Scholars Academic Journal of Pharmacy (SAJP)
Abbreviated Key Title: Sch. Acad. J. Pharm.
ISSN 2347-9531 (Print)
ISSN 2320-4206 (Online)

A Unit of Scholars Academic and Scientific Society, India
www.saspublisher.com

Pharmacology

Phytochemical Analysis and Evaluation of Antidiabetic Activity of *Rhus aromatica* Mother Tincture by *Invitro* Methods

Deepa P*, Jayachandran TP
Department of Pharmacology, Department of Pharmaceutical Sciences Cheruvandoor, Kerala, India

Original Research Article

***Corresponding author**
Deepa P

Article History
Received: 25.06.2018
Accepted: 06.07.2018
Published:30.07.2018

DOI:
10.21276/sajp.2018.7.7.2

Abstract: Diabetes is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. *Rhus aromatica* is a deciduous shrub in the family Anacardiaceae native to Canada and the United states. The aim of the current study was to screen the *Rhus aromatica* mother tincture for its in vitro antidiabetic activity. One antidiabetic therapeutic approach is to reduce gastrointestinal glucose production and absorption through the inhibition of carbohydrate digesting enzymes such as α- amylase and α-glucosidase. Inhibition of amylase and glucosidase enzymes involved in digestion of carbohydrates can significantly decrease the post prandial increase of blood glucose after a mixed carbohydrate diet and therefore can be an important strategy in management of blood glucose. Free radicals are atoms or molecules that have at least an unpaired electron in their outer most shell or orbital,which are not contributing to molecular bonding and are capable of independent existence. Free radicals involving oxygen atoms are known as reactive oxygen species (ROS). In diabetic complication free radicals are generated, some of the most important ROS and RNS in vascular cells. The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species can initiate degenerative diseases. The best described pharmacological property of flavonoids is their capacity to act as potent antioxidant that has been reported to play an important role in the alleviation of diabetes mellitus. Antioxidant compounds like phenolic acids, polyphenols and flavonoids are commonly found in plants have been reported to have multiple biological effects, including antioxidant activity. The antioxidant potential of Rhus aromatica mother tincture was examined by DPPH free radical scavenging assay, nitric oxide (NO) free radical scavenging assay and Hydrogen peroxide free radical scavenging assay.
Keywords: in vitro antidiabetic; *Rhus aromatica*, α-glucosidase, α-amylase enzymes, antioxidant, DPPH, nitric oxide, flavonoids, mother tincture.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorder characterized by hyperglycemia; altered metabolism of lipids, carbohydrates and proteins; and an increased risk of vascular complications. Diabetes occurs when the pancreas is not producing insulin or produced insulin cannot be used by the body, these may lead to raise blood glucose levels. Hyperglycemia for the long-term are associated with damage to the various organs and tissues. Blood glucose level is maintained within a range of 80-120mg/dl. Elevation of the blood sugar is known to bring about an increase in the secretion of insulin which results in an increased uptake of glucose by the cells and also its conversion into glycogen within the cells that results in the reduction of the blood sugar level[1].

Rhus aromatica is a deciduous shrub in the family Anacardiaceae native to Canada and the United states. It is a woody plant that can grow to around 2-4 meters tall with a rounded form. It is also known as fragrant sumac. It contained a wide range of constituents including flavonoids, sterols, alkaloids, tannins, glycosides, carbohydrates etc .*Rhus aromatica* exerted antiviral, antihaemorragic and antidiabetic effects. Homeopathy is a holistic method of treatment that uses micro doses of natural substances originating from plants, minerals or animal parts. Homeopathic mother tincture is a combination of botanical extract with specified amount of alcohol[2].

Free radicals are atoms or molecules that have at least an unpaired electron in their outer most shell or orbital, which are not contributing to molecular bonding and are capable of independent existence. Free radicals involving oxygen atoms are known as reactive oxygen species (ROS), they are formed when oxygen is partially reduces and non-radicals are formed likewise with it. In diabetic complication free radicals are generated. Oxygen molecule can be regarded as a free radical because it contains two unpaired electrons. In diabetic complication free radicals are generated, some

of the most important ROS and RNS in vascular cells[3].

