Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2020.0604.10**

PHYTOCHEMICAL ANALYSIS AND EVALUATION OF *ZANTHOXYLUM RHETSA* (ROXB) FOR IT'S *IN VITRO* PHARMOCOLOGICAL PROPERTIES

Thejashwini B. R, Shilpashree M, Kiran Kumar D. J, Rama T*

Department of PG studies in Biotechnology, Government science college (Autonomous)

Bengaluru-560001, Karnataka, India.

ABSTRACT: *Zanthoxylum rhetsa* (*Z. rhetsa*) commonly known as Indian pepper or Indian prickly ash belongs to Rutaceae family, which is widely distributed in Western Ghats and subtropical areas. Various research studies on Z.*rhetsa* reveals that different parts of the tree have been used in traditional medicines. In this regard the phytochemical profiling of extracts prepared from dried fruits of*Z. rhetsa*using different solvent systems such as methanol, water and acetone was carried out. The methanol extract showed the maximum yield of phytochemicals when compared to other solvent systems. Five compounds were detected when fatty acid profiling of dried fruit sample of *Z. rhetsa*wasperformed using gas chromatography technique. The total antioxidant content of methanol extract of *Z. rhetsa*was determined and then further, investigated for its antioxidant property using, 2, 2-diphenyl-1-picrydrazly (DPPH) and reducing power assays. The total antioxidant content of methanol extract was found to be 5.86±0.015 µg/mL. The DPPH results showed IC_{50} value as 359 µg/mL and the increased value of absorbance of reducing power assay indicated the increased antioxidant activity of methanol extract. Further, the methanol extract of *Z. rhetsa* was also investigated for its anti-inflammatory activity using protein denaturation method. The anti-inflammatory activity was observed only at concentration of 10 and 25 mg of methanolic extract. Hence, our study reveals that the dried fruit of *Z. rhetsa* could be used as a natural source of anti-inflammatory and antioxidant compounds that helps to combat the diseases associated with increased level of reactive oxygen species

KEYWORDS: Antioxidant activity, Anti-inflammatory activity, GC analysis, Phytochemicals, *Zanthoxylumrhtesa*.

Article History: Received: July 08, 2020; Revised: July 26, 2020; Accepted: August 10, 2020.

Corresponding Author: Dr. Rama T* Ph.D.
Department of PG studies in Biotechnology, Government science college (Autonomous)
Bengaluru-560001, Karnataka, India.

1. INTRODUCTION

Plants are considered as boon for the mankind because of their unique properties. The plants are directly or indirectly used in the traditional medicine preparations. Now a day the traditional medicine is used worldwide because of their minimal side effects and thus gaining an economic importance day by day. There are more than 80,000 species of plants are reported to have at least some medicinal values [1]. The use of traditional medicines for treating humans and animals for various diseases are documented since ancient times. These medicines are not only used to treat the diseases but also have a great potential to maintain the good health of the mankind. Even today 80 % of our world population depends on the traditional medicines for initial treatments [2]. Plants have established the chemical defence against environmental threats like microbial attack, UV radiations, and reactive oxygen species. The chemicals which are involved in plant defense mechanism are known as phytochemicals and they are less toxic and are biologically active [3]. Phytochemicals are key ingredients in preparation of drugs. Plant based drug are increasing worldwide because of their biodegradable nature and they are also considered as safe drugs. Currently used drugs in treating various diseases such as aspirin, atropine, artimesinin, colchicine, digoxin, ephedrine, physostigmine, pilocarpine, morphine, reserpine, toxol, tubocurarine, quinine, quinidine vincristine and vinblastine are believed to be acquired from the plant material[4].*Zanthoxylum* is the largest genera of the family of Rutaceae, consisting of more than 200 species distributed worldwide in tropical and temperate regions of Eastern and Southeast Asia, America and Africa [2]. *Zanthoxylum* are deciduous or evergreen woody plants. *Zanthoxylum* species are reported to be used as raw materials in pharmaceutical and cosmetic industries. Traditionally, *Zanthoxylum* fruits and leaves are used as mouth fresheners and for tooth care, while bark and fruits are used as spices. The different parts of the species *Z. rhetsa* have been used to treat different diseases. Even in Indian traditional medicine system, *Z. rhetsa* has been used to treat the dental caries, cardiac diseases, respiratory diseases, tooth infection, stomach infection, rheumatism, intestinal worms, asthma, bronchitis, urinary disease and venereal diseases [5]. There are many studies revealing the presence of phytochemicals in bark, leaves and fruits of this plant. The antimicrobial, antioxidant, anti-inflammatory, antinociceptive, antidiabetes and anti-diarrheal activities of *Z. rhetsa*are attributed to various phytochemicals present within it [3, 6].

