



## Evaluation of Antioxidant and Anti-Inflammatory Activity of Methanolic Extract of *Rhus chinensis* Seed: *In-vitro* Studies

G. Pallavi Rao<sup>a#</sup>, Pavitra Chettri<sup>a#</sup>, C. Vinutha<sup>b</sup> and G. Sowmyashree<sup>a\*</sup>

<sup>a</sup> Department of Biochemistry, Mount Carmel College, Autonomous, Bengaluru- 560052, Karnataka, India.

<sup>b</sup> Department of Biochemistry, College of Agriculture, University of Agricultural Sciences, Dharwad, Hanumanamatti, Haveri-581115, Karnataka, India.

### Authors' contributions

Authors GPR and PC shares equal authorship and this work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/AJRB/2022/v10i330226

### Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/89601>

Original Research Article

Received 14 May 2022  
Accepted 18 July 2022  
Published 25 July 2022

### ABSTRACT

*Rhus chinensis* is a dioecious shrub, found in warm temperate zone across Asia. In India it is found in Northeastern part like South Assam, Arunachal Pradesh, West Bengal and Tamil Nadu. [16] The roots, stem, leaves and seed have various therapeutic effects on ailments like hemoptysis, jaundice, snakebites, diabetes. Phytochemical analysis of *Rhus chinensis* extracts revealed the presence of various biochemical compounds such as alkaloids, flavonoids, tannins, triterpenoids, amino acids, proteins and carbohydrates. These compounds have well proven remarkable anti-oxidant and anti-inflammatory activity.

The adverse effect of oxidative stress results in many disorders such as aging, cardiovascular disorders and so on. Inflammation is yet again another problem which causes many disorders like rheumatoid arthritis, asthma and Crohn's disease etc. This research was conducted to evaluate the antioxidant and anti-inflammatory effect of *Rhus chinensis* seed (Methanolic Extract). The *in-vitro* antioxidant activity was carried out by testing 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and ferric chloride reducing activity. Further, effect on protein denaturation, inhibition of proteinase and Human Red Blood Cell (HRBC) membrane stabilization was carried out to screen for anti-inflammatory activity. The total phenolic content was found to be 5 mg/mL of

#Contributed equally;

\*Corresponding author: E-mail: g.sowmyashree@gmail.com, g.shree.aj@gmail.com;

Gallic acid equivalent (GAE) and flavonoid content was found to be 335 µg/mL of Quercetin equivalent (QE). Aspirin and Diclofenac sodium were used as standard drug. All the assays showed positive results and it was concentration dependent. Thus, *Rhus chinensis* seed is a promising anti-oxidant and anti-inflammatory agent with significant activity. Moreover, the use of natural products like *Rhus chinensis* can be highly efficient, beneficial, cost effective, safer alternative with lesser side effects. Altogether, studies on *Rhus chinensis* seeds have huge scope as an alternative ethno-pharmaceutical compound for inflammations.

**Keywords:** *Rhus chinensis*; anti-oxidant; anti-inflammatory; therapeutics and ethno-pharmaceutical.

## 1. INTRODUCTION

In recent times, degenerative diseases like arthritis, atherosclerosis, cancer and inflammatory joint disease etc., adversely trouble humanity has their origin in deleterious free radical reactions. In normal cellular metabolism the cells utilize oxygen for Adenosine Triphosphate(ATP) synthesis, Reactive oxygen species (ROS) and reactive nitrogen species are produced as the by products from these cellular reactions [1]. Reactive oxygen species consists of free radicals such as hydroxyl free radical, lipid peroxyl, superoxide free radical and lipid peroxide along with oxygen intermediates non-free radical such as hydrogen peroxide and singlet oxygen [2]. Oxidative stress results due to imbalance between reactive oxygen species production and detoxification in cells and tissues. The environmental factors such as UV radiations, pollutants and heavy metals are majorly involved in increasing production [3]. Endogenous antioxidant defense mechanism is naturally present in human beings. However, 1% of reactive oxygen species escapes this mechanism every day and causes oxidative damage to the surrounding tissue [4]. Humans are not genetically evolved to survive oxidative stress for past middle age populations, hence antioxidant supplements should be given through diet to ensure healthier population [5]. Synthetic antioxidants such as propyl gallate, butylatedhydroxy toluene and butylatedhydroxy anisole are used to reduce oxidation but studies shows that these synthetic antioxidants are suspected to cause cancer [6].

Inflammation is the response of immune system to injury, microorganisms or irritants, which increases vascular permeability, protein denaturation and causes membrane alteration [7]. Inflammation ranges from acute to chronic based on severity. Inflammation is good in short term but in long term may results many adverse health effect. Oxidative stress and inflammation are like a paradox. Studies shown, during

inflammation leucocytes and mast cells causes respiratory burst which results in increased uptake of oxygen and generates huge amount of reactive oxygen species. Release inflammatory mediators and ROS also increase which in turn results in oxidative-stress induced inflammation [8,9].

Non-steroidal Anti-inflammatory Drugs (NSAIDs) are the most common medications used in the world for inflammation and its related disorders. These drugs when taken for a long-term cause various side effects [10]. Considering all these facts, this study is focused to use natural source containing bioactive compounds to deal with free radicals and inflammation associated diseases with minimum side effects.

*Rhus* sp. are widely used in medicine and food as they are rich in flavonoids, phenolics and other phytochemicals [11,12]. These compounds act as primary source of antioxidants. *Rhus toxicodendron*, is used as homeopathic remedy for symptoms such as rheumatic pains, skin irritation and inflammation [13,14]. Barka and co-workers reported, *Rhus tripartita*, is shown to have antioxidant, antimicrobial and anti-inflammatory [15,16]. Ganj Renuka Rani and co-workers studies shown that the flavonoids isolated from *Rhus mysorens* having anti-inflammatory activity [17,18].

## 2. MATERIALS AND METHODS

### 2.1 Collection and Preparation of the Extract

The *Rhus chinensis* was collected from Makhel village in Mao Maram (Senapati) Manipur, the seeds of *Rhus chinensis* were washed & air-dried for 30 days at room temperature. Extraction was carried out as per do et al. [19]. Briefly, the dried seeds were finely homogenized using mortar and pestle. Then, 10gm of the powder was weighed and transferred into a fresh beaker. Further, 80% methanol was added to the sample

and was incubated at room temperature for 30 mins. The prepared solution was filtered by using filter paper (What man filter paper No. 42). The sample was transferred in a fresh beaker and placed in a magnetic stirrer for overnight for mixing. The mixed solution was centrifuged to 4500 rpm for 10 mins and the supernatant was collected. The collected extract was used throughout the whole study.

## 2.2 Methods

### 2.2.1 Estimation of total phenolic content

#### 2.2.1.1 Standard gallic acid

Total phenolic content was estimated by Folin Ciocalteu's (FC) method by Kaur et al.,;Javanmardi, J et al., [20,21] with minor modifications. The reaction mixture of different concentrations was made and 0.2-1.0 mL aliquots of standard gallic acid (0.25 mg/mL) and was made up to 1.0 mL by using a solvent mixture (methanol: water 1:1). 5.0 mL FC (1:10 dilution FC reagent is used) is added to all the test tubes and shaken. To all the test tubes 4.0 mL of sodium carbonate was added and all the test tubes were incubated for 5 mins at room temperature. After incubation, the absorbance was read at 765 nm using UV- visible spectrophotometer. The extracts were performed in duplicates in order to obtain the concordant values. The blank was performed using the solvent mixture.

### 2.2.2 Estimation of total flavonoids

#### 2.2.2.1 Standard quercetin

The total flavonoid content in the extracts was determined using quercetin as standard by Wuttisin et al., Pekel Anna et al., [22,23] with minor modifications, 0.2-1.0 mL aliquots of standard quercetin (0.1 mg/mL) prepared in methanol was pipetted out into different test tubes. The volume in each test tubes is made up to 2.0 mL. Ethanol (2.0 mL) is taken as blank. 0.1 mL of 10 % of  $AlCl_3$  (aluminium chloride) is added to all the test tubes, followed by 0.1 mL of potassium acetate ( $CH_3COOK$ ) in all test tubes, 2.8 mL of distilled water was added to all the test tubes and these tubes were incubated at room temperature for 30 mins. After incubation, the absorbance was read at 425 nm using a colorimeter. The extracts were performed in duplicates in order to obtain the concordant values. The total flavonoid content in the extracts

was expressed as mg equivalents of Quercetin in mg/mL of extract.

### 2.2.3 In vitro antioxidant activity

#### 2.2.3.1 DPPH-Assay (2,2-diphenyl-1-picrylhydrazyl)

The reducing power of the plant extract was determined by the method proposed by Mishra et al., [24] with minor modifications. Different aliquots of standard ascorbic (1 mg/mL) were taken. The volume was made up to 1.0 mL using distilled water, followed by 3.0 mL of DPPH solution in all the test tubes similarly different concentrations of the sample were taken and was allowed to stand at room temperature for 30 mins. After incubation, the absorbance was read at 517 nm using a colorimeter. A mixture of distilled water and methanol was taken as negative control and 1 mL of distilled water and 3 mL of DPPH was taken as positive control. The experiment was performed in triplicate.

The percentage inhibition of DPPH by the samples was calculated using the formula:

$$\% \text{ Inhibition of DPPH} = 100 * (1 - A2/A1)$$

Where, A1= Absorbance of control sample, and A2= Absorbance of the test sample.

#### 2.2.3.2 $FeCl_3$ Assay for antioxidant activity

The total antioxidant activity can be measured using the ferric reducing antioxidant power assay (FRAP) proposed by Maruthamuthu Vijayalakshmi et al., [25]. Different concentrations of the sample was taken to this 2.5 mL of 0.2M sodium phosphate buffer of pH 6.6 was added, followed by 2.5 mL of 1% potassium ferri cyanide solution is added. The reaction mixture was shaken well and is vortexed well and is then incubated at 50-degree Celsius for 20 minutes using a vortex shaker. After incubation, 2.5mL of 10% trichloroacetic acid was added and is centrifuged at 3,000 rpm for 10 mins. The supernatant was extracted and to this 2.5 mL of distilled water is added to the reaction mixture and 0.5 mL of 0.1% ferric chloride is added. The color of the solution was observed and the absorbance was read at 700 nm using a UV-visible spectrophotometer. The ascorbic acid (1 mg/ mL) was used as the reference standard; the reducing power of the samples was compared with the reference standard.

The percentage inhibition of FeCl<sub>3</sub> by the samples was calculated using the formula:

$$\% \text{ Inhibition} = 100 * (1 - A2/A1)$$

Where, A1= Absorbance of the control sample, and A2= Absorbance of the test sample.

## 2.2.4 *In-vitro* Anti-inflammatory activity

### 2.2.4.1 Effect on Protein denaturation

Protein denaturation assay was done according to the method described by Gunathilake et al., Sakat S et al., [26,27] with minor modifications. The reaction mixture (5 mL) consisted of 0.2 mL of 1% Bovine Serum Albumin (BSA), 4.78 mL of Phosphate Buffer Saline (PBS, pH 6.4), and 0.02 mL of the extract, this mixture was incubated in a water bath 37°C for 15 mins and then the reaction mixture was heated at 70°C for 5 mins after cooling the turbidity was measured at 660 nm in a UV-Vis Spectrophotometer. Phosphate buffer saline was used as negative control and the mixture containing BSA and PBS was used as the positive control. Diclofenac sodium and Aspirin were used as standard anti-inflammatory drugs. The experiment was performed in triplicate.

The percentage of inhibition of Protein Denaturation was calculated using the formula

$$\% \text{ Inhibition of denaturation} = 100 * (1 - A2/A1)$$

Where, A1= Absorbance of the control sample, and A2= Absorbance of the test sample.

### 2.2.4.2 Proteinase inhibition assay

Proteinase inhibition assay was performed according to the method described by Sakat S et al., [28,29] with minor modifications. The reaction mixture 2 mL consisted of 0.06mg trypsin, 1 mL of 20 mM Tris HCl buffer (pH 7.4), and 1 mL of extract of different concentrations. The mixture was incubated at 37°C for 5 mins and then 1 mL of 0.8%(W/V) casein was added. The mixture was further incubated for 20 more mins, 2 mL of 70% perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged and the absorbance was read at 210 nm against the blank. Tris HCl Buffer was used as negative control 2 mL of Tris HCl with 0.06mg of trypsin and 1 mL of casein was used as the positive control. The experiment was performed in triplicates.

The percentage inhibition of proteinase inhibitory activity was calculated:

$$\% \text{ Inhibition of proteinase} = 100 * (1 - A2/A1)$$

Where, A1= Absorbance of the control sample, and A2= Absorbance of the test sample.

### 2.2.4.3 HRBC Membrane stabilization Assay

HRBC membrane stabilization assay was performed as described by Saleem et al., [30] with minor modifications. Preparation of Blood cell suspension: Around 10mL of fresh human blood was taken and was transferred to the centrifuge tube containing equal volume of 3.2% Trisodium citrate (w/v), this was centrifuged at 3000 rpm for 10 mins and the tubes were washed three times with normal saline. The volume of blood was measured and reconstituted as 10% (v/v) suspension with normal saline.

### 2.2.4.4 Hypotonicity induced haemolysis

This assay was performed by the modified method of Shinde et al., [31,32]. The reaction mixture consisted of different concentrations of the extract which was made up to 0.5 mL with phosphate buffer, 2 mL of hyposaline, and 0.5 mL of HRBC suspension. This was incubated at 37°C for 30 mins and centrifuged at 3000 rpm for 20 mins. The absorbance of the supernatant was measured at 560 nm. Aspirin was used as a standard drug for inflammation. Phosphate buffer was used as negative control and positive control. was made omitting the sample. The experiment was performed in triplicate.

The percentage inhibition of haemolysis was calculated:

$$\% \text{ Inhibition of haemolysis} = 100 * (1 - A2/A1)$$

Where, A1= Absorbance of the control sample, and A2= Absorbance of the test sample.

## 3. RESULTS AND DISCUSSION

### 3.1 Estimation of Total Phenolic Content

#### 3.1.1 Standard gallic acid

The total phenolic content expressed in terms of GAE of 1 to 100 diluted of the methanolic seed extract of *Rhus chinensis* (MERC) was found to be 0.05 mg/mL of GAE. The total phenolic

content in the extract was found to be 5 mg/mL of GAE. The total phenolic were calculated using the following linear equation based on the calibration curve of gallicacid;(y=12.233x, R<sup>2</sup>=0.9976).

