraquinone glycoside

The principle of determination of total anthraquinone glycoside by initial acidic treatment is useful to break the aglycone glycone acetal linkage present in anthraquinone glycosides. Take the accuratelyweighed quantity of plant material and powder to coarse size. Reflux the powder with water for 2 hrs. filter and concentrate to yield solid residue. Take 1 g of this residue and dissolve in 30 ml water .mix and reflux on water bath for 15 minutes. Cool make up the volume to 30ml with water. centifuge this solution at 4000 rpm for 10 minutes. Take 20 ml of supernatant liquid and acidify with sufficient quantity of 2 M hydrochloric acid. Extract this acidic solution with 3 different portions of 15ml of chloroform, mix well separate and discard chloroform layer. Combine the aqueous layer and add 0.10g of sodium bicarbonate.mix well shake for 3 minutes and centrifuge at 4000 rpm for 10 minutes. Take 10 ml of supernatant and add 20 mlof 10.5% w/v ferric chloride hexahydrate.mix reflux in water bath for 20 minutes add 1 ml Concentrated HCL . heat for 20 minutes with shaking to obtain a clear solution. Cool and shake with 25 ml diethyl ether in a separating funnel. Repeat the step until anthaquinones are exhaustively extracted and tested by Bontrager's reaction.separate the diethyl extract and combine wash combined diethyl ether exactly with 15 ml distilled water twice. take the diethyl extract in 100 ml volumetric flask and adjust the volume with diethyl ether .take 15 ml of this

solution and evaporate to dryness. Dissolve the residue in 10 ml ,0.5 w/v magnesium acetate in methanol yielding a red solution measure the absorbance at 515nm.[10]

Antioxidant Activity by DPPH Scavenging

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant. The free radical scavenging activity of all the extract was evaluated by 1, 1-diphenyl-2-picryl hydrazyl (DPPH) according to the previously reported method. Briefly, 0.1mM solution of DPPH in methanol was prepared, and 1mL of this solution was added to 3 mL of the solution of all extracts in methanol at different concentration (10µg/ml,20µg/ml,30µg/ml,40µg/ml,50µg/ml,60µg/ml,70µg/ml,80µg/ml,90µg/ml, and100µg/ml) The mixtures were shaken vigorously and allowed to stand in dark room at temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (UV-1800 SHIMAZU). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula.DPPH scavenging effect (% inhibition) = $(A_0 - A1)/A_0)*100$Where, A_0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged. The % scavenging was then plotted against concentration and regression equation was obtained to calculate IC50 (micro molar concentration required to inhibit DPPH radicalformation by 50%) values.[18,19]

Nitric oxide scavenging activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitric ions that can be estimated by using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduce production of nitric oxide. Equal volume of Sodium nitroprusside (5mM) in standard phosphate buffer solution was incubated with different concentration of test solutions (10 µg/ml to 100 µg/ml) and the tubes were incubated at 25° C for 150 min. then 2 ml incubated solution was removed and diluted with equal volume of Griess reagent. The absorbance of chromophore formed was read at 546nm. The experiment was repeated for three times. Ascorbic acid was used as standard. IC_{50} value was calculated from % inhibitionwhich was calculated by following formula.% Inhibition = $(A_{blank} - A_{sample}) / A_{blank} X \ 100$[20,21]

Reducing power assay

This method is based on the principle of increase in the absorbance of the reaction mixtures. Increase in the absorbance indicates an increase in the antioxidant activity. In this method, antioxidant compound forms a colored complex with potassium ferricyanide, tri-chloro acetic acid and ferric chloride, which is measured at 700 nm.[22,23]1 ml of different concentration of test solutions

(10 µg/ml to 100 µg/ml) was added in a mixture of 2.5 ml of phosphate buffer solution (pH 6.6) and 2.5 ml of potassium ferricyanide solution (1% w/v) and incubated at 50 °C for 20 min. Then 2.5 ml of tri-chloroacetic acid (10% w/v) was added to the mixture, which was then centrifuged for 10 min at 1000 rpm. The 2.5 ml of upper layer of solution was mixed with equal volume of distilled water and 0.5 ml of $FeCl_3$solution (0.1% w/v), and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reductive potential.[24, 25]

Results and Discussion

Qualitative analysis

Phytochemical Analysis of different extracts of *Begonia picta*showed the presence of different group of active constituents in petroleum ether, chloroform and methanol extracts. The results obtained were tabulated as follows. Petroleum ether extract contained Carbohydrates, steroids Alkaloids, Flavonoids, Tannins, Phenols .Chloroform extract contained Carbohydrates, alkaloids Flavonoids, Tannins, Phenols etc. Methanolic extract containedCarbohydrates, Proteins,Saponins,Steroids, alkaloids Flavonoids, Tannins, Phenols Glycosides, glycosides,Anthraquinone glycosides.