2. MATERIALS AND METHODS

Plant material

The sample (Figure 1) for the study was collected from Nepal. The sample was authenticated as *Zanthoxylumrhetsa* by Dr. A. N. Srineswara of Mahatma Gandhi botanical garden, University of Agriculture sciences, GKVK campus, Bengaluru-65.

Figure 1: *Z. rhetsa* fruit

Standardization of extraction protocol for the *Z. rhetsa*fruit samples

The Dried fruit sample of *Z. rhetsa*fruits were shade dried for 4-5 days. The dried sample was weighed and pounded into fine power. The powdered sample was further subjected to soxhlet extraction using three different solvents namely, methanol, acetone and water. Each solvent was run through soxhlet for 24 hours. The filtrate which was obtained after the extraction process was evaporated using hot air oven. The obtained extract was collected in stopper bottles and stored in refrigerator till further use.

Qualitative analysis of extracts prepared from *Z. rhetsa* fruit extracts

The different solvent extracts of *Z. rhetsa* fruits were assessed for the presence of the phytochemicals by following standard methods described by Harbone [7] and Kokate [8].

Test for Alkaloids: About 2 mL of each solvent extract (acetone, methanol, water) was added with 1 mL Mayer's reagent. Upon mixing well, the change in the colour from yellow to cream was observed.

Test for Carbohydrates: Solvent extracts (2 mL each) were treated with 1 mL of Molisch's reagent and then few drops of con. H_2SO_4 was added slowly to the sides of the test tubes. Formation of purple or reddish colour was noticed.

Test for Flavonoids: About 2 mL of each solvent extract was added with few drops of conc. H_2SO_4 and mixed well. After mixing the observation was made for the change in colour of the extracts.

Test for Phenols: Solvent extracts (10 mL each) were treated with few drops of ferric chloride solution. The extracts appeared bluish black colour.

Test for Proteins: About 0.5 mL of each solvent extract was treated with equal volume of 40% NaOH solution and two drops of one percent copper sulphate solution was added. Colour change of

solution to violet indicates the presence of protein.

Test for Saponins: The distilled water (5 mL) was added to 0.5 mL of each solvent extract, mixed well and later the observation was made for the appearance of foam.

Test for Steroids: About of 5 mL of each solvent extract was treated with 2 mL of acetic anhydride and 2 mL of conc. H_2SO_4. After mixing well the formation of blue or green colour was observed.

Test for Tannins: The small amounts of each extract was mixed with water and heated on a water bath. Later, mixture was filtered and to the filtrate ferric chloride was added. The colour of the extract was turned into dark green colour.

Quantitative estimation of phytochemicals presents in the *Z. rhetsa* fruit extracts

The three different solvent extracts were estimated quantitatively using standard protocols

Total Tannin Content

An aliquot (1 mL) of each extract was taken in different test tubes. 0.5 mL of FC reagent was added to each tube, followed by addition of 2.5 mL of sodium carbonate and then incubated for 40 minutes at room temperature. Absorbance was recorded at 725 nm [9]. Tannin content was expressed in mg of tannic acid equivalents (TE) per gram of extract. Standard graph of tannic acid was obtained by plotting the concentration of tannic acid against the absorbance and then the total tannins content of the extract was determined from the standard graph of tannic acid.

Total Flavonoid Content

An aliquot (0.5 mL) of each extract was taken in different test tubes. 2 mL of distilled water was added followed by the addition of 0.15 mL of sodium nitrate (5% $NaNO_2$w/v) and then allowed to stand for 6 minutes. 0.15 mL of Aluminium chloride (10% $AlCl_3$w/v) was added to each tube and was incubated for 6 minutes. 2 mL of sodium hydroxide (NaOH4% w/v) and 3 mL of distilled water was added to each tube. Absorbance at 510 nm was recorded using a spectrophotometer (Sky technology)Flavonoid content was expressed in mg of quercetin equivalent (QE) per gram of extract. The standard graph of quercetin was obtained by plotting the concentration of quercetin against absorbance values [10]. The flavonoids content of the extracts was determined from the standard graph.

