International Journal of Research in Pharmacy and Life Sciences

Journal Home Page: www.pharmaresearchlibrary.com/ijrpls

Research Article Open Access

In-vitro evaluation of Anti-oxidant and Anti-diabetic activity of ethanolic extract of *Psychotria octosulcata.*

K. Naveen*, P. Naresh Babu

Department of Pharmacology, Ratnam Institute of Pharmacy, Pidathapolur, SPSR Nellore, A.P, India.

A B S T R A C T

The present study was undertaken to evaluate the antidiabetic and antioxidant effect of extract, namely ethaonol of whole plant by *in vitro* assays. *Psychotria octosulcota* whole plant were dried and subjected to ethanolic extraction. Antidiabetic activity was measured by α- glucosidase inhibitor and α- amylase inhibitor assay. Antioxidant activity was studied by measuring the total antioxidant activity by 1-1-Diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH) free radical scavenging assay, Superoxide anion radical scarvenind assay and Nitric oxide radical inhibition assay method. IC50 values of both extracts were calculated for all the assays. The results showed that ethanolic extract of whole plant of *Psychotria octosulcota* have the potential to inhibit alpha glucosidase with IC50 value of 0.2 5mg/ml. Similar to antidiabetic effect. Therefore,it is suggested that ethonolic extract of *Psychotria octosulcota* (EEPO) is a potential source for natural antidiabetic and antioxidant compounds and could have potential use in the management of diabetesmellitus**.**

Keywords: *Psychotria octosulcota*, α-glucosidase, α-amylase, antidiabetic, antioxidant

A R T I C L E I N F O

CONTENTS

Article History: Received 28 September 2016, **Accepted** 22 October 2016, **Available Online** 24 November 2016

***Corresponding Author**
K. Naveen
Department of Pharmacology,
Ratnam Institute of Pharmacy,
Pidathapolur, Nellore, A.P, India.
Manuscript ID: IJRPLS3181

PAPER-QR CODE

Citation: K. Naveen. In-vitro evaluation of Anti-oxidant and Anti-diabetic activity of ethanolic extract of Psychotria octosulcata.. *Int. J. Res. Pharm, L. Sci.*, 2016, 4(2): 81-85.

1. Introduction

India continues to be the 'Diabetic Capital' of the world with 50.8 million diabetics. Similar trends have also been found in the companion animals, that is, dogs and cats.[1] Diabetes is a growing problem in dogs and cats and

prevalence is increasing over time due to several reasons such as genetics, environmental, obesity, physical inactivity, etc.[2] Diabetes mellitus may be categorized into several types but the two major types are type 1 and type II. Drugs are used primarily to save life and alleviate symptoms.[3] Secondary aims are to prevent long-term diabetic complications and, by eliminating various risk factors, to increase longevity.[4] According to WHO, it is estimated that 3% of the world's population haven diabetes and the prevalence is expected to double by the year 2025 to 6.3% [2]. Management of diabetes without any side effect is still a challenge to the medical community.[5]

Oral hypoglycaemic agents are also useful in the treatment of type II DM. Oral hypoglycaemic agents include sulphonylureas, biguanides, alpha glucosidase inhibitors, meglitinide analogues, and thiazolidenediones.[6] The main objective of these drugs is to correct the underlying metabolic disorder, such as insulin resistance and inadequate insulin secretion.[7] They should be prescribed in combination with an appropriate diet and lifestyle changes.[8] Diet and lifestyle strategies are to reduce weight, improve glycaemic control and reduce the risk of cardiovascular complications, which account for 70% to 80% of deaths among those with diabetes.[9]Plant species of the genus Psychotria (Rubiaceae) have been extensively studied, particularly due to the presence of bioactive alkaloids.[10] Literature review showed that the extracts of many Psychotria species showed anti-inflammatory and analgesic activity. [11]As the plant was enriched with antioxidant constituents it may be used for relieving free radical induced pathogenesis. The whole plant of *Psychotria octosulcata* will be extracted with ethanol and investigated for its antidiabetic activity.[12]

