ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

Determination of Total Phenolic Content and Antioxidant Activity of the Whole Plant Extracts of *Argyreia imbricata* (Roth) Sant. & Patel

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Article History:

Received on: 02 Feb 2021
Revised on: 06 Mar 2021
Accepted on: 10 Mar 2021

Keywords:

Argyreia imbricata extracts,
Total phenolic content,
Antioxidant activity

ABSTRACT

Plants of the genus *Argyreia* posses' ethno medicinal importance and various pharmacological activities are also reported. In this study, phenolic content, anti-oxidant potential of different extracts of *Argyreia imbricata* was evaluated. Extraction of powdered whole plant material was done with different solvents viz., petroleum ether, chloroform, ethyl acetate and methanol by soxhelation. The estimation of phenolics in the selected extracts of *Argyreia imbricata* was done by using a Folin-Ciocalteau reagent. The antioxidant potential of different extracts was evaluated by total anti-oxidant capacity, DPPH, hydroxyl and nitric oxide radical scavenging and reducing power assay methods. In the phenolics estimation, the methanol extract showed a significant presence of phenolic content followed by ethyl acetate extract. In case of anti-oxidant activity, the methanol and ethyl acetate extract showed a significant activity based on their concentration. The findings of this study clearly revealed the potential of methanol extract of *A. imbricata* in phenolic content and anti-oxidant activity. Also, the ethyl acetate extract showed significant results. Further studies on identification, isolation of the active constituents, toxicity evaluations may give useful results.

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ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v12i1.4469

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INTRODUCTION

The genus *Argyreia* belongs to the Convolvulaceae family is a larger one, consist of more than 200 species that have a wide distribution in Asian countries, including all over India (Traiperm *et al.*, 2017; Sebastin *et al.*, 2019). Various species of this genus have ethnomedicinal importance and diverse pharmacological activities of the *Argyreia* plants were described in various reports (Modi *et al.*, 2010; Joseph *et al.*, 2011; Jaiswal and Tailang, 2018). *Argyreia imbricata*, another one plant of this genus, is commonly found in south India at an altitude up to 300m mean sea level, which has not been previously explored scientifically to our knowledge, was selected for our research.

Initially, extraction, preliminary phytochemical evalutaion and the spectral evaluation of active constituents in the extracts of the whole plant material of *Argyreia imbricata* was done (Sebastin *et al.*, 2019). As a part of the investigation of different pharmacological activities, the anti-inflammatory activity (Sebastin *et al.*, 2020) and the anti-diabetic activity (Sebastin *et al.*, 2021) of the extracts were evaluated successfully. Now, it was focused on the evaluation of total phenolic content and anti-oxidant activity of the prepared extracts of the whole plant *Argyreia imbricata*.

MATERIALS AND METHODS

Collection and extraction of plant material

The entire plant *Argyreia imbricata* was collected from the Mekkarai, a village located nearby the hillocks of the Western Ghats in the Tirunelveli District of Tamil Nadu, India. The collected material was dried and powdered after the proper identification and authentication. The powdered material of the whole plant was extracted by soxhelation with the solvents in the rising order of polarity, such as petroleum ether, chloroform, ethyl acetate, and methanol (Sebastin *et al.*, 2019). The dried extracts obtained were utilized for further experiments.

Total phenolic content estimation

In our preliminary phytochemical evaluation (Sebastin *et al.*, 2019), only the ethyl acetate and methanol extract of the *Argyreia imbricata* showed the presence of phenolic compounds. Based on this finding, these two extracts were selected for the estimation of total phenolic content, which was done in accordance with the procedure of (Francis and Andrew, 2010; Senguttuvan *et al.*, 2014; Baba and Malik, 2015) with slight modification.

Test extracts (0.5ml) of test extracts in different concentration were mixed with an equal volume of Folin-Ciocalteau reagent and 1ml of 20% sodium carbonate solution. The volume of the mixture was made in to 10ml by distilled water. Shaken well and kept at the dark condition for 45min., followed by the absorbance of the solution was measured spectrophotometrically (725nm). Blank was prepared. A calibration curve of gallic acid was prepared, and the linearity was obtained. The total phenolic content in the test extracts was expressed as milligram of gallic acid equivalent (mg GAE/g extract) by using the standards curve.

In vitro antioxidant activity

Antioxidant activity of all the extracts of *A. imbricata* was evaluated by total antioxidant capacity, DPPH, hydroxyl and nitric oxide radical scavenging and reducing power assay methods.

