Evaluation of Total Phenolic, Total Flavonoid Content And Antioxidant Activity of Rhus Vulgaris

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Evaluation of Total Phenolic, Total Flavonoid content and Antioxidant activity of Rhus vulgaris

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Abstract

Background: *Rhus vulgaris* is rich in various classes of polyphenols and flavonoids that act as free radical scavengers and reduce oxidative stress and cure various harmful human diseases. *The plant* is a traditionally known medicinal plant which is used against a number of diseases including cancer.

Methods: The current investigation points towards the investigating quantitative phenolic contents, flavonoid contents and the free radical scavenging activity & antioxidant activity of *Rhus vulgaris* extract in n-hexane, acetone, and 80% of aqueous methanol. The phenolic contents were estimated by Folin–Ciocalteu procedure and gallic acid as a reference molecule whereas the total flavonoid contents were determined by using aluminum chloride and catechin. In addition, the dilution serial method was used to evaluate the leaves extract of *rhus vulgaris*, and the DPPH (2,2-diphenyl-1-picrylhydrazyl) method was utilized to assess the above-mentioned extracts against oxidative stress.

Results: The result revealed that the ranges of total phenolic content from 5.82 ± 4.6 to 83.15 ± 7.6 mg GAE/g of the dry weight of extract, expressed as gallic acid equivalents. The total flavonoid concentrations were varied from 2.21 ± 7.34to 23.47 ± 4.87 mg CE/g, expressed as catechin equivalents. Antioxidant activity of extracts was expressed as the concentration of DPPH radical's inhibition ranges from 1.2 ± 0.32 to 22.86 ± 3.71 mg AAE/ g.

Conclusion: The 80% aqueous methanolic extract of *Rhus vulgaris* showed the highest phenolic and flavonoid contents and strong antioxidant potential and it could be used as antibiotics for different curable and incurable diseases.

Keywords: *Rhus vulgaris*, Total phenolic contents, Total Flavonoid contents, Antioxidant activity

1. Introduction

Phenolic compounds are associated with a high number of biological activities and one with special interest is their antioxidant capacity and may help to protect the cells against the oxidative damage caused by free radicals[1]. Antioxidants are compounds that can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction. Synthetic antioxidants are widely used, but their use is being restricted nowadays because of their toxic and carcinogenic side effects [2].

Sumac is the common name for a genus (Rhus) that contains over 250 individual species of flowering plants in the family Anacardiaceae[3]. Traditional species of *Rhus* used to manage several ailments including influenza, wounds, diarrhea, abdominal pain, indigestion, diabetes, malaria, rheumatism, aching gums, toothaches, swollen legs, dog bites, peptic ulcer, kidney stones, skin eruptions, bruises, and boils [5,6]. *R. vulgaris* in Ethiopia is used to treat various diseases according to Ethno-botanically or Ethno-pharmacological studies? For instance, it is used in the treatments of diarrhea, gonorrhea, inflammation, evil eye, wound, and lung TB in the Amhara region[7,8].

Previous studies on phytochemical investigation of the stem bark of *Rhus vulgaris* revealed the presence of secondary metabolites such as tannins, saponins, flavonoids, terpenoids, glycosides, alkaloids, and phenol. *R. vulgaris* methanolic extract (1000 mg/kg) showed greater anti-inflammatory activity compared to indomethacin (10 mg/kg), the standard anti-inflammatory drug, with a decrease in inflammation for up to 90 min. The dichloromethane, ethyl acetate, and

aqueous extracts of *R. vulgaris* stem bark, root, and leaves have exhibited moderate to toxic toxicity against brine shrimp with LC50 values ranging from 3.55 μg/ml to 734.06 μg/ml while cyclophosphamide, the positive control, demonstrated an LC50 value of 15.28 μg/ml [9]. The other reports describe isolations of new biflavonoids on the genus *Rhus*. Such as agathisflavone, amentoflavone, hinokiflavone, *rhus* flavanone, and succedanea flavone have been sourced from *Rhus* species and evaluated for activity against a range of pathologically significant viruses [10,11].

The present work to conduct a quantitative analysis of the antioxidant activity, total phenolic content, and total flavonoids in hexane, acetone, and 80% aqueous methanolic extracts of different varieties of *Rhus vulgaris* leaves and to correlate the total phenolic contents and flavonoid contents with the antioxidant activities as such comparative antioxidant study of these different varieties of the genus *Rhus* has been reported.

