In Vitro Nephroprotective Activity and Potential Antioxidant Activity of Martynia Annua Fruit Extract

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Abstract—**Background: Kidney disorders caused by drug toxicity, oxidative stress, diabetes, and environmental pollutants are increasing worldwide. Synthetic nephroprotective drugs are often associated with adverse effects and high cost. Therefore, there is a growing need for safe and effective plant-based nephroprotective agents. Martynia annua Linn., belonging to the family Martyniaceae, is traditionally used for various medicinal purposes and is known to possess antioxidant and therapeutic properties.**

Objective: The present study aimed to evaluate the in vitro nephroprotective and antioxidant activity of ethanolic extract of Martynia annua fruit.

Materials and Methods: The fruits of Martynia annua were collected, shade dried, powdered, and extracted using 95% ethanol by Soxhlet extraction. Preliminary phytochemical screening was carried out to identify the presence of bioactive constituents. Antioxidant activity was evaluated using DPPH radical scavenging assay with ascorbic acid as standard. Nephroprotective activity was assessed against cisplatin-induced toxicity using HEK-293 cell lines by MTT assay.

Results: Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, carbohydrates, saponins, and phenolic compounds. In the DPPH assay, the extract showed concentration-dependent antioxidant activity with maximum inhibition of 62.69% at 100 µg/mL and IC50 value of 74.04 µg/mL. In the MTT assay, the extract exhibited significant nephroprotective activity by increasing cell viability against cisplatin-induced toxicity. Maximum cell viability of 82.76% was observed at 100 µg/mL.

Conclusion: The findings of the study indicate that the ethanolic extract of Martynia annua fruit possesses significant antioxidant and nephroprotective activity, which may be attributed to the presence of flavonoids and phenolic compounds. The plant can be considered a potential natural source for the development of nephroprotective agents, although further pharmacological and clinical studies are required.

Index Terms—**Martynia annua, nephroprotective activity, antioxidant activity, DPPH assay, HEK-293 cell line, MTT assay, cisplatin.**

I. INTRODUCTION

Renal disorders have become a major global health concern due to their increasing incidence and complications associated with long-term illness. Diseases such as acute kidney injury and chronic kidney disease are frequently associated with diabetes mellitus, hypertension, exposure to environmental pollutants, and continuous administration of certain therapeutic drugs (1). The kidneys perform essential physiological functions including filtration of blood, maintenance of electrolyte and fluid homeostasis, regulation of blood pressure, and elimination of metabolic waste products. Because of these important functions, renal tissues are highly susceptible to toxic and chemical injury (1). Drug-associated nephrotoxicity is considered one of the leading causes of kidney dysfunction, particularly during prolonged treatment with antibiotics, analgesics, anticancer agents, and other medications (4). Among anticancer drugs, cisplatin is widely reported to induce renal damage by promoting oxidative stress, inflammatory responses, and degeneration of renal tubular cells (2, 3). Overproduction of reactive oxygen species during oxidative stress plays an important role in the progression of renal injury. Elevated levels of free radicals can damage cellular components by inducing lipid peroxidation, mitochondrial impairment, DNA

damage, and apoptosis of renal cells (5). Although several synthetic medicines are currently used for the treatment and management of kidney disorders, their prolonged use may produce adverse effects and increase the economic burden on patients (6). Owing to these limitations, medicinal plants are receiving considerable attention as potential therapeutic alternatives because they possess naturally occurring phytoconstituents with antioxidant and cytoprotective activities (7). Plant-derived bioactive compounds are known to reduce oxidative stress and protect tissues from cellular injury caused by free radicals (5,7). A large number of medicinal plants have shown promising effects in preventing or minimizing renal damage because of the presence of various phytochemical constituents, including flavonoids, phenolic compounds, tannins, alkaloids, and glycosides (7,8). These naturally occurring compounds possess significant antioxidant and anti-inflammatory properties, which help in scavenging free radicals and protecting cellular components against oxidative stress–induced injury (5,7). Among the plants used in traditional medicine, Martynia annua Linn. has attracted scientific interest due to its wide range of pharmacological activities (9). The plant belongs to the family Martyniaceae and has been traditionally utilized for the management of inflammation, wounds, skin diseases, epilepsy, and poisonous bites or stings (9,10). Different parts of the plant, especially the fruits, are reported to contain several bioactive secondary metabolites that may be responsible for its therapeutic potential (10,11). The present investigation was undertaken to evaluate the antioxidant and nephroprotective activities of the ethanolic extract obtained from the fruits of Martynia annua. The extract was prepared using the Soxhlet extraction technique and subsequently subjected to preliminary phytochemical screening for identification of major bioactive constituents (11). Antioxidant potential of the extract was assessed by the DPPH free radical scavenging method, whereas nephroprotective activity was determined using HEK-293 cell lines against cisplatin-induced cellular toxicity through MTT assay (12,13). The results demonstrated considerable free radical scavenging activity along with enhanced cell viability in extract-treated groups, suggesting a protective effect on renal cells exposed to toxic insult. The nephroprotective effect observed during the study may be attributed to the antioxidant properties of phytoconstituents present in the extract, which possibly reduce oxidative stress–mediated cellular damage (5,7). These findings indicate that Martynia annua fruit possesses potential as a natural source for the development of plant-based nephroprotective formulations. However, additional pharmacological, toxicological, and clinical investigations are necessary to establish its safety profile, therapeutic efficacy, and precise mechanism of action.

II. MATERIAL AND METHOD

Collection and authentication of Plant material

The plant material of Martynia annua was collected from District- Solapur, Maharashtra during winter season and washed thoroughly. The plant specimens where recognized and verified by Mrs. Mulani mam the department of botany at Ishwarrao tatya More-Patil Arts, commerce, science mahila mahavidyalay Ektanagar, Dighanchi. The collected material was washed thoroughly with tap water to remove adhering dust and impurities, shade dried room temperature, and pulverized into coarse powder using a mechanical grinder. The powdered material was stored in an airtight container for further study.

Fig no. 1 Martynia annua fruit

Preparation of aqueous extract:

The plant material shade dried for period for two weeks and it was finely coarse powdered using mortal pestle. 110 gm of coarse powder extracted with 95% ethanol in a soxhlet apparatus at 75 % C^{o} till complete extraction was achieved. The extract was concentrated in water bath. The resulting substance was an oily dark colour liquid. The final extract was stored in a tightly closed container and

kept in a cool place for studies. (14)

Fig no. 2 Extraction of Martynia annua fruit

Preliminary phytochemical screening:

The aqueous extract of Martynia annua was subjected to preliminary phytochemical evaluation using standard qualitative testing methods to detect the presence of different classes of secondary metabolites. The screening procedures were carried out for identification of alkaloids, carbohydrates, flavonoids, tannins, saponins, and phenolic compounds (14,15). Development of characteristic colour changes or formation of precipitates during the reactions was considered as evidence for the presence of the corresponding phytochemical constituents.

Sr.no.	Test	Inference
1.	Alkaloid	Present
2.	Carbohydreate	Present
3.	Tannin	present
4.	Saponin	present
5.	Flavonoid	present
6.	Phenol	present

Table no .1 Phytochemical screening

Fig no. 3 Phytochemical tests

- DPPH Radical Scavenging Assay (Antioxidant Activity)

- Principle: -

The DPPH test relies on the conversion of the purple-colored DPPH radical to a yellow-colored diphenyl picryl hydrazine when antioxidants are present.Radical scavenging activity is indicated by the drop in absorbance at 517 nm.

- Preparation of Solutions: -

a) DPPH Solution:

A freshly prepared 0.1 mM DPPH solution was made by dissolving 3.94 mg of DPPH in methanol. The solution was protected from light and stored in dark conditions until use.

b) Standard Solution:

A standard stock solution of ascorbic acid was prepared by dissolving 100 mg of ascorbic acid in 100 mL of distilled water to obtain a concentration of 1000 µg/mL.

c) Sample Solution:

The plant extract/sample solution was prepared in distilled water to obtain a stock concentration of 200 µg/mL. From this stock solution, different concentrations such as 20, 40, 60, 80, and 100 µg/mL were prepared for antioxidant analysis.

- Procedure: -

1. The antioxidant potential of the test samples was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging method.
2. In a microplate, 100 µL of each prepared concentration (20–100 µg/mL) of the sample solution was added separately.
3. To each well, 100 µL of methanolic DPPH solution was added and the reaction mixture was incubated in dark conditions for 30 minutes at room temperature.
4. A change in color from deep violet to pale yellow indicated free radical scavenging activity. The absorbance of the reaction mixtures was measured at 510 nm using a colorimeter.
5. The percentage of radical scavenging activity was determined using the standard formula. (16)

DPPH radical scavenging activity (%) = (Absorbance of control – Absorbance of test sample) / Absorbance of control x 100

- Nephroprotective Activity by MTT Assay

The nephroprotective potential of the test sample was evaluated using the HEK-293 cell line through the MTT assay method. The cells were cultured in DMEM/F-12 medium supplemented with 10% fetal bovine serum and 1% antibiotic solution, and maintained at 37°C in a humidified atmosphere containing 5% CO_2.

