ejpmr, 2022,9(7), 436-442

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DIURETIC ACTIVITY OF EXTRACTS OF LEAVES AND PREMATURE FLOWERS OF VITIS VENIFERA (LINN.) ON EXPERIMENTAL ANIMAL

Shaikh Sohel*, Jugal Kishor Vyas, Anjali Wankhade and Vivek Paithankar

Vidyabharti College of Pharmacy, CAMP, Amravati-444602 Maharashtra.

***Corresponding Author: Shaikh Sohel**

Vidyabharti College of Pharmacy, CAMP, Amravati-444602 Maharashtra.

Article Received on 09/05/2022 **Article Revised on 30/05/2022** **Article Accepted on 20/06/2022**

ABSTRACT

Grapes (*Vitis vinifera* L.) are commonly known grape species that belong to the Vitis genus in the Vitaceae family and come from western Asia and southern Europe. This review consists of traditional uses, phytochemical compounds, nutritional constituents, pharmacological activities, genotoxicological studies, and toxicity studies of *V. vinifera*. The data were obtained from scientific databases and search engines such as PubMed, Elsevier, Springer, Frontiers, Google Scholar, Scopus, Science Direct, and MDPI. In some countries, grapes used for traditional uses, such as drug therapy for blood-forming, anemia, allergies, wound care, colds and flu, carminative, bronchitis, diarrhea, and anti-phlegm. The main phytochemical compounds in *V. vinifera* are phenolic compounds, aromatic acids, flavonoids, proanthocyanidins, and stilbenoids. Nutritional constituents can be found in grapevines, i.e., proteins, lipids, carbohydrates, minerals, and vitamins. Parts of the grapevines had a wide variety of biological activities, i.e., antioxidant, antiviral, antiplatelet, antifungal, anticataract, antiobesity, anticholinergic, anti-sunburn, anti-inflammatory, and wound-healing activities. The phytochemical compounds content in each part of the grapevines were different. Each pharmacological activity depends on the grapevine's phytochemical compounds, components used, and extraction type. However, more studies are needed regarding the genotoxicity and toxicity of *V. vinifera.*

KEYWORDS: Diuresis, Vitis Venifera, Mechanism of Urine formation, Pharmacological studies, Screening, etc.

INTRODUCTION

Medicinal plants have been widely used as a source for the treatment of human disorders since the ancient times to this date.[1] Between 70 and 95% of people in developing nations rely on herbal medicines for managing different disease ailments and their health benefits are growing rapidly in recent time.[2] One of the application areas of botanicals is their diuretic effect. Therefore, herbal medicines are employed to tackle edematous condition such as such as cardiac failure, cirrhosis, and nephritic syndrome that lead to fluid overload in the body.[3]

1.1 Diuresis

Diuresis refers to increased urine production and excretion by the kidneys, and sometimes it is accompanied by loss of electrolytes such as sodium, chloride and potassium. It could be caused by high blood sugar, diabetes mellitus, diabetes insipidus, medications, polydipsia, acute renal failure, etc. Some side effects of diuresis include fatigue, headache, weakness, lethargy, hypokalemia, hypomagnesaemia, hypernatremia, metabolic acidosis and hypotension.[4] Diuresis is a condition in which the kidneys filter too much bodily fluid. That increases your urine production and the frequency with which you need to use the bathroom.

Most adults will urinate about four to six times a day, with average output between 3 cups and 3 quarts of urine. People with diuresis urinate more often than that, even though their fluid intake may not have changed. Despite the side effect of diuresis, intentional diuresis is necessary in the treatment or management of kidney disorders, hypertension and congestive heart failure, in order to remove water from patients' body. Few examples of diuretics include furosemide, hydrochlorothiazide, mannitol, bumetanide, chlorothiazide and amiloride. Cardiovascular diseases are responsible for approximately one-third of all deaths throughout the world.[5] Conditions such as hypertension lead to other types of diseases, such as stroke, and kidney and other heart diseases. Common clinical strategies to achieve lowering of blood pressure include the use of angiotensin converting enzyme inhibitors, beta blockers, calcium-channel blockers and diuretics. Diuretics mostly work by stimulating urine output together with urinary excretion of sodium from the body. Diuretics such as the high-ceiling loop and thiazides diuretic have been associated with several side effects, such as electrolyte and metabolic changes, new-onset diabetes development, renin-angiotensin system activation and weakening of sexual function [6].This fact necessitate that there is a

strong need for novel diuretics which are relatively safe with better or equivalent diuretic activity.

