Current Trends in Biotechnology and Pharmacy
Vol. 18(4) 2015-2023, October 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2024.4.47

Identification of Phytoconstituents and investigation of nephroprotective potential of seeds of *Rumex vesicarius* in experimental animals

Adikay Sreedevi*, Gayathri, Kaveripakam Sai Sruthi

Division of Pharmaceutical Chemistry, Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Visvavidyalayam, Tirupati.

Corresponding author: sreedeviadikay8@gmail.com

Abstract

The present study is planned for phytochemical analysis and to evaluate the nephroprotective effect of methanol seed extract of *Rumex vesicarius* on cisplatin-induced nephrotoxicity. The study initiated with pharmacognostic studies of seeds followed by preparation of methanol extract by using hot extraction method. Then the preliminary phytochemical studies were performed followed by HPLC and GC-MS analysis. Further *in vitro* antioxidant studies were performed by adopting DPPH, ABTS and NO techniques. Further Pharmacological studies were initiated with acute toxicity studies. Nephroprotective activity in cisplatin induced model is evaluated by the determination of levels of serum markers and urinary functional parameters. Phytochemical analysis revealed the presence of bioactive phytoconstituents. HPLC revealed in the presence of rutin, whereas GC-MS analysis of methanolic extract resulted in 108 compounds. Upon *in vitro* antioxidant studies the seed extract exhibited markable antioxidant activity. Animal studies showed that methanol extract successfully ameliorated the nephrotoxicity induced by cisplatin in dose dependent passion. Hereby the current study resulted in identification of phytochemical compounds in seeds of *Rumex vesicarius* and also scientifically validated the ethnomedicinal use of seeds of *Rumex vesicarius* in attenuation of renal problems.

Keywords: Nephrotoxicity, Cisplatin, *Rumex vesicarius,* Phytochemical.

Introduction

Even with the rapid advancement of molecular medicine, the search for natural remedies and the discovery of novel phytochemicals remain vast areas of study. Since antiquity, family or cultural tradition has dictated how to consume therapeutic plants. This practise has been transmitted through the centuries and is now common in folk medicine. (1,2). Numerous studies on herbal medicines are being undertaken constantly because they are widely accepted by people as a healthy alternative to treatments based on chemicals or genetics (3). There are numerous functional secondary metabolites found in medicinal plants throughout the plant, but many of these compounds' biological activities are still unknown (4).

In traditional medicine wide number of medicinal plants are used to treat many diseases and disorders including renal problems and many of them are not evaluated scientifically. *Rumex vesicarius* L., is one such plant. It is a

Current Trends in Biotechnology and Pharmacy
Vol. 18(4) 2015-2023, October 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2024.4.47

member of the Polygonaceae family and has green, edible leaves. The abundance of ß-carotenes, vitamins, proteins, lipids, organic acids, and minerals in this shrub makes it a valuable nutritional supplement (5). The plant is used to treat tumours, hepatic disorders, bad digestion, constipation, heart problems, aches, illnesses of the spleen, bloating, hiccough, asthma, bronchitis, dyspepsia, dermatitis, toothache, and nausea (6). The entire plant is medicinally noteworthy and heals a number of maladies.

Prominently seeds of this plant is used to cure urinary and renal troubles by tribal people of various regions of India (7). Hence the current study was aimed at the identification of phytoconstituents and evaluation of the nephroprotective activity of seeds of *Rumex vesicarius* in cisplatin- induced rat model.

Materials and Methods

Plant material collection and authentication

Plant material (Seeds of *Rumex vesicarius*) was obtained from a local market in Tirupati, Andhra Pradesh, then authenticated by botanist Dr. K. Madhava Chetty, an assistant professor in the department of botany at S.V. University, Tirupati. A voucher specimen (No. 0911) was subsequently submitted.

Pharmacognostic studies

Powder microscopy was carried out as per the standard methodology by treating with different chemical reagents and identification of diagnostic characters (8). Seed powder was subjected for determination of varied physicochemical parameters as per the procedures mentioned in Ayurvedic Pharmacopoeia 1996 (9).

Extract preparation

The seeds were ground up into a coarse powder, and the extract was made by macerating them with methanol and then extracting them using a hot extraction process (10).

Preliminary phytochemical screening

Preliminary phytochemical studies was performed for the methanol extract of seeds of *Rumex vesicarius* (MERV) to screen the presence of various phytoconstituents as per standard procedures (11).