HEK-293 cells were seeded into a 96-well plate and allowed to attach for 24 hours. After incubation, the cells were treated with different concentrations of the test sample (20, 40, 60, 80, and 100 µg/mL). Nephrotoxicity was induced by treating the cells with cisplatin (20 µM), followed by incubation for another 24 hours.

After completion of treatment, 20 µL of MTT reagent (5 mg/mL) was added to each well and the plates were incubated for 3–4 hours to allow formation of formazan crystals by viable cells. The supernatant was then removed carefully, and the crystals formed were dissolved using 100 µL of DMSO.

The absorbance was measured at 550 nm using a microplate reader. The nephroprotective activity of the sample was determined based on its ability to improve cell viability against cisplatin-induced toxicity. The percentage of cell viability was calculated using the standard formula (17,18,19,20)

Cell Viability (%) = (Absorbance of treated cells / Absorbance of control cells) × 100

Result: -

- For Antioxidant Assay: The results of antioxidant assay is given in table 09 and % inhibition curve of ascorbic acid and ethanolic extract is given in the figure 2. The maximum % inhibition for ascorbic acid and Martynia annua was 82.90% and 62.69% respectively at 100 µg/ml.The minimum % inhibition for ascorbic acid and Martynia annua was 29.02% and 20.21% respectively at 20µg/ml. The IC50 value of ascorbic acid and Martynia annua was 59.23 and 74.04 respectively. Results of antioxidant assay indicated that the extract of Martynia annua possess antioxidant activity.

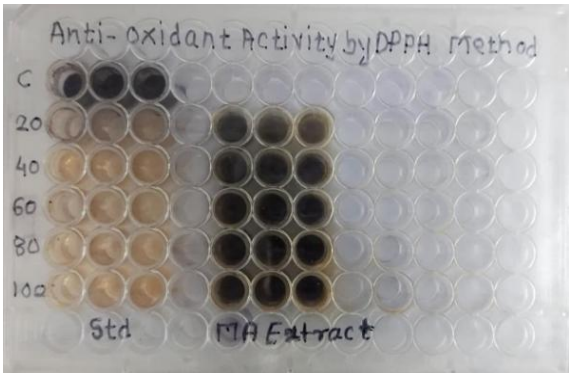


Fig no. 4 DPPH Antioxidant assay 96- well microplate

SR.No.	Sample Code	Concentration (µg/mL)	Absorbance at 510nm				% Inhibition	IC50 (µg/m L)
			Test 1	Test 2	Test 3	Mean		
1	Control	-	1.93	1.93	1.93	1.93	-	59.23
2	Standard (Ascorbic Acid)	20	1.34	1.37	1.39	1.37	29.02 %	
		40	1.31	1.29	1.33	1.31	32.12 %	
		60	0.95	0.97	0.93	0.95	50.77%	
		80	0.82	0.85	0.79	0.82	57.51%	
		100	0.35	0.32	0.32	0.33	82.90%	
3	Martynia annua Extract	20	1.52	1.54	1.55	1.54	20.21%	74.04
		40	1.34	1.35	1.37	1.35	30.05%	
		60	1.07	1.11	1.14	1.11	42.49%	
		80	0.82	0.85	0.87	0.85	55.96%	
		100	0.68	0.72	0.75	0.72	62.69%	