1.2 PATHOPHYSIOLOGY

1.2.1 Urine[8]

The urine produced is 95% water and 5% nitrogenous wastes. Wastes such as urea, ammonia, creatinine are excreted in the urine. Apart from these, the potassium, sodium and calcium ions are also excreted. Urine is a liquid by-product of metabolism in humans and in many other animals. Urine flows from the kidneys through the ureters to the urinary bladder. Urination results in urine being excreted from the body through the urethra.

Cellular metabolism generates many by-products that are rich in nitrogen and must be cleared from the bloodstream, such as urea, uric acid, and creatinine. These by-products are expelled from the body during urination, which is the primary method for excreting water-soluble chemicals from the body. A urinalysis can detect nitrogenous wastes of the mammalian body.

Characteristics of urine[11]

Quantity

Average urine production in adult humans is around 1.4 L of urine per person per day with a normal range of 0.6 to 2.6 L per person per day, produced in around 6 to 8 urinations per day.

Constituents

About 91-96% of urine consists of water. The remainder can be broadly characterized into inorganic salts, urea, organic compounds, and organic ammonium salts. Urine also contains proteins, hormones, and a wide range of metabolites, varying by what is introduced into the body.

Color

Urine varies in appearance, depending principally upon a body's level of hydration, interactions with drugs, compounds and pigments or dyes found in food, or diseases.

pH

The pH normally is within the range of 5.5 to 7 with an average of 6.2. In persons with hyperuricosuria, acidic urine can contribute to the formation of stones of uric acid in the kidneys, ureters, or bladder. Urine pH can be monitored by a physician or at home.

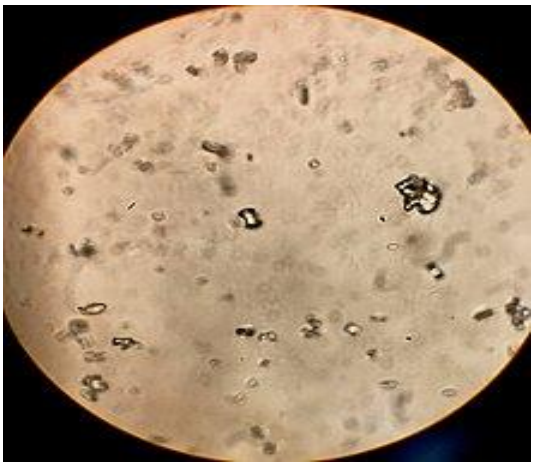

Fig. 2: Microscopic image of urine.

Density

Human urine has a specific gravity of 1.003–1.035.

Urine formation mechanism[9]

Waste is excreted from the human body, mainly in the form of urine. Our kidneys play a major role in the process of excretion. Constituents of normal human urine include 95 percent water and 5 percent solid wastes. It is produced in the nephron, which is the structural and functional unit of the kidney. Urine formation in our body is mainly carried out in three phases namely

1. Glomerular filtration
2. Reabsorption
3. Secretion

Table no1: hormones involved in Urine production

Hormones That Affect Osmoregulation and kidney function		
Hormone	**Where produced**	**Function**
Epinephrine and Norepinephrine	Adrenal medulla	Can decrease kidney function temporarily by vasoconstriction
Renin	Kidney nephrons	Increases blood pressure by acting on angiotensinogen
Angiotensin	Liver	Angiotensin II affects multiple processes and increases blood pressure
Aldosterone	Adrenal cortex	Prevents loss of sodium and water
Anti-diuretic hormone – ADH (vasopressin)	Hypothalamus (stored in the posterior pituitary)	Prevents water loss

1.3 DIURESIS

Diuresis is increased urination (polyuria) or, in the related word senses more often intended, the physiologic process that produces such an increase or the administration of medications to encourage that process. It involves extra urine production in the kidneys as part of the body's homeostatic maintenance of fluid balance. Diuresis is a condition in which the kidneys filter too much bodily fluid. That increases your urine production

and the frequency with which you need to use the bathroom.

