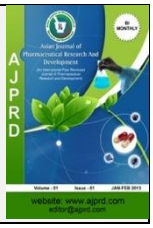
Available online on 15.08.2020 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research



Open Access

Research Article

Repurposing of Dipeptidyl Peptidase-4 Inhibitors

Sravanthi Reddy Nulaka, Anusha Nimmala, Rakesh Pasupuleti, Sripriya Ieppala, Narender Boggula, Vasudha Bakshi, Naveen Pathakala*

School of Pharmacy, Anurag University, Venkatapur, Ghatkesar, Medchal, Telangana, India.

ABSTRACT

Back ground: Anti-oxidants play an important role to protect damage caused by oxidative stress (OS). Free radical and Reactive Oxygen Species (ROS) are basically the main cause of several disorders in humans that are generated as an imbalance between formation and neutralization of pro-oxidants resulting in oxidative stress. They cause oxidative damage to lipids, proteins, and DNA, eventually leading to many chronic diseases, such as cancer, diabetes, aging and other degenerative diseases in humans. Various studies have been done to identify anti-oxidants from plant sources and efforts have been taken to incorporate it in conventional therapy.

Objective: The aim of the present research work is to assess the repurposing potential of dipeptidyl peptidase-4 inhibitor by checking its anti-oxidant activity.

Methods: Anti-oxidant potential of the vildagliptin was studied using different *in vitro* methods. The anti-oxidant activity was estimated by using DPPH radical scavenging activity, nitric oxide scavenging assay, hydrogen peroxide scavenging assay and reducing power assay methods. The ascorbic acid used as a standard reducing agent. All the analysis was made with the use of UV-Visible spectrophotometer.

Results: The extract showed good dose dependent free radical scavenging property in both the models used in this study. The results of the assay showed that it possesses significant free radical scavenging and reducing power properties at concentration-dependent manner.

Conclusion: This study suggests that vildagliptin exhibit great potential for anti-oxidant activity and may be useful for their medicinal functions. However, further exploration is necessary for effective use in both modern systems of medicines. This will further trigger extensive research for better understanding of the impact of vildagliptin on health.

Key words: DPP-4 inhibitors, DPPH assay, ascorbic acid, vildagliptin, *in-vitro* anti-oxidant activity, UV-Visible spectrophotometer.

ARTICLE INFO: Received 04 July 2020; Review Completed 25 Sept. 2020; Accepted 02 Oct. 2020; Available online 15 Oct. 2020

Cite this article as:

Nulaka SR, Nimmala A, Pasupuleti R, Ieppala S, Boggula N, Bakshi V, Pathakala N, Repurposing of Dipeptidyl Peptidase-4 Inhibitors, Asian Journal of Pharmaceutical Research and Development. 2020; 8(5):59-66.
DOI: http://dx.doi.org/10.22270/ajprd.v8i5.838

***Address for Correspondence:**

Naveen Pathakala, Assoc. Professor, Department of Pharmacology, School of Pharmacy, Anurag University,Venkatapur, Ghatkesar, Medchal, Hyderabad, Telangana, INDIA-500088

INTRODUCTION

Drug discovery is a time-consuming, laborious, costly and high-risk process. According to a report by the Eastern Research Group (ERG), it usually takes 10-15 years to develop a new drug. However, the success rate of developing a new molecular entity is only 2.01%, on average[1]. As demonstrated in a report by the Food and Drug Administration (FDA), the number of drugs approved by the FDA has been declining since 1995. Moreover, investment in drug development has been gradually increasing, as reported by Pharmaceutical Research and Manufacturers of America (PhRMA). This indicates that the cost of new drug development will continue to grow. Hence, it is urgent to find a new strategy to discover drugs[2].Drug repurposing holds the potential to bring medications with known safety profiles to new patient populations. The term "drug repositioning" has been used interchangeably with "drug repurposing" or "drug reprofiling"[3]. Drug repurposing is viewed as an approach to rediscovering value in "old molecules" and finding new therapeutic uses, particularly in areas with high risk of failure, such as psychiatry[4]. All these expressions are relatively synonymous for describing the process that seeks

to discover new applications for an existing drug numerous examples exist for the identification of new indications for existing molecules, most stemming from serendipitous findings or focused recent efforts specifically limited to the mode of action of a specific drug[5].

This decreases the overall cost of bringing the drug to market because the safety and pharmacokinetic profiles of the repositioned candidates are already established. It is in fact quite common but it has been mostly done by serendipity over the years. It is only recently that more systematic approaches based on computational analyses are being used[6]. Faced with scientific and economical challenges the possibility of finding new indications for drugs is an attractive proposition for the industry. Drug repurposing could be applied at many phases of drug discovery and development but has a greater potential when the drug has already been tested for safety. Recently there has been a significant growth of publications for novel, more systematic and non-obvious approaches for computational drug discovery[7].