2. Materials and methods

Plant Material and Extraction

The whole plant of *Psychotria octosulcata* was collected from Seshachalam forest, Tirumala, Chitoor Dt and identified by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, Chitoor Dt, A.P. The collected whole plant was immediately dried at room temperature for one month, powdered mechanically sieved (10/44) and stored in air tight containers. After collection of the plant, shade drying the whole plant of *psychotria octosulcata* were then blended in to fine powder with a blender and used for the aqueous and ethanol extracts.[14] Ethanol extract was extracted by using soxhlet extractor for 18-20 h. The extract obtained, was concentrated under reduced pressure at controlled temperature (40-50° C) and finally made powdered.[15]

Experimental animals:

Male Wistar albino rats (130-160gm) were used in the study. Animals were housed individually in polypropylene cages in a ventilated room under ambient temperature of 22±2° C and 45-65 % relative humidity, with a 12 hour light followed by 12 hour dark. All the animals were acclimatized at least 7days to the laboratory conditions prior to experimentation. Tap water and standard food pellets were provided ad libitum. Food pellet was with held overnight prior to dosing. All rats were handled and maintained strictly as per guidelines of Guide for the care and Use of Laboratory animals.[16]

Phytochemical analysis:

The ethanoilc extract of whole plant of *psychotria octosulcata* was subjected to different chemical tests separately for the identification of various active constituents.[17]

Acute oral toxicity study

The acute oral toxicity study was performed as per the Organisation for Economic and Cooperation and Development (OECD) 423 guidelines. Six female rats (nulliparous and non pregnant; 130-160 gm bwt) were divided into two groups (3 per group) ie., control and test groups. Control group received 0.5 % carboxy methyl cellulose as vehicle at a dose of 10ml/kg b wt while the test group received an oral dose of 2000mg/kg b wt of ethanolic extract of whole plant of *Psychotria octosulcata* [EEPO] (10ml/kg b wt in 0.5% CMC).[18]

Pharmacological studies:

In vitro antioxidant activity: EEPO was evaluated for its anti oxidant activity by selecting the following activities. 1, 1-Diphenyl-2-picrylhydrazy (DPPH) free radical scavenging activity, Superoxide anion radical scavenging activity and Nitric oxide radical inhibition assay.[19]

1,1-Diphenyl-2-pycrylhydrazy l (DPPH) free radical scavenging activity and Superoxide anion radical scavenging activity:[20,21] DPPH free radical scavenging activity and superoxide anion scavenging was measured by the formula given as follows

DPPH scavenging effect (%) = $[(A_x - A_y)/ A_x] \times 100$

A_x = Absorbance of the control reaction

A_y = Absorbance in the presence of extracts or standards.

The amount of the samples needed to inhibit 50% of the radical (IC_{50}) had been then calculated.

Nitric oxide radical inhibition assay[22]

Sodium nitroprusside (10mM) of 2ml was added to 0.5ml of phosphate buffer saline. This mixture was then added to 0.5ml of extracts or 0.5 ml of standard solution and at 25^0C it was incubated for 150min. Then reaction mixture of 0.5ml had been pipette away and blended with 1ml of sulphanilic acid reagent (0.33 percent in 20 percent of glacial acetic acid) and kept apart 5min for diazotization reaction. N-(1-*Naphthyl*) *ethylenediamine dihydrochloride* (1%) of 1ml was mixed to it and allowed to stand for 30min.The resultant index was formation of a pink colour chromophore in incandescent light. Finally, all the solutions were measured for absorbance at 540 nm. Gallic acid and rutin were taken as standard reference.The IC_{50} value has been measured for all the solutions.

***In-vitro* antidiabetic activity:**

Inhibition of carbohydrate digesting enzymes

Diabetes is a multifactorial illness to a wide range of problems and, therefore, it needs numerous healing approaches. Acting as a key enzyme for carbohydrate digestion is abdominal α-glucosidase, α-glucosidase secreted in the epithelium of the small intestine. α-glucosidase has acknowledged as a healing target for the modulation of postprandial hyperglycemia, which could be

the early metabolic disturbance that develops in NIDDM. In this respect, inhibitors can prevent the uptake of nutritional carbohydrates, suppress post-prandial hyperglycemia and could be helpful for treating patients with diabetes or obesity[54].α-glucosidase inhibitors such as acarbose, miglitol, and voglibose are known to reduce postprandial hyperglycemia mainly by interfering with the activity of carbohydrate-digesting enzymes and post poning glucose absorption. In addition, many inhibitors that are α-glucosidase actually been removed from plants, which are of medical value.[23]

α-glucosidase inhibitory assay [24]

The inhibition rate was calculated by the formula that is following

Inhibition rate (%) = [{(amount of glucose produced by the control that is positive - (amount of glucose produced by the addition of EEPO) - (glucose production value in blank)/ (amount of glucose produced by the positive control)}] x 100.

α-amylase inhibitory assay[25]

Both test samples and nojirimycin (20-120 µg/ml) of 500 µl concentration were put into 500 µl of 0.02 moles of sodium phosphate buffer of pH 6.9 with 0.006 M sodium chloride containing 0.5 µg/ml porcine pancreatic α-amylase solution and were incubated at 25˚C for 10 minutes. After the pre-incubation, 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer of pH 6.9 with 0.006 M salt chloride had been added to each tube at pre specified intervals. The response mixtures were then incubated at 25˚C for ten minutes. The response was stopped by adding 1 ml of 3, 5-dinitrosalicylic acid color reagent. The test tubes had been then incubated in a water bath that is boiling for five minutes and cooled down to room temperature. The reaction mixture was then diluted by the addition of 10 ml of distilled absorbance and water was measured at 540 nm.

3. Results and Discussion

Preliminary phytochemical screening

The data corresponding to table.1 describes the preliminary phytochemical investigation report of EEPO. Phenols, flavonoids. Saponins, phytosterols, steroids and terpenoids are present in EEPO.

Acute oral toxicity study: The EEPO treated rats throughout the study. Rubbing of nose and mouth on the floor of the cage and restlessness were the only behavioral signs of toxicity shown by the animals and these disappeared with in 24 hrs of extract administration. During the study there were no significant changes in body weights of treated rats compared to control group. Further there were no gross pathological abnormalities in both control and treated rats. Thus the LD_{50} value was found to be greater than 2000mg/kg b.wt. with reference to the Globally harmonized system of classification and labeling the chemicals, *psychotria octosulcata* can be classified as Category -5 and this provides the relevance for protecting human and animal health.[26]

***In-vitro* antioxidant activity**

1-diphenyl-2-picryl hydrazyl [DPPH], superoxide [O_2•]), hydrogen peroxide [H_2O] and nitric oxide [NO] scavenging activity[27,28]

The ability of EEPO to scavenge DPPH, superoxide and nitric oxide were measured in vitro. The IC_{50} values of the samples are mentioned in **table 1** respectively. EEPO significantly reduced DPPH levels. In similar manner, Superoxide and nitric oxide scavenging activity for EEPO was found to be high as witnessed by their low IC_{50} values.

In vitro studies on EEPO for its antidiabetic activity

α- glucosidase and α-amylase inhibitory activities[29,30]

The IC_{50} values of EEPO for sucrase inhibitory activity had been 256.10 ± 0.26µg/ml. The outcomes in table 5.11 fig 5.5 showed that EEPO exhibited strong activity almost in a dose-dependent way and is hence inferred to be an effective α-glucosidase inhibitor. In addition, EEPO inhibited the activity of α-amylase with IC_{50} of 70.29 ± 0.43µg/ml, the outcome of which is shown in table 2 and figure 5.6. In this research, EEPO exhibited strong inhibitory activity against α-glucosidase and α-amylase, which is similar with standards ie., acarbose and nojirimycin, correspondingly. Nevertheless, EEPO revealed moderate α-glucosidase and α-amylase inhibitory activity contrasted with acarbose and nojirimycin, correspondingly. These outcomes verify that EEPO have α-glucosidase and α-amylase inhibitory properties.

4. Conclusion

The present investigation concluded that whole plant possess antidiabetic and act by inhibit α-amylase and α-glucosidase enzyme and scavenging free radicals. Morever, ethonolic extract of *Psychotria octosulcota* was found more effective and thus could have potential for application in the management of diabetes mellitus. Further studies on *in-vivo* action.

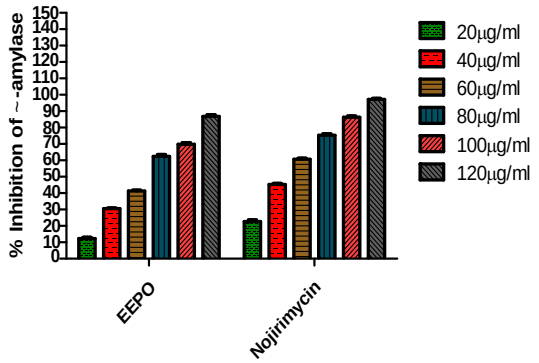

Figure 1: α-amylase inhibitory activity of EEPO

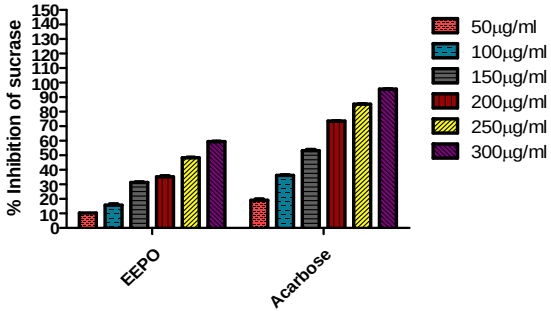

Figure 2: α-glucosidase inhibitory activity of EEPO

Table 1: Phyto-chemical screening of aerial parts extract of *Walsura piscidia* Roxb

S No	Constituent	Ethanolic extract
1	Alkaloids	-ve
2	Glycosides	+ve
3	Saponin glycosides	+ve
4	Flavonoids	+ve
5	Tannins	-ve
6	Steroids	+ve
7	Triterpenoids	+ve
9	Phenols	+ve
10	Proteins	-ve
11	Carbohydrates	-ve

+ve sign indicates presence; - ve sign indicates abscence

Table 2: *In-vitro* antioxidant activity of EEPO

Test material	IC_{50} (µg/ml) ± SEM[a]		
	DPPH	Super oxide	Nitric oxide
EEPO	9.21±0.42	32.80±2.05	150.12±1.54
Ascorbic acid	3.21±0.54	-	-
Rutin	4.32±0.48	-	82.03±2.87

[a]Average of 3 determinations.

Table 3: α-glucosidase (sucrase) inhibitory activity of EEPO

Concentration (µg/ml)	Percentage inhibition (%) of sucrase by		IC_{50} (µg/ml)	
	EEPO	Acarbose	EEPO	Acarbose
50	10.30 ± 0.04	19.12 ± 0.68	256.10 ± 0.26	140.01± 0.03
100	15.64 ± 0.74	36.23 ± 0.26		
150	31.28 ± 0.31	53.24 ± 0.72		
200	35.19 ± 0.62	73.50 ± 0.09		
250	48.34 ± 0.25	85.24 ± 0.01		
300	59.48 ± 0.13	95.52 ± 0.03		

Table 4: α-amylase inhibitory activity of EEPO

Concentration (µg/ml)	Percentage inhibition (%) of α-amylase by		IC_{50} (µg/ml)	
	EEPO	Nojirimycin	EEPO	Nojirimycin
20	12.26 ± 0.85	22.68 ± 0.95	70.29 ± 0.43	44.08 ± 0.63
40	30.59 ± 0.31	45.37 ± 0.53		
60	41.30 ± 0.54	60.74 ± 0.62		
80	62.46 ± 1.06	75.34 ± 0.85		
100	69.82 ± 0.96	86.28 ± