Phytochemical analysis and determination of antioxidant and antibacterial activities of extracts of leaf and fruit of Ruta chalepensis (tenadam)

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Research Article

Keywords: Ruta chalepensis,Total phenolic content, Total flavonoid content, antioxidant

Posted Date: July 15th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1822359/v1

Abstract

Background: *Ruta chalepensis* is a plant belongs to the family Rutaceae, originally native to the Mediterranean region. In Ethiopia, it is used as a flavoring agent in foods and beverages and also used as food preservative. It is also used as antisplasmodic, diuretic, sedative, and analgesic effects, and externally for its antirheumatic effect for treatment of menstrual problems as well as it is used as antiinflammatory, antifungal, antibacterial, antiplatelet, antihelminthic agent.

Results: Phytochemical analysis of the methanol extracts of the fruit and leaf of the *Ruta chalepensis* showed the presence of some important bioactive compounds including flavonoids, alkaloids, steroids, etc. In addition to this, the extracts also showed good total phenolic content, total flavonoid content, antioxidant activity and antibacterial activities. For example, the total phenolic content of leaf and fruit extracts of *R. chalepensis* is 142.159 ±0.02 and 156.477 ±0.15 mgGAE/g dry weight respectively whereas the total flavonoid content of the extracts is 118.246±0.059 and 135.952± 0.116 mgQE/ g of dry weight respectively.

Conclusions: Fruit and leaf extracts of *R. chalepensis* showed a good total phenolic and total flavonoid contents. The two extracts also showed weak to moderate antibacterial activities against both gram positive and gram negative bacteria at higher concentrations.

Introduction

Conventional medication has remained as the foremost reasonable and effortlessly accessible source of treatment within the essential wellbeing care framework of asset for poor communities. The nearby individuals have a long history of conventional plant utilization for restorative purposes [1]. The ethnobotany gives a wealthy asset for natural drug research and improvement [2]. *Ruta chalepensis* may be a plant belongs to the family Rutaceae, originally native to the Mediterranean region [3]. It is evergreen shrubs with bluish-green takes off that transmit an effective odor and have a biting taste. It is additionally characterized by an especially wide range of distinctive plant constituents [4]. In Ethiopia, it is used as a flavoring agent in foods and beverages and also used as food preservative. In traditional medicine, it is used for its antisplasmodic, diuretic, sedative, and analgesic effects, and externally for its anti-rheumatic effect for treatment for menstrual problems, anti-inflammatory, antipyretic, antifungal, antibacterial, antiplatelet, antihelminthic agent, and also as food flavoring agent [5]. It also recommended in herbal treatment of insomnia, headaches, nervousness, abdominal cramps, and renal troubles. *Ruta* species contain many bioactive compounds including flavonoids, phenolic compounds like ellagic acid, sulphoraphane, limonene, indoles, allium compounds, alkaloids and tannins [6]. Herein we reported the comparison of the determination of antioxidant and antibacterial activities of the extracts of fruit and leaf of *R. chalepensis* along with the total flavonoid and phenolic contents.

Experimental

Chemicals and reagents

The chemicals and reagents used for this study were distilled water, deionized water, methanol, 10% ferric chloride, Wagner's reagent (Iodine in potassium iodide), aluminum chloride ($AlCl_3$), sodium nitrite ($NaNO_{2)}$, hydrochloric acid (HCl), sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), nitric acid (HNO_3), sodium carbonate, iodine, NaH_2PO_4, Na_2HPO_4, phosphoric acid, sodium molybdate, sodium tungstate, trichloroacetic acid, potassium hexacyanoferrate (II), iron chloride, bromine, ascorbic acid, Gallic acid, DPPH, quercetin and ammonia solution.

Plant materials

Fresh leaves and fruits of *Ruta chalepensis* were collected from Addis Kidame, which is located in Awi administrative zone and 100 km away from Bahir Dar, capital city of Amhara Regional State, Western Ethiopia in May 2019. The plant material was identified and authenticated by a botanist at biology department, Bahir Dar University, Bahir Dar, Ethiopia. The plant materials were collected according to the national guide line of the country as well as the plant materials were collected with the permission of the Amhara Regional State Agricultural Bureau.

Extraction of the plant materials

The fresh leaves and fruits of *R. chalepensis* were dried at room temperature for a week under a shed. The dried plant materials were ground to a powder and 200 g of powdered leaves and 200 g of fruits were extracted using 1000 mL of methanol each. The extracts were filtered by Whitman (no.1) filter paper and the extract was concentrated using rotary evaporator under reduced pressure at 40 ℃ to obtain the crude extracts.

Phytochemical analysis

The phytochemical analysis of methanol extract of the leaves and the fruits of *R. chalepensis* was done by slight modifications based on the standard procedures described on different studies and literatures [7.8.9].

Determination of total phenolic Content

Total phenolic content for the methanol extract of the fruits and the leaves of *R. chalepensis* was analyzed by using Folin-Ciocalteu method as described before with slight modification [10]. In this strategy, 5 mg of the methanol extract of the test was weighed independently and broken up in 10 mL of distilled water. 1 mL of this arrangement was transferred to isolated test tube and mixed with 5 mL of distilled water and at that point 0.5 mL of Folin-Ciocalteu reagent was included. After 5 min, 1.5 mL of 20% of Na_2CO_3 arrangement was included and eventually the volume was made up to 8 mL with distilled water and at last incubated for 60 minutes at room temperature. After incubating, the absorbance of the extract against the clear was measured at 765 nm. These information were utilized to estimate the entire phenolic substance by using a standard calibration curve gotten from different concentrations of Gallic acid (20, 40, 60 and 80 µg/mL) and it was expressed as Gallic acid equivalent.

Determination of total flavonoid content

The total flavonoid content of the extract of the plant material was analyzed by using aluminum chloride assay as described before in different studies [11]. By using this method, 1 mL of methanol extract of the sample (2 mg/4 mL) was diluted with 2 mL of water in separate test tube. Then 0.3 mL of 5% $NaNO_2$ solution was added to the mixture and after 5 min, 10% of 0.3 mL $AlCl_3$ was added and allowed to stand for 6 min. 2 mL of 1M NaOH and 2.4 mL of water were added to the reaction flask and mixed well. Absorbance of the mixture was measured at 510 nm against a blank and the data were used to estimate the total flavonoid content using a standard calibration curve obtained from various concentrations of quercetin (20, 40, 60 and 80 µg/mL). The analysis was performed in triplicate and total flavonoid content was determined as quercetin equivalents.

Antioxidant capacity assay

This assay was determined by using two different ways as described and briefly explained below. These include

DPPH radical scavenging assay

Free radical scavenging assay of the extracts was determined by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) by measuring the reduction of the absorbance of the extracts by using a UV spectrophotometer at 517 nm as described before by different studies [12]. In this study, the extracts of the fruit and leaf of *R. chalpepensis* were prepared in the amounts of 25, 50, 100 and 200 µg/mL and the assay mixture contains a total volume of 6 mL which consists of 4.5 mL of the extract and 1.5 mL of freshly prepared DPPH solution (1 mM in methanol).The contents were mixed vigorously in a vortex mixer for 10 seconds and incubated at room temperature in the dark for 30 minutes and then measured at 517 nm and the experiment was performed in triplicates. In each experiment, the test sample alone in methanol will be used as control (blank).

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was determined by using ascorbic acid as a standard and the results were expressed as percentage inhibition as described before in different studies [13]. In this study, methanol extracts of fruits and leaves of *R. chalpepensis* were mixed with PBS (Phosphate Buffered Saline) and potassium ferric cyanide [$K_3Fe(CN)_6$] complex and then incubated at 50°C for 20 minutes. The assay was done by using 2.5 mL of different concentrations of the extracts and standards (10, 20, 40, 80 ppm) which were mixed with phosphate buffer (2.5 mL, 0.2 M, p^H = 6.6) and potassium ferricyanide (2.5 mL, 1%). This was incubated at 50°C for 20 minutes. After the incubation, 2.5 mL of 10% trichloroacetic acid was added to a mixture and then 4 mL of the reaction mixture was mixed with distilled water (4 mL) and ferric chloride (0.5 mL, 0.1%) solution. The absorbance of the solution was measured at 700 nm and the experiment was performed in triplicates.

Antimicrobial activity assay

Antimicrobial assay was determined by using agar well diffusion method in microbiology laboratory of biology department at Bahir Dar University. Four bacteria were used for the determination in which two of them were gram negative bacteria (*E. coli* and *S. typhi*) and the remaining two were gram positive bacteria (*S. aurous* and *S. pyogenes*). By using the method, a series of plant extracts with concentrations of (100, 200, 400, 800 ppm) were added to each test tube by using the sterile micro pipette and diffused at room temperature for 2 hrs. The respective extracts were maintained and the experiment was done in triplicates and average values of zone of inhibition were recorded in mm for antimicrobial activity as described before in different studies [14, 15].

Data analysis

The results were reported as mean ± standard deviation (SD). The calibration curves were constructed by using Microsoft excel window 10 and origin 8.

Results And Discusion

The results obtained during the phytochemical analysis and the determination of antioxidant and antimicrobial activities as well as total phenolic and total flavonoid contents of methanol extract of the leaf and fruit of *Ruta chalepensis* were discussed as follows.

Qualitative phytochemical analysis

The qualitative phytochemical analysis of bioactive compounds present in the methanol extracts of fruits and leaves of *R. chalepensis* had been analyzed and showed the presence of different compounds. The presence of bioactive compounds in the extracts was detected by using color change as a confirmatory test and shown in the Table 1 below. Both extracts mostly showed from weak to moderate bioactive compounds however, the fruit extract of *R. chalepensis* showed relatively greater steroids and saponins compared to that of the leaf extract according to our investigation as shown in the Table 1.

Table 1
Qualitative analysis of phytochemicals of leaf and fruit extracts of *R. chalepensis*.

Phytochemicals	Type of test (reagent)	Fruit of extract	Leaf of extract	Color observed
Alkaloids	Mayer's Test	++	++	Yellow precipitate-
Flavonoids	H_2SO_4 test	++	++	Yellow color
Phenols	Ferric Chloride Test	++	++	Blue black color
Tannins	Lead acetate test	+	+	White precipitate
Steroids	Salkowski's Test	++	+	Red color
Saponins	Honey comb test	++	+	Formation of Froth
Terpinoids	Salkowski's Test	+	+	Reddish brown
Glycosides	NaOH test	+	+	Yellow color
Key:- (++) = highly present, (+) = present, (-) = not present				

Determination of total phenolic content

The Folin-Ciocalteu reagent (FCR) or Folin phenol reagent or folin-Denis reagent, also called Gallic acid equivalent method (GAE) is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric *in vitro* assay of phenolic and polyphenolic antioxidant activity [10]. The assay is simple and relies on the transfer of electron in alkaline medium from phenolic /antioxidant/ to phosphomolybdic and phosphotungstic acid complexes, manifested in the formation of blue color complexes [possibly $(PMoW_{11}O_{40})^{4-}$] which can be determined by measuring the absorbance at 765 nm using UV-Vis spectrophotometer [12]. As shown in the Table 2 below, Gallic acid standard solution ranging from 20 to 80 ppm were used to construct a calibration curve for the determination of the total phenolic content of the sample. The equation obtained from the calibration curve of a Gallic acid was $Y = 0.0088x + 0.1605$ and the linear regression coefficient (R^2) value equals to 0.9878 (Fig. 1).

Table 2
Absorbances of the standard solution Gallic acid.

Concentration	Absorbance at 765 nm
20 ppm	0.318 ± 0.18
40 ppm	0.549 ± 0.05
60 ppm	0.673 ± 0.13
80 ppm	0.864 ± 0.09

The total phenolic content of the leaf and fruit extracts was reported in terms of milligram Gallic acid equivalent per dry sample (mgGAE/dry weight). The absorbance of each extracted sample found in the range of Gallic acid standard solution. The total phenolic content of the methanol extract of the leaf and fruit of *R. chalepensis* was observed (142.159 ± *0.02*, and 156.477 ± *0.15*) mgGAE/g dry extract respectively as shown in the Table 3. From the two results, the total phenolic content of the fruit extract of *R. chalepensis* is greater compared to that of the leaf extract of the plant. From this finding, it is possible to conclude that the total phenolic content of different extracts is different of the same plant.

Table 3
Total phenolic content of leaf and fruit extracts of *R. chalepensis*.

Plant extract	Absorbance at 765 nm	mgGAE/g dry extract
leaf	0.786 ± 0.17	*142.159* ± 0.02
Fruit	0.849 ± 0.23	*156.477* ± 0.15

Determination of total flavonoid content

The total flavonoid content of methanol extracts of the leaf and fruit of *R. chalepensis* was determined by using aluminum chloride colorimetric assay using quercetin as a standard to construct a calibration curve. The calibration curve (Fig. 2) of quercetin was constructed by using various concentrations (20, 40, 60, and 80 ppm) of quercetin versus absorbance (Table 4) and by using this calibration curve, the total flavonoid content of methanol extracts of the leaf and fruit of *R. chalepensis* was determined using a straight line with regression equation, Y = 0.0042x + 0.0455 and a linear regression coefficient (R^2) = 0.9898 which was obtained from calibration curve of quercetin (Fig. 2).