The process of oxidation in the human body damages cell membranes and other structures, including cellular proteins, lipids and DNA. When oxygen is metabolised, it creates unstable molecules called 'free radicals'[8], which steal electrons from other molecules, causing damage to DNA and other cells. The body can cope with some free radicals and needs them to function effectively. However, the damage caused by an overload of free radicals over time may become irreversible and lead to certain diseases, including heart disease, liver disease and some cancers[9] (such as oral, oesophageal, stomach and bowel cancers). Oxidation can be accelerated by stress, cigarette smoking, alcohol, sunlight, pollution and other factors. You can find these antioxidants in many different foods that you're probably eating right now[10]. The recommendations based on epidemiological studies are such that fruits, vegetables and less processed staple foods ensure the best protection against the development of diseases caused by oxidative stress, such as cancer, coronary heart disease, obesity, type 2 diabetes, hypertension and cataract[11]. The explanation consists in the beneficial health effect, due to antioxidants present in fruit and vegetables. There are numerous antioxidants in dietary plants: carotenoids, phenolic compounds, benzoic acid derivatives, flavonoids, proanthocyanidins, stilbenes, coumarins, lignans, and lignins. Of the 50 analysed food products with high antioxidant content, 13 were spices, 8 were fruits and vegetables, 5 were berries, 5 were chocolate based, 5 were breakfast cereals, and 4 were nuts or seeds. Considering the typical serving sizes, blackberries, walnuts, strawberries, artichokes, cranberries, brewed coffee, raspberries, pecans, blueberries, ground cloves, grape juice and unsweetened baking chocolate were at the top of the classification[12].

Anti-oxidants are involved in the defense mechanism of the organism against the pathologies associated to the attack of free radicals[13]. Endogenous antioxidants are enzymes, like superoxide dismutase, catalase, glutathione peroxidase or non-enzymatic compounds, such as uric acid, bilirubin, albumin, metallothioneins[14]. When endogenous factors cannot ensure a rigorous control and a complete protection of the organism against the reactive oxygen species, the need for exogenous anti-oxidants arises, as nutritional supplements or pharmaceutical products, which contain as active principle an anti-oxidant compound[15]. Amongst the most important exogenous anti-oxidants, vitamin E, vitamin C, β-carotene, vitamin E, flavonoids, mineral & Se are well exogenous anti-oxidants can derive from natural sources (vitamins, flavonoids, anthocyanins, some mineral compounds), but can also be synthetic compounds, like butylhydroxyanisole, butylhydroxytoluene, gallates etc[16].

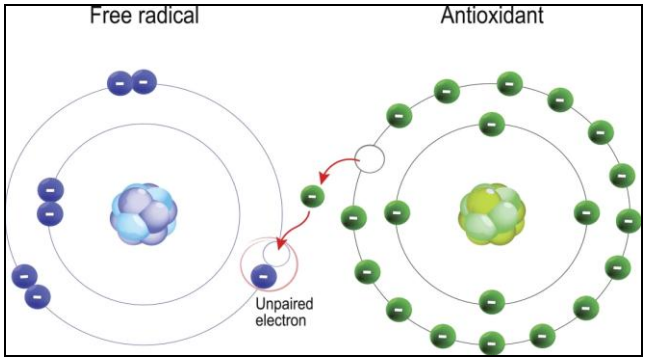


Figure 1: Effect of free radicals

Free radicals are unstable atoms that can damage cells, causing illness and aging. To become more stable, they take electrons from other atoms[17]. This may cause diseases or signs of aging. According to the free radical theory of aging, first outlined in 1956, free radicals break cells down over time[18].

As the body ages, it loses its ability to fight the effects of free radicals. The result is more free radicals, more oxidative stress, and more damage to cells, which leads to degenerative processes, as well as "normal" aging[19]. The free radical theory of aging is relatively new, but numerous studies support it. For example, studies on rats showed significant increases in free radicals as the rats aged. These changes matched up with age-related declines in health. Over time, researchers have tweaked the free radical theory of aging to focus on the mitochondria[20]. Mitochondria are tiny organelles in cells that process nutrients to power the cell. Research on rats suggests that free radicals produced in the mitochondria damage the substances that the cell needs to work properly. This damage causes mutations that produce more free radicals, thus accelerating the process of damage to the cell. This theory helps explain aging, since aging accelerates over time[20]. The gradual, but increasingly rapid build-up of free radicals offers one explanation for why even healthy bodies age and deteriorate over time[21].