Recent evidences suggest that diets rich in polyphenolic compounds play a significant role against oxidative stress related disorders

because of their antioxidant activities [33]. Hence, polyphenolic constituents of seed of *Rhus chinensis* may have the property to counteract oxidative stress related disorders. There exists a positive correlation between the total phenolic content and free radical scavenging property of the seed extract. There is a linear relationship between the concentration of phenolic content and the antioxidant property.

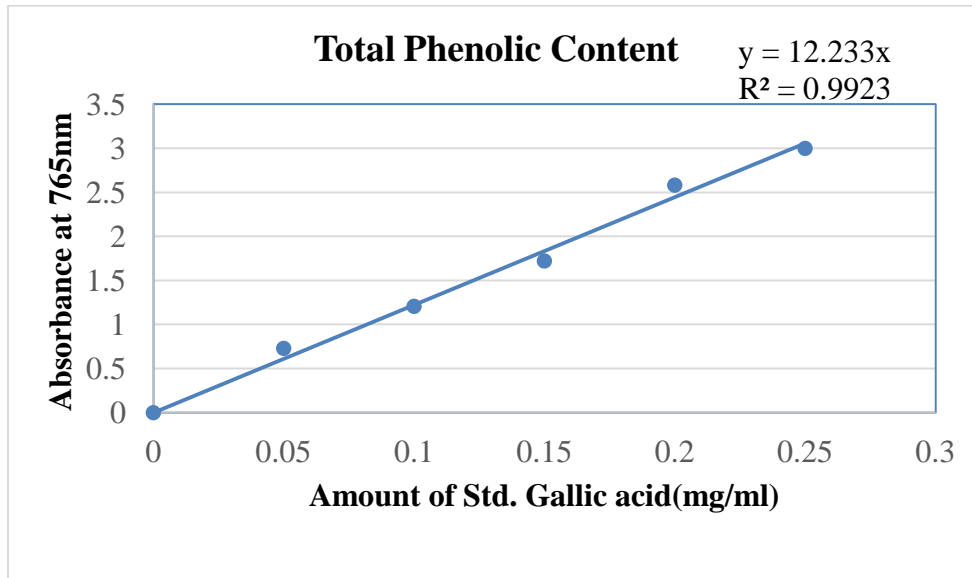


Fig. 1. Total phenolic content by standard gallic acid

### 3.2 Estimation of Total Flavonoids

#### 3.2.1 Standard quercetin

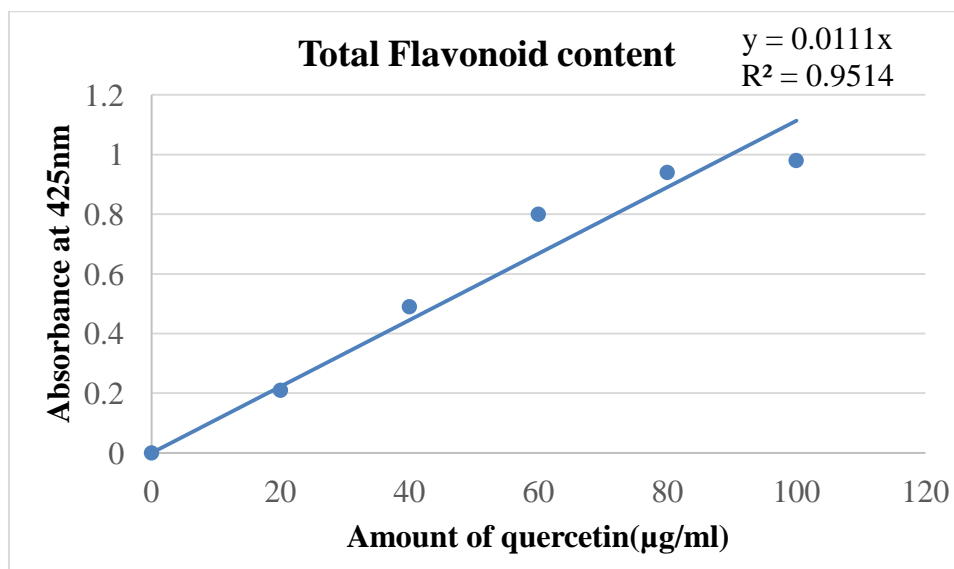


Fig. 2. Total flavonoid content by standard quercetin

The amount of total flavonoid content was calculated using the standard quercetin ( $y=0.0111x$ ,  $R^2 =0.9976$ ) and is expressed as quercetin equivalent (QE) per gram of the extract. The total flavonoid content present in the methanolic extract seed of *Rhus chinensis* was found to be 335 µg/mL of QE.

Flavonoids are secondary metabolites produced from the plants, which is a majority phenolic acid in nature. Studies have shown that it has a role in antioxidant activity [34].