Notes: (+) indicates presence of phytochemical (-) indicates absence of phytochemical

Table 1: Result of qualitative phytochemical analysis

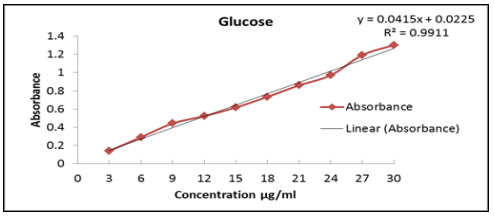
Sr.no	Test	Pet Ether	Chloroform	Methanol
1	Carbohydrate	-	-	+
2	Protein	-	-	+
3	Saponins	-	-	+
4	Steroids	+	-	+
5	Alkaloids	+	-	+
6	Flavonoids	-	+	+
7	Tannins	-	+	+
8	Phenol	-	+	+
9	Cardiac Glycosides	-	-	-
10	Anthraquinone glycosides	-	-	+

Quantitative Analysis

Total Carbohydrate Content

Calibration curve of standard Glucose was obtained by Microsoft Excel where graph was plotted by keeping concentration in x-axis and absorbance in y-axis as shown in figure.

Figure 1: Calibration curve of Glucose

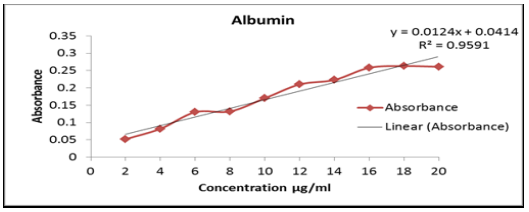


Calculation of total carbohydrate content of the extract was done by using calibration curve equation: y= 0.0415x+0.0225, R^2= 0.9911 obtained by plotting calibration curve of standard Glucose where y was the absorbance and x was the concentration.

Total Protein Content

Calibration curve of standard Albumin was obtained by Microsoft Excel where graph was plotted by keeping concentration in x-axis and absorbance in y-axis as shown in figure.

Figure 2: Calibration curve of Albumin

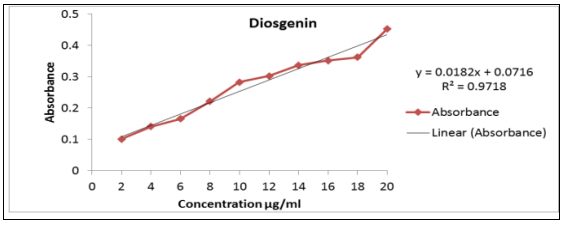


Calculation of total Protein content of the extract was done by using calibration curve equation: y= 0.0124x+0.0414, R^2= 0.9591obtained by plotting calibration curve of standard Glucose where y was the absorbance and x was the concentration.

Total Saponin Content

Calibration curve ofstandard Diosgeninwas obtained by Microsoft Excel where graph was plotted by keeping concentration in x-axis and absorbance in y-axis as shown in figure.

Figure 3: Calibration curve of Diosgenin

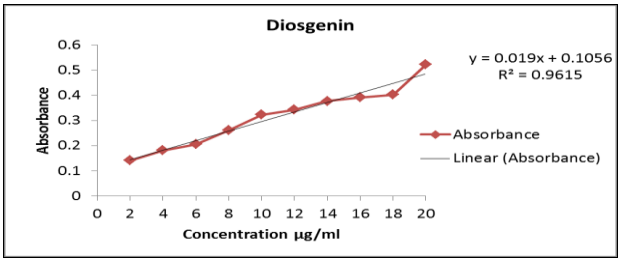


Calculation of total saponincontent of the extract was done by using calibration curve equation: y= 0.0182x+0.0716, R^2= 0.9718obtained by plotting calibration curve of standard Diosgeninwhere y was the absorbance and x was the concentration.