METHODOLOGY

Homeopathic mother tincture was collected from Central Research Institute for Homeopathy; kottayam .I first evaporated away the alcohol content from mother tincture under reduced pressure in a rotary evaporator to obtain a semisolid mass that weighed 2.5g.

Preliminary phytochemical screening of Rhus aromatica[5]

Molish's test: The filtrate was subjected to Molisch's test. Formation of reddish brown ring indicated the presence of carbohydrates.

Fehling's test: Dissolve a small portion of extract in water and treat with Fehling's solution [brown color indicated the presence of carbohydrate.]

Phenols test: The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapors. Blue coloration of the spot indicated the presence of phenols.

Test for flavonoids: Shinoda test: To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added .A pink or red coloration of the solution indicated the presence of flavonoids in the drugs.

Lead acetate test: To 5ml of extract 1ml of lead acetate solution was added. Flocculent white precipitate indicated the presence of flavonoids.

Test for tannins: To a 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added. Dark blue or greenish grey coloration of the solution indicated the presence of tannins in the drug.

Test for steroid/terpenoid • Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added. Dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicated the presence of terpenoids.

Test for alkaloids

- Draggendorf's test: A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Draggendorf's reagent. Orange coloration of the spot indicated the presence of alkaloids.
- Hager's test: The extract was treated with few ml of Hager's reagent. Yellow precipitation indicated the presence of alkaloids.
- Wagner's test: The extract was treated with few ml of Wagner's reagent. The reddish brown precipitation indicated the presence of alkaloids.

Tests for Glycosides

- **Legal's test**: Dissolved the extract [0.1g] in pyridine [2ml], added sodium nitroprusside solution [2ml] and made alkaline with Sodium hydroxide solution. Pink to red color solution indicates the presence of glycosides.

Test for Saponins

- **Foam test**: 1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes.A1cm layer of foam formation indicates the presence of Saponins

Test for Anthraquinones

- **Borntrager's test**: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. Pink or red coloration of aqueous layer indicated the presence of Anthraquinones.

Test for Amino acids

- **Ninhydrin test**: Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent. Blue color indicated the presence of amino acids.

Invitro methods for antidiabetic activity

α-amylase inhibition assay

Different concentration of extract was taken into different test tubes. Make the volume to 0.5ml with phosphate buffer of pH 6.9, Blank was measured by taking 1 ml of phosphate buffer. Control was measured by taking 0.5ml of phosphate buffer. The solution was then treated with 0.5ml of alpha amylase (0.5mg/ml). The solution was incubated at 25^{o}C for 10 minutes. Added 0.5ml of 1% starch solution in 0.02 M sodium phosphate buffer of pH 6.9 to all the tubes, and then incubate at 25^{o}C for 10 minutes. The reaction was stopped by adding 1.0 ml of DNS and the reaction mixture was kept in boiling water bath for 5 minutes, cooled to room temperature. The solution was mixed with 8 ml distilled water. Read the absorbance of the solution in calorimeter at 570 nM against blank solution [5]. Percentage Inhibition= (Optical density of control – Optical Density of test)/ Optical density of control}× 100]

α-glucosidase inhibition assay

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 1ml with 0.2 M Tris buffer pH 8.0

and various concentration of sample for 5min at 37°C.The reaction is initiated by adding 1ml of α-glucosidase enzyme (1U/ml) to it followed by incubation for 10 min at 37°C. Then, the reaction mixture was heated for 2 min in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidase peroxidase method [6].

Calculation of 50% Inhibitory Concentration (IC50). The concentration of the plant extracts required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by

I % = (Ac-As)/Ac X 100, [16]

Where Ac is the absorbance of the control and as is the absorbance of the sample.

In vitro data were expressed as mean percentage inhibition ± SD. IC_{50} value of percentage inhibition of enzymes was determined using nonlinear regression graph (log_{10} concentration versus percentage enzyme inhibition. All statistical analysis and IC_{50} value determination were carried out in Graph Pad Prism (Version 5.0) software.

Antioxidant assays

DPPH scavenging assay

To different volume of extract, 0.5 ml of methanolic solution of DPPH was added and made up to 2ml using methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and a tube without the extracts served as the positive control. After 30 minutes of incubation, the discolouration of the purple colour was measured at 518nm in a spectrophotometer [1]. The assay was calculated as:

$$\text{Radical scavenging activity} = \frac{\text{Control-Test}}{\text{Control}} \times 100$$

Nitric oxide scavenging capacity assay

The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO•. Under aerobic condition, NO• reacts with oxygen to produce stable products (nitrate and nitrite), which can be determined using Griess reagent. The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with Naphthylethylenediamine dihydrochloride can be immediately read at 550 nm. Four ml of mother tincture or standard solution of different concentration were taken in different test tubes and 1.0 ml of Sodium nitroprusside, (5 mM) solution was added into the test tubes. Then they were incubated for 2 h at 30oC to complete the reaction. Two ml solution was withdrawn from the mixture and mixed with 1.2 ml of Griess reagent (1% Sulfanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H3PO4) and the absorbances of the solutions were measured at 550 nm using a spectrophotometer against blank [7] .Ascorbic acid was used as standard. The percentage (%) inhibition activity was calculated from the following equation: [(A0 – A1)/A0] x 100. Where, A0 is the absorbance of the Control and A1 is the absorbance of the extract or standard. IC50 was calculated by linear regression method[1]**.**

Hydrogen peroxide free radical scavenging assay

Each test tube containing different concentrations of sample was added with 0.6 ml hydrogen peroxide solution and it made up to 5ml with phosphate buffer. After 10m the absorbance of the solution was measured at 230nm using UV against blank solution containing phosphate buffer without using hydrogen peroxide. The % scavenging of hydrogen peroxide for sample and standard compound were determined [8].

I % = (Ac-As)/Ac X 100, [16]

Where Ac is the absorbance of the control and as is the absorbance of the sample

Calculation of IC_{50}

Using 6 different concentrations of sample and standard solution

RESULTS

Phytochemical analysis

The phytochemical analysis of the *Rhus aromatica* mothertincture is shown in below table 1. The antidiabetic and antioxidant properties of Rhus aromatica due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, steroids, tannins etc

Table-1: Phytochemical analysis

Phytochemical components	Rhus aromatica mother tincture
Carbohydrates	+
Proteins	+
Alkaloids	+
Flavonoids	+
Glycosides	+
Terpenoids	+
Steroids	+
Phenols	+
Saponins	+

Table-2: α glucosidase enzyme inhibition assay

Sl No	Concentration (µg/ml)	OD of Std	% inhibition of Std	OD of Test	% inhibition of Test
1	100	0.18±0.02	53.8±1.5	0.71±0.02	26.78±1.47
2	200	0.17±0.01	56.41±1.47	0.64±0.02	34.03±1.44
3	300	0.16±0.02	58.97±1.47	0.51±0.01	47.43±3.67
4	400	0.14±0.01	64.10±1.48	0.15±0.02	61.30±1.48
5	500	0.04±0.01	89.74±2.56	0.08±0.01	79.48±2.96

Values are mean±S.D of triplicate

There was a dose dependent increase in percentage of inhibitory activity against α glucosidase enzyme. At a concentration 100µg/ml of Rhus MT showed a % inhibition 26.78±0.02 and for 500µg/ml it was 79.48±0.01. The IC_{50} Value of standard drug acarbose was found to be 94.28µg/ml and test was found to be 303.03µg/ml.

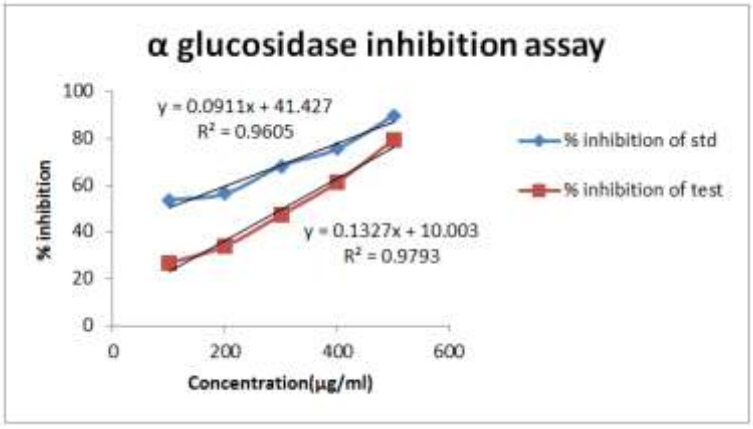

Fig-1: α glucosidase enzyme inhibition assay

Alpha glucosidase enzyme inhibition assay statistical evaluation showed that there was no statistical significant difference between percentage inhibitions of std and test, corresponding to each concentrations ie.*Rhus aromatica* MT possess significant alpha glucosidase enzyme inhibition activity as compared with acarbose.

Table-3: Statistical summary of α glucosidase enzyme inhibition assay

Man whitney t test summary	
P value	0.3095
P value	ns

Table-4: α amylase enzyme inhibition assay

Sl No	Concentration (µg/ml)	OD of Std	% inhibition of Std	OD of test	% inhibition of test
1	100	0.26±0.02	24.62±1.02	0.28±0.01	17.64±4.02
2	200	0.22±0.01	35.29±3.39	0.26±0.01	23.52±1.02
3	300	0.21±0.01	38.23±1.69	0.25±0.01	26.4±4.48
4	400	0.15±0.02	47.05±3.39	0.24±0.02	29.84±3.14
5	500	0.12±0.01	64.70±3.39	0.20±0.01	41.17±3.39

Values are mean± S.D of triplicate

There was a dose dependent increase in percentage of inhibitory activity against α amylase enzyme. At a concentration 100µg/ml of Rhus MT showed a % inhibition 17.64±0.01 and for 500µg/ml it was 41.17±.02. The IC_{50} value of standard drug acarbose was found to be 369.8µg/ml and test IC_{50} value was found to be 722.64µg/ml.

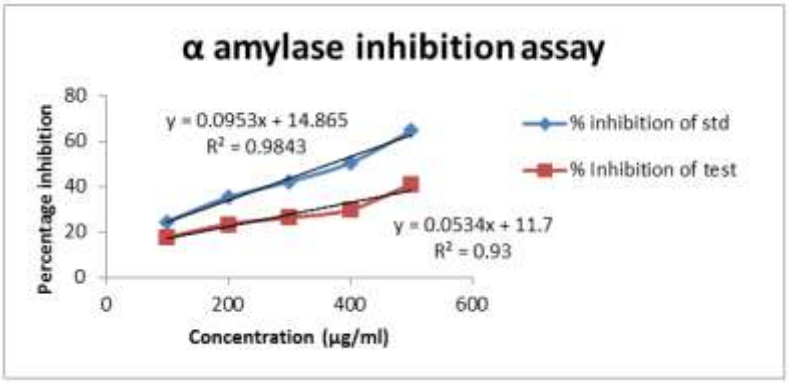

Fig-2: α amylase enzyme inhibition assay

Alpha amylase enzyme inhibition assay statistical evaluation showed that there was no statistical significant difference between percentage inhibitions of std and test, corresponding to each concentrations ie.*Rhus aromatica* MT possess significant alpha glucosidase enzyme inhibition activity as compared with control.

Table-5: Statistical summary of alpha amylase enzyme inhibition assay

Man whitney t test summary	
P value	0.1732
P value	ns

Table-6: DPPH free radical scavenging assay

Sl No	Concentration (µg/ml)	OD of Std	% inhibition of Std	OD of test	% inhibition of test
1	100	0.59±0.01	53.68±1.50	0.68±0.02	46.62±1.4
2	200	0.51±0.01	59.96±1.47	0.62±0.01	51.33±1.2
3	300	0.50±0.02	60.75±1.47	0.60±0.02	52.90±1.6
4	400	0.46±0.01	63.89±1.48	0.59±0.02	53.68±1.5
5	500	0.26±0.01	79.59±2.56	0.58±0.01	54.47±1.8

Values are mean±S.D of triplicate.