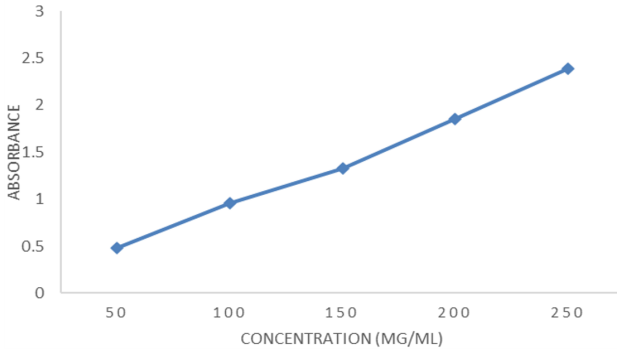

Figure 1: Calibration curve of gallic acid in the total phenolic estimation

Total antioxidant capacity (TAC)

Evaluation of the total antioxidant capacity of the test extracts was done by Phosphomolybdate assay method by reference with the procedure of (Mbinda and Musangi, 2019).

0.1ml of test extracts (100μg/100μl) was mixed with the reagent (0.6M sulphuric acid, 4mM ammonium molybdate and 28mM sodium phosphate). The tubes containing the reaction mixture were covered with silver foil and kept in the water bath (95°C) for 1.5h. Cooled to room temperature and the absorbance of reaction mixtures was measured spectrophotometrically at 695nm. Gallic acid was used as the standard. The total antioxidant capacity was calculated by the formula

$$T = C\frac{V}{M}$$

T – Total antioxidant content (mg/g) of the extracts; C – Concentration of Gallic acid (mg/ml) obtained from the calibration graph; V – Volume of the extract taken (ml); M –the weight of the extract (g); TAC is expressed as milligram Gallic acid equivalents (mg GAE)/g of extract.

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

DPPH assay of the tests was carried out with reference of (Mol *et al.*, 2016; Jemli *et al.*, 2016). Reaction mixture contains 1ml of test extracts in different concentration (50, 100, 250, 500 and 1000μg/ml) and 1ml of methanolic solution of DPPH (0.1mM). It was kept in 30min. Incubation in dark at room temperature. Then, the absorbance of the reaction mixtures was measured at 517nm, using methanol as the blank, DPPH in methanol as the control and ascorbic acid as the standard control. The inhibition percentage of DPPH radical by the tests was identified by

$$\%\ inhibition = \frac{Control\ Abs. - Sample\ Abs.}{Control\ Abs.} \times 100$$

The percentage of inhibition against concentration was plotted as a graph. The IC_{50} values of the samples were assessed from the regression equation of the graph.

Hydroxyl radical scavenging assay

It was assessed in reference with (Batool *et al.*, 2019). Deoxyribose (0.1ml; 2.8mM) prepared in potassium phosphate buffer (20mM; pH 7.4). To this EDTA (0.1mM), ferric chloride (200μl; 100mM), H_2O_2 (100μl; 200mM) and 100μl of the test extracts in different concentration were added. After the addition of ascorbic acid (100μl; 300mM) to this mixture, it was kept in 37°C for 1h. then trichloroacetic acid (1ml; 2.8%w/v) and 1%w/v thiobarbituric acid (1ml) prepared in 50mM NaOH were added, and the whole reaction mixture was kept in the water bath (15min) for boiling. Then the absorbance of cooled was measured spectrophotometrically (532nm). Normal and standard control were prepared. Quercetin was applied as the standard control. Suppression of deoxyribose degradation was calculated by

% inhibition =

$$\frac{Control\ Abs. - Sample\ Abs.}{Control\ Abs.} \times 100$$

Nitric oxide radical scavenging assay

The nitric oxide radical scavenging potential of the tests was evaluated in reference with the procedure of (Francis and Andrew, 2010; Batool *et al.*, 2019). The reaction mixture (5ml) was prepared by mixing sodium nitroprusside (5mM) in phosphate buffer (pH 7.3) and test extracts in different concentration. After the addition of 1ml of Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% naphthyl ethylene diamine dihydro chloride) to the reaction mixture, it was kept in 25°C (in front of a 25W tungsten lamp) for 3h. The nitric oxide radical thus formed was interacted with oxygen to produce nitrite ion, which was measured spectrophotometrically (546nm). Normal and standard control were prepared. Ascorbic acid was employed as the standard control. The percentage inhibition nitric oxide radical formation was determined by

% inhibition =

$$\frac{Control\ Abs. - Sample\ Abs.}{Control\ Abs.} \times 100$$

Reducing power assay

Evaluation of the antioxidant activity of test extracts by reducing power assay method was done in reference with the procedure of (Jemli *et al.*, 2016; Moonmun *et al.*, 2017). 500μl of the test extracts in different concentration (50, 100, 250, 500, 1000μg/ml) was mixed with 1.5ml of sodium phosphate buffer (0.2M; pH 6.6) and 1.5ml of 1% potassium ferricyanide. This mixture was incubated at 50ºC for 20min. Then, 5ml of 10% trichloroacetic acid was added to the incubated mixture and centrifuged at 3000rpm for 6min (4°C) and the upper layer solution was collected. 1.5ml of the collected upper layer solution was mixed with an equal volume of distilled water and 300μl of 0.1% fresh ferric chloride solution and kept for 10min. Then, the absorbance of the reaction mixture was read at 700nm. Ascorbic acid was used as the standard for comparative evaluation.