### 3.3 *In vitro* Antioxidant Activity

#### 3.3.1 DPPH Assay (2,2-diphenyl-1-picrylhydrazyl)

The DPPH assay was performed to screen for antioxidant activity of MERC. The results obtained shows that the antioxidant activity was maximum at 500 µg/mL which had 99.3% of radical scavenging activity which was higher than the standard Ascorbic acid (500 µg/mL) which showed 96.3% of scavenging activity (Fig. 3).

#### 3.3.2 FeCl<sub>3</sub> assay for antioxidant activity

The Ferric chloride reducing assay was performed to analyse the reducing power of MERC and thus investigate the antioxidant

property. The results showed that reducing power increased with the increase in concentration. The maximum reduction was seen at 500 µg/mL which had 92.4% of reduction potential which was higher than the standard ascorbic acid (500 µg/mL) which showed 91.5% of reduction potential (Fig. 4).

Thus, by analysing the results of both the assays we can say that the MERC has extremely good antioxidant capacity.

### 3.4 *In-vitro* Anti-Inflammatory Activity

#### 3.4.1 Effect on protein denaturation

The inhibition of protein denaturation by MERC increased with the increase in concentration. Maximum inhibition of 88.8% was shown at 500 µg/mL. The standard drugs Aspirin (150 µg/mL) and Diclofenac sodium (100 µg/mL) showed 83.3% and 69.4% of inhibition respectively (Fig. 5).

#### 3.4.2 Proteinase inhibition assay

Significant proteinase inhibition activity of 68.1% and 60.35% was shown at 500 µg/mL and 250 µg/mL in whereas the Standard drugs Aspirin (150 µg/mL) and Diclofenac sodium (100 µg/mL) showed 81.3% and 67.92% of inhibition respectively (Fig. 6).