Total Phenolic Content

Different volumes with varying concentrations (0, 1, 2.5, 5, 7.5, 10 µg/mL) of Gallic acid was mixed with 5 mL of FC reagent (1:10) and 4 mL of 7.5% sodium carbonate was added. Then the mixture was incubated for 30 minutes at 20 °C and the absorbance was measured at 765 nm using a spectrophotometer. The working concentration of each extracts 1 mg/mL was prepared. To the 1 mL of each solvent extract 5 mL of FC reagent and 4 mL of sodium bicarbonate was added and then the mixture was incubated for at 20 °C for 30 minutes. The absorbance was measured at 765 nm using a spectrophotometer [11]. The standard graph of Gallic acid was obtained by plotting the concentration of Gallic acid against the absorbance values. The phenolic content of the sample was

determined from the standard graph.

Total Protein Content

Different volumes of BSA with varying concentrations (0, 0.2, 0.4, 0.6, 0.8,1 µg/mL) was taken in a series of test tubes. 0.1 mL and 0.2 mL of each solvent extract was taken in other two test tubes and the volume of all the tubes was made up to 1 mL with distilled water. A tube with 1 mL of water was used as a blank. 5 mL reagent C (reagent A + reagent B) was added to all the test tubes. Including the blank, all the test tubes were allowed to stand for 10 minutes. Then 0.5 mL of reagent D (FC reagent) was added and mixed well. The test tubes were then incubated at room temperature in dark for 30 minutes. Absorbance was recorded at 660 nm using a spectrophotometer once the blue colour was developed [12].

Total Carbohydrate Content

Pounded dried fruit sample of about 100 mg was taken in the test tubes. The sample were hydrolyzed by keeping it in hot water bath for 3 hours after adding 5 mL of 2.5 NHCl. Then the sample were cooled to room temperature and were neutralized with solid sodium carbonate until the formation of effervescence was ceased, the volume was made up to 10 mL with distilled water and centrifuged. The supernatant was collected and 0.5 mL and 1 mL aliquots were used for analysis. The aliquots of glucose ranging from 0.1 to 1 mL were taken in the series of test tubes. The volume of all tubes was made up to 1 mL using distilled water. A tube with 1mLof water was used as a blank. 4 mL of Anthrone reagent was added to the test tubes and were heated for 8 minutes in hot water bath (90-95 °C). The tubes were cooled rapidly and the colour obtained was read at 630 nm using a spectrophotometer [13].

Analysis of chemical properties

Extraction of oil

The 10g of powered fruit sample was taken in a flask and then the sample was immersed in the mixture of chloroform and methanol (2:1) solvents and the flask were left undisturbed for 24 hours. Next the extract obtained was filtered and evaporated using hot air oven to get sample oil. The oil residue obtained finally was used to analyze its chemical properties and for GC analysis.

The saponification value, acid value and iodine value of the sample oil was determined using the titration method.

Acid Value (AV)

About 0.3-0.5 g of oilwas taken into conical flask, 10 mL of ethanol was added and this mixture was titrated against 0.1N KOH using phenolphthalein as indicator till pink colour was appeared.

$$AV = \frac{\text{Burette reading} \times 0.1 \times 56.11}{\text{Weight (g)}}$$

Esterification Value (EV)

About 0.5 g of oil was taken in a conical flask, 25 mL of 0.5 N alcoholic KOH was added and the

mixture was refluxed for 30 minutes. The mixture was added with 4 drops of phenolphthalein indicator and was titrated against 0.5 N HCl.

$$EV = \frac{(\text{Blank value} - \text{Sample value}) \times 0.5 \times 56.11}{\text{Weight (g)}}$$

Saponification Value (SV)

Saponification value was obtained by adding the acid value and esterification value.

$$SV = AV + EV$$

Evaluation of pharmacological activities of *Z. rhetsa*fruit extract

Estimation of total antioxidant content

The reagent solution (1 mL) consisting Sodium phosphate and Ammonium molybdate dissolved in water was added to 0.1 mL of each solvent extract. The mixture was incubated at 95 °C for 90 minutes. The absorbance of the mixed solution was measured at 765 nm using 350-800 nm range UV-spectrophotometer against distilled water as blank control [14]. The standard graph was plotted using the ascorbic acid.