RESULTS AND DISCUSSION

Results of total phenolic estimation are shown in Tables 1 and 2 and Figure 1. The results clearly indicated a significant presence of phenolic content in the methanol extract followed by the ethyl acetate extract (Table 2).

Regarding with antioxidant evaluation, the result of the total antioxidant capacity of the extracts tested by phosphomolybdate assay is shown in Table 3.

Generally, a higher absorbance value indicated the greater antioxidant capacity of test extracts. In this evaluation, the ethyl acetate and methanol extracts of *A. imbricata* showed a significant activity comparing with others, such as petroleum ether and chloroform extracts. The ethyl acetate extract showed the absorbance value of 1.788 and TAC of 87.63mgGAE/g followed by methanol extract with an absorbance value of 1.767 and TAC of 1.767mgGAE/g comparing with the absorbance value of 2.115 of the standard gallic acid.

In the case of evaluation of antioxidant activity by DPPH assay, the result is shown in Table 4. The results indicated a concentration based rise of percentage inhibitory activity of test extracts. The tested methanol extract showed a significant percentage inhibition score of 84.78, followed by the ethyl acetate extract showed 83.95 as the score of percentage inhibition. The IC_{50} value of both the extracts was found as 108.85μg/ml. These results are significant one when comparing with the scores of the standard ascorbic acid.

In the case of hydroxyl radical scavenging assay, a concentration depended rise of percentage inhibition was found in the results. It was strongly revealed by the methanol extract in 1000μg/ml concentration with the score of percentage inhibition of 89.43$\pm$0.345 followed by the ethyl acetate extract in the same concentration with the percentage inhibition score of 76.34$\pm$0.078.

Table 1: Scores in the calibration curve of gallic acid in the total phenolic estimation

Conc. of gallic acid (μg/ml)	Gallic acid (ml)	Folin-Ciocalteu reagent (ml)	Sat. sodium carbonate (ml)	Distilled H20(ml)	Absorbance at 725nm
50	0.5	0.5	1	8	0.4852±0.026
100	0.5	0.5	1	8	0.9561±0.019
150	0.5	0.5	1	8	1.3276±0.003
200	0.5	0.5	1	8	1.8532±0.017
250	0.5	0.5	1	8	2.3892±0.243

Table 2: Estimation of the total phenolic content of *A. imbricata* extracts

Extracts	Concentration of extract (μg/ml)	Absorbance at 725nm	From graph gallic acid equivalent (μg/ml)	Gallic acid equivalent in 1g of extract (mg)	Average (mg/g)
Ethyl acetate	200	0.2280±0.005	25	125	116.25
	400	0.4322±0.009	43	107.5	
Methanol	200	0.2833±0.008	30	150	143.75
	400	0.5288±0.002	55	137.5	

Table 3: Antioxidant activity of test extracts by phosphomolybdate assay

Standard (Gallic acid)		Test (1μg/1μl)		
Conc. (mg/ml)	Absorbance	Extracts	Absorbance	TAC (mg/GAE/g)
0.02	0.577±0.048	Petroleum ether	0.858±0.036	39.78
0.04	0.823±0.068	Chloroform	1.281±0.026	61.54
0.06	1.105±0.021	Ethyl acetate	1.788±0.028	87.63
0.08	1.634±0.088	Methanol	1.767±0.047	86.55
0.1	2.115±0.034			

TAC - total antioxidant capacity; GAE – gallic acid equivalent

Table 4: Antioxidant activity of test extracts by DPPH assay

Conc. (μg/ml)		% inhibition				
		Std.	Test extracts			
Std.	Test	A. A	1	2	3	4
10	50	53.19+0.326	21.82+0.245	24.41+0.214	37.42+0.325	37.50+0.312
25	100	88.37+0.482	41.06+0.654	43.27+0.345	49.57+0.356	49.61+0.375
50	250	90.11+0.531	45.67+0.324	51.29+0.243	63.22+0.276	62.13+0.465
75	500	90.55+0.231	55.68+0.432	60.18+0.213	71.35+0.473	72.63+0.367
100	1000	91.13+0.439	66.93+0.423	68.84+0.343	83.95+0.285	84.78+0.563
IC50		5.67+0.567	301.87+0.345	235.09+0.243	108.85+0.678	108.85+0.486

Conc.–Concentration; Std.–Standard; A.A–Ascorbic acid; 1–Petroleum ether; 2–Chloroform; 3–Ethyl acetate; 4–Methanol

Table 5: Antioxidant activity of test extracts by hydroxyl radical scavenging assay