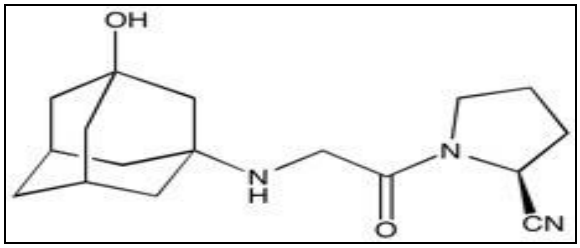


Figure: 2 Chemical structure of vildagliptin

The Molecular formula of vildagliptin is $C_{17}H_{35}N_3O_2$[22]. The Molecular weight of vildagliptin is 303.406g/mol, and

protein binding is about 9.3%. Vildagliptin, previously identified as LAF237, is a new oral anti-hyperglycemic agent (anti-diabetic/hypoglycemic drug) of the new dipeptidyl peptidase-4 (DPP-4) inhibitor class of drugs. Vildagliptin inhibits the inactivation of GLP-1 and GIP by DPP-4, allowing GLP-1 and GIP to potentiate the secretion of insulin in the beta cells and suppress glucagon release by the alpha cells of the islets of Langerhans in the pancreas. It is currently in clinical trials in the US and has been shown to reduce hyperglycemia in type 2 diabetes mellitus[23]. While the drug is still not approved for use in the US, it was approved in Feb 2008 by European Medicines Agency for use within the EU and is listed on the Australian PBS with certain restrictions. It will cause some adverse effects like nausea, hypoglycemia, tremor, headache, dizziness, weakness, constipation, weight gain, sore throat, excessive sweating, heart burn and swelling of face, lips, eyelids, tongue, hands and feet. And it is used to treat type 2 diabetes mellitus and also reduces appetite[24].

The aim of the present research work is to investigate the repurposing potential of dipeptidyl peptidase-4 inhibitor by checking its anti-oxidant activity.

***In-vitro* anti-oxidant activity screening**

In the present study, the commonly used anti-oxidant evaluation methods such as DPPH radical scavenging activity, nitric oxide scavenging assay, hydrogen peroxide scavenging assay and reducing power assay methods were chosen to determine the anti-oxidant potential.

2,2-Diphenyl 1-picryl hydrazyl [DPPH] radical scavenging assay:

0.1mM DPPH solution was prepared by dissolving DPPH 1.9mg in 50 ml of ethanol. This stock solution was prepared freshly and kept in the dark at ambient temperature when not in used. About 2mg of vildagliptin (test samples) were dissolved in 20 ml ethanol to obtain a solution of 100µg/ml[25]. From this stock solution, different working dilution were prepared to get concentration of 10, 20, 30, 40, 50 µg/ml with ethanol as a solvent. The standard stock solution was prepared by dissolving ascorbic acid (standard sample) in ethanol with a final concentration of 100µg/ml and different concentration of 10, 20, 30, 40, 50 µg/ml were prepared by using ethanol.

0.1mM solution of DPPH in ethanol was prepared and 0.5 ml of this solution was added to 1.5 ml of sample solution and incubate it for 30 min, and the absorbance was taken at 517nm. The difference in the absorbance between test and control of DPPH was calculated and expressed as percent scavenging of DPPH radical[26]. The difference in the absorbance between test and control of DPPH was calculated and expressed as percent scavenging of DPPH radical.

$$IC_{50}\ \% = 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \text{ X } 100$$

Nitric oxide scavenging assay:

About 2mg of vildagliptin (test samples) were dissolved in 20 ml with ethanol to obtain a solution of 100µg/ml. From this stock solution, different working dilution were prepared to get concentration of 10, 20, 30, 40, 50 µg/ml with ethanol[27]. The standard stock solution was prepared by dissolving ascorbic acid (standard sample) in ethanol with a final concentration of 100µg/ml and different concentration of 10, 20, 30, 40, 50 µg/ml were prepared by ethanol.

Sodium nitroprusside 5mM in phosphate buffer at pH 7.4 saline was mixed with different concentrations of the test sample or standard and incubated at 35 ^{0}C for 150 min. At regular intervals, 1.5 ml of samples (incubated solution) was removed and diluted with 1.5 ml of Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% naphthyl ethylenediamine dihydrochloride). The absorbance was read at 546nm. The difference in the absorbance between test and control on nitric oxide was calculated and expressed as percent scavenging of nitric oxide radical[28]. Capability to scavenge the nitric oxide radical was calculated by using equation:

$$IC_{50}\ \% = 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \text{ X } 100$$

Hydrogen peroxide scavenging assay:

About 2mg of vildagliptin (test samples) were dissolved in 20 ml with ethanol to obtain a solution of 100µg/ml. From this stock solution, different working dilution were prepared to get concentration of 10, 20, 30, 40, 50 µg/ml with ethanol. The standard stock solution was prepared by dissolving ascorbic acid (standard sample) in ethanol with a final concentration of 100µg/ml and different concentration of 10, 20, 30, 40, 50 µg/ml were prepared by ethanol[29]. 4 ml of standard and test solution was added to 0.6 ml hydrogen peroxide solution. After 10 min, the absorbance of the solution was measured at 230nm using UV-Vis spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging of hydrogen peroxide of both plant fraction and standard compound were determined[30]. The percentage inhibition was calculated for the samples and standard using the following:

$$IC_{50}\ \% = 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \text{ X } 100$$

Reducing power assay:

About 2mg of vildagliptin (test samples) were dissolved in 20 ml with ethanol to obtain a solution of 100µg/ml. From this stock solution, different working dilution were prepared to get concentration of 10, 20, 30, 40, 50 µg/ml with ethanol. The standard stock solution was prepared by dissolving ascorbic acid (standard sample) in ethanol with a final concentration of 100µg/ml and different concentration of 10, 20, 30, 40, 50 µg/ml were prepared by ethanol[31]. To 1 ml of prepared sample solution, add 2.5 ml of phosphate buffer (pH 7.4). To this, add 2.5 ml of 1% potassium ferricyanide solution, after incubation at 50 ^{0}C for 20 min, add 2.5 ml of 10% trichloroacetic acid the contents were centrifuged at 3000rpm for 10 min and about 1.5 ml of supernatant liquid was collected and mixed with 2.5 ml of distilled water and 0.5 ml of 1% ferric chloride. The absorbance was read at 700nm using UV-Vis

spectrophotometer. Difference in the absorbance between test and control was calculated and expressed as reducing power in percentage[32,33]. Ability of reducing power of test and control in percentage was calculated by:

$$IC_{50}\ \% = 1 - \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \text{ X } 100$$

RESULTS AND DISCUSSION

A drug repurposing strategy could be a potential approach to overcoming the economic costs for diabetes mellitus (DM) treatment incurred by most countries. DM has emerged as a global epidemic, and an increase in the outbreak has led developing countries like Mexico, India, and China to recommend a prevention method as an alternative proposed by their respective healthcare sectors. Incretin-based therapy has been successful in treating diabetes mellitus, and inhibitors like sitagliptin, vildagliptin, saxagliptin, and alogliptin belong to this category. As of now, drug repurposing strategies have not been used to identify existing therapeutics that can become dipeptidyl peptidase-4 (DPP-4) inhibitors.

Glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 (DPP-4) inhibitors have been successful in clinical use in regulating glucose metabolism in diabetes mellitus patients apart from other therapies like insulin, sulfonylureas, biguanides, meglitinides, thiazolidinediones, and alpha-glucosidase inhibitors. The mechanism of DPP-4 inhibitors is to stop the degradation of incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) by the DPP-4 enzyme to regulated glucose metabolism. DPP-4 also degrades regulatory factors like chemokines and growth factors revealing the role of DPP-4 inhibitors in controlling inflammatory diseases**.**

Reactive oxygen species such as hydroxyl, superoxide and peroxyl radicals are formed in human cells by endogenous factors and exogenously result in extensive oxidative damage that in turn lead to geriatric degenerative conditions, cancer and a wide range of other human diseases. Anti-oxidant research is an important topic in the medical field as well as in the food industry. Many plants, particularly medicinal ones, have been extensively studied for their anti-oxidant activity in recent decades. The anti-oxidants act either by scavenging various types of free radicals derived from oxidative processes, by preventing free radical formation through reduction precursors or by chelating agents. The anti-oxidant results were illustrated in the following tables and figures.

Table 1: DPPH scavenging assay

S. No.	Conc. of sample (µg/ml)	Absorbance of ascorbic acid	% Inhibition	Absorbance of vildagliptin	% Inhibition
1	10	0.32	41	0.13	28
2	20	0.69	60	0.15	37
3	30	1.1	69	0.17	44
4	40	2.6	80	0.57	54
5	50	2.8	86	1.2	65

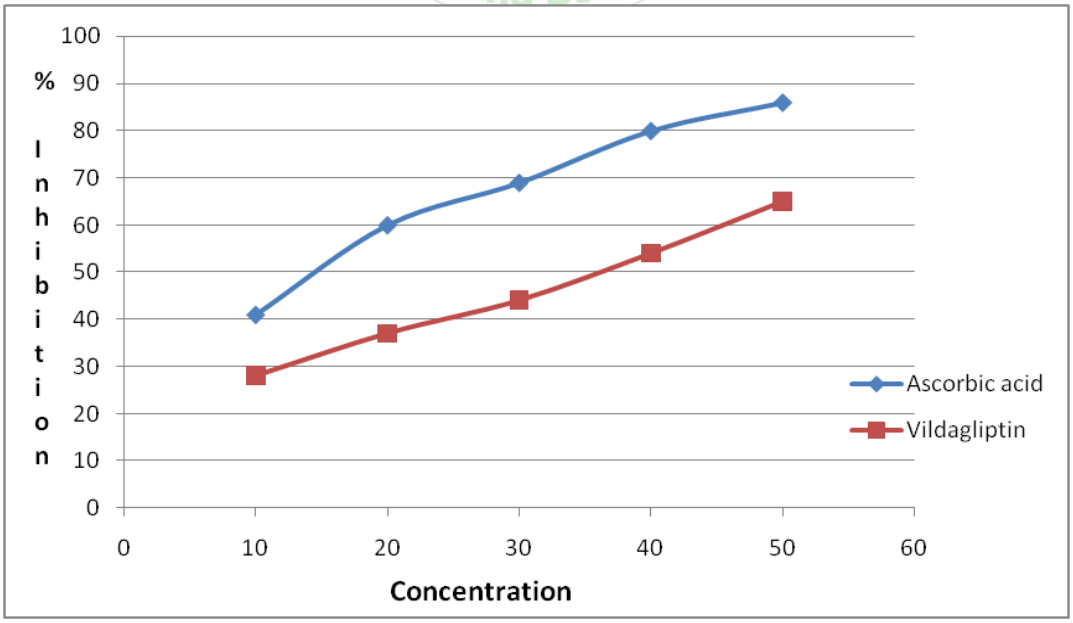


Figure 3: Percentage inhibition of vildagliptin against ascorbic acid by DPPH scavenging assay