Total Steroid Content

Calibration curve ofstandard Diosgeninwas obtained by Microsoft Excel where graph was plotted by keeping concentration in x-axis and absorbance in y-axis as shown in figure.

Figure4: Calibration curve of Diosgenin

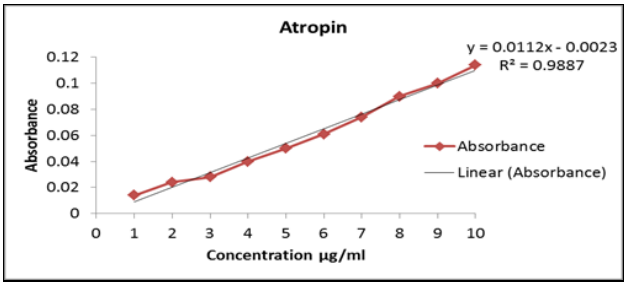


Calculation of total steroid content of the extract was done by using calibration curve equation: y= 0.019x+0.1056, R^2= 0.9615 obtained by plotting calibration curve of standard Diosgeninwhere y was the absorbance and x was the concentration.

Determination of Total Alkaloid content

Calibration curve ofstandard Atropinewas obtained by Microsoft Excel where graph was plotted by keeping concentration in x-axis and absorbance in y-axis as shown in figure.

Figure 5: Calibration curve of Atropine

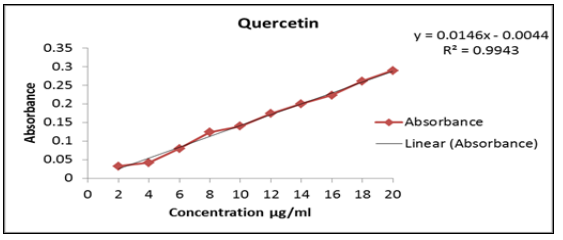


Calculation of total Alkaloid content of the extract was done by using calibration curve equation: y= 0.0112x-0.0023, R^2= 0.9887 obtained by plotting calibration curve of standard Atropine where y was the absorbance and x was the concentration.

Determination of Total Flavonoid content

Calibration curve ofstandard Quercetin was obtained by Microsoft Excel where graph was plotted by keeping concentration in x-axis and absorbance in y-axis as shown in figure.

Figure 6 : Calibration curve of Quercetin

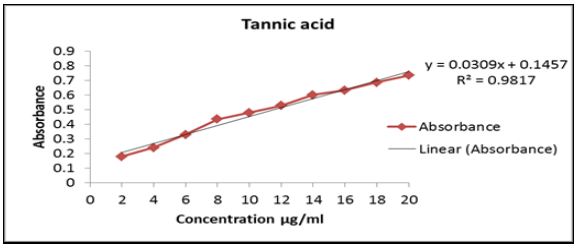


Calculation of total flavonoid content of the extract was done by using calibration curve equation: y= 0.0146x-0.0044, R^2= 0.9943 obtained by plotting calibration curve of standard Atropine where y was the absorbance and x was the concentration.

Determination of Total Tannin content

Calibration curve of standard Tannic acid was obtained by Microsoft Excel where graph was plotted by keeping concentration in x-axis and absorbance in y-axis as shown in figure.

Figure 7: Calibration curve of Tannic acid

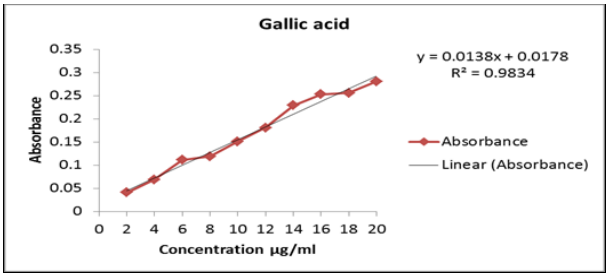


Calculation of total steroid content of the extract was done by using calibration curve equation: y= 0.0309x-0.1457, R^2= 0.9817 obtained by plotting calibration curve of standard Tannic acid where y was the absorbance and x was the concentration.

Determination of Total Phenolic content

Calibration curve of standard Gallic acid was obtained by Microsoft Excel where graph was plotted by keeping concentration in x-axis and absorbance in y-axis as shown in figure.

Figure 8: Calibration curve of Gallic Acid

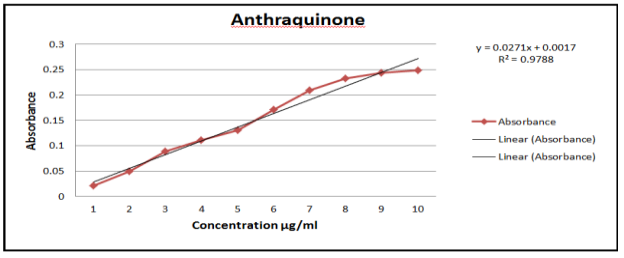


Calculation of total Phenolic content of the extract was done by using calibration curve equation: y= 0.0138x-0.00178, R^2= 0.9834 obtained by plotting calibration curve of standard Gallic acid where y was the absorbance and x was the concentration.

Determination of total Anthraquinone Glycoside content

Calibration curve of standard Anthraquinone was obtained by Microsoft Excel where graph was plotted by keeping concentration in x-axis and absorbance in y-axis as shown in figure.

Figure 9: calibration curve of Anthraquinone



Calculation of total Anthraquinone content of the extract was done by using calibration curve equation: y= 0.0271x-0.0017, R^2= 0.9788 obtained by plotting calibration curve of standard Anthraquinone where y was the absorbance and x was the concentration.

Table 2: Quantitative Analysis of *Begonia Picta smith* leaves (The values are means of three replicates with standard deviations (mean ± S.D)

Sr. No.	Parameter	Part of Plant Leaves (Mean ± SEM)
		Leaves
1	Carbohydrate	23.56 ± 0.98
2	Protein	8.58 ± 0.13
3	Saponins	21.62 ± 0.05
4	Steroids	22.67 ± 0.54
5	Alkaloids	3.19 ± 0.03

6	Flavonoids	20.32± 0.29
7	Tannins	13.23 ± 055
8	Total Phenolic	13.29 ± 0.18
9	Anthraquinone glycosides	12.32 ± 0.16

Quantitative phytochemical analysis for leaves of*begonia picta smith* showed that plant have high concentration of carbohydrate,steroids flavonoids, tannins and total phenolic. mentioned in table no 2.It also reveals that leaf high concentration of most of phytochemicalsQuantitative phytochemical analysis for extracts of leaves showed that petroleum ether extract contain maximum concentration of steroids (17.20 ± 0.07) and methanolic extract contain maximum concentration of Carbohydrate (07.39 ± 0.06)Protein (04.92 ± 0.41) Saponin (8.05 ± 0.31) Alkaloids (4.89 ± 0.18) Flavonoids (24.25± 0.02)Tannins (11.02 ± 0.05) Total Phenol (17.36 ± 0.09)Anthraquinone glycosides (14.35 ± 0.03) mentioned in table no 3

Residual Solvent Analysis

The residual solvents used in the extraction of herbal extract were estimated by the GCHS techniques. The residual solvents content & chromatogram found in the *Begonia picta smith* leaves extract were as follows

Table4: Residual solvent in various extract of *Begonia Picta Smith* Leaves

Sr.No	Sample Name	Petroleum ether (ppm)	Chloroform (ppm)	Methanol (ppm)
1	Pet ether extract	67.97	--	--
2	Chloroform extract	--	5.77	--
3	Methanol extract	--	--	12.92

Table 3:Quantitative phytochemical analysis of *Begonia picta smith* leaves extracts (The values are means of three replicates with standard deviations (mean ± S.D)

Sr. No.	Parameter	Pet. ether Extract	Chloroform Extract	Methanol Extract
1	Carbohydrate	---	---	07.39 ± 0.06
2	Protein	---	---	04.92 ± 0.41
3	Saponin	---	---	8.05 ± 0.18
4	Steroids	17.20 ± 0.07	---	7.05± 0.18
5	Alkaloids	4.01 ± 0.04	---	4.89 ± 0.18
6	Flavonoids	---	10.18 ± 0.08	24.25 ± 0.02
7	Tannins	---	9.75 ± 0.16	11.02 ± 0.05
8	Total Phenol	---	15.05 ± 0.02	17.36 ± 0.09
9	Anthraquinone glycosides	---	---	14.35 ± 0.03

Figure10 : HSGC Chromatogram (A)standard Pet ether(B)Standard Chloroform(C) standard methanol (D)Pet ether extract (E)Chloroform Extract(F)Methanol extract