Table no. 2 Observation of Antioxidant activity

➢ Graphical presentation

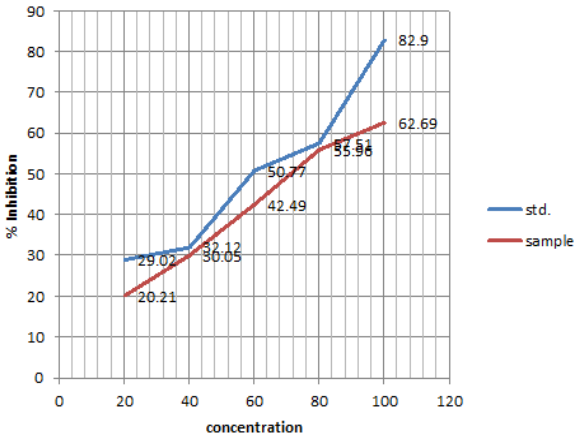


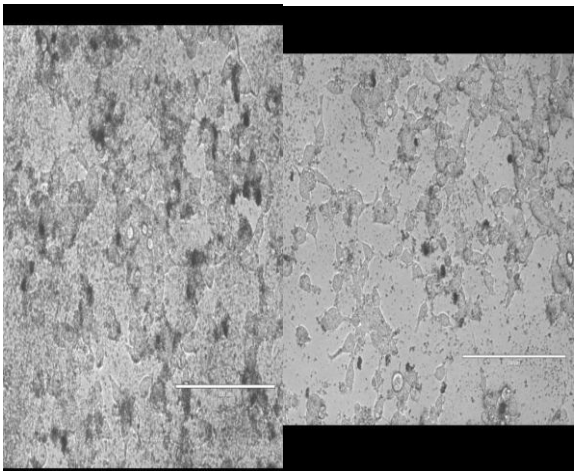
Fig no. 5 Graphical presentation of martynia annua fruit Ethanolic extract

➢ For nephroprotective activity: -

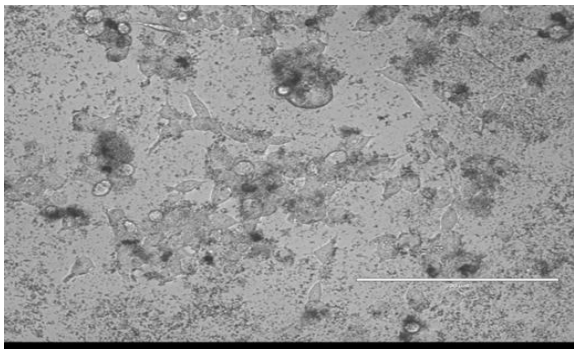
Observation Table:

SR. NO.	Sample code	Concentrations. (µg/mL)	Optical density at 550nm			Mean	% of Inhibition	% of Viability
1	Control (Cisplatin)		1.491			-	-	-
2	Standard (N-acetylcysteine0	20	1.001	1.003	1.005	1.003	32.73%	67.27%
		40	1.112	1.115	1.118	1.115	25.22%	74.78%
		60	1.209	1.211	1.216	1.212	18.71%	81.29%
		80	1.323	1.326	1.329	1.326	11.07%	88.93%
		100	1.407	1.409	1.414	1.41	5.43%	94.57%
3	Martynia annua Extract	20	0.782	0.787	0.789	0.786	47.28%	52.72%
		40	0.881	0.883	0.888	0.884	40.71%	59.29%
		60	1.003	1.005	1.007	1.005	32.60%	67.40%
		80	1.174	1.176	1.175	1.175	21.19%	78.81%
		100	1.232	1.234	1.236	1.234	17.24%	82.76%

Table no. 3 Effect of test compound against HEK-293 cell line



Control Sample



Standard

Fig. no 6 Microscopic evaluation of Nephroprotective activity on HEK-293 cell

- Graphical Representation-

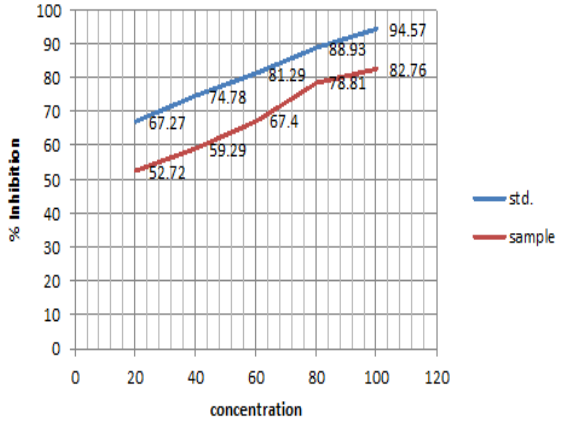


Fig no. 7 Graphical presentation of effect of concentration on % cell viability

➢ Interpretation :-

- Antioxidant activity-

The Martynia annua extract's antioxidant activity as determined by the DPPH free radical scavenging test is shown in the data above. For both the standard and the Martynia annua extract, the results showed a concentration-dependent rise in % inhibition across all tested concentrations. (20–100µg/mL). The Martynia annua extract showed moderate radical scavenging activity at lower concentrations, with approximately 20–30% inhibition at 20 and 40 µg/mL, respectively. As the concentration increased, the antioxidant activity improved significantly, reaching around 55–63% inhibition at 80 and 100 µg/mL. Despite the standard's somewhat increased scavenging activity, the Martynia annua extract displayed substantial antioxidant potential, indicating

the presence of bioactive phytoconstituents capable of neutralizing free radicals. Overall, the findings suggest that the Martynia annua extract possesses significant antioxidant activity and may help protect against oxidative stress related cellular damage.