2. Material and methods

2.1. Chemicals and reagents

Folin & Ciocalteu's Folin-Ciocalteu reagent was obtained from Loba Chemie (India), Sodium hydroxide (NaOH), aluminum chloride (AlCl3.6H2O), $AlCl_3$, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Catechin, ascorbic acid (AA), Sodium nitrite ($NaNO_2$), and gallic acid were procured from QualiChem (India), Sodium carbonate (Na_2CO_3). All chemicals used were of analytical grade.

2.2. Plant materials

Fresh and healthy leaves of *R. vulgaris* were collected from a local farm in the Region of Amara; Central Gondar city in February 2020. The plant was taxonomically identified by the Department

of Biology (Botany), University of Gondar. The plant was washed thoroughly with tap water to remove dust particles from the leaves. The collected plant material was dried under shade at room temperature for 10 days. Then the dried leaves were powdered using an electrical grinder uniformly and kept in a dry & cool place for further use.

2.3. Extraction procedure

The exhaustive maceration was used to extract *Rhus vulgaris* plant materials by soaking 1 g of powdered samples sequentially into 25 ml of *n*-hexane, acetone, and 80% aqueous methanol in an enclosed glass bottle with occasional shaking at room temperature for 24 hrs. Then the extracts were filtered through a Whatman filter paper No. 1. The extracts were concentrated using a rotary evaporator then stored at -4ºC for further usage.

2.4. Determination of total phenolic content

The concentration of total phenol present in *Rhus vulgaris* extracts was determined by Folin–Ciocalteu (FC) reagent method described by Munro et al. [1].

In brief, (0.50) ml of each of the following, namely: 80% aqueous methanol solution, acetone, and n-hexane were poured into the three different test tubes. Then, 3ml of distilled water and 250µL of Folin-Ciocalteu reagent were added and hand shaken. After 5 minutes, 1 ml of 7.5 % sodium carbonate was added. It was allowed to incubate for 90 min at room temperature. The intense blue color was developed due to the reaction between the phenols and Folin-Ciocalteu reagent. After incubation, absorbance was measured at a 760 nm spectrophotometer using a UV/Visible spectrophotometer. The amounts of total phenolic compounds in the *Rhus vulgaris* extract were determined using equation 1.

The total phenolic content was calculated from the calibration curve using gallic acid as a standard and the results were expressed as milligram of gallic acid equivalents per gram dry weight of extract (mg GAE/g DW). All of the studied samples were tested in triplicate for each extract and the mean values of absorbance were reported. The same method was repeated for the standard molecule of gallic acid, and then the required calibration curve was constructed (Figure 1).

$$TPC = \frac{C.V.DF}{m} \quad \text{..... (1)}$$

Where:

- TPC = the total content of phenolic compounds, mg/g plant extract in GAE
- C = concentration of gallic acid obtained from the curve (mg/L)
- V= the volume of the sample solution (L)
- m= mass of extract in grams.
- DF= Dilution factor

2.5. Determination of total flavonoid contents

Catechin was used as a standard to express total flavonoid contents of samples as mg catechin equivalent per gram of dry weight (mg CE/g of DW)[12]. The amounts of total flavonoid in the extract were determined as follows: Briefly, an aliquot (0.50 ml) of each plant extract was added to a 10 ml volumetric flask containing 4 ml of distilled water. To this flask, 0.3 ml of 5% $NaNO_2$ was added. After 5 min of incubation, 0.3 ml of 10% $AlCl_3$ was added. At the 6^{th} min, 2 ml of 1 M NaOH was added, and finally distilled water was added till the mark. An Orange yellowish color was developed. After 10 min of incubation, the absorbance was measured at 510 nm using a UV- vis spectrophotometer. Each sample was tested in triplicate. The total flavonoid contents in the *extract* were determined using equation 2.

$$TPC = \frac{C.V.DF}{m} \quad \text{..... (2)}$$

where:

- TFC = the total content of flavonoids compounds, mg/g plant extract in CE
- C = concentration of catechin obtained from the curve (mg/L)
- V = the volume of the sample solution (L)
- m = mass of extract in grams.
- DF = Dilution factor

2.6. Determination of antioxidant activity-DPPH

The 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH) radical scavenging assay is an easy, rapid and sensitive method to screen the antioxidant activities of plant extracts**.** Several methods are available for the determination of free radical scavenging activity but the assay employing the stable DPPH has received great attention owing to its ease of use and convenience [13].