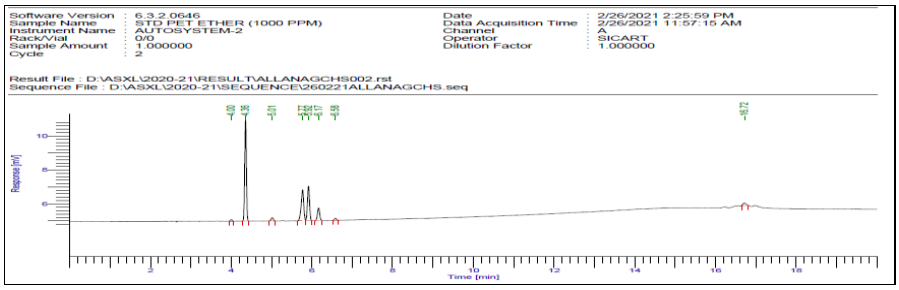


Fig10A

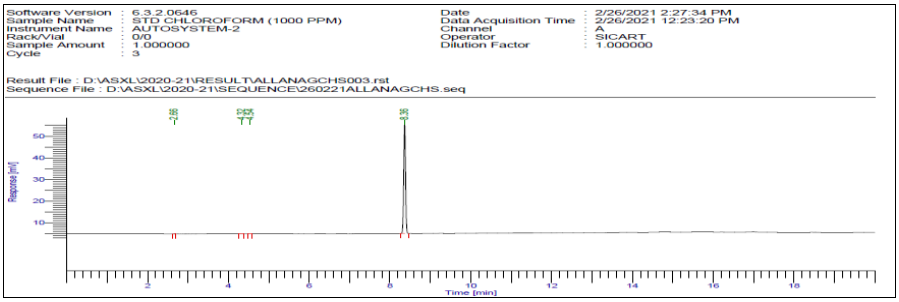


Fig10B

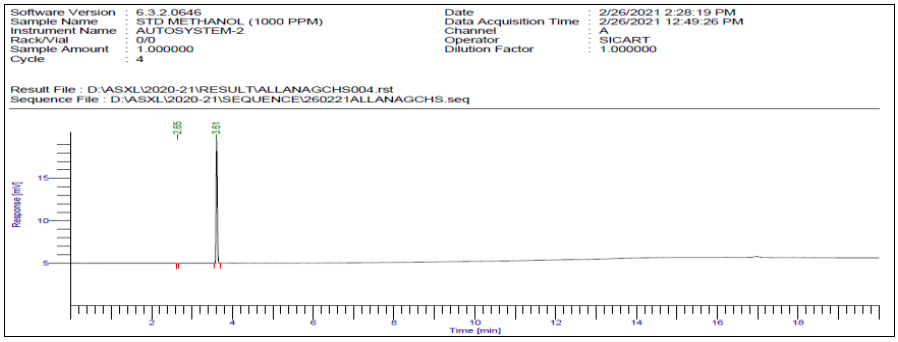


Fig10C

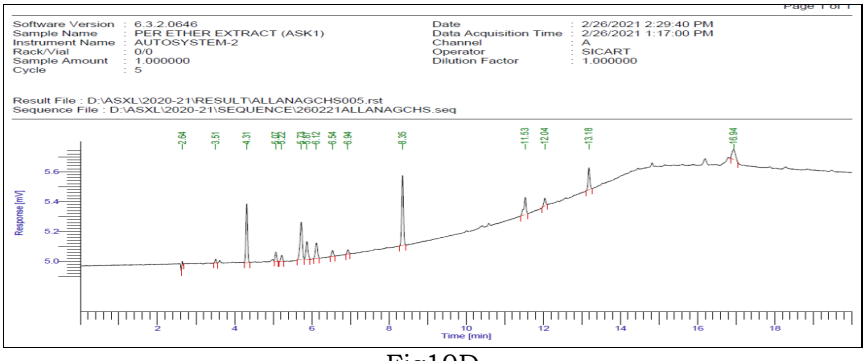


Fig10D

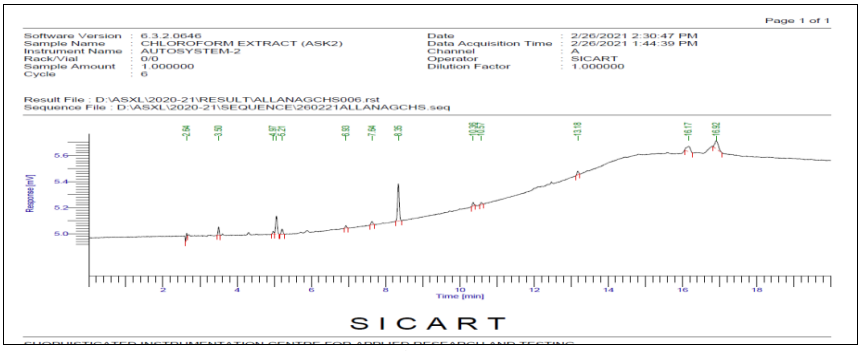


Fig10E

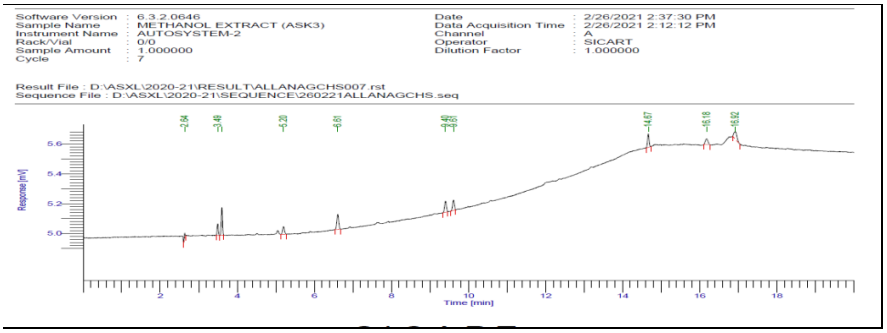


Fig 10F

Table 5: Retention time and area under the curve of solvents and various extract of *Begonia picta smith* Leave

Sr. No.	Sample	Retention Time (min)	Area [uV*sec]	Height
1	Petroleum ether	4.356	17315.80	5933.47
2	Chloroform	8.363	159453.45	50159.10
3	Methanol	3.608	31589.70	14537.00
4	Pet. ether extract	4.315	1176.75	394.34
5	Chloroform extract	8.345	921.12	286.03
6	Methanolic extract	3.600	408.79	185.48

These results clearly demonstrate that the HSGC, is a reliable method for the accurate quantitative determination of residual solvents in herbal extracts.