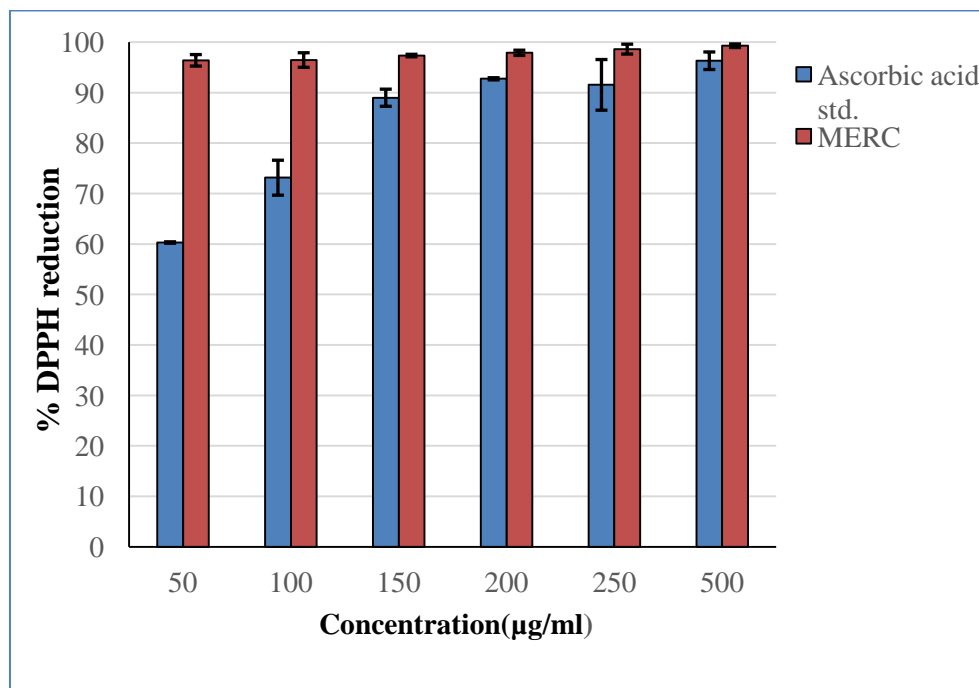


Fig. 3. DDPH radical scavenging activity

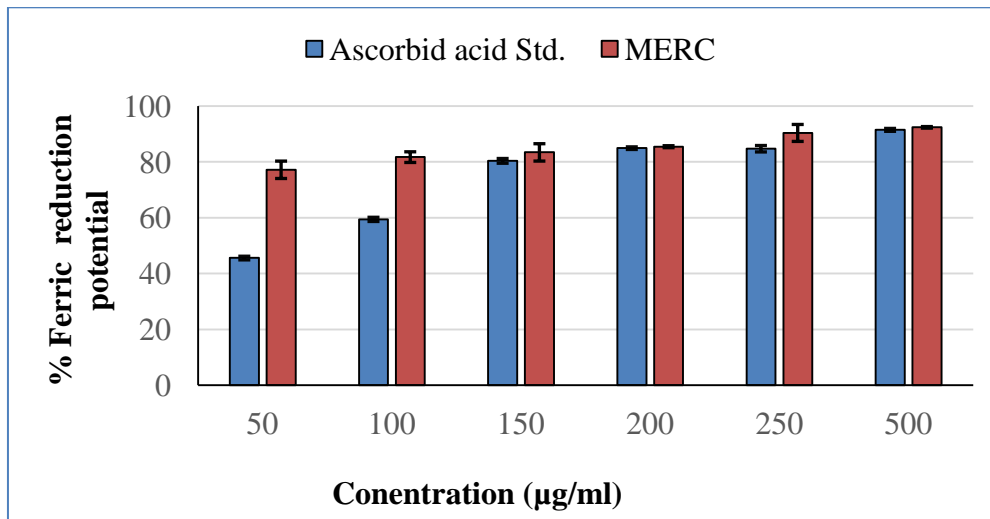


Fig. 4. FeCl<sub>3</sub> reduction potential

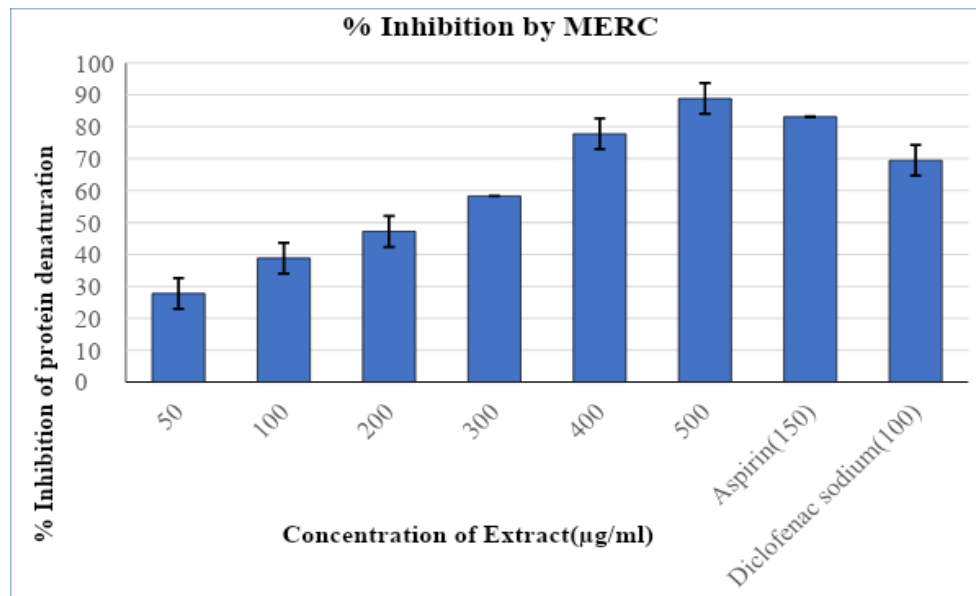


Fig. 5. Inhibition of protein denaturation

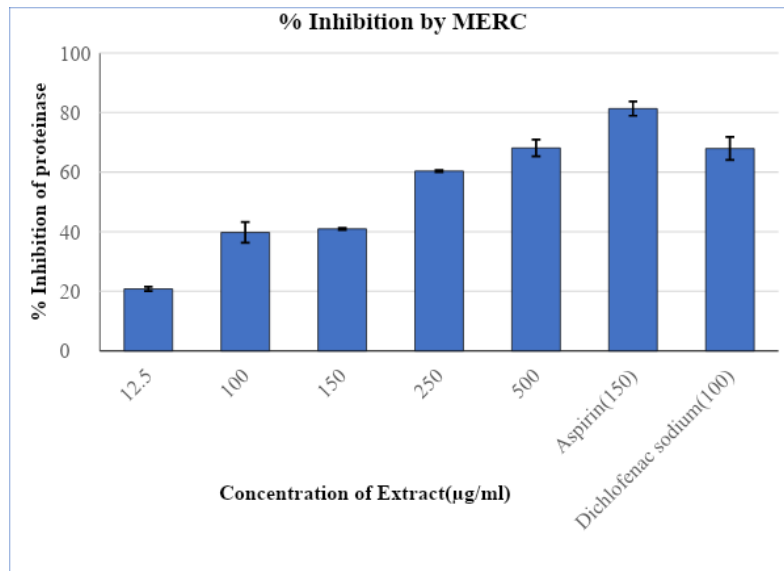
### 3.4.3 HRBC membrane stabilization assay

Membrane stabilisation assay is taken as a measure of in vitro anti-inflammatory activity [33]. The results of membrane stabilization of MERC were concentration dependent; the maximum activity was shown by 250 µg/ mL of the sample with 77.7% inhibition (Fig. 7).