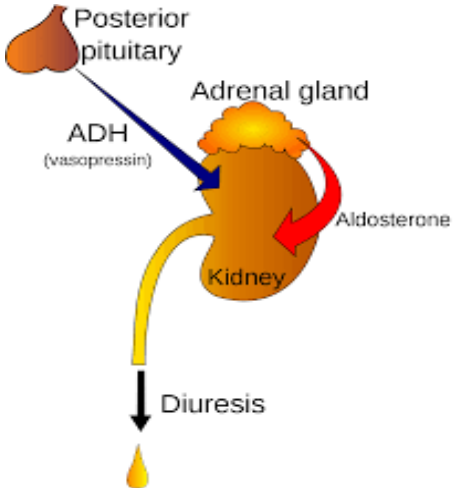

Fig 3: diuresis.

1.3.1 Types of diuresis[10]

1. Osmotic diuresis
2. Forced diuresis
3. Rebound diuresis
4. Immersion diuresis
5. cold-induced diuresis

MATERIALS AND METHODS

Pharmacognostic study

Identification, collection and authentication of plant material

The parts of plant were collected from Amravati in month of September. The plant specimen was authenticated by Professor Mrs. Ghurde, Botanist, Vidyabharati Mahavidyalaya. After cleaning parts of plant from foreign particles they spread over trays and separately dried in shade, pulverized by a mechanical grinder and passed through 40-mesh sieve to get the fine powder, finally subjected to extraction.

Extraction parts of Vitis Vinifera Linn

The commonly employed technique for the separation of the active constituents from the crude drug is called extraction which involves the use of different solvents. Many of the complex substances metabolized by the plants have therapeutic importance. But these are always found in association with other substances. Therefore in order to study these active constituents alone it has to be separated from other unwanted substances produced.

Preparation of extract

Leaves and flowers of plant were collected, washed, dried in shade and pulverized in a grinder mixer to obtain a coarse powder and then passed through 40 mesh sieves. The powdered drug was subjected to solvent extraction by soxhlet apparatus.

Extraction procedure

About 100g of powdered drug was extracted successively with 70% ethanol using soxhlet apparatus. The extraction was carried out for 72 hours. Then the solvent was completely removed by evaporating in rotatory flask evaporator. The dried extract thus obtained was kept in refrigerator until the further experiment.

Experimental Animals

Female wistar rats (120 -150 gm) were maintained in an air-conditioned room at 25°C±2°C, with a relative humidity of 55%±5%, and a 12-h light/dark cycle. A basal diet and tap water were provided ad libitum. Male and female rats were assigned to each dose group by stratified random sampling based on body weight. The animals were kept under laboratory conditions for an acclimatization period of 7 days before carrying out the experiments.

Experimental procedure: 1000mg/kg Dose was administered to overnight fasted Rats .Food was withheld for a further 3-4 hours after administration of Vitis Vinifera leaves and flower extract and observed for signs for toxicity. The body weight of the rats's before and after administration were noted that changes in eyes and mucous membranes, skin and fur, respiratory, and also motor activity and behavior pattern. Special attention was directed to observations of convulsions, tremors, diarrhea, salivation; lethargy, sleep, and coma were noted. The onset of toxicity and signs of toxicity of LD50 values are noted.

Pharmacological screening

Animals

Healthy female wistar rats were procured from animal house and weigh about 120-125gms were used for the study. Every experimental animal was clinically examined preoperatively for any disease. The animals were kept under observation in laboratory and allowed acclimatize, for 7 days before experimentation. Animals kept in separate spacious clean cages under controlled room temperature (25±1)°c & relative humidity (50±15)%; in a 12hrs light-dark cycles. They fed with a standard diet and water ad-libitum. Before the experiment the rats were divided into groups.

Table no 5. Groups of Animals.

Sr. no.	Group	No. of Animals	Treatment and Dose	Route of Administration
1	I	6	Saline only	Oral
2	II	6	Standard drug (furosemide 10mg/kg body weight)	Oral
3	III	6	Test extract of Vitis Vinifera leaves extract	Oral
4	IV	6	Test extract of Vitis Vinifera flower extract	Oral

Procedure

Rats were divided into four groups contain six animal each. Group I serves normal which receives normal saline (5ml/kg b wt). Group two serves as standard which receives furosemide (10mg/kg). Group three and four receives leaves 500 mg and flower extract 500 mg respectively through oral route. The animals were deprived of food and water for 18hrs prior to the experiment. Route of administration used for all groups was oral using gavage. Food and water were withdrawn 8 h before the administration of drug. Immediately after dosing, all the animals were placed individually in metabolic cages and urine passed by the animals over a period of 24 hours was collected in a jar. Total urine output, Urinary Osmolality, pH was determined.

Determination of Diuretic Activity

Diuretic activity was determined following the methods used by Lipschitz, with slight modification .Each rat was placed in an individual metabolic cage 24 h prior to initiation of the experiment for acclimatization and then fasted for 18 h with free access to water ad libitum.

Before treatment, all animals were given oral saline with a volume of 15 mL/kg body weight. Each group then received standard drug, water and various doses of extract. Immediately after dosing, the rats were placed individually in a metabolic cage. The urine was then collected, measured and the pH determined after five hours. Urinary electrolytes concentrations, Na+, K+ and Cl- were estimated. The following parameters were calculated in order to compare the effects of extracts with those of the vehicle and standard.

RESULTS AND DISCUSSION

Extract

Table No 6: Extract of shows Vitis Vinifera yield, colour, nature.

Sr.No	Extract	Result	
		leaves	flower
1	Yield	13.8%	12.3%
2	Colour	Greenish yellow	green

PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical analysis revealed the presence of carbohydrates, alkaloids, flavonoids, tannins, proteins, gums and phenolic compounds in Vitis Vinifera extract.

Table No 7: Preliminary Phytochemical analysis.

SR.NO.	COMPOUNDS	VVLE	VVFE
1	Carbohydrates	+	+
2	Tannins	+	+
3	Alkaloids	+	+
4	Saponin	-	_
5	Flavonoid	+	+
6	Fixed oil	+	-

+: presence of compounds

-: absence of compounds

Acute toxicity study

In acute toxicity study, all the animals were found to be surviving after 72 hrs. This indicates that extracts were found to be safe upto the dose levels studied. Since all animals survived at a dose of 1000 mg/kg body weight of both extracts, the LD_{50} Of the extract will be >1000mg/kg body weight. No major behavioral changes were observed during this period of study.

DIURETIC STUDIES

Urine volume excretion after 5 hrs

The urine excretion induced by the Vitis Vinifera was dose dependent, however only 500 mg/kg of VVFE revealed slight significant incease ($p < 0.05$) in the volume of urine excreted when compared with the control. The extract produced significant diuresis with VVLE ($p<0.05$). Standard drug (FURO10) also produced apparent urine output. The volume of urine excreted by furosemide group was more than twice the volume of urine excreted by the control. Diuretic index of FURO10 showed highest followed by VVFE 500.

Table No 8: Diuretic activity of Vitis Vinifera extracts in rats after 5 hrs of oral administration.

Group	Volume of urine(ml)	Diuretic index	Diuretic Activity
NC	5.16±0.21	1.00	-
Standard(furo)	10.15±0.17	1.96	1
VVFE 500 mg/kg	7.35±0.15**	1.42	0.72
VVLE 500 mg/kg	8.05±0.23**	1.56	0.79

Notes: Each value represents mean ± S.E.M; n=4; Analysis was performed by one way ANOVA; acompared to negative control; **p<0.05; number followed by FURO, VVLE and VVFE indicates dose/kg. Diuretic index = volume problem group/volume control group.

Urinary pH

Urinary pH measurement revealed that the different treatment groups of both extracts produced relatively alkaline urine. The NC group produced the lowest pH and the standard group an intermediate pH (7.50) between vehicle and extract-treated groups. Group comparison revealed that the pH of urine from rats treated with the higher doses of both extracts (VVFE and VVLE) displayed a significant ($p<0.05$) increase compared to negative control.

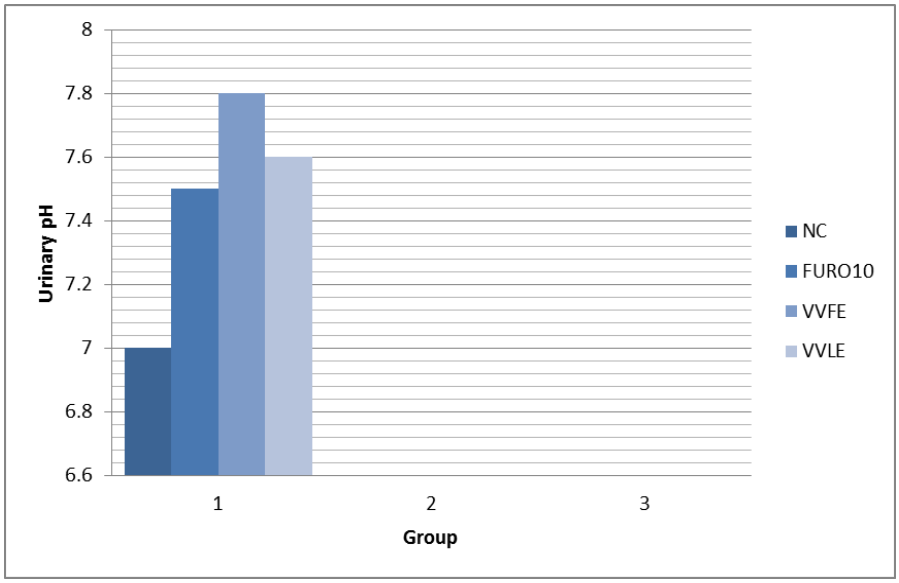

Fig. No.9: Urinary pH of rats treated with extracts of the leaves of Vitis Vinifera.

DISCUSSION

Diuresis could be beneficial for treatment of a number of disease conditions such as congestive heart failure, nephritic syndrome, hypertension, liver cirrhosis, poisoning, and certain kidney diseases. It is also important in kidney stone treatment. An increased fluid volume flowing through the kidney could dissolve the stones, and flush out the deposits.[50] In addition to conventional drugs, numerous herbal preparations are used as diuretics. There are also scientific studies that have been carried out to support the diuretic effects of many traditional medicinal herbal products.[51] The various parts of Vitis Vinifera has a range of promising medicinal claims among which its diuretic potential is yet to be supported by scientific evidence. Therefore, the aim of this study was to evaluate the diuretic activity of extract.

The present study revealed that Vitis Vinifera has notable diuretic effects in the given animal model. As diuretics are employed clinically in treatment of edema, it would be most important to demonstrate effectiveness in the presence of electrolyte and water. Accordingly, saline was given to impose water and salt load or simulate edema. Regarding acute toxicity, leaf extract of Vit. Vinifera does not produce a toxic response in rats at a dose of 1000 mg/kg in extracts.

Vitis Vinifera was studied for presence of a variety of secondary metabolites. Procyanidin, gallic acid, epicatechin, catechin, quercetin, white grapes has flavonol glycosides. In a previous study, the quantitative phytochemical analysis carried out on leaf extract of F showed presence of flavonoids terpenoids (1280.39 mg/100g), tannins and alkaloids in larger amounts.

CONCLUSION

The aim of the study is to evaluate the Diuretic activity of the extract of Leaves and flowers of Vitis Vinifera.

Rats were divided into four groups contain six animal each. Group I serves normal which receives normal saline (5ml/kg b wt). Group two serves as standard which receives furosemide (10mg/kg). Group three and four receives 250 mg and 500 mg respectively through oral route. The animals were deprived of food and water for 18hrs prior to the experiment. Route of administration used for all groups was oral using gavage. Food and water were withdrawn 8 h before the administration of drug. Immediately after dosing, all the animals were placed individually in metabolic cages and urine passed by the animals over a period of 24 hours was collected in a jar. Total urine output, Urinary Osmolality, pH was determined.

The present finding, therefore, supports the traditional claim of the plant as a diuretic agent. The urinary PH and electrolytes analysis results hint that the extracts to have multiple modes of action. The safety profile of the extract was an added advantage that calls for conducting further research to ascertain the findings reported in this study.

ACKNOWLEDGEMENTS

I thank all who in one way or another contributed in the completion of this thesis. First, I give thanks to the Almighty Allah for protection and ability to do work.
I am grateful to all of those with whom I have had the pleasure to work during this and other related projects.
I have gratitude to Mr. Raosahed Shekhawat, Chairman of Vidyabharati Educational society for keen interest, moral support and providing all the facilities to carry out the present study to successful completion.
I would like to acknowledge my indebtedness and render my warmest thanks to my supervisor, Professor Mr. Jugalkishor V.Vyas Sir, Head of Pharmacology Department, who made this work possible. His friendly guidance and expert advice have been invaluable throughout all stages of the work. I would also wish to

express my gratitude to Professor for discussions and valuable suggestions which have contributed greatly to the improvement of the thesis under whose active guidance, innovative ideas, constant inspiration and encouragement of the work entitled “Diuretic activity of extracts of leaves and premature flowers of vitis venifera (linn.) On experimental animal” has been carried out.

The thesis has also benefited from comments and suggestions made by Dr. Anjali Wankhade Maam and Dr.V.V. Paithankar Sir who have read through the manuscript. I take this opportunity to thank them.

I take this opportunity to express my thanks to Dr.M.U.Ghurde Botanist, for identification and evaluation of the plant material used in this study.

I would like to express my utmost thanks to my batchmates Rutuja Nistane, Janhavi Burade, Shubham Marbade, Shivam Sable, Pravin Paikrao, Shubhangi Bhagat, Alka Zade for their support during my project work.

I express my sincere thanks to lab technicians and attenders for their help throughout the study.

I am also deeply thankful to my informants. Their names cannot be disclosed, but I want to

Acknowledge and appreciate their help and transparency during my research. Their information have helped me complete this thesis. I am also so thankful to my fellow students whose challenges and productive critics.

Nobody has been more important to me in the pursuit of this project than the members of my family. I would like to thank my parents, whose love and guidance are with me in whatever I pursue. They are the ultimate role models.

I thank my family who encouraged me and prayed for me throughout the time of my research. May the Almighty Allah richly bless all of you.

REFERENCES

1. Yadav RH. Medicinal plants in folk medicine system of Ethiopia. J Poisonous Med Plant Res., 2013; 1(1): 7–11.
2. Organization WH. WHO Traditional Medicine Strategy 2002–2005.
3. Geneva: World Health Organization; 2002: 2015. 3. Vazir A, Cowie MR. The use ofdiuretics in acute heart failure: evidence based therapy World J Cardiovascular Diseases, 2013; 03: 25–34. doi:10.4236/wjcd.2013.32A004
4. Woodrow, P. Assessing fluid balance in older people: fluid needs: In the first of two articles about assessing fluid balance in older people, Philip Woodrow reviews physiology and how to assess fluid needs. Nursing Older People, 2002; 14(9): 31-32.
5. World Health Organization ISoHWG: World Health Organization (WHO)/International Society of Hypertension (ISH) statement on management of hypertension. J. Hypertens, 2003; 21: 1983-1992.
6. Gasparotto JA, Boffo MA, Lourenço ELB, Stefanello MEA, Kassuya CAL, Marques MCA. Natriuretic and diuretic effects of Tropaeolum majus (Tropaeolaceae) in rats. J. Ethnopharmacol, 2009; 122(3): 517-522.
7. Moh. Adil Tahseen*, Garima Mishra Ethnobotany and Diuretic Activity of Some Selected Indian Medicinal Plants: A Scientific Review; the pharma innovation – journal 2013; ISSN: 2277- 7695, 2013; 2(3).
8. Rose, C.; Parker, A.; Jefferson, B.; Cartmell, E. "The Characterization of Feces and Urine: A Review of the Literature to Inform Advanced Treatment Technology". Critical Reviews in Environmental Science and Technology, 2015; 45(17): 1827–1879.
9. Shils, Maurice Edward; Shike, Moshe. Modern Nutrition in Health and Disease. Lippincott Williams & Wilkins, 2006. ISBN 9780781741330. 2015-05-11.
10. Ganong, William F. Review of Medical Physiology. McGraw-Hill Professional, 2005; 719. ISBN 978-0-07-144040-0.https://en.wikipedia.org/wiki/Diuretic
11. Ajay K. Singh; Gordon H. Williams. Textbook of Nephro-Endocrinology. Academic Press, 12 January, 2009; 250–251. ISBN 978-0-08-092046-7.
12. C.I. Wright, L. Van-Buren, C.I. Kroner, M.M.G. Koning,Herbal medicines as diuretics: A review of the scientific evidence, Journal of Ethnopharmacology, 2007; 114(1): 131. ISSN03788741, https://doi.org/10.1016/j.jep.2007.07.023.(https://www.sciencedirect.com/science/article/pii/S0378874107003662).
13. C.V. Pantoja et al Diuretic and natriuretic effects of chromatographically purified fraction of garlic (Allium sativum) Journal of Ethnopharmacology, 1996.
14. D.C. BraterPharmacology of diuretics American Journal of the Medical Sciences, 2000.
15. Shweta Parihar & Devender Sharma. A Breif Overview on Vitis Vinifera. Sch Acad J Pharm, Dec, 2021; 10(12): 231-239.
16. Aouey, B., Samet, A. M., Fetoui, H., Simmonds, M. S., & Bouaziz, M. Anti-oxidant, antiinflammatory, analgesic and antipyretic activities of grapevine, 2016.
17. Esatbeyoglu, T., Ewald, P., Yasui, Y., Yokokawa, H., Wagner, A. E., Matsugo, S., ... & Rimbach, G. Chemical characterization, free radical scavenging, and cellular antioxidant and antiinflammatory properties of a stilbenoid-rich root extract of Vitis Vinifera. Oxidative medicine and cellular longevity, 2016.
18. Goufo, P., Singh, R. K., & Cortez, I. A reference list of phenolic compounds in grapevine (Vitis Vinifera L.) roots, woods, canes, stems, and leaves. Antioxidants, 2020; 9(5): 398.
19. Cádiz-Gurrea, M. D. L. L., Borrás-Linares, I., Lozano-Sánchez, J., Joven, J., Fernández-Arroyo, S., & Segura-Carretero, A. Cocoa and grape seed byproducts as a source of antioxidant and anti-inflammatory proanthocyanidins. International journal of molecular sciences, 2017; 18(2): 376.

20. Pérez-Navarro, J., Cazals, G., Enjalbal, C., Izquierdo-Cañas, P. M., Gómez-Alonso, S., Saucier, C. Flavanol glycoside content of grape seeds and skins of Vitis Vinifera varieties grown in Castilla-La Mancha, Spain. Molecules, 2019; 24(21): 4001.