DPPH scavenging activity of different extracts *begonia picta* leaves

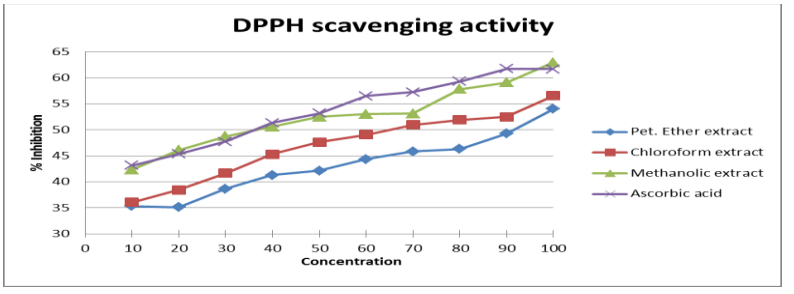
DPPH scavenging activity was performed on different extracts (10 µg/ml to100 µg/ml) of *begonia picta smith* using ascorbic acid as a standard antioxidant (10 µg /mlto100ug/ml) the result shows that The result shows that DPPH scavenging activity is directly proportional to all extracts and ascorbic acid shown in(Table no 5)and The sequence of antioxidant activity of Begonia picta extracts were;

Ascorbic acid (IC50 value 37.98µg/ml) > Methanol(with IC50 value 42.11.µg/ml) > Chloroform (IC50 value 69.25µg/ml) > Pet. Ether (IC50 value 90.15.µg/ml)

Table 6: DPPH scavenging activity of different extracts *begonia picta smith* leaves (The values are means of three replicates with standard deviations (mean ± S.D)