Antioxidant assay

DPPHassay

The free radical scavenging effect of methanol fruit extract was assayed *in vitro* by 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Different volume (0. 6, 6, 15, 30, 60 µL) with varying concentration (1, 10, 25, 50, 100 µg/mL) of the methanol fruit extract was taken in different tubes. The volume was made to 3 mL with distilled water in respective test tubes. 1 mL of solution was taken from each tube and 3 mL of DPPH solution was added. The reaction mixture was shaken well and incubated at room temperature for 15 minutes in dark. The absorbance of the mixed solution was measured at 517 nm using UV-spectrophotometer against methanol as blank control and standard graph was plotted using the ascorbic acid [15, 16]. The free radical scavenging activity was measured as a decrease in the absorbance of DPPH and calculated using the following formula.

$$\text{DPPH scavenging effect (\%)} = \frac{AC - AT}{AC} \times 100$$

AC=absorbance of control

AT=absorbance of test sample

Reducing power assay

Different volumes (0.6, 6, 15, 30, 60 µL) with varying concentrations (1, 10, 25, 50, 100 µg/mL) of the methanol fruit extract was taken in different tubes. The volume of the tubes was made up to 3 mL. An mL of solution was taken from each tube into different test tubes and 2.5 mL of phosphate buffer with 2.5 mL of potassium ferricyanide was added. Then mixture was incubated for 20 minutes at 50 °C. After the incubation, 2.5 mL of TCA was added and the mixture was again incubated for

10 minutes at room temperature. The upper layer of the mixture was taken into different test tubes, 2.5 mL of distilled water was added and 0.5 mL of iron (III) chloride solution was also added. The absorbance was measured at 700 nm using 380-800nm range UV-spectrophotometer against distilled water as blank control [17].

Anti-inflammatory assay

Protein denaturation protocol

To evaluate the Anti-inflammatory activity of methanol fruit extract, the protocol was described by Elias [18]and Padmanabhan [19] was followed with small modifications. 0.2 mL of egg albumin (fresh hen`s egg) was taken in different test tubes. 1.4 mL of phosphate buffered saline (PBS) was added to tubes. The mixture was incubated at 37 °C for 15 minutes and then heated at 70 °C for a minute. After cooling the absorbance was noted using spectrophotometer. Different concentrations (100, 500, 1000 µg/mL) of Diclofinac sodium, a standard drug was used. An egg albumin with buffer was maintained as control. The percentage inhibition of protein denaturation activity of Methanol fruit extract was calculated by using following formula [17, 18].

$$\text{Percentage inhibition} = \frac{AC-AT}{AC} \times 100$$

AC=absorbance of control

AT=absorbance of test sample

Methyl esterification of sample

About 0.15 g of oilwas taken in 50 mL volumetric flask; 4 mL of 0.5N methanolicNaOH was added and was heated in a steam bath until the fat globules turns to solution which took about 5 minutes. 5 mL of boron trifluoride was added and boiled for 2 minutes then 2 mL of saturated NaCl and hexane was added. This mixture was directly injected onto the injector port of gas chromatography (Shimadzu).

GC analysis

Quantitative analysis of *Z. rhetsa* fruit sample was carried out using gas chromatography (Shimadzu) equipped with a capillary column of length 30.0 m, 0.25 mm diameter and 0.25 µm film thickness. The injector was maintained at 320 °C. The GC was programmed to maintain thecarrier gas-nitrogen (N_2), linear velocity, injection volume-1 µL and split ratio of 1:20. Oven temperature was maintained initially at 70 °C for 3 minutes, and then the temperature was increased to 270 °C. The sample was directly injected to GC. The injector and flame Ionized detector were maintained at 250 °C, signals were recorded and analyzed further**.**

Statistical analysis

Quantitative analysis of phytochemical and total antioxidant content assay was performed in triplicates, and the values are presented as average values along with their standard derivations.

3. RESULTS AND DISCUSSION

Qualitative estimation of phytochemicals

The fruit extracts of *Z. rhetsa* prepared from acetone, methanol and water were screened for phytochemical analysis using standard protocol. These extracts showed the positive results for the presence of alkaloids, carbohydrates, flavonoids, phenols, proteins, saponnins and tannins. The steroids were absent in all these extracts (Table 1).

Table 1: Qualitative phytochemicals analysis of *Z.rhetsa* fruit extracts

Sl. No	Phytochemicals	Acetone extract	Methanol extract	Water extract
1	Alkaloids	+	+	+
2	Carbohydrates	+	+	+
3	Flavonoids	+	+	+
4	Phenols	+	+	+
5	Proteins	+	+	+
6	Saponins	+	+	+
7	Steroids	-	-	-
8	Tannins	+	+	+

(+) indicates the Presence of phytochemicals; (-) indicates the Absence of phytochemicals

Quantitative estimation of phytochemicals

The quantitative phytochemical analysis of acetone, methanol and water extract of *Z. rhetsa* fruit result reveals that methanol extract exhibits higher amount of phytochemicals than acetone and water extract (Table 2). The carbohydrate present in sample was found to be 22% and total protein content was found to be 0.96±0.03 and 1.93±0.098 µg/mL at samples weighing 0.1 and 0.2 mL respectively. Hence the methanol extract was further investigated for the pharmacological properties.

Table 2: Quantitative phytochemical analysis of *Z. rhetsa* fruitextracts

Samples	Flavonoids(µg/mL)	Tannins (µg/mL)	Phenols (µg/mL)
Methanol	0.63±0.134	11.3±0.620	0.78±0.045
Acetone	0.14±0.020	10.4±0.321	0.59±0.04
Water	0.27±0.005	9.5±0.050	0.395±0.005

Values are mean± SD of triplicates

Antioxidant assay

Total antioxidant content

The total antioxidant content of sample was determined using Phosphomolybdeum method. The total antioxidant content of *Z. rhetsa* fruit was found high in methanol extract than other solvent systems. The total antioxidant content was found to be 5.86±0.015 µg/mL in methanol extract

followed by 5.32±0.01 µg/mL in acetone extract and 5.30±0.015 µg/mL in water extract (Table 3).

Table 3: Total antioxidant content of the *Z. rhetsa*fruit extracts

Extract sample	Concentrationµg/mL
Methanol	5.86±0.015
Acetone	5.32±0.01
Water	5.30±0.015

Values are mean± SD of triplicates

DPPH assay

DPPH radical scavenging activity of methanol extract of the dried fruits was measured as percentage inhibition of free radical. The results of DPPH were calculated to get the half maximal inhibitory concentration (IC_{50}) value (Table 4). The IC_{50} valueof *Z. rhetsa* was obtained as359 µg/mL, indicating the best antioxidant property of the sample.

Table 4: DPPH radical scavenging activity of *Z. rhetsa* methanol fruit extract

Sl. No	Concentration µg/mL	Inhibition %	IC_{50} Value
C1	1	16.04	
C2	10	14.19	
			359
C3	25	22.4	
C4	50	19.1	

Reducing power assay

The reducing potentiality of *Z. rhetsa*methanol fruit extract was found to be significant. The reducing power of extract was increased with the increase in the concentration of the extract (Figure 2). The rise in the absorbance in accordance to the sample concentration (1, 10, 25, 50, 100 mg/mL) indicated the antioxidant activity of the sample.

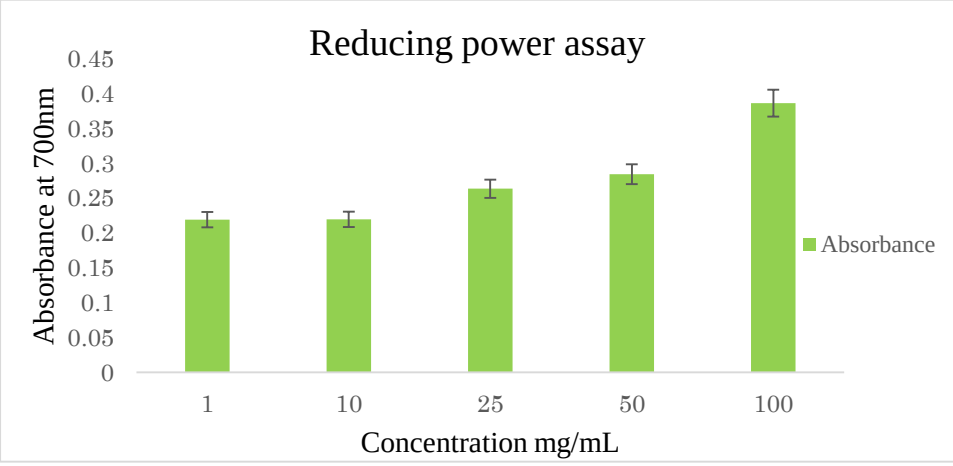

Figure 2: Reducing Power Assay of *Z. rhetsa*methanol fruit extract

Anti-inflammatory assay

An *in vitro* anti-inflammatory activity of methanol extract of *Z. rhetsa* fruit sample was evaluated using protein denaturation method. The highest percentage of inhibition (85%) of protein denaturation was observed at concentration of 10 mg/mL. A significant anti-inflammatory activity of extract was also observed at concentration of 25 mg/mL (Table 5). Different concentrations (100, 500, 1000 µg/mL) of diclofinac sodium, a standard drug was used. The diclofinac sodium showed 43.91, 41.56, and 45.56 % of inhibition at concentrations 100, 500 and 1000 µg/mL respectively.

Table 5: *In Vitro* Anti-inflammatory activity of methanol fruit extract of *Z. rhtesa*

Sample	Concentration of Sample	% Inhibition of Protein Denaturation
Standard drug		
	100 (µg/mL)	43.91
Diclofinac sodium	500 (µg/mL)	41.56
	1000 (µg/mL)	45.56
Z. rhetsa fruit extract		
	400 (mg/mL)	-74.9
Methanol extracts	100 (mg/mL)	-73
	25 (mg/mL)	77.30
	10 (mg/mL)	85

GC analysis

Fatty acid composition of *Z. rhetsa* fruit was analysed using GC. About five compounds were detected in the *Z. rhetsa* extract (Table 6). The results revealed that palmitic acid (12.86 %) was found as a major component followed by linolenic acid (7.07 %), oleic acid (5.64 %) and stearic acid (1.32 %).

Table 6: Fatty acid profiling of *Z. rhetsa*fruit extract

NO	Retentions time(Min)	Compounds found	Molecular formula	Molecular weight	Area	Area peak%
1	9.226	PALMITIC ACID	$C_{16}H_{32}O_2$	256.43	138969	12.86
2	14.588	LINOELIC ACID	$C_{18}H_{32}O_2$	280.44	38177	3.53
3	14.784	LINOLENIC ACID	$C_{18}H_{30}O_2$	278.43	76379	7.07
4	14.969	OLEIC ACID	$C_{18}H_{34}O_2$	282.47	60931	5.64
5	15.620	STEARIC ACID	$C_{18}H_{36}O_2$	284.48	14325	1.32

DISCUSSION

The fruits of *Z. rhetsa* have been widely used as spice and are also used the in preparation of traditional medicine since ancient times. The natural products are the main source for the plant based pharmacological industries. The bioactive constituents in plant are responsible for their pharmacological properties. These bioactive compounds are produced in plants by secondary metabolism. The bioactive compounds that are found in fruit extract of *Z. rhetsa* in the present study are tannins, flavonoids, phenols, saponins, carbohydrate and proteins (Table 1). The quantitative analysis of phytochemical resulted higher concentrations of phytochemicals in methanol extract than acetone and water extracts. This variation in yields of phytochemicals is due to the polarity of the extraction solvents. The plant materials contain higher concentration of polar compounds that are soluble in solvents with higher polarity. Presence of higher levels of phytochemicals in methanol extracts indicates that extraction efficiency depends on the polarity of the solvents [20]. Tannins are water-soluble polyphenols mainly present in plant-based materials. Some important activities of tannins are antiseptics, anticarcinogenic and anti-inflammatory which are reported in many studies which makes them suitable for pharmaceutical and nutraceutical industries [21]. Flavonoids are reported to have some of important properties such as skin protection from UV light exposure; DNA protection, strengthening of capillaries, anti-inflammatory activity and protective action against radiation, moistening, softening, soothing, and antiseptic and many other[22, 10]. Phenolic compounds in plants acts as antioxidant, structural polymers, attractants, UV screens, signal compounds and defence response chemicals. Phenolic compounds are vital in defense response, like such as anti-aging, anti-inflammatory, antioxidant and anti-proliferative activities [23]. Carbohydrates are immediate energy sources for our body whereas lipids are long-term energy. The protein functions as growth factors, gene activators, acts as antibodies, maintaince osmotic balance, catalyse biochemical reactions and provide mechanical support to cells [12].To measure the antioxidant activity of *Z. rhetsa* fruit extract DPPH, and reducing power assay methods were followed; DPPH radical scavenging activity revealed that *Z. rhetsa* fruit methanol extract possesses moderate antioxidant activity (Table 3). Results of this study suggest that methanol fruit extract of *Z. rhetsa* possesses the phytochemical compounds that are capable of donating hydrogen to a free radical to scavenge. The total antioxidant content of the sample was measured using Phosphomolybdenum method. Our study demonstrates that methanol extract of *Z. rhetsa* fruit exhibited the highest antioxidant capacity for phosphomolybdate reduction. Some of studies have shown that flavonoids and some of compounds that are related to polyphenols contribute to the Phosphomolybdate scavenging activity of the medicinal plants [24]. In our present study, reducing potentiality of methanol fruit extract of *Z. rhetsa* was found to be significant and the reducing potentiality of the extract was increased with increasing the extract concentration. Rise in the absorbance of the sample is due to increase in concentration of hydrogen donating compounds

present in the extract [24].The various concentration of *Z. rhetsa* fruit methanol extract, ranging from 10 to 400 mg/mL was tested for its protein denaturation property which in turn indicates the anti-inflammatory activity of the fruit sample. The obtained results clearly demonstrate that the methanol have an anti-inflammatory activity at specific concentration. The anti-inflammatory activity was observed at concentration of 10 and 25 mg/mL (Table 5). Percentage of inhibition by the extract exhibited results in reverse concentration manner, highest anti-inflammatory activity was observed at concentration of 10 mg/mL. Denaturation mechanism of proteins involves alteration in electrostatic, hydrophobic, hydrogen and disulphide bonding [25]. Results of anti-inflammatory assay of some drugs were obtained in dose dependent manner, but variation that observed in the results of this study is may be due to change in turbidity or viscosity of the sample [26]. Five compounds were detected by fatty acid profiling of *Z.rhetsa* fruit extract. The compounds detected in the fatty acid profiling are linoelic acid, linolenic acid, oleic acid, stearic acid and palmitic acid (Table 6). These compounds have the pharmacological importance and are employed in pharmacological industries. Palmitic acid that was detected in fatty acid profiling of *Z. rhetsa* exhibits the antitumor activity they can be employed as a lead compound in anticancer drugs[27]. Linoleic acid exhibits antioxidant property[28]. Linoleic, stearic, oleic and linolenic acids are used as food supplements and presence of these fatty acids may reduce the risk of cardiovascular diseases [29, 30].

4. CONCLUSION

Since ancient times, *Z. rhetsa* has greater value in Ayruvedic medicines; traditionally this plant has been involved in treating various diseases. In this study standardization of extraction protocol for *Z. rhetsa* fruit sample was carried out using three different solvents- methanol, acetone and water. The highest quantity of phytochemicals such as carbohydrate, protein, tannins, phenols and flavonoids were extracted in methanol. Methanol extract was further investigated for its antioxidant and anti-inflammatory activities. The methanol extract of *Z. rhetsa* exhibited moderate antioxidant activity with DPPH and reducing power assay. The anti-inflammatory activity of methanol fruit extract showed significant activity at concentrations 10 and 25 mg. The Fatty acid profiling of *Z. rhetsa* using Gas chromatography results revealed the presence of five pharmacologically important bioactive compounds. Based on the results of this study, it can be concluded that the fruits of *Z. rhetsa* can be used as a potential source of antioxidant and anti-inflammatory product. Therefore, further investigation is required to isolate and use the lead compounds in Z. *rhetsa* fruits for the anti-inflammatory and antioxidant therapies.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

FUNDING

None

ACKNOWLEDGEMENT

Authors Remains thankful to the Head of Department and faculties of Biotechnology department, Government science college (Autonomous). Authors also acknowledge Mrs Priyanka and Mr. B. V. Srinivas from Aspartika Biotech for day to day support in laboratory and we also thank MrsLakshmi for her support. We thank Dr. A. N. Sringeswara of Mahatma Gandhi botanical garden, University of Agriculture sciences, GKVK campus, Bengaluru-65 for authenticating the sample.

CONFLICT OF INTEREST

The Authors declare that there is no conflict of interest.

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