Quantitative estimation of Phenols and Flavonoids

Tannic acid was used as a reference to evaluate the extracts' total phenolic content using the Folin-Ciocalteu reagent (12). Utilizing aluminium chloride colorimetry, it was possible to quantify the total flavonoid concentration. Using established techniques, the extract's flavonoid content (quercetin equivalent) was determined (13).

HPLC analysis

HPLC of the crude extract was performed on column C18 Phenomenox 5u,4.6×250 mm. The mobile phase used for the analysis was methanol and water; injection volume: 20 **µ**l; flow rate: 1ml/min and detected at 254nm.

GC-MS analysis

The "Clarus 680 GC. Gas chromatograph fitted and linked to a mass detector Turbo mass gold-Perkin Elmer with turbomass version 5.2.0 spectrometer with an Elite-5MS (5% Phenyl 95% dimethyl Polysilioxane), 30m x 500m id capillary column" was used to conduct the GC-MS analysis of the MERV. The instrument's initial temperature setting was 600C. The oven's temperature was then increased to 300^0C. at the rate of hike of 10^0C/min, and maintained for 6min. Helium flow rate: 1.0ml/min; Ionization voltage: 70eV. Injection of samples in split mode at 1:10. The component spectrums were compared to the database of component spectrums in the GC-MS NIST library. Further bioactivity of the compounds was predicted based on Dr. Dukes ethnomedicinal database (14).

In vitro antioxidant studies

DPPH antioxidant activity

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

MERV in concentrations from 1 to 5 mg/mL was taken and method adopted for DPPH antioxidant activity was as per Olamide et al., 2017 (15). Ascorbic acid (Vitamin C) was used standard.

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity

The extract's ability to scavenge ABTS was compared to that of ascorbic acid, and % inhibition was calculated using the accepted techniques (16).

Nitric oxide scavenging (NOS) assay

The NOS assay was conducted by using reagents like sodium nitroprusside, Griess reagent etc by methods of Reddy et al., 2020 (17).

Pharmacological screening of nephroprotective activity

Animals

The current study made use of healthy Wistar strain albino rats that were 2 to 3 months old and weighed 150 to 200g. The experiment was conducted in accordance with the Institutional Animal Ethical Committee's (IAEC) approval and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of India's requirements (Registration No. Ref No. 1677/PO/Res/S/2012/CPCSEA/IAEC/14/23-02-19).

Acute toxicity studies

Acute toxicity studies were performed as per OECD 423 guidelines (18).

Treatment protocol [19]:

Five groups of six animals each were formed out of the animals. Animals in Group I were given the vehicle orally from days 1 through 9 and were retained as a standard control. Animals in the Group-II were kept as disease controls and received a single dose of cisplatin (6 mg/kg/b.w., i.p.) on day 5 and vehicle (water) orally from days 1 through 9. Animals in Group III were given methanol extract (200 mg/kg b.w.) from Day 1 through Day 9, as well as cisplatin (6 mg/kg b.w., i.p.) on Day 5 and were retained as Lower dose treated animals. Animals in Group IV were given methanol extract (400 mg/kg b.w.) from days 1 through 9, as well as cisplatin (6 mg/kg b.w., i.p.) on days 5 and were kept as higher dose treated animals. Animals in Group V were given Cystone (5 ml/kg b.w.) from Days 1 through 9 and Cisplatin (6 mg/kg b.w., i.p.) on Day 5 and were treated as Standard.

At the end of treatment, assessment of nephrotoxicity is done by estimation of urinary functional parameters, serum biochemical tests and Anti-oxidant studies to the isolated kidney tissue as per standard methodology (10). Statistical analysis carried out and the mean $\pm$ standard error was used to express the data. Tukey-Kramer multiple comparison tests and one-way ANOVA were conducted, and mean values with $p<0.05$ were regarded as significant.

Results and Discussion

Pharmacognostic studies

The morphological characters were examined and seeds found to be light brown in colour, odourless, slight sweet in taste and conical in shape. Powder microscopy of seed powder showed palisade cells, pitted xylem vessels, starch grains, annular xylem vessels, pitted tracheids and parenchyma cells with cotyledons (Figure 1). Different physicochemical parameters, such as ash values, extractive values, and loss on drying, were calculated and tabulated in Table 1.

Current Trends in Biotechnology and Pharmacy
Vol. 18(4) 2015-2023, October 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2024.4.47

Figure 1: Powder microscopy of seed powder of *Rumex vesicarius*

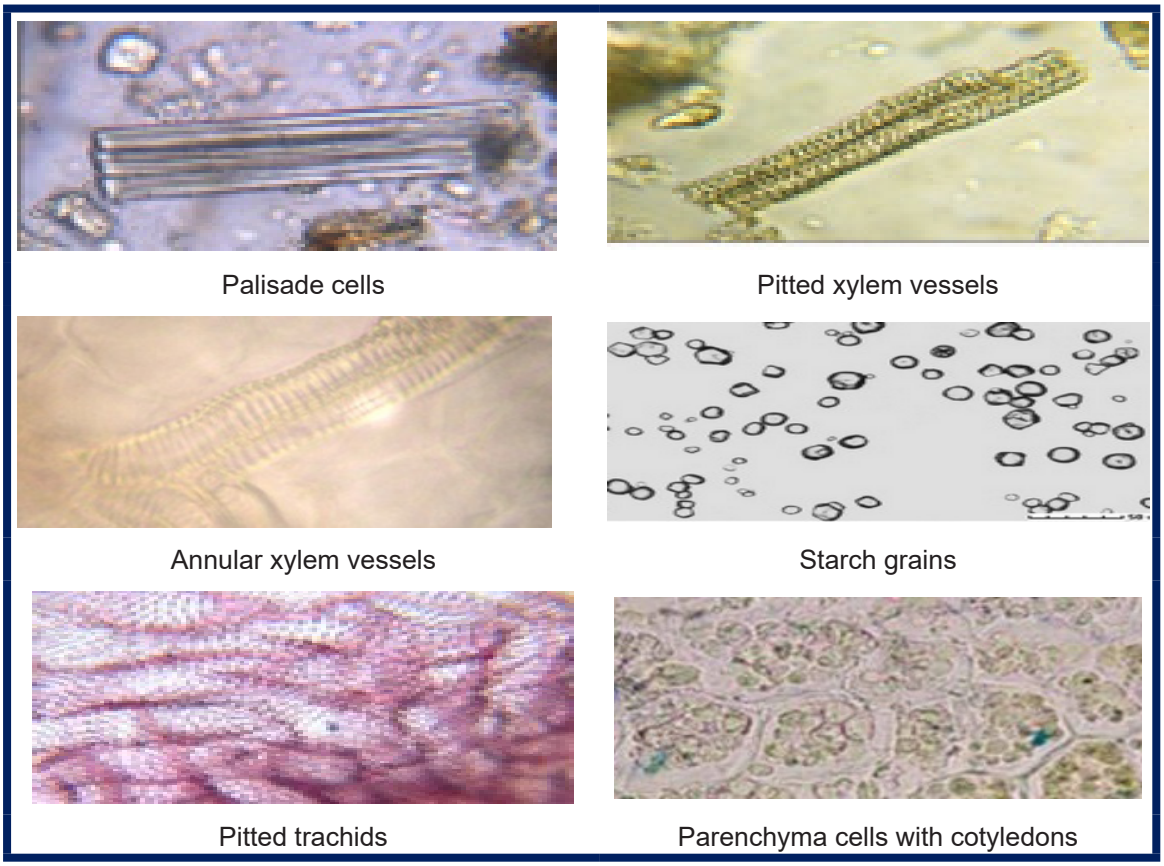


Table 1: Physicochemical analysis of seeds of *Rumex vesicarius*

S. No	Parameters	Values (%w/w)
1.	Ash values a)Total ash value b)Acid insoluble ash c)Water insoluble ash	1.4 0.504 1.14
2.	Extractive values a)Alcohol soluble extractive b)Water soluble extractive c)chloroform soluble extractive	 **72** **42** **20**
3.	Loss on drying	**4.2**

Screening for preliminary phytochemicals and quantitative estimate

Preliminary phytochemical screening of MERV revealed the presence of phenolic substances such as saponins, triterpinoids, glycosides, and flavonoids. The Phenolic content was calculated as mg of gallic acid /g of plant material and it was found to be 64.80±1.50. The total flavonoid content of MERV were demonstrated as quercetin equivalents per gram and was discovered to be 50.08±0.12 mg of quercetin equivalent /g.