Concentration (µg/ml)	% Scavenging activity			
	Leaf extract			
	Pet. Ether extract	Chloroform extract	Methanolic extract	Ascorbic acid
10	35.33 + 0.54	36.08 + 0.25	42.33 + 0.77	43.15 + 0.41
20	35.13 + 0.54	38.45 + 0.46	46.08 + 0.63	45.34 + 0.61
30	38.65 + 0.48	41.66 + 0.28	48.75 + 0.75	47.74 + 0.73
40	41.29 + 0.41	45.28 + 1.18	50.6+ 1.27	51.34 + 0.74
50	42.15 + 0.52	47.62 + 0.36	52.47 + 0.56	53.19 + 0.50
60	44.33 + 0.61	49.05 + 0.26	53.02 + 1.38	56.52 + 0.43
70	45.84 + 0.68	50.94 + 0.69	53.14 + 0.79	57.21 + 0.58
80	46.33 + 0.35	51.92 + 0.80	57.82 + 0.94	59.3 + 0.26
90	49.27 + 0.26	52.5 + 0.57	59.1 + 0.59	61.72 + 0.85
100	54.03 + 0.49	56.57 + 0.38	62.91 + 0.68	61.64 + 1.03

Figure No: 11 DPPH scavenging activity of different extracts *begonia picta smith*leaves



Nitric oxide scavenging activity

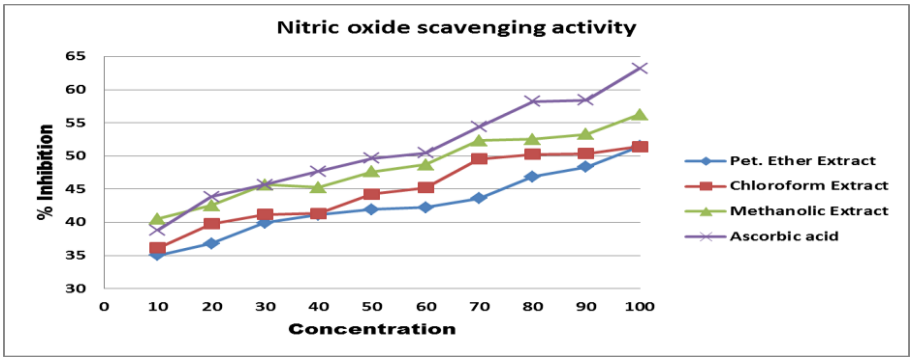
Nitric oxide scavenging activity was performed on different extracts (10 µg/ml to100 µg/ml) of *begonia picta smith* using ascorbic acid as a standard antioxidant (10 µg /ml to100ug/ml) the result shows that The result shows that nitric oxide

scavenging activity is directly proportional to all extracts and ascorbic acid shown in (Table no 6) The result shows that Nitric oxide scavenging activity is directly proportional to all extracts and ascorbic acid. The IC_{50} of petroleum ether, chloroform, methanolic extracts and ascorbic acid was found to be 71.72, 79.28, 60.74 and 52 µg/ml respectively,

Table 7 :Nitric oxide scavenging activity of different extracts *begonia picta smith*leaves (The values are means of three replicates with standard deviations (mean ± S.D)

Concentration (µg/ml)	% Scavenging activity			
	Leaves extract			
	Pet. Ether extract	Chloroform extract	Methanolic extract	Ascorbic acid
10	35.06 + 0.73	36.16 + 1.02	40.51 + 1.22	38.82 + 0.35
20	36.82 + 1.06	39.76 + 0.75	42.55 + 0.70	43.86 + 1.02
30	39.91 + 1.07	41.18 + 0.43	45.68 + 0.33	45.66 + 0.29
40	41.13 + 0.39	41.32 + 0.54	45.29 + 0.64	47.67 + 0.35
50	41.95 + 0.11	44.24 + 0.58	47.65 + 0.21	49.65 + 0.29
60	42.25 + 0.81	45.24 + 0.57	48.68 + 1.27	50.46 + 0.59
70	43.62 + 0.73	49.56 + 0.82	52.31 + 0.23	54.37 + 0.47
80	46.86 + 0.64	50.25 + 0.55	52.53 + 0.84	58.23 + 0.63
90	48.36 + 0.71	50.37 + 0.56	53.26 + 0.58	58.44 + 0.48
100	51.48 + 0.42	51.43 + 0.91	56.26 + 1.16	63.21 + 1.21

Figure No: 12 Nitric oxide scavenging activity of different extracts *begonia picta smith*leaves



Reducing power assay

Reducing power assay was performed on different extracts (10 µg/ml to100 µg/ml) of *begonia picta smith* using ascorbic acid as a standard antioxidant (10 µg /ml to100ug/ml) The result shows that absorbance of Reducing power assay is directly proportional to concentration in all extracts and ascorbic acid. The increase in absorbance indicates highest reducing power which also describe graphically as follows