Conc. (μg/ml)		% inhibition				
		Std.	Test extracts			
Std.	Test	Quercetin	1	2	3	4
10	50	41.70+0.132	21.12+0.112	25.81+0.213	34.34+0.245	36.33+0.344
25	100	70.32+0.212	40.12+0.121	43.56+0.134	48.12+0.183	49.43+0.342
50	250	78.44+0.155	45.34+0.212	48.55+0.023	59.32+0.277	60.67+0.632
75	500	85.46+0.132	53.16+0.176	60.12+0.214	68.43+0.243	72.23+0.267
100	1000	91.13+0.174	66.22+0.129	67.33+0.145	76.34+0.078	89.43+0.345
IC50		14.77+0.034	431.42+0.366	409.72+0.234	189.47+0.126	150.8+0.473

Conc.–Concentration; Std.–Standard; 1–Petroleum ether; 2–Chloroform; 3–Ethylacetate; 4–Methanol

Table 6: Antioxidant activity of test extracts by nitric oxide radical scavenging assay

Conc. (μg/ml)		% inhibition				
		Std.	Test extracts			
Std.	Test	Ascorbic acid	1	2	3	4
10	50	53.19+0.326	21.72+0.322	22.31+0.233	32.44+0.056	38.77+0.122
25	100	88.37+0.482	40.12+0.212	41.53+0.067	40.65+0.089	49.53+0.043
50	250	90.11+0.531	45.36+0.214	48.15+0.132	57.44+0.189	61.62+0.134
75	500	90.55+0.231	55.16+0.321	56.32+0.147	69.22+0.155	72.73+0.134
100	1000	91.33+0.439	60.65+0.068	61.35+0.144	78.33+0.213	86.32+0.097
IC50		4.45+0.032	457.72+0.234	375.23+0.435	162.63+0.432	124.22+0.324

Conc.–Concentration; Std. –Standard; 1–Petroleum ether; 2–Chloroform;3–Ethyl acetate; 4–Methanol

Table 7: Antioxidant activity of test extracts by reducing power assay

Conc. (μg/ml)		Absorbance				
		Std.	Test extracts			
		A.A	1	2	3	4
10	50	0.604±0.032	0.195±0.043	0.283±0.043	0.316±0.038	0.289±0.045
25	100	1.580±0.032	0.297±0.086	0.385±0.034	0.477±0.091	0.457±0.032
50	250	2.194±0.065	0.387±0.012	0.555±0.097	0.865±0.012	0.844±0.033
75	500	2.593±0.032	0.872±0.056	1.131±0.033	1.586±0.099	1.576±0.078
100	1000	2.970±0.021	1.194±0.033	1.779±0.044	2.623±0.056	2.625±0.056
IC50		8.94±0.032	289.73±0.354	176.06±0.564	104.46±0.234	110.68±0.451

Conc.–Concentration;Std. – Standard; A.A–Ascorbic acid; 1–Petroleumether; 2–Chloroform; 3–Ethyl acetate; 4–Methanol

The score of methanol extract is a significant one comparing with the score of the standard, quercetin, which showed 91.13±0.174 percentage inhibition in the concentration of 100μg/ml (Table 5)

The results of the nitric oxide radical scavenging assay also showed a concentration based increase in percentage inhibition activity.

In this method also the methanol extract (1000μg/ml) showed a significant score next to that the same concentration ethyl acetate extract showed a significant score comparing with other tested extracts and the standard (Table 6).

The result of an antioxidant evaluation of *A. imbricata* extracts by reducing power assay is shown in Table 7.

Generally, increased absorbance of the reaction mixture indicated increased reducing power. From the results, it was found that the methanol extract in 1000μg/ml concentration showed 2.625 as the absorbance score followed by the ethyl acetate extract in the same concentration showed 2.623 absorbance score, which clearly indicated

the antioxidant potential of above said extracts comparing with the standard, ascorbic acid.

CONCLUSION

In the present study, the whole plant *Argyreia imbricata* was collected and after the identification and authentication, the collected material was dried, powdered and extracted by soxhelation with solvents of ascending order of polarity viz., petroleum ether, chloroform, ethyl acetate, and methanol. Selected extracts were subjected to the estimation of phenolic content and all the extracts prepared were evaluated for the anti-oxidant activity by different methods. In both the estimation of phenolic content and evaluation of antioxidant activity, the methanol extract of the whole plant *A. imbricata* showed significant results and next to that the ethyl acetate extract. Our future studies in the direction of identification and isolation of the active constituents and toxicity evaluations of these extracts may give valuable results for further researches.

ACKNOWLEDGEMENT

Authors are thankful to Mr. Kumaran J., M. Pharm., (Pharmaceutical Biotechnology), for his help in the preparation of this manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

Funding Support

The authors declare that they have no funding support for this study.

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