HPLC analysis of of MERV

HPLC analysis of MERV resulted in 20 number of peaks at different retention times ranging from 3.13 to 23.86 (Figure 2). Out of all

Current Trends in Biotechnology and Pharmacy
Vol. 18(4) 2015-2023, October 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2024.4.47

the peaks, peak eluted at 5.16 (retention time) in MERV was matched with rutin (Std) **(**Figure:2).

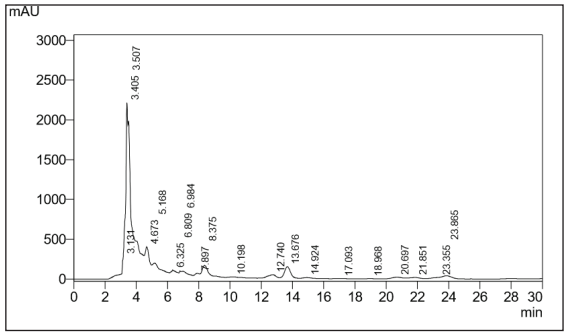
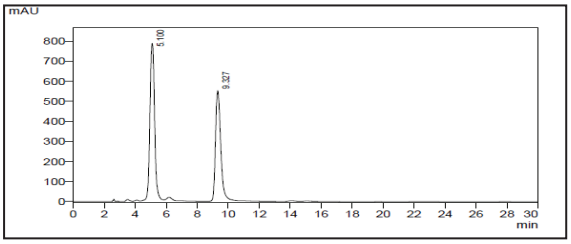


Figure 2a: HPLC chromatogram of MERVFig-



ure 2b: HPLC chromatogram of RUTIN

In Vitro antioxidant studies

Antioxidant studies of MERV was determined by three different assays and results were tabulated in Table 4. IC_{50} values of MERV is 3.33 (DPPH), 2.57 (NOS) and 3.58 (ABTS). The scavenging activity was increased with the escalation in the concentrations of MERV (Table 2).

GC-MS analysis of MERV

The GC-MS analysis of MERV resulted in 108 compounds (Figure 3). Among them many of the compounds like 2,3-Anhydro-d-mannosan, N-butyl-N-vinylacetamide, 2-hydroxy-gamma-butyrolactone, P-Dioxane-2,3-diol, 4-Tosyl-diformal-1-rhamnitol are biologically active and may strongly provide innate defense against oxidative stress.

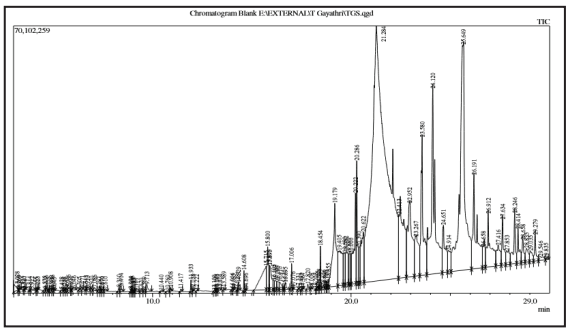


Figure 3: GC-MS chromatogram of MERV

Table :2 Effect of MERV on *in vitro* antioxidants

Concentration (mg/ml)	% inhibition of DPPH (Mean±SEM)	% inhibition NOS (Mean±SEM)	%inhibition ABTS (Mean±SEM)
1	41.84±0.30	42.63±0.48	36.56±0.48
2	42.28±0.52	50.93±0.59	41.16±0.56
3	52.90±0.67	52.4±0.66	52.43±0.69
4	52.92±0.77	53.2±0.69	53.2±0.74
5	54.22±0.69	54.26±0.75	54±0.86
Standard (Vitamin C)	43.19±0.14	55.2±0.12	55.66±0.15
IC_{50}	3.33	2.57	3.58

Table 4: Effect of MERV on oxidative stress

Group	**Catalase (**uM/min/mg protein)	**SOD** **(**Units/mg protein)	**GSH** (µmol/mg protein)	**MDA (**nmol/mg protein)
I	45.58±0.52	26.75±0.66	12.42±0.48	43.58±1.46
II	17.38±0.37[a*]	8.25±0.30 [a*]	3.50±0.26 [a*]	98.75±0.54 [a*]
III	31.45±0.51[b*]	11.95±0.19 [b*]	4.1±0.33 [b*]	79.14±0.13 [b*]
IV	38.91±0.38[b*]	15.13±0.31 [b*]	6.9±0.20 [b*]	69.53±0.58 [b*]
V	43 .67±0.40 [a* b*]	17.25±0.61 [a* b*]	8.66±0.24 [a* b*]	55.82±0.13 [a* b*]

All values were expressed as mean±SEM of 6 observations
a*=P<0.05, considered statistically significant when compared to the normal sgroup.
b*=P<0.05, considered statistically significant when compared to the disease control.