DPPH radical is a stable radical with a maximum absorption at 517nm that can readily undergo reduction by an anti-oxidant. The vildagliptin were found to possess concentration dependent scavenging activity on DPPH radicals and the results were given in Table 1 and Figure 3. The percentage inhibition (% inhibition) at various concentrations (10-50 µg/ml) of vildagliptin and standard ascorbic acid (10-50 µg/ml) were calculated. The % inhibition of vildagliptin is compared with standard ascorbic acid. The results showed that the test sample shows half of the activity of standard. It needs to be tested for repeatability and reproducibility. The maximum IC_{50} value for vildagliptin is found at 50µg/ml (65%).

Table: 2 Nitric oxide scavenging assay

S. No.	Conc. of sample (µg/ml)	Absorbance of ascorbic acid	% Inhibition	Absorbance of vildagliptin	% Inhibition
1	10	0.75	40	0.06	31
2	20	0.56	49	0.13	39
3	30	0.78	69	0.17	45
4	40	1.6	78	0.29	53
5	50	2.3	88	0.4	70

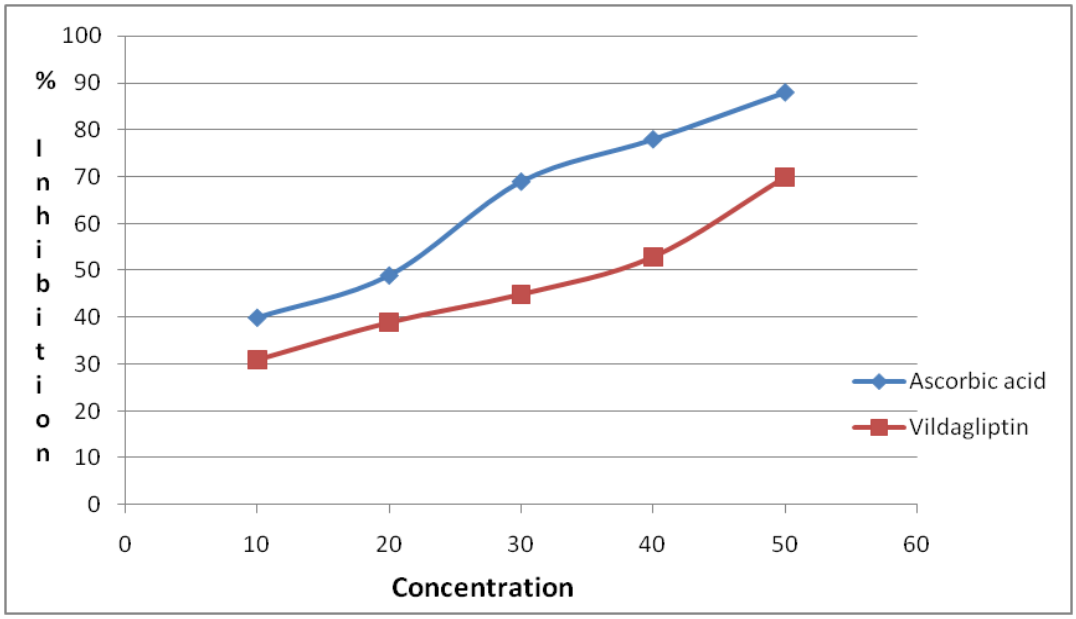


Figure: 4 Percentage inhibition of vildagliptin against ascorbic acid by nitric oxide scavenging assay

The nitric oxide scavenging activity of vildagliptin were detected and compared with ascorbic acid. The percentage inhibition (% inhibition) at various concentrations (10-50 µg/ml) of vildagliptin and standard ascorbic acid (10-50 µg/ml) were calculated. The % inhibition of vildagliptin is compared with standard ascorbic acid. The results showed that the test sample shows half of the activity of standard. It needs to be tested for repetability and reproducibility. The maximum IC_{50} value for vildagliptin is found at 50µg/ml (65%).

Table: 3 Hydrogen peroxide scavenging assay

S. No.	Conc. of sample (µg/ml)	Absorbance of ascorbic acid	% Inhibition	Absorbance of vildagliptin	% Inhibition
1	10	0.33	39	0.25	25
2	20	0.59	57	0.59	36
3	30	1.9	78	0.89	49
4	40	2.55	87	1.5	60
5	50	3.1	95	1.7	79

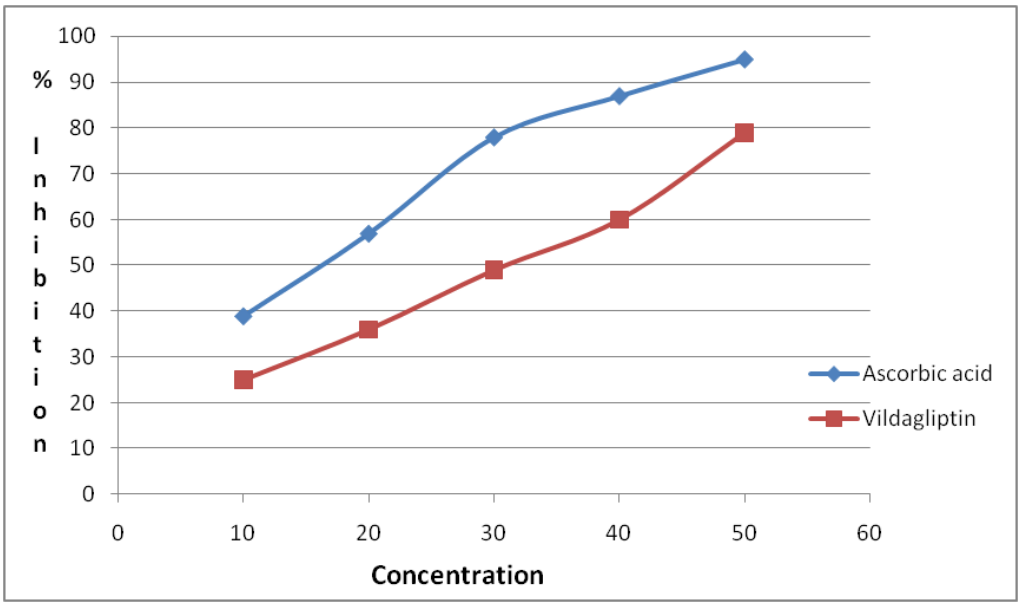


Figure 5: Percentage inhibition of vildagliptin against ascorbic acid by hydrogen peroxide scavenging assay

The scavenging ability of vildagliptin on hydrogen peroxide scavenging assay is shown in the above table and compared with reference standard drug ascorbic acid. Vildagliptin was capable of H_2O_2 scavenging dose dependent manner. The percentage inhibition (% inhibition) at various concentrations (10-50 µg/ml) of vildagliptin and standard ascorbic acid (10-50 µg/ml) were calculated. The % inhibition of vildagliptin is compared with standard ascorbic acid. The results showed that the test sample shows half of the activity of standard. It needs to be tested for repetability and reproducibility. The maximum IC_{50} value for vildagliptin is found at 50µg/ml (79%).

Table: 4 Reducing power assay

S. No.	Conc. of sample (µg/ml)	Absorbance of ascorbic acid	% Inhibition	Absorbance of vildagliptin	% Inhibition
1	10	0.48	29	0.033	19
2	20	0.55	41	0.049	30
3	30	0.64	66	0.072	44
4	40	0.86	80	0.098	59
5	50	0.98	88	0.146	75

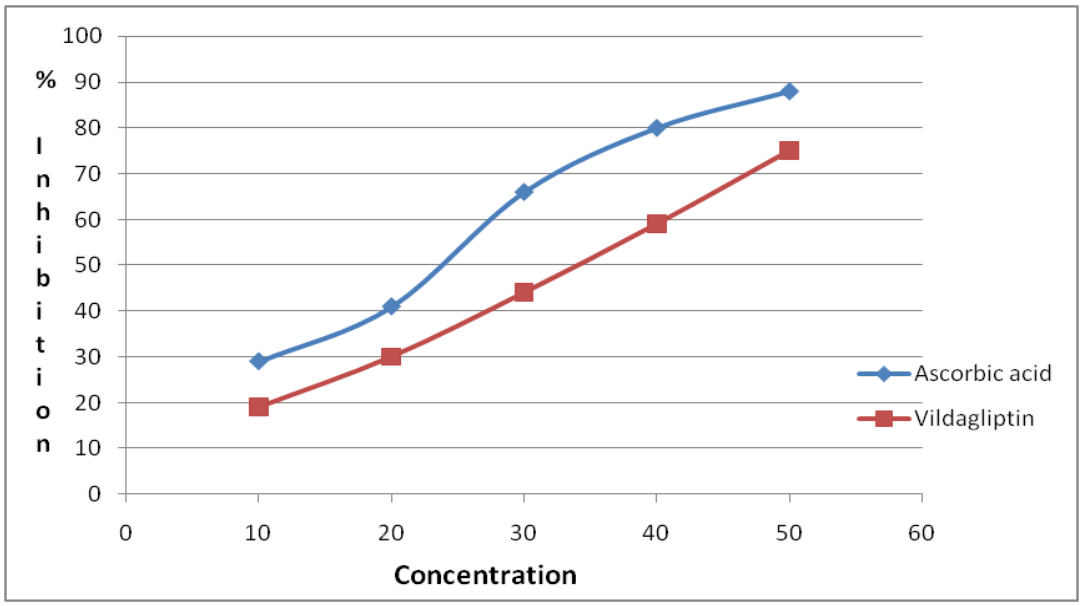


Figure: 6 Percentage inhibition of vildagliptin against ascorbic acid by reducing power assay

The ability of vildagliptin to reduce ferric to ferrous was determined by accoding to this method. The reducing capacity of a compound may serve as a significant indicator of its potential anti-oxidant activity. The % inhibition at various concentrations (10-50 µg/ml) of vildagliptin and standard ascorbic acid (10-50 µg/ml) were calculated. The % inhibition of vildagliptin is compared with standard ascorbic acid. The results showed that the test sample shows half of the activity of standard ascorbic acid. It needs to be tested for repeatability and reproducibility. The maximum IC_{50} value for vildagliptin is found at 50µg/ml (75%).

CONCLUSION

Anti-oxidants are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions. Free radicals or ROS formed in the body as a result of biological oxidation. Anti-oxidants are the compounds that, when added to food products, especially to lipids and lipid-containing foods, can increase the shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food products during processing and storage[33,34].

A DPPH method is most frequently used one for *in-vitro* anti-oxidant activity evaluation. On the basis of the results obtained from the present study, it is concluded that our sample vildagliptin exhibits nearly half of the activity of standard ascorbic acid. This concludes that the given sample vildagliptin can be used for anti-oxidant activity. However, the components responsible for the anti-oxidative activity are currently unclear. Therefore, further investigations need to be carried out to isolate and identify the anti-oxidant compounds present in the samples. These results are novel and provide new insights into the role of anti-oxidants as a therapeutic strategy for treating various disorders. Future studies will focus on understanding the mechanism.

Declarations

ACKNOWLEDGEMENT

The authors wish to thank the management of School of Pharmacy, Anurag University, Venkatapur, Ghatkesar, Medchal, Telangana, India for providing necessary equipment for research, praiseworthy inspiration, constant encouragement, facilities and support.

Author contributions

All authors contributed to data collection, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

All authors declare that there is no conflict of interests regarding publication of this paper.

Additional information

No additional information is available for this paper.

Financial support and Sponsorship

None.

Ethical approval

No.

REFERENCES

1. Y Cha, T Erez, IJ Reynolds, D Kumar, J Ross, G Koytiger, R Kusko, B Zeskind, S Risso, E Kagan, S Papapetropoulos, I Grossman, D Laifenfeld. Drug repurposing from the perspective of pharmaceutical companies. British Journal of Pharmacology. 2018; 174(2):168-180.
2. HanqingXue, Jie Li, HaozheXie, and YadongWang. Review of Drug Repositioning Approaches and Resources. International Journal of Biological Sciences. 2018; 14(10):1232-1235.
3. Christine Roder, Melanie J. Thomson. Auranofin: Repurposing an Old Drug for a Golden New Age. Drugs in R&D. 2015; 15:13-20.
4. Erin E. Mulvihill, Daniel J. Drucker. Pharmacology, Physiology, and Mechanisms of Action of Dipeptidyl Peptidase-4 Inhibitors. Endocrine Reviews. 2014; 35(6):992-1019.
5. Sarita Bajaj. RSSDI clinical practice recommendations for the management of type 2 diabetes mellitus 2017. Int J Diabetes Dev Ctries. 2018; 38(Suppl 1):1–115.
6. Aurelia Magdalena Pisoschi, Gheorghe Petre Negulescu. Methods for total antioxidant activity determination biochemistry & analytical biochemistry Pisoschi and Negulescu. Biochem & Anal Biochem. 2011; 1:01-03.
7. Sunil Kumar, The importance of antioxidant and their role in pharmaceutical science Asian Journal of Research in Chemistry and Pharmaceutical Sciences. 2014; 1(1):27-30.
8. Adrianvella. Mechanism of Action of DPP-4 Inhibitors. The Journal of Clinical Endocrinology & Metabolism. 2012; 97(8):2626–2628.
9. Satish Babu, Vildagliptin - A New Prospect in Management of Type 2 Diabetes. Indian Journal of Clinical Practice. 2012; 22(8):377.
10. Shirisha K, Priyanka B, Habibur Rahman, Dipankar Bardalai, Fulch an Ali. Review on *Albizia lebbeck* (L.) Benth: A Plant Possessing Diverse Pharmacological Activities. Research Journal of Pharmacognosy and Phytochemistry. 2013; 5(5):263-268.
11. Priyanka B, Anitha K, Shirisha K, Janipasha SK, Dipankar B, Rajesh K. Evaluation of antioxidant activity of ethanolic root extract of *Albizia lebbeck* (L.) Benth. International Research Journal of Pharmaceutical and Applied Sciences. 2013; 3(2):93-101.
12. Akhtar SN, Dhillon P. Prevalence of diagnosed diabetes and associated risk factors: evidence from the large-scale surveys in India. Journal of Social Health and Diabetes. 2017; 5(1):28.
13. Mohan V, Sandeep S, Deepa R, Shah B, Varghese C. Epidemiology of type 2 diabetes: Indian scenario. Indian Journal of Medical Research. 2007; 125(3):217.
14. Kodama K, Tojjar D, Yamada S, Toda K, Patel CJ, Butte AJ. Ethnic differences in the relationship between insulin sensitivity and insulin response. Diabetes care. 2013; 36(6):1789–1796.
15. S Chandra Mohan, V Balamurugan, S Thiripura Salini, R Rekha. Metal ion chelating activity and hydrogen peroxide scavenging activity of medicinal plant *Kalanchoe pinnata*. Journal of Chemical and Pharmaceutical Research. 2012; 4(1):197-202.

16. Joseph NM, Sabharwal Monika, Shashi Alok, Mahor Alok, Rawal Shruti. *In-vitro* and *in-vivo* models for antioxidant activity evaluation: A review. International Journal of Pharmaceutical Sciences and Research. 2010; 1(1):1-11.

17. Naithani V, Singhal AK, Chaudhary M. Comparative evaluation of Metal Chelating, Antioxidant and Free Radical Scavenging activity of TROIS and six products commonly used to control pain and inflammation associated with Arthritis. Int. J. Drug Dev. & Res. 2011; 3(4):208-216.

18. Emanuel Almeida Moreira de Oliveira, Karen Luise Lang. Drug Repositioning: Concept, Classification, Methodology, and Importance in Rare/Orphans and Neglected Diseases. Journal of Applied Pharmaceutical Science. 2018; 8(08):157-165.

19. Abdul-Lateef Molan, Abbas Mohammed Faraj, Abdulkhaliq Saleh Mahdy. Antioxidant activity and phenolic content of some medicinal plants traditionally used in Northern Iraq. Phytopharmacology. 2012; 2(2):224-233.

20. Bhuiyan MAR, Hoque MZ, Hossain SJ. Free Radical Scavenging Activities of *Zizyphus mauritiana*. World J. Agr. Sci. 2009; 5(3):318-322.

21. Maesden S. Blois. Antioxidant determinations by the use of a stable free radical. Nature. 1958; 181:1199-1200.

22. R. Govindarajan, M. Vijaykumar, A.K. Rawat, S. Mehrotra. Free radical scavenging potential of *Picrorhiza kurrooa* Royle ex Benth. Indian J Exp Biol. 2003; 41:875-879.

23. M Oyaizu. Studies on Products of Browning Reaction, Antioxidant Activities of Products of Browning Reaction Prepared from Glucose Amine. Jap. J. Nutr. 1986; 44:307-315.

24. Hatano T, Edamatsu R, Mori A. Effect of interaction of tannins and related poyphenols on superoxide anion radical and on DPPH radical. Chem Pharm Bull. 1989; 37:2016-2021.

25. Hausladen A, Stamer JS. Nirosative stress. Method in Enzymology. 1999; 300:389-395.

26. Huang DJ, Chen HJ, Lin CD, Lin YH. Antioxidant and antiproliferative activities of water spinach (*Ipomea aquatic* Frosk.) constituents. Bot Bull Acad Sin. 2005; 46:99-106.

27. Jayaprakash GK, Rao LJ. Phenolic constituents from lichen *Parmontrema stuppeum*. Food Control. 2000; 55: 1018-1022.

28. Koksal E, Bursal E, Dikici E, Tozoglu F, Gulcin I. Antioxidant activity of *Melissa officinalis* leaves. J. Med. Plant. Res. 2011; 5(2):217-222.

29. Koleva II, Van Beek TA, Linssen JPH, de G root A, Evstatieva LN. Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem Anal. 2002; 13(1):8-17.

30. Meir S, Kanner J, Akiri B, Hada SP. Determination and involvement of aqueous reducing compounds in oxidative defense system of various senescing leaf. J. Agri. Food chem. 1995; 43:1813-1819.

31. Roberta Re, Nicoletta Pellegrini, Anna Proteggente, Ananth Pannala, Min Yang Catherine Rice-Evans. Antioxidant activity applying an improved ABTS Radical cationdecolorization assay. Free Radical Biology and Medicine. 1999; 26:1231-1237.

32. Umamaheswari M, Chatterjee TK. *In vitro* antioxidant activities of the fractions *of Coccinia grandis* L. leaf extract. Afr. J. Traditional, complementary and Alternative Medicines. 2008; 5(1):61-73.

33. Narender Boggula, Himabindu Peddapalli. Phytochemical analysis and evaluation of *in vitro* anti oxidant activity of *Punica granatum* leaves. International Journal of Pharmacognosy and Phytochemical Research. 2017; 9(8):1110-1118.

34. Yanala Suresh Reddy, Krishna Mohan Chinnala, K. Vamshi Sharath Nath, E. Madhan Mohan, B. Preethi and B. Narender. *In vitro* evaluation of anti-oxidant activity of different extracts of *Justicia gendarussa* leaf. Der Pharma Chemica. 2015; 7(8):21-24.