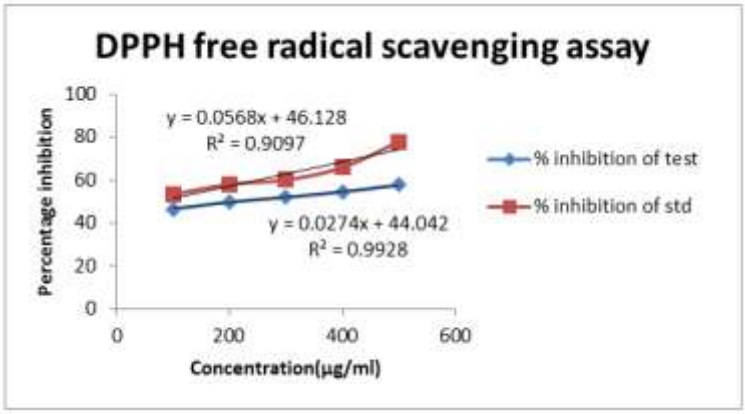

Fig-3: DPPH free radical scavenging assay

From the above graphical method, the IC_{50} value of standard and test was found to be 69.28µg/ml and 220.74µg/ml respectively. Statistically it was showed that there was no significant difference between % inhibition of test and standard corresponding to each concentration ie.sample possess good DPPHfree radical scavenging activity as compared with standard.

Table-7: Statistical summary of DPPH free radical scavenging assay

Man whitney t test summary	
P value	0.0278
P value	ns

Table-8: Nitric oxide free radical scavenging assay

Sl No	Concentration (µg/ml)	OD of Std	% inhibition of Std	OD of Test	% inhibition of test
1	100	0.12±0.06	57.40±4.11	0.19±0.01	32.62±4.093
2	200	0.10±0.06	64.53±2.049	0.17±0.06	39.71±4.093
3	300	0.09±0.02	68.08±2.049	0.15±0.06	46.80±4.09
4	400	0.08±0.05	71.63±2.043	0.14±0.02	53.90±2.02
5	500	0.02±0.06	92.90±2.049	0.07±0.02	75.17±2.52

Values are mean±S.D of triplicate

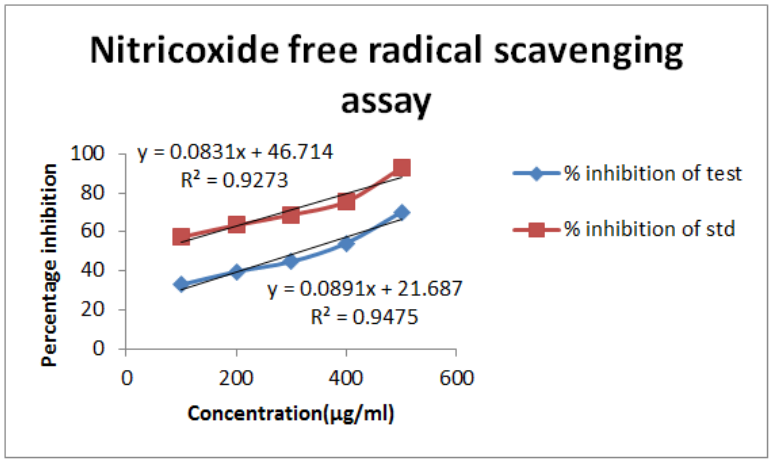

Fig-4: Nitric oxide free radical scavenging assay

From the above graphical method, the IC_{50} value of standard and test was found to be 39.63µg/ml and 318.20µg/ml respectively. Statistically it was showed that there was no significant difference between standard and test corresponding to each concentration ie.sample possess good nitric oxide scavenging activity as compared with ascorbic acid.

Table-9: Statistical summary of Nitric oxide free radical scavenging assay

Man whitney t test summary	
P value	0.0952
P value	ns

Table-10: Hydrogen peroxide free radical scavenging assay

Sl No.	Concentration (µg/ml)	OD of test	% inhibition of test	OD of std	% Inhibition of std
1	100	0.091±0.02	16.66±1.47	0.059±0.02	24.3±0.77
2	200	0.056±0.02	25.64±0.77	0.048±0.04	38.46±2.39
3	300	0.049±0.06	34.32±4.60	0.045±0.02	42.30±2.21
4	400	0.038±0.02	51.28±1.47	0.026±0.04	61.53±3.7
5	500	0.011±0.02	85.89±0.73	0.012±0.02	84.61±0.73