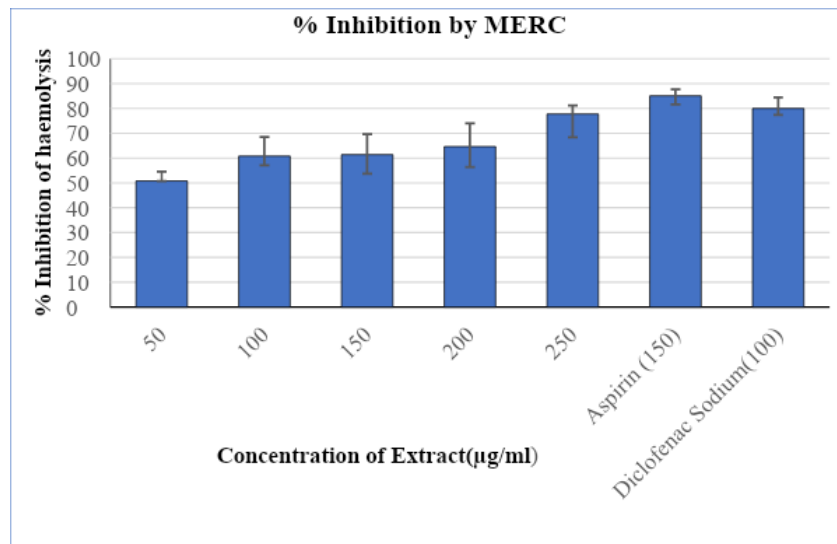
The standard drugs Aspirin (150 µg/mL) showed 85.02% of inhibition of haemolysis and Diclofenac sodium (100 µg/mL) showed 79.94% of inhibition in comparison to this the MERC showed significant results as 61.36% and 60.76% of inhibition at 150 µg/mL and 100 µg/mL

concentration. In comparison to the Standard drug the Methanolic extract of *Rhus chinensis* seed has shown significant values and anti-inflammatory activity.

The study of the *Rhus chinensis* proved to show promising results with less degree of toxicity. All the efficient drugs available in the market show some degree of toxicity in an individual due to various components present in it. The idea was to find a drug from natural source which may provide increased therapeutic care and decreased toxicity, which can be beneficial for the individual and is not compulsive.



**Fig. 6. Inhibition of Proteinase**



**Fig. 7. Inhibition of haemolysis**

Thus, *Rhus chinensis* rich in phytochemicals like flavonoids, phenolic compounds which helps in controlling all of the inflammatory mediators like histamine, heparin and various cytokines, and these components also have antioxidant and radical scavenging activity.

#### 4. CONCLUSION

The idea of the study was to find a drug from natural source which may provide increased therapeutic care, decreased toxicity and can be beneficial for the individual and is not compulsive. The antioxidant, anti-inflammatory property of methanolic extracts of *Rhus chinensis*

was evaluated efficiently using *in-vitro* studies. The study revealed the presence of flavonoids, terpenoids, phenols in the extract and shown less degree of toxicity compare to available commercial drugs used in this study. Further, by performing various experiment the results proved a wide variety of chemical ingredients which were available that might be responsible for various therapeutic activities like antioxidant, anti-inflammatory.

#### ACKNOWLEDGEMENT

The authors wish to acknowledge the Department of Biochemistry and the



management of Mount Carmel College, Autonomous, Bengaluru for providing facility to conduct this research work.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