Pharmacological evaluation

Acute toxicity studies

There was no morbidity and animals had not shown any changes in behavior and no signs of toxicity. Hence, the MERV was found to be safe at dose of 2000 mg/kg, b.w.

Effect of MERV on cisplatin-induced nephrotoxicity

When compared to normal animals, the administration of cisplatin led to a significantly higher (p 0.05) level of BUN, serum creatinine, urinary total protein, lipid peroxidation, and a lower level of urinary creatinine, as well as lower levels of reduced glutathione, catalase, and superoxide dismutase. However, treatment of the MERV considerably counteracted the effects of cisplatin in dose-dependent passion at both doses of 200 and 400 mg/kg,b.w (Table 5 and 6). Furthermore, the illness control group also showed a considerable reduction in body weight. When compared to disease control, the extract-treated animals displayed a significant reduction in the decline of body weight.

Table 3: Effect of MERV on biochemical parameters of Cisplatin –induced nephrotoxicity

Group	Serum creatinine(mg/dl)	Blood urea nitrogen(mg/dl)	Urine creatinine(mg/dl)	Total urinary protein(g/dl)
I	0.84±0.01	11.67±0.44	8.89±0.28	4.16±0.24
II	2.57±0.06 [a*]	28±0.38 [a*]	2.49±0.26 [a*]	11.93±0.26 [a*]
III	1.73±0.05[b*]	18.35±0.29 [b*]	5.65±0.27 [b*]	8.89±0.21 [b*]
IV	1.27±0.05 [b*]	12.50±0.24 [b*]	10.67±0.21 [b*]	5.56±0.27 [b*]
V	1.18±0.04[a*b*]	15.42±0.35 [a*b*]	9.33±0.44 [a*b*]	5.192±0.21 [a*b*]

All values were expressed as mean±SEM of 6 observations
a*=P<0.05, considered statistically significant when compared to the normal group.
b*=P<0.05, considered statistically significant when compared to the disease control.

Current Trends in Biotechnology and Pharmacy
Vol. 18(4) 2015-2023, October 2024, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2024.4.47

Table 5: Effect of MERV on body weight in cisplatin- induced nephrotoxicity

Group	Body Weight	
	Initial	Final
I	163.8±0.60	169.85±0.60
II	155±0.96[a*]	133.8±0.61 [a*]
III	145±0.98 [b*]	139±0.96 [b*]
IV	161±0.86 [b*]	152±0.89 [b*]
V	159.2±0.60 [a* b*]	151.8±0.62 [a* b*]

All values were expressed as mean±SEM of 6 observations
a*=P<0.05, considered statistically significant when compared to the normal group.
b*=P<0.05, considered statistically significant when compared to the disease control.

Discussion

Rumex vesicarius is one of several anti-oxidant-rich medicinal herbs. Tribes from several parts of India utilize its seeds as folk medicine to cure urological and renal issues. The goal of the current study was to assess the nephroprotective potential of *Rumex vesicarius* seeds. To evaluate the quality and purity of plant material, the work began with powder microscopy and was followed by physico-chemical analysis of *Rumex vesicarius* seeds. The presence of flavonoids and phenolic chemicals, which are known to have effective antioxidant and nephroprotective properties, was discovered in methanol extract by preliminary phytochemical analyses (20).

The phenolic and flavonoid content of MERV was found to be high overall. Rutin was detected in the MERV extract after additional HPLC analysis. According to earlier scientific research, rutin protects the kidneys against drug-induced nephrotoxicity (21).

A variety of bioactive chemicals were found in the *Rumex vesicarius* seed extract after GC-MS analysis and were discovered to have powerful antioxidant characteristics. The existence of phytochemical antioxidants was also confirmed by other in vitro antioxidant tests. According to earlier findings, plants with a high concentration of antioxidant phytochemicals are beneficial in reducing nephrotoxicity.