Values are mean±S.D of triplicate

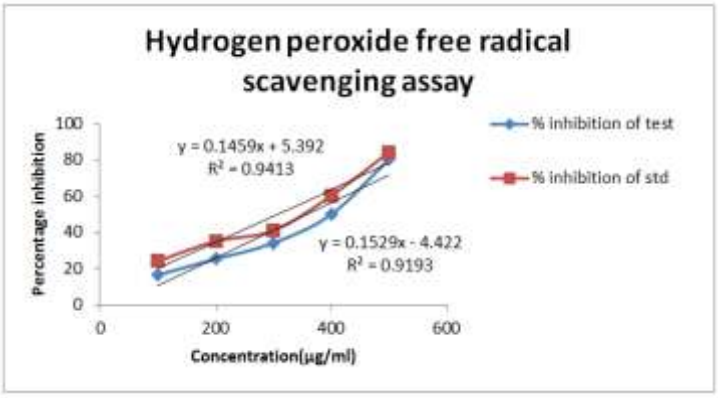

Fig-5: Hydrogen peroxide free radical scavenging assay

From the above graphical method, the IC_{50} value of standard and test were found to be 307.64µg/ml and 357.89µg/ml respectively. Statistically it was showed that there was no significant difference between % inhibition of standard and test corresponding to each concentration.ie. Sample possesses good hydrogen peroxide scavenging activity as compared with standard.

Table-11: Statistical summary of Hydrogen peroxide free radical scavenging assay

Man whitney t test summary	
P value	0.6905
P value	ns

DISCUSSIONS

Diabetes mellitus is mainly due to lack of insulin secretion or action. The carbohydrate metabolic disorder may cause various health problems such as diabetes. Alpha amylase and alpha glucosidase are the carbohydrate metabolizing enzymes. These enzymes are mainly involved in the degradation of oligosaccharides and disaccharides. The inhibition of these enzymes increases the carbohydrate digestion time and reduces the glucose absorption. The mother tincture of *Rhus aromatica* has an invitro antidiabetic activity in a dose dependent manner by the inhibitory activity of alpha amylase and alpha glucosidase enzymes.

Nitric oxide scavenging activity is useful for the prevention from disease caused by excessive NO generation in the human body. Hydroxy radical is capable to damage a vast number of biological molecules in the human body and to join nucleotides in DNA which causes carcinogenesis, mutagenesis and cytotoxicity. Therefore reducing capacity of Rhus aromatica mother tincture may be used as indicator of the antioxidant activity.

REFERENCES

1. Chakraborty D, Samadder A, Dutta S, Khuda-Bukhsh AR. Antihyperglycemic potentials of a threatened plant, Helonias dioica: antioxidative stress responses and the signaling cascade. Experimental Biology and Medicine. 2012 Jan;237(1):64-76.
2. Reichling J, Neuner A, Sharaf M, Harkenthal M, Schnitzler P. Antiviral activity of Rhus aromatica (fragrant sumac) extract against two types of herpes simplex viruses in cell culture. Die Pharmazie-An International Journal of Pharmaceutical Sciences. 2009 Aug 1;64(8):538-41.
3. Trevor AJ, Katzung BG, Masters SB. Basic &

clinical pharmacology. McGraw-Hill Medical; 2009.
4. Raja M & Muthu D. (n.d.). Screening of preliminary phytochemical analysis for Homoeopathic Drugs. 1–6.
5. Kishore L, Singh R. Effects of different homeopathic potencies of Cephalendra indica in treatment of neuropathic pain in streptozotocin induced diabetes. Bulletin of Faculty of Pharmacy, Cairo University. 2017 Dec 1;55(2):273-80.
6. Tomar RS, Sisodia SS. Antidiabetic activity of Annona squamosa Linn. in alloxan-induced diabetic rats. International Journal of Green Pharmacy (IJGP). 2014;8(4).
7. Ahmed MF, Kazim SM, Ghori SS, Mehjabeen SS, Ahmed SR, Ali SM, Ibrahim M. Antidiabetic activity of Vinca rosea extracts in alloxan-induced diabetic rats. International Journal of Endocrinology. 2010;2010.
8. Journal I, Review PS, Unit DZ. In vitro Antioxidant and in vivo Antidiabetic Potential of n-butanol extract of Chrysanthemum fuscatum in Streptozotocin Induced..., (January). 2017.