- Halliwell Barry. Role of free radicals in the neurodegenerative diseases. *Drugs & Aging*. 2001;18(9):685-716.
- Halliwell B. Cellular responses to oxidative stress: Adaptation, damage, repair, senescence and death. *Free Radicals in Biology and Medicine*. 2007;187-267.
- Malekzadeh Kebria M, Zangiabadi I, Ghantabpour T, Heydari M. Oxidative Stress and Effect of Herbal Antioxidants in Animal Reproduction: Future and Perspective. *Plant Biotechnol Persa*. 2021; 3(2):82-91.
- Fusco Domenico, et al. Effects of antioxidant supplementation on the aging process. *Clinical Interventions in Aging*. 2007; 2(3):377-87.
- Barja Gustavo. Free radicals and aging. *TRENDS in Neurosciences*. 2004; 27(10):595-600.
- Shylaja MR, Peter KV. In *Handbook of Herbs and Spices*. 2004;2.
- Omoigui Sota. The biochemical origin of pain—proposing a new law of pain: The origin of all pain is inflammation and the inflammatory response. Part 1 of 3—A unifying law of pain. *Medical Hypotheses*. 2007;69(1):70-82.
- Coussens LM, Werb Z. Inflammation and cancer. *Nature*. 2002;420(6917):860–867.
- Weiss RB. Hypersensitivity reactions. *Semin Oncol*. 1992 Oct;19(5) 458-477. PMID: 1384149.
- Hart F Dudley, Huskisson EC. Non-steroidal anti-inflammatory drugs. *Drugs*. 1984;27(3):232-255.
- Wu T, Mc Callum JL, Wang S, et al. Evaluation of antioxidant activities and chemical characterisation of staghorn sumac fruit (*Rhus hirta* L.). *Food Chemistry*. 2013;138:1333-1340.
- Abu-Reidah, Ibrahim M, Rana M Jamous, Mohammed S Ali-Shtayeh. *Phytochemistry, Pharmacological Properties and Industrial Applications of Rhus coriaria* L. (Sumac). *Jordan Journal of Biological Sciences*. 2014;7(4).
- Lee, Kyung Jin, Myeong Gu Yeo. Homeopathic Rhus toxicodendron has dual effects on the inflammatory response in the mouse preosteoblastic cell line MC3T3-e1. *Homeopathy*. 2016;105(01):42-47.
- Barka ZB, Lahbib K, Aouadhi C, Ladjimi MH, Miled HB, Rhouma KB, Sakly M, Maaroufi A, Schneider YJ, Tebourbi O. Antioxidant, Antimicrobial and Anti-Inflammatory Activities of Edible Rhus Tripartita (Ucra) Hydromethanolic Extracts. *J Pharm Pharmacol Res*. 2019; 3:074-087.
- Ganjirenuka Rani, et al. *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*. 2017;12(3):37-40. e-ISSN:2278-3008, p-ISSN:2319-7676.
- Djakpo, Odilon, Weirong Yao. Rhus chinensis and Galla Chinensis—folklore to modern evidence. *Phytotherapy Research*. 2010;24(12):1739-1747.
- Zhang, Chengting, et al. Phenolic composition, antioxidant and pancreatic lipase inhibitory activities of Chinese sumac (*Rhus chinensis* Mill.) fruits extracted by different solvents and interaction between myricetin- 3- O- rhamnoside and quercetin- 3- O- rhamnoside. *International Journal of Food Science & Technology*. 2018;53(4):1045-1053.
- Tan Daopeng, Qixin Yan, Xiaojun Ma. Chemical constituents of *Rhus chinensis*. *Chemistry of Natural Compounds*. 2015; 51(3):542-543.
- Truong, Dieu-Hien, Dinh Hieu Nguyen, Nhat Thuy Anh Ta, Anh Vo Bui, Tuong Ha Do, Hoang Chinh Nguyen. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of Severiniabuxifolia. *Journal of Food Quality*. 2019;2019.
- Kaur, Charanjit, Harish C Kapoor. Anti-oxidant activity and total phenolic content of some Asian vegetables. *International Journal of Food Science & Technology*. 2002;37(2):153-161.
- Javanmardi J, et al. Antioxidant activity and total phenolic content of Iranian Ocimum accessions. *Food Chemistry*. 2003;83(4):547-550.
- Wuttisin, Narunan, Warakarn Boonsook. Total phenolic, flavonoid contents and antioxidant activity of siraitiagrosvenorii

- fruits extracts. Food and Applied Bioscience Journal. 2019;7(3):131-141.
23. Pękal, Anna, Krystyna Pyrzynska. Evaluation of aluminium complexation reaction for flavonoid content assay. Food Analytical Methods. 2014;7(9):1776-1782.
  24. Mishra, Krishnanand, HimanshuOjha, and Nabo Kumar Chaudhury. Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. Food Chemistry. 2012;130(4):1036-1043.
  25. Vijayalakshmi, Maruthamuthu, Kandasamy Ruckmani. Ferric reducing anti-oxidant power assay in plant extract. Bangladesh Journal of Pharmacology. 2016;11(3):570-572.
  26. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HP. *In vitro* anti-inflammatory properties of selected green leafy vegetables. Biomedicines. 2018;6(4):107.
  27. Sakat S, Juvekar AR, Gambhire MN. *In vitro* antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int. J. Pharm. Pharm. Sci. 2010;2:146–155.
  28. Leelaprakash G, Mohan Dass S. *In vitro* anti-inflammatory activity of methanol extract of *Enicostemmaaxillare*. International Journal of Drug Development and Research. 2011;3(3):189-196.
  29. Saleem, Ammara, Mohammad Saleem, Muhammad Furqan Akhtar. Antioxidant, anti-inflammatory and antiarthritic potential of *Moringaoleifera* Lam: An ethnomedicinal plant of Moringaceae family. South African Journal of Botany. 2020;128:246-256.
  30. De Subrata, Ravishankar B, Bhavsar GC. Investigation of the anti-inflammatory effects of *Paederiafoetida*. Journal of Ethnopharmacology. 1994'43(1):31-38.
  31. Shinde UA, et al. Membrane stabilizing activity—a possible mechanism of action for the anti-inflammatory activity of *Cedrusdeodara* wood oil. Fitoterapia. 1999;70(3):251-257.
  32. Lallianrawna, Samuel, et al. Determination of total phenolic content, total flavonoid content and total antioxidant capacity of *Ageratinaadenophora* (Spreng.) King & H. Rob. Science Vision. 2013;13(4):149-156.
  33. Xu Baojun J, Chang SKC. A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. Journal of Food Science. 2007;72(2):S159-S166.
  34. Anosike, Chioma A, Onyechi Obidoa, Lawrence US Ezeanyika. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanumaethiopicum*). DARU Journal of Pharmaceutical Sciences. 2012; 20(1):1-7.

© 2022 Rao et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*

*The peer review history for this paper can be accessed here:*  
<https://www.sdiarticle5.com/review-history/89601>