Table 8: Absorbance of reducing power assay of different extracts of *Begonia picta smith* leaves (The values are means of three replicates with standard deviations (mean ± S.D)

Concentration (µg/ml)	% Scavenging activity			
	Leaves extract			
	Pet. Ether extract	Chloroform extract	Methanolic extract	Ascorbic acid
10	0.05+ 0.001	0.051 + 0.002	0.043+ 0.004	0.054 + 0.002
20	0.053+ 0.001	0.056 + 0.003	0.053 + 0.002	0.05 + 0.004
30	0.054 + 0.001	0.060 + 0.001	0.058 + 0.001	0.067+ 0.002
40	0.056 + 0.001	0.064 + 0.002	0.062 + 0.003	0.071+ 0.000
50	0.056+ 0.002	0.066 + 0.009	0.067+ 0.009	0.078 + 0.001
60	0.063 + 0.002	0.070 + 0.008	0.067+ 0.001	0.081+ 0.002
70	0.064 + 0.001	0.073 + 0.001	0.078+ 0.000	0.085 + 0.001
80	0.065+ 0.001	0.083 + 0.002	0.080 + 0.001	0.097 + 0.002
90	0.078 + 0.002	0.085 + 0.004	0.098+ 0.001	0.103 + 0.003
100	0.080 + 0.003	0.090 + 0.000	0.102 + 0.005	0.108 + 0.006

Figure No: 12 Absorbance of reducing power assay of different extracts of *Begonia picta smith* leaves

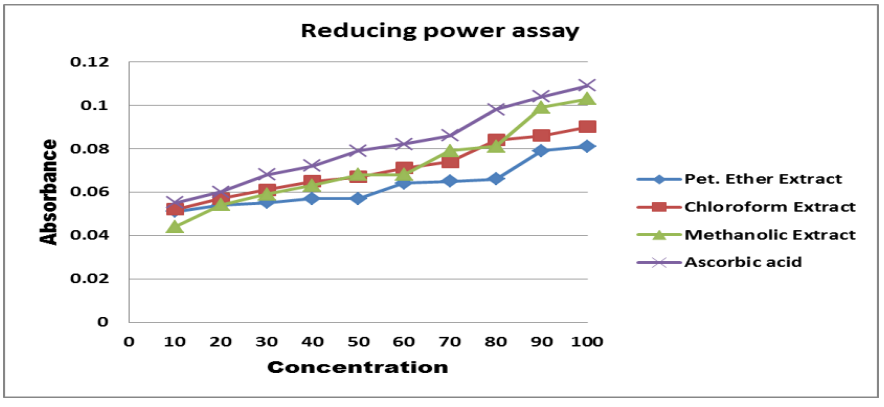


Table 9:IC50 values of different Extracts of *Begonia picta smith* leaves

Method of evolution	IC_{50} Value			
	Leaves extract			
	Pet. Ether extract	Chloroform extract	Methanolic extract	Ascorbic acid
DPPH Scavenging activity	90.15	69.25	42.11	37.98
DPPH Scavenging activity	71.72	79.28	60.74	52
Nitric oxide scavenging activity	0.081	0.09	0.103	0.109

The above results indicate that methanolic extract have significant anti-oxidant activity as compare to other. Phytochemical analysis and antioxidant activity determination in present study revealed that the phytochemicals, including polyphenol and flavonoid may be responsible for the observed antioxidant activity.

Conclusion

The present study deals with the studies on phytochemical and antioxidant activity on leaves of *Begonia picta.* Literatures regarding the present study were found very less**.**Different phytochemical have different therapeutic value. The plant material *Begonia picta* was successively extracted with Petroleum ether, chloroform and methanol. The phytochemical test result showed that the methanolic extract has large number of phytochemicals. so detail study of quantitativeestimation reveals that leaves contained high concentration of phytochemicals,the residual solvents present in the three herbal extract also in current specified limits. Evaluation of In Vitro Anti-Oxidant activity reveals that methanolic extract is most potent as compare to other extracts by taking Ascorbic acid as standard. This information clearly represent that the plant *Begonia picta* is the good source of dietary antioxidant.

Acknowledgement

The authors are grateful to the management of Ali-Allana College of Pharmacy, Akkalkuwa and SICART vallabhnagar Gujarat for the support and guidance.

Conflict of interest: Nil

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