A solution of 0.1 mM DPPH in 80% of aqueous methanol was prepared, and 1.6 mL of DPPH solution was mixed with different concentrations of the extract in 80% of methanol (0.05, 0.1, 0.4, 0.8, 1.6, and 2.4 μg/ml). Afterward, the volume of each solution was adjusted to 4 ml with 80% aqueous methanol and the incubation was made for 30 min at room temperature in a dark cupboard. The control solution was prepared by mixing 3 ml of 80% aqueous methanol and 2 ml of DPPH solution. A stock solution of ascorbic acid (1000 mg/L) in 80% aqueous methanol was prepared; from which 500 mg/L of the intermediate standard ascorbic acid solution was prepared. A series of the standard ascorbic acid solution, viz. (1, 2.5, 5, 10, 15, 20, 25, 50, 100, 150, 200, 500 mg/L) were then prepared and 2.4 ml from each of these solutions were mixed with 1.6 ml of DPPH and incubated at room temperature for 30 min. A mixture of 2.4 ml 80% aqueous methanol and 1.6 ml of DPPH was used as a blank solution. The reaction was carried

out in triplicate and the decrease in absorbance was measured at 517 nm using a UV-vis spectrophotometer with slight modification. The percent in inhibition is calculated based on equation 3.

$$\% \text{ Inhibition} = \frac{(A_{Control} - A_{Sample}) \times 100\ \%}{A_{Control}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where $A_{control}$ is the absorbance of DPPH solution without extract and A_{sample} is the absorbance of a sample with DPPH solution.

3. Result and discussions

3.1. Total polyphenols

The results obtained from the assay were expressed as means ± standard deviation of triplicate analyses and are presented in Table 1

Table 1. Extraction yield, TPC, TFC, and antioxidant activity of n-hexane, acetone, and 80% of methanol the leaves extract of *Rhus vulgaris*.

Extracts	Total Phenolic Content (mg GAE/g of DW)	Total Flavonoid Content (mg CE/g)	DPPH Inhibition (mg AAE/ g)
n-hexane extract	5.82 ± 4.6	2.21 ± 7.34	1.2 ± 0.32
Acetone extract	43.23 ± 6.98	12.56 ± 0.12	4.63 ± 5.43
80 % aqueous methanol extract	83.15 ± 7.6	23.47 ± 4.87	22.86 ± 3.71

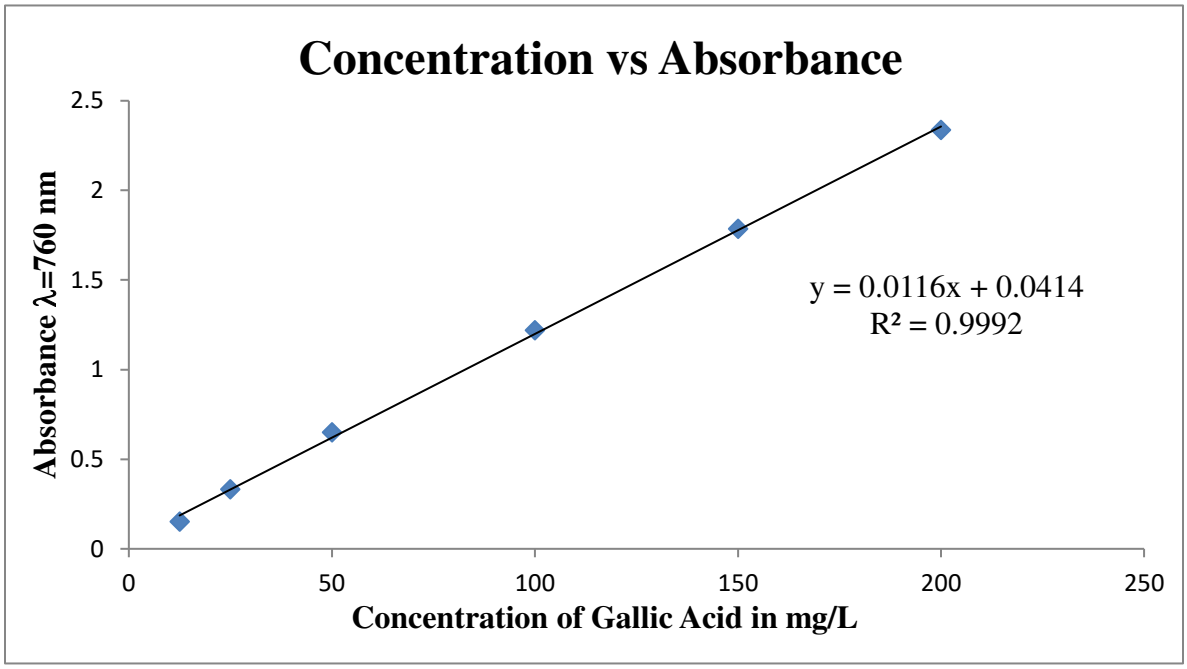
Key: - GAE = gallic acid equivalent; CE = Catechin Equivalent; TFC = total flavonoid content; TPC = total phenol content; AAS= Ascorbic Acid Equivalent; DPPH= 2, 2-diphenyl-1-picryl-hydrazyl radical.

The total phenolic content in n-hexane extract, acetone extract, and 80% of aqueous methanolic extract was 5.82 ± 4.6 mg GAE/g, acetone, extract 43.23 ± 6.98 mg GAE/g, and 83.15 ± 7.6 mg

GAE/g respectively. The highest value of total phenol was obtained in the aqueous methanol extract.

The amount of total phenolic content *in R. vulgaris* samples collected from *Rhus vulgaris* was influenced significantly by extracting solvent ($p < 0.05$), and the contents were varied within the range of 5.82 to 83.15 mg of GAE/100 g in dry weight for n-hexane to 80% aqueous methanol, respectively. Among the solvents, 80% aqueous methanol was the most efficient extracting solvent for TPC, followed by acetone, and n-hexane, indicating that the TPC extracted in ginger was higher in polar solvents compared with less polar solvents (Table 1).

The amount of total phenol was done based on the standard calibration plot of gallic acid. It was constructed in the concentration range of (12.5-200) mg/L and the coefficient of determination (R^2) was found to be 0.9992 (**Figure 1**). Based on the standard plot of gallic acid



(y=0.0116x+0.0414).

Figure 1. Standard curve of gallic acid for the determination of total phenolic content.

Phenolic compounds are known as powerful antioxidants due to their ability to scavenge free radicals, singlet oxygen, and superoxide radicals. The radical scavenging activity is attributed to hydroxyl groups replacing the aromatic ring of the phenolic components [14]. Various investigations have linked a high phenolic diet to the prevention of several cardiovascular, metabolic, infectious, and cancer diseases due to their pharmacological and biological activities [15].

The high phenolic content recorded in this study in the presence of methanol fraction *is* consistent with the research in [16], which reported that the highest phenolic content in the methanol extract of *R.vulgaris.* The result is responsible for powerful antioxidants due to their ability to scavenge free radicals, singlet oxygen, and superoxide radicals. Other studies also reported phenolic contents in plant extracts. The methanolic extract of leaves, stem, and fruit of the *Rhus pentaphylla were* 71.16, 141.79, 76.88, and *Rhus tripartite* 64.13, 108.83, and 75.16 respectively in (mg GAE g^{-1} DW) [17]. On the contrary, the ethanolic extracts of *Rhus javanica* from leaves (51.91±0.01), steam (28.5 ± 0.03), greenish fruit (53.66 ± 0.01), and blackish-grey fruit (32.74 ± 0.03) using Gallic acid standards [18] is less than the value obtained in this study. This difference could be the difference in the species, environmental (e.g., season, temperature, light, soil), and genetic factors that determine the variation of the antioxidant activity of these compounds.

This finding indicates that 80% of aqueous methanolic extract of *R. vulgaris* can be used as a suitable candidate in the manufacturing of natural antioxidants and synthetic antibiotics. In line with previous studies, the current study found a strong positive correlation between antioxidant activities of plant extracts and total phenolic content, which implies that the studied plant's phenols are the major contributor to the antioxidant activity in these extracts.

3.2. Total flavonoids

The total content of flavonoids in 80% of methanol, acetone, and n-hexane extract of *R. vulgaris* was determined from the regression equation of the calibration curve (, R^2 = 0.9987) and expressed as milligrams of catechin equivalents (CE). In the case of flavonoids, high content was observed in 80% of aqueous methanol extracts (23.47 ± 4.87 CE/100g of DW) and acetone extracts (12.56 ± 0.12), whereas n-hexane has the lowest content (2.21 ± 7.34 CE/100g). It was observed that the effect of solvents on TFC is similar to that on TPC.

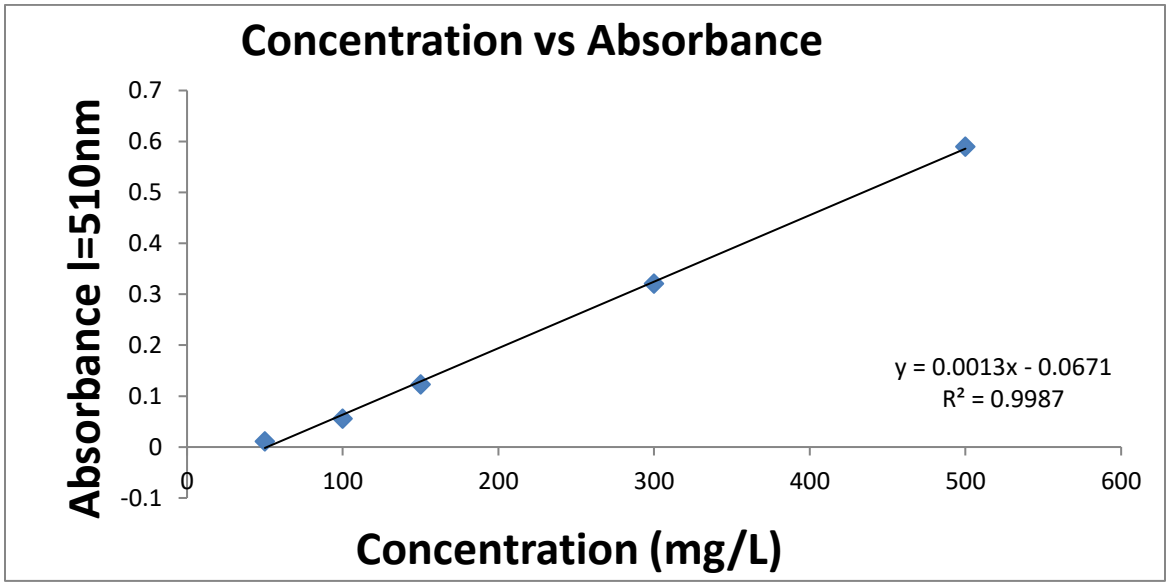


Figure 2. Standard curve of Catechin for the determination of total flavonoids.

Flavonoids are naturally occurring polyphenol compounds with benzo-pyrone structures. The flavonoid content was determined by the aluminum chloride method. Aluminum chloride forms acid-stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxide group of flavones and flavonols [19]. The total flavonoids of *R. vulgaris* were also compared with the species of *Rhus javanica* from leaves (17.72±0.02), steam (4.94±0.04), greenish fruit (9.1±0.012), and blackish-grey fruit (11.0± 0.02). RL exhibited the highest total flavonoid

content (17.78±0.002 mg/ 1g of extract), whereas RBF contained a lower amount of flavonoid (11.3±0.02 mg/ 1 g of extract), followed by RGF (9.4±0.012 mg/1g of extract), and RS (5.06±0.004mg/ 1 g of extract) using the calibration of Quercetin standard. Other reports showed that the species of *Rhus tripartite* leaves *58.91,* stem cortex *9.66, and* Fruit *9.96* and *Rhus pentaphylla* leaves *62.91,* steam cortex 5.88 and 10.41 total flavonoids were expressed as mg Rutin equivalents per gram of extract (mg RE g^{-1} DW).

3.3. Total antioxidant activity

The calibration curve was plotted as percentage inhibition versus different concentrations of ascorbic acid and the value of the absorbance obtained corresponding to concentrations. The percentage inhibitions against concentration are given in **Figure 3**. Ascorbic acid equivalent antioxidant capacity is expressed as mg of AAE per 100g of *Rhus vulgaris* leaves extracts.

The free radical-scavenging activity of *R. vulgaris* leaves extract, measured by DPPH assay, is shown in Table 1. It was observed that DPPH free radical-scavenging activity of the extract (1.2, 4.63, and 22.86 mg AAE/g n-hexane, acetone, and 80% aqueous methanol respectively) due to the presence of a relatively higher value of total phenol and total flavonoid contents as shown in **Figure 3**. Its percent inhibition was increased with increasing concentration. This means, as the concentration increased, the DPPH radical scavenging activity by the extract was also increased. The concentration and absorbance of the sample are inversely related. The absorbance was decreased as the concentration of the sample increased. Except for n-hexane and acetone extracts, there were significant differences ($p < 0.05$) of DPPH radical scavenging abilities between all extracts.

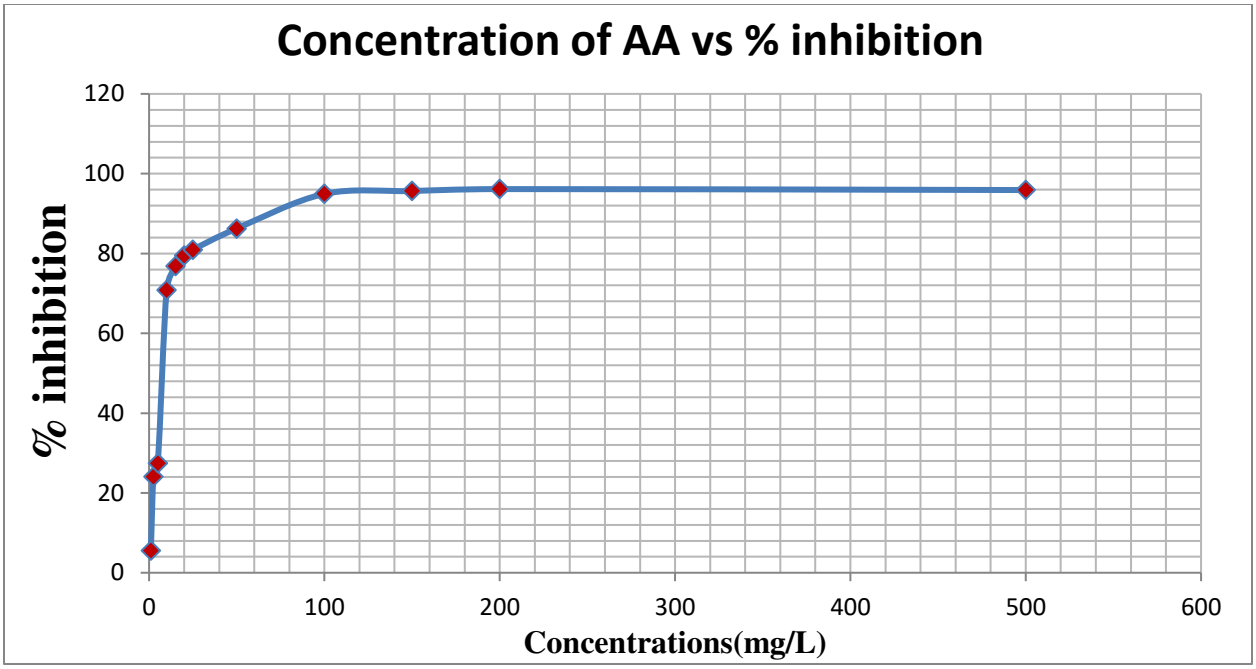
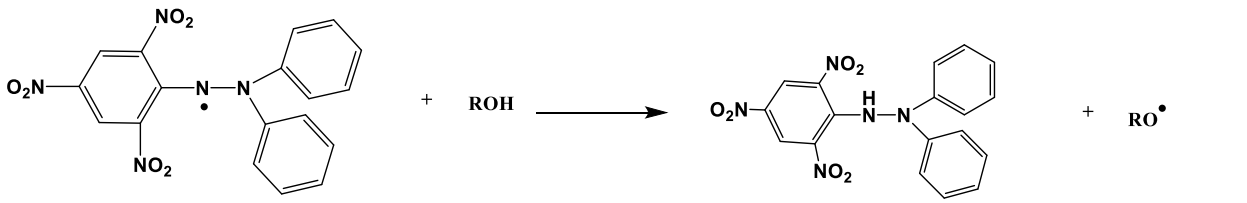


Figure 3. Calibration curve of Ascorbic acid standard, showing IC_{50} value.

DPPH method was developed by Blois (1958) with the viewpoint to determine the antioxidant activity in a like manner by using a stable free radical 2,2-diphenyl-1- picrylhydrazyl (DPPH; $C_{18}H_{12}N_5O_6$, M=394.33). The assay is based on the measurement of the scavenging capacity of antioxidants towards it. As shown in **Fig.4** the odd electron of a nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine [20]. DPPH is a stable free radical which presents a deep purple color and a strong absorption band in the range of 517 nm. In the presence of antioxidant compounds, DPPH can accept an electron or a hydrogen atom from the antioxidant scavenger molecule, to be converted to a more stable DPPH molecule. As the reduced form of DPPH is pale yellow, it is possible to determine the antioxidant activity by studying the change of color spectrophotometrically. The greater the free radical scavenging capacity of an antioxidant compound, the more reduction of DPPH and the less purple color there is in the sample [21].



1: Diphenylpicrylhydrazyl (Free radical) **2: Diphenylpicrylhydrazyl (Non- radical)**

Figure 4. Reduction of DPPH by antioxidants.

Previous studies showed the plants of the genus *Rhus* used in this study, namely *Rhus typhina* and *Rhus javanica* reported. The result of *Rhus typhina* is much better than DPPH radical-scavenging activities reported by on the ethanol fruit extract (3.99, 6.67, and 81.86% at 12.5, 25, and 50 mg/ml, respectively) [4] and the ethanolic extracts of *Rhus javanica* using DPPH the IC_{50} values leaves (157.699), steam (561.046), greenish fruit (189.31), and blackish-grey fruit (239.276) using Gallic acid standards [18]. Our results confirm those of Odongo et al.(2017) reporting substantial antioxidant activity plant parts from this species. They have displayed good antioxidant activity because they contain phenols, flavonoids, and tannins [16].

4. Conclusion

In the present study, the phenolic, flavonoid, and antioxidant activity was evaluated from *Rhus vulgaris. Rhus vulgaris* possessed high phenolic and flavonoid content and exhibited good antioxidant activity by DPPH. It is noticed that the highest concentration of phenolic compounds in the extracts were obtained using solvents of high polarity; 80% aqueous methanolic extract manifested greater power of extraction for phenolic compounds from *Rhus vulgaris.* The high contents of phenolic compounds and significant linear correlation between the values of the concentration of phenolic compounds and antioxidant activity indicated that these compounds contribute to the strong antioxidant activity. The results of this work indicated that *Rhus vulgaris*, when a proper extraction solvent is established, could serve as a medicine against free-radical-associated oxidative damage. further investigations on the different mechanisms shall be studied further.

Ethics approval and consent to participate

Approval to carry out this work was obtained from the University of Gondar Scientific and Research Center of the University of Gondar.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AA - Study design, Literature search, data collection, data analysis, data interpretation, writing manuscript, GG - Research supervision, Study design, editing manuscript, DS – Research supervision, editing manuscript, ML- Research supervision, Provision of reagents and materials used in the study. All authors read and approved the final manuscript.

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5. Reference

[1] Ezeiza D, Tefera M. Effects of Solvents on Total Phenolic Content and Antioxidant Activity of Ginger Extracts. J Chem 2021; 2021: 1–5

[2] Plaza CM, De Torres LED, Lücking RK, et al. Antioxidant activity, total phenols and flavonoids of lichens from Venezuelan Andes. J Pharm Pharmacogn Res 2014; 2: 138–147

[3] Mohamed, Khedr FG, Mohammed EI. Phenolic Compounds, Antioxidant and Antibacterial Activities of Rhus flexicaulis Baker. 2019; 12: 17–21

[4] Korah R, Zhang H, Chen W. Antimicrobial and antioxidant activities of Chinese sumac (Rhus typhina L.) fruit extract. Food Control 2011; 22: 128–132 I'm Internet: http://dx.doi.org/10.1016/j.foodcont.2010.06.002

[5] Njoroge PW, Opiyo SA. Antimicrobial Activity of Root Bark Extracts of Rhus natalensis and Rhus Rispoli. Basic Sci Med 2019; 8: 23–28

[6] Alam P, Parvez MK, Arbab AH, et al. Inter-species comparative antioxidant assay and HPTLC analysis of sakuranetin in the chloroform and ethanol extracts of aerial parts of Rhus rhinorrhoea and Rhus tripartita. Pharm Biol 2017; 55: 1450–1457 I'm Internet: https://doi.org/10.1080/13880209.2017.1304428

[7] Gebeyehu G, Asfaw Z, Enyew A, et al. Ethnobotanical study of traditional medicinal plants and their conservation status in Mecha Woreda. Int J Pharm Heal Care Res 2014; 02: 137–153

[8] Seble WY, Zemede A, Ensermu K. Ethnobotanical study of medicinal plants used by local people in Menz Gera Midir District, North Shewa Zone, Amhara Regional State, Ethiopia. J Med Plants Res 2018; 12: 296–314

[9] Mutuku A, Mwamburi L, Keter L, et al. Evaluation of the antimicrobial activity and safety of Rhus vulgaris (Anacardiaceae) extracts. BMC Complement Med Ther 2020; 20: 1–12

[10] Abegaz BM. Novel phenyl anthraquinones, isoflurano naphthoquinones, homoisoflavonoids, and biflavonoids from African plants in the genera Bulbine, Scilla, Ledebouria, and Rhus. Phytochem Rev 2002; 1: 299–310

[11] Mdee LK, Yeboah SO, Abegaz BM. Rhus chalcones II-VI, five new bichalcones from the root bark of Rhus pyroides. J Nat Prod 2003; 66: 599–604

[12] Haile K, Mehari B, Atlabachew M, et al. Phenolic composition and antioxidant activities of cladodes of the two varieties of cactus pear (opuntia ficus-indica) grown in Ethiopia. Bull Chem Soc Ethiop 2016; 30: 347–356

[13] Abebe M, Abebe A, Mekonnen A. Assessment of antioxidant and antibacterial activities of crude extracts of Verbena officinalis Linn root or Atuch (Amharic). Chem Int 2017; 3: 172–184

[14] Farahmandfar R, Esmaeilzadeh Kenari R, Asnaashari M, et al. Bioactive compounds, antioxidant and antimicrobial activities of Arum maculatum leaves extracts as affected by various solvents and extraction methods. Food Sci Nutr 2019; 7: 465–475

[15] Khalid A. Shadid , Ashok K. Shakya , Rajashri R. Naik , Nidal Jaradat , Husni S. Farah , NaeemShalan , NoomanA.Khalaf AO. Phenolic Content and Antioxidant and Antimicrobial Activities of Malva sylvestris L., Malva oxyloba Boiss., Malva parviflora L., and Malva aegyptia L. Leaves Extract Khalid. J Chem 2021; 2021: 1–10

[16] Odongo E, Mungai N, Mutai P, et al. Antioxidant and anti-inflammatory activities of selected medicinal plants from western Kenya. African J Pharmacol Ther 2017; 6: 178–182

[17] Itidel C, Chokri M, Mohamed B, et al. Antioxidant activity , total phenolic and flavonoid content variation among Tunisian natural populations of Rhus tripartita (Ucria) Grande and Rhus pentaphylla Desf . Ind Crop Prod 2013; 51: 171–177 Im Internet: http://dx.doi.org/10.1016/j.indcrop.2013.09.002

[18] Anggraini NB, Elya B. Anti-elastase , Antioxidant , Total phenolic and Total Flavonoid Content of Macassar Kernels (Rhus javanica L) from Pananjung Pangandaran Nature Tourism Park- Indonesia. J Nat Remedies 2020; 20: 61–67

[19] Kamtekar S, Keer V, Patil V. Estimation of phenolic content, flavonoid content, antioxidant and alpha amylase inhibitory activity of marketed polyherbal formulation. J Appl Pharm Sci 2014; 4: 61–65

[20] Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. J Food Technol 2011; 48: 412–422

[21] Carmona-Jiménez Y, García-Moreno MV, Igartiburu JM, et al. Simplification of the DPPH assay for estimating the antioxidant activity of wine and wine by-products. Food Chem 2014; 165: 198–204