Table 4
Absorbance of quercetin at different concentration.

Concentration of quercetin	Absorbance at 510 nm
20 ppm	0.131 ± 0.0054
40 ppm	0.216 ± 0.0120
60 ppm	0.279 ± 0.0035
80 ppm	0.387 ± 0.0038

The total flavonoid content of the extracts was determined by using aluminum chloride calorimetric method where the Al^{3+} in $AlCl_3$ was bind with the hydroxyl groups of the flavonoids present in the plant extract through electron transfer reaction and produce a yellow color solution [16]. Then when NaOH was added on the mixture the color was changed to pink color which was an indication of the presence of

flavonoids in methanol extracts of fruit and leaf of *R. chalepensis* as shown below in the Table 5. Quantitatively the total flavonoid content of methanol extracts of the fruit and leaf of *R. chalepensis* was expressed in terms of quercetin equivalent (mgQE) per dry extract/sample. As it is observed in Table 5, both methanol extracts of fruit and leaf of *R. chalepensis* contains flavonoids. The total flavonoid content of methanol extract of fruit was 135.952 ± 0.116 mgQE/g dry sample while that of methanol extract of leaf was 118 ± 0.059 mgQE/g dry extract which is fruit extract contains relatively more flavonoids as compared to that of the leaf extract.

Table 5
Total flavonoid content of the leaf and fruit extracts of *R. chalepensis*.

Extracts	Absorbance at 510 nm	mgQE/ g of dry weight
Fruit	0.331 ± 0.129	135.952 ± 0.116
leaf	0.295 ± 0.046	118.246 ± 0.059

Antioxidant activity determination

Antioxidant activities of methanol extracts of fruits and leaves of *R. chalepensis* were evaluated by using FRAP and DPPH assays.

DPPH radical scavenging capacity test

The DPPH free radical scavenging ability of methanol extract of fruit and leaf of *R. chalepensis* was evaluated by constructing calibration curve of ascorbic acid (AA) (Fig. 3). The calibration curve was plotted as absorbance versus different concentrations (25, 50, 100, and 200 ppm) of ascorbic acid which was based on the standard procedures (Table 6). A straight line with a linear regression equation, Y= -0.002x + 0.5979 and linear regression coefficient, R^2 = 0.9867 was obtained from this curve which was used to express DPPH radical scavenging capacity of each extract. Based on the calibration curve of AA shown in (Fig. 3), the DPPH free radical scavenging ability of methanol extracts of the fruit and leaf part of *R. chalepensis* were evaluated by using color change as the reagent was added and recording the absorbance of each extract at a different concentrations. Qualitatively DPPH radical scavenging ability of methanol extracts of fruit and leaf part of *R. chalepensis* were evaluated by using color change as described before in other studies [17]. In this study, when 1 mL a 0.1 mM DPPH solution in methanol was added into standard solution and each extracts, a pink color was formed in each extracts, standard and blank solutions and after some time this pink color was disappeared and changed to yellow color in all test samples. From this, we can conclude that the change of color from pink to yellow in each extracts as well as standard solution confirmed that they have DPPH radical scavenging capacity in this test

solution. As shown in Table 7 below, as the concentration of the extracts increases, percent inhibition of the extracts also increases which implies that both fruit and leaf extracts have greater potential to scavenge DPPH free radical at a higher concentrations. As shown in the Table below, methanol extract of the fruit extract had greater percentage inhibition than that of the leaf extract at given concentrations.

Table 6
% Inhibition and absorbance of AA.

Concentration of AA	Absorbance of AA at 517 nm
25 ppm	0.565 ± 0.054
50 ppm	0.471 ± 0.112
100 ppm	0.401 ± 0.045
200 ppm	0.192 ± 0.028

Table 7
Percent Inhibition and absorbance of the extracts and the standard.