Nephrotoxicity is a serious consequence of the effective anticancer medication cisplatin and a dose-limiting side effect (22). An increase in serum indicators, urine total protein, and malondialdehyde levels, as well as a decrease in urinary creatinine, GSH, SOD, and catalase levels, were all signs of renal impairment brought on by the administration of cisplatin. According to studies by Fang et al. (2021), Yadav et al. (2019), these results are consistent (23,24).

As described in earlier investigations on different plants, treatment with MERV reduced the effects of cisplatin on dose-dependent passion. This might be brought on by MERV's "free radical scavenging activity, enhanced glomerular filtration, and rejuvenation in renal tubular cells." Innate protection against oxidative stress brought on by cisplatin may be significantly attributed to the presence of antioxidant principles such flavonoids, particularly rutin, and other phytoconstituents.

Conclusion

The current scientific research resulted in identification of various phytochemical compounds in seeds of *Rumex vesicarius.* Furthermore, the current study presents scientific evidence that supports the ethnomedicinal use of *Rumex vesicarius* seeds in the amelioration of renal issues.

References

1. Mohammadhosseini M., Frezza C., Venditti A., Mahdavi B.(2021) An overview of the genus Aloysia Paláu (Verbenaceae): Essential oil composition, ethnobotany and biological activities. *Nat. Prod. Res.*1–17. [PubMed] [Google Scholar]

2. Omer Iqbal M, Bashir Yahya E, Andleeb S, Masood Ahmed M, Umar Javaid M, Sha-

keel W, Iqbal I.(2021) In vivo assessment of reversing Cisplatin-Induced nephrotoxicity using *Jatropha mollissima* crude extract and its potential cytotoxicity. Saudi J Biol Sci. 28(12):7373-7378. doi: 10.1016/j.sjbs.2021.08.057. PMID: 34867040; PMCID: PMC8626311.

3. Barbosa H.M., Albino A.M., Cavalcante F.S.A., Lima R.A.(2017) Abordagem fitoquímica de metabólitos secundários em Solanum acanthodes (Solanaceae) Hook. *South Am. J. Basic Education, Technical Technological*;4(1) [Google Scholar]

4. Abogmaza, A. F., Keer, K. F., Ayad, A. T., & Yahya, E. B. A Review on the Medicinal and Aromatic Plants Growing in Libya and Their Therapeutic Properties. Int. Res. J. Sci. Technol., 2(1).

5. Beddou F, Bekhechi C, Ksouri R, Chabane Sari D, Atik Bekkara F.(2015) Potential assessment of Rumex vesicarius L. as a source of natural antioxidants and bioactive compounds. J Food Sci Technol;52(6):3549-60. doi: 10.1007/s13197-014-1420-9. PMID: 26028737; PMCID: PMC4444908.

6. Gopal R, Vijayakumaran M, Venkatesan R, Kathiroli S (2008) Marine organisms in Indian medicine and their future prospects. Nat Prod Radiance 7(2):139–145. http://nopr.niscair.res.in/bitstream/123456789/5658/1/NPR%207(2)%20139-145.pdf

7. Mostafa HAM, ElBakry AA, Alam EA. (2011) Evaluation of antibacterial and antioxidant activities of different plant parts of *Rumex vesicarius* L. (Polygonaceae) *Int J Pharm Pharm Sci*;3(2):109–118. [Google Scholar]

8. Prakash, A., Janmeda, P., Pathak, P. *et al.* (2019) Development and standardization of quality control parameters of different parts of *Trianthema portulacastrum* L.. *SN Appl. Sci.* **1:**1108 . https://doi.org/10.1007/s42452-019-1074-3

9. The Ayurvedic Pharmacopoeia of India, Part- I, Volume – III, Government of India, Ministry of Health and Family Welfare. Department of Ayush; New Delhi; 123.

10. Adikay S, Pravallika BV and Sruth KS (2021) Phytochemical analysis and pharmacological evaluation of ethanol extract of fruits of Grewia tiliaefolia for nephroprotective activity. Int J Pharm Sci & Res 12(9): 4933-41. doi: 10.13040/IJPSR.0975-8232.12(9):4933-41.

11. Harborne J B,(1973) Phytochemical Methods, Chapman & Hall: International Edition, Japan: Toppan Company Ltd, 49-188.

12. Sumana Dutta, Sanjib Ray(2020) Comparative assessment of total phenolic content and in vitro antioxidant activities of bark and leaf methanolic extracts of Manilkara hexandra (Roxb.) Dubard, Journal of King Saud University - Science,32: 643-647, ISSN 1018-3647, https://doi.org/10.1016/j.jksus.2018.09.015.

13. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N(2019) Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. Plants (Basel);8(4):96. doi: 10.3390/plants8040096. PMID: 30978964; PMCID: PMC6524357.

14. Matulyte I, Marksa M, Ivanauskas L, Kalvėnienė Z, Lazauskas R, Bernatoniene J(2019) GC-MS Analysis of the Composition of the Extracts and Essential Oil from *Myristica fragrans* Seeds Using Magnesium Aluminometasilicate as Excipient. Molecules ;24(6):1062. doi: 10.3390/molecules24061062. PMID: 30889896; PMCID: PMC6471610.

15. Olamide E. Adebiyi, Funsho O. Olayemi,

Tan Ning-Hua, Zeng Guang-Zhi(2017) In vitro antioxidant activity, total phenolic and flavonoid contents of ethanol extract of stem and leaf of Grewia carpinifolia, Beni-Suef University Journal of Basic and Applied Sciences, 6(1):10-14,ISSN:2314-8535,https://doi.org/10.1016/j.bjbas.2016.12.003. (https://www.sciencedirect.com/science/article/pii/S2314853516301007)

16. Shahinuzzaman, M., Yaakob, Z., Anuar, F.H. *et al*(2020) In vitro antioxidant activity of *Ficus carica* L. latex from 18 different cultivars. *Sci Rep* **10,** 10852 . https://doi.org/10.1038/s41598-020-67765-1

17. Reddy KS, Likithasree P, Peraman R, Jyothi MV, Babu CN, Pradeepkumar B, Sudheer A(2020) Spatial long-term memory retention by banana and papaya peel extract: In silico and in vivo evaluation. International Journal of Pharmaceutical Investigation;10(2):202-7.

18. Organization for Economic Cooperation and Development (OECD). Guideline 423 for testing chemicals: Paris 2001: 1-14.

19. Zahra Eslamifar, Abbas Moridnia, Susan Sabbagh, Reza Ghaffaripour, Leila Jafaripour, Mahin Behzadifard(2021) "Ameliorative Effects of Gallic Acid on Cisplatin-Induced Nephrotoxicity in Rat Variations of Biochemistry, Histopathology, and Gene Expression", BioMedResearch International, Article ID 2195238, 11 https://doi.org/10.1155/2021/2195238

20. Hossain MA, AL-Raqmi KA, AL-Mijizy ZH, Weli AM, Al-Riyami Q(2013) Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. Asian Pac J Trop Biomed;3(9):705-10. doi: 10.1016/S2221-1691(13)60142-2. PMID: 23998010; PMCID: PMC3757278.

21. Qu S, Dai C, Lang F, Hu L, Tang Q, Wang H, Zhang Y, Hao Z(2018) Rutin Attenuates Vancomycin-Induced Nephrotoxicity by Ameliorating Oxidative Stress, Apoptosis, and Inflammation in Rats. Antimicrob Agents Chemother;63(1):e01545-18. doi: 10.1128/AAC.01545-18. PMID: 30397060; PMCID: PMC6325182.

22. McSweeney KR, Gadanec LK, Qaradakhi T, Ali BA, Zulli A, Apostolopoulos V(2021) Mechanisms of Cisplatin-Induced Acute Kidney Injury: Pathological Mechanisms, Pharmacological Interventions, and Genetic Mitigations. Cancers (Basel);13(7):1572. doi: 10.3390/cancers13071572. PMID: 33805488; PMCID: PMC8036620.

23. Fang, Cy., Lou, Dy., Zhou, Lq. *et al*(2021) Natural products: potential treatments for cisplatin-induced nephrotoxicity. *Acta Pharmacol Sin* **42:**1951–1969 https://doi.org/10.1038/s41401-021-00620-9

24. Yadav AV and Upasani CD(2019) Nephroprotective activity of Plumeria rubra against cisplatin induced nephrotoxicity in experimental rats. International Journal of Pharmacy and Pharmaceutical Sciences; 11: 108-13.