- Nephroprotective activity

Both the standard and Martynia annua extract clearly enhance cell viability in concentration-dependent manner when tested for nephroprotective efficacy against cisplatin-induced damage in HEK-293 cells using the MTT assay. Cisplatin significantly reduces cell viability due to its cytotoxic effects; however, treatment with the test samples provides notable protection. At lower concentrations (20–40 µg/mL), the Martynia annua extract exhibits moderate protection compared to the standard, indicating lower initial efficacy. As the concentration increases (60–100 µg/mL), a significant improvement in cell viability is observed, with the Martynia annua extract demonstrating substantial protective activity, although still slightly lower than the standard. At the highest concentration, near-maximal cell viability is achieved, confirming strong cytoprotective effects. Overall, the findings suggest that the Martynia annua extract possesses significant nephroprotective potential against cisplatin-induced cellular damage, likely through antioxidant and cell-protective mechanisms, with scope for further optimization to enhance its efficacy.

III. DISCUSSION

The present study demonstrated that the ethanolic extract of Martynia annua fruit possesses significant antioxidant and nephroprotective activity. Preliminary phytochemical screening confirmed the presence of flavonoids, phenolics, tannins, alkaloids, and saponins, which may contribute to its therapeutic effects. The extract showed concentration-dependent antioxidant activity in the DPPH assay and significantly improved cell viability against cisplatin-induced toxicity in HEK-293 cell lines. These findings suggest that the antioxidant phytoconstituents present in the extract may help protect renal cells from oxidative damage. Therefore, Martynia annua fruit may serve as a potential natural source for the development of nephroprotective agents. However, further pharmacological and clinical studies are necessary to confirm its safety and mechanism of action.

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REFERENCE

[1] Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO clinical practice guideline for acute kidney injury. Kidney Int Suppl. 2012;2(1):1–138.
[2] Pabla N, Dong Z. Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. Kidney Int. 2008;73(9):994–1007.
[3] Miller RP, Tadagavadi RK, Ramesh G, Reeves WB. Mechanisms of cisplatin nephrotoxicity. Toxicology. 2010;276(1):1–8.
[4] Perazella MA. Drug-induced nephropathy: an update. Expert Opin Drug Saf. 2005;4(4):689–706.
[5] Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 5th ed. Oxford: Oxford University Press; 2015.
[6] Rang HP, Dale MM, Ritter JM, Flower RJ, Henderson G. Rang and Dale's Pharmacology. 8th ed. Edinburgh: Elsevier Churchill Livingstone; 2016.
[7] Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. ScientificWorldJournal. 2013; 2013:162750.
[8] Krishnaiah D, Sarbatly R, Bono A. Phytochemical antioxidants for health and medicine: a move towards nature. Biotechnol Mol Biol Rev. 2007;1(4):97–104.
[9] Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Dehradun: International Book Distributors; 2006.

[10] Gupta RK, Deogade MS. Antioxidant activities of Martynia annua Linn. root extract. Int J Ayurvedic Med. 2020;10(4):326–328.
[11] Palaramb RS, Thenmozhi M, Prakash B. Phytochemical screening and analysis of antioxidant activity from the botanical extract of the plant Martynia annua. J Adv Zool. 2023.
[12] Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958; 181:1199–1200.
[13] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55–63.
[14] Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. 3rd ed. London: Chapman and Hall; 1998.
[15] Kokate CK. Practical Pharmacognosy. 4th ed. New Delhi: Vallabh Prakashan; 1994.
[16] Basic Biology and Clinical Assessment, Stephen-2005, Pages 343-349
[17] Siddartha Baliyan, Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa, Molecules 2022.
[18] MTT Assay Protocol for Cell Viability and Proliferation by Sigma pvt.Ltd
[19] Sohn, S. J., Kim, S. Y., Kim, H. S., Chun, Y. J., Han, S. Y., Kim, S. H., & Moon, A. (2013).In vitro evaluation of biomarkers for cisplatin-induced nephrotoxicity using HK-2 human kidney epithelial cells. Toxicology Letters, 217(3), 235–242.
[20] Qiu, X., Zhou, X., Miao, Y., & Li, B. (2018). An in vitro method for nephrotoxicity evaluation using HK-2 human kidney epithelial cells combined with biomarkers of nephrotoxicity. Toxicology Research, 7(6), 1205–1213.https://doi.org/10.1039/c8tx00095f