Concentration	Absorbance at 517 nm			% Inhibition		
	AA	Fruit	Leaf	AA	Fruit	Leaf
25 ppm	0.56 ± 0.05	0.81 ± 0.09	0.69 ± 0.09	70.17 ± 0.13	57.08 ± 0.00	63.57 ± 0.11
50 ppm	0.47 ± 0.11	0.73 ± 0.15	0.60 ± 0.12	75.14 ± 0.05	61.73 ± 0.10	68.27 ± 0.03
100 ppm	0.40 ± 0.05	0.65 ± 0.08	0.52 ± 0.07	78.83 ± 0.01	65.90 ± 0.23	72.81 ± 0.23
200 ppm	0.19 ± 0.03	0.50 ± 0.01	0.40 ± 0.02	89.86 ± 0.17	73.76 ± 0.08	78.99 ± 0.05

Ferric reducing antioxidant power (FRAP) assay

In this method, calibration curve of ascorbic acid was used as standard to determine antioxidant activities of methanol extract of fruits and leaves of *R. chalepensis*. Calibration curve of ascorbic acid (Fig. 4) was constructed using different concentrations (10, 20, 40, and 80 ppm) of ascorbic acid with their corresponding absorbance. The absorbance measured by using UV-VIS spectrometer at 700 nm was performed in triplicate and the mean values (Table 8) were used to construct the calibration curve. The

equation of the calibration curve was obtained from the given absorbance versus concentration graph and the equation was, y = 0.0131x + 0.5404 with a liner regression coefficient R^2 = 0.9456.

Table 8
Percent reducing power and absorbance of AA at different concentrations.

Concentration of Ascorbic acid (ppm)	Absorbance of ascorbic acid at 700 nm
10	0.56 ± 0.05
20	0.87 ± 0.09
40	1.16 ± 0.03
80	1.54 ± 0.08

The ferric reducing antioxidant power (FRAP) assay was used to check single electron donating potential of methanol extracts of fruit and leaf of *R. chalepensis*. The method was performed by using potassium ferric cyanide. The antioxidant ability of methanol extracts of fruits and leaves were analyzed based on the existence of reluctant or appearance of color change. After incubation the color of a mixture was changed from yellow to near colorless solution which indicates the reduction of Fe^{3+} to Fe^{2+}. From this we understand that the methanol extracts of the fruits and leaves were act as an antioxidant which was able to donate electrons for the reduction of Fe^{3+} to Fe^{2+}. A greenish blue color was observed, when a 0.1% $FeCl_3$ solution was added to the mixture which act as an indication for the formation of Prussian blue complex $Fe_4[Fe(CN)_6]$ as described before [18]. As shown in Table 9, the absorbance and % reducing power of each extract increases as the concentration of the extract increases. In addition to this, from the Table 9, it is possible to conclude that the ferric reducing antioxidant power of the fruit extract is slightly greater compared to that of the leaf extract of *R. chalepensis*

Table 9: % Reducing power and absorbance of fruit and leaf extracts of *R. chalepensis.*

Concentration	Absorbance at 700 nm			% reducing power		
	Fruit	Leaf	AA	Fruit	Leaf	AA
10 ppm	0.61±0.08	0.47±0.15	0.56 ± 0.05	64.70±0.14	61.7 ± 0.21	67.85 ± 0.14
20 ppm	0.65±0.16	0.52±0.06	0.87 ± 0.09	72.31 ± 07	65.3 ± 0.62	79.31 ± 0.34
40 ppm	0.73±0.28	0.60±0.19	1.16 ± 0.03	75.34±0.24	70.01±0.23	84.48 ± 0.27
80 ppm	0.81±0.11	0.69±0.23	1.54 ± 0.08	77.78±0.18	73.91±0.30	88.31 ± 0.16

Antibacterial activity assay of leaf and fruit extracts

As shown in the Table 10 below, higher inhibition zone was recorded for methanol extract of the fruit than that of the leaf extract in all four bacteria. All extracts had lowest antibacterial potential as compared with that of antibiotics used as standard (Gentamycin). In this study, good antibacterial results were recorded at higher concentrations (800 ppm). Zone of inhibition of methanol extract of the fruit against a positive bacteria *Staphylococcus aureus* and *Streptococci pyogenes and* two negative bacteria *Escherichia coli* and *Salmonella typhi* were (14.5 ± 0.46, 15.8 ± 0.16, 12.7 ± 0.17, 10.1 ± 0.11) respectively. Zone of inhibition methanol extract of leaf against a positive bacteria *Staphylococcus aureus* and *Streptococci pyogenes and* two negative bacteria *Escherichia coli* and *Salmonella typhi* were *(12.9 ± 0.33, 14.8 ± 0.23, 10.7 ± 0.14, 9.5 ± 0.13)* respectively. In general the results indicated that methanol extracts of fruit have slightly stronger antibacterial activity compared to that of the methanol extract of leaf but the difference is not much significant.

Table 10
Comparison of zone of inhibition among leaf extracts of *Ruta chalepensis.*

Sample extract	Concentration of extracts (mg/mL)	Zone of inhibition (mm) for bacteria			
		Gram positive		Gram negative	
		Staphylococcus aureus	*Streptococci pyogenes*	*Escherichia coli*	*Salmonella typhi*
Fruit	100	11.3 ± 0.03	12.1 ± 0.18	8.9 ± 0.23	8..6 ± 0.25
	200	12.6 ± 0.14	13.3 ± 0.16	9.7 ± 0.35	9.5 ± 0.29
	400	13.2 ± 0.18	14.3 ± 0.11	11.3 ± 0.19	9.9 ± 0.04
	800	14.5 ± 0.46	15.8 ± 0.16	12.7 ± 0.17	10.1 ± 0.11
Leaf	100	10.8 ± 0.25	11.1 ± 0.22	6.9 ± 0.08	6..2 ± 0.03
	200	11.9 ± 0.21	12.3 ± 0.20	7.5 ± 031	7.7 ± 0.43
	400	12.3 ± 0.10	13.3 ± 0.16	9.8 ± 0.29	8.1 ± 0.27
	800	12.9 ± 0.33	14.8 ± 0.23	10.7 ± 0.14	9.5 ± 0.13
Standard	Gen.	24.5 ± 0.21	27.7 ± 0.11	25.0 ± 0.49	23.4 ± 0.24
Data expressed as mean of three determinations ± standard deviation					

Conclusion

Based on this study, methanol extract of fruit and leaf of *R. chalepensis* contained some of important bioactive compounds as discussed in phytochemical analysis. In addition to this, both fruit and leaf extracts of *R. chalepensis* showed a good antioxidant capacity in both DPPH and FRAP assays. The two extracts of *R. chalepensis* also showed weak to moderate antibacterial activities against both gram positive and gram negative bacteria at higher concentrations. In both cases as the concentration of the extracts increases, both antioxidant and antimicrobial activities of the extracts also increases.

Declarations

Acknowledgements

The authors would like to acknowledge the Department of Chemistry, College of Science, Bahir Dar University for research facility and other important things. HGW also acknowledge Amhara Regional State Education Bureau for the study leave.

Authors' contributions

ZYD designed, supervised the experimental work, analyzed the experimental data and wrote the manuscript. HGW conducted the entire experimental work, analyzed the experimental data, interpretation of the data, and manuscript review. All authors read and approved the final manuscript.

Funding

No funding was received for this research work from outside source.

Availability of data and materials

The data sets supporting the findings of this article are all presented in the main manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflicts of interest.

References

1. Prakash P' Gupta N. Therapeutic uses of *Ocimum sanctum* Linn (Tulsi) with a note on eugenol and its pharmacological actions: a short review. *Indian journal of physiology and pharmacology*, 2005; 49(2):125-131.
2. Vishwakarma AP, Vishwe A, Sahu P, Chaurasiya A. Magical remedies of *Terminalia arjuna* (ROXB.). *International Journal of Pharmaceutical Archive*, 2013;*2*(8):189-201.
3. Asgarpanah J, Khoshkam R. Phytochemistry and pharmacological properties of *Ruta graveolens* L. *Journal of Medicinal Plants Research*, 2012); *6*(23): 3942-3949.
4. Asgarpanah J, Khoshkam R. Phytochemistry and pharmacological properties of *Ruta graveolens* L. *Journal of medicinal plants research*, 2012; 6(23):3942-3949.
5. Khouri NA, El-Akawi Z. Antiandrogenic activity of *Ruta graveolens* L in male Albino rats with emphasis on sexual and aggressive behavior. *Neuroendocrinology letters*, 2005; *26*(6): 823-829.
6. Arora S, Tandon S. DNA fragmentation and cell cycle arrest: a hallmark of apoptosis induced by *Ruta graveolens* in human colon cancer cells. *Homeopathy*, 2015;104(01): 36-47.
7. Osibemhe M, Onoagbe IO. Qualitative and quantitative phytochemical evaluations of Strophanthus hispidus stem bark. *J Pharm Biol Sci*., 2015; 10: 120-124.
8. Ram S, Sinha VS. Qualitative phytochemical analysis of some plants uses to cure malaria in Kolhan region of Jharkhand, India. *J Med Plants Stud*., 2015; 3: 60-62.
9. Bhatt PR, Patel UD, Modi CM, Pandya KB, Patel HB. Thin-layer chromatography and *in vitro* free radical scavenging activity of few medicinal plants from the surroundings of Junagadh, Gujarat, India*. Ann. Phytomed*., 2019; *8*(1): 45-55.
10. Mitra K, Uddin N. Total phenolics, flavonoids, proanthrocyanidins, ascorbic acid contents and in-vitro antioxidant activities of newly developed isolated soya protein. *Discourse Journal of Agriculture and Food Sciences*, 2014; 2(5): 160-168.
11. Katsube T, Tabata H, Ohta Y, Yamasaki Y, Anuurad E, Shiwaku K, Yamane Y. Screening for antioxidant activity in edible plant products: comparison of low-density lipoprotein oxidation assay, DPPH radical scavenging assay, and Folin-Ciocalteu assay. *Journal of agricultural and food chemistry*, 2004; *52*(8), 2391-2396.
12. Leaves L. Antioxidant activity by DPPH radical scavenging method of ageratum conyzoides. *American Journal of Ethnomedicine*, 2014; 1(4): 244-249.
13. Naik R, Acharya RN, Borkar SD, Shukla, VJ. Evaluation of phytochemical content, nutritional value and antioxidant activity of *Olax scandens* (Roxb.) leaves. *International Ayurvedic Medical Journal*, 2015; 3: 702-9.
14. Enujiugha VN, Talabi JY, Malomo SA, Olagunju AI. DPPH radical scavenging capacity of phenolic extracts from African yam bean (*Sphenostylis stenocarpa*). *Food and Nutrition Sciences*, 2012; 3: 7-13.

15. Lalas S, Gortzi O, Athanasiadis V, Tsaknis J, Chinou I. Determination of antimicrobial activity and resistance to oxidation of *Moringa peregrina* seed oil. *Molecules*, 2012; 17(3): 2330-2334.
16. Proestos C, Boziaris IS, Nychas GJ, Komaitis M. Analysis of flavonoids and phenolic acids in Greek aromatic plants: Investigation of their antioxidant capacity and antimicrobial activity. *Food Chemistry*, 2006; *95*(4): 664-671.
17. Prior RL. Oxygen radical absorbance capacity (ORAC): New horizons in relating dietary antioxidants/bioactives and health benefits. *Journal of functional foods*, 2015; 18: 797-810.
18. Shalaby EA, Shanab SM. Antioxidant compounds, assays of determination and mode of action. *African journal of pharmacy and pharmacology*, (2013; 7(10): 528-539.

Figures

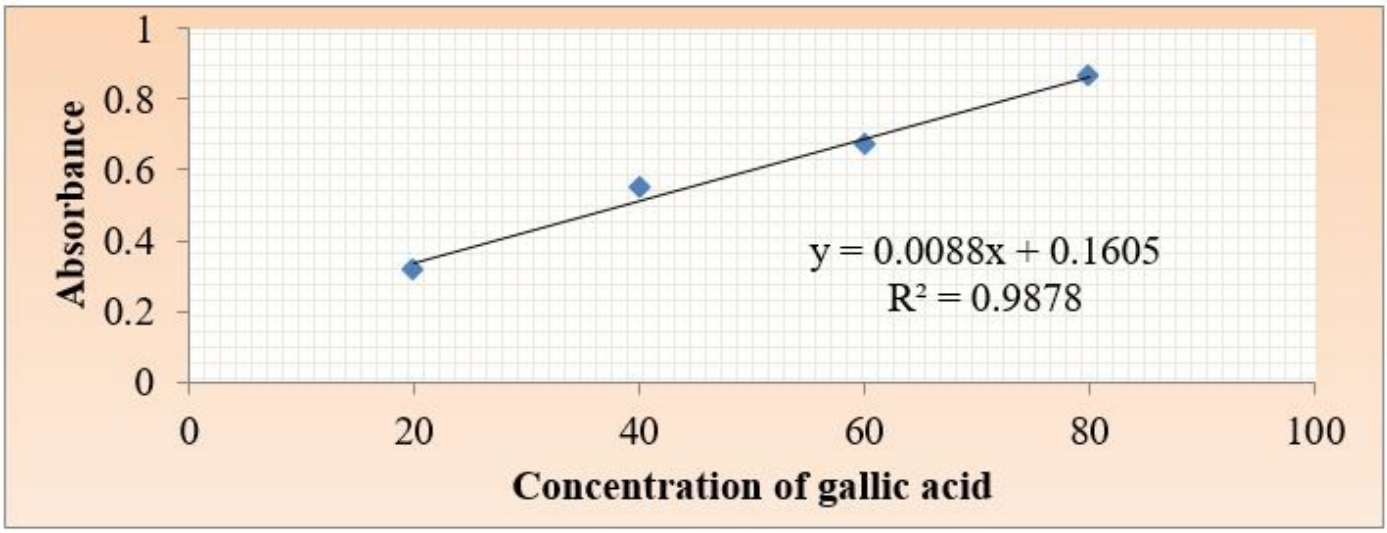

Figure 1

Calibration curve of Gallic acid standard.

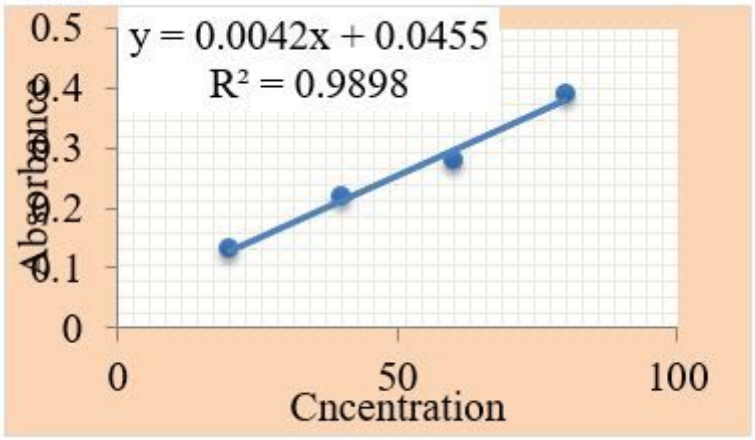

Figure 2

Calibration curve of quercetin.

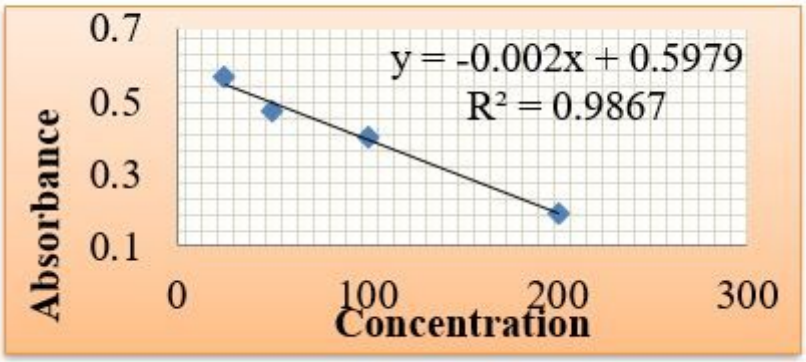

Figure 3

Calibration curve of AA for DPPH assay.

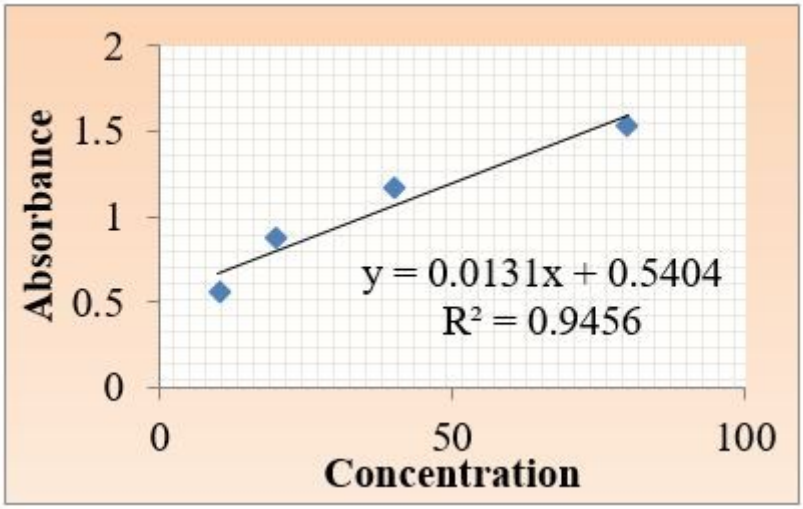

Figure 4

Calibration curve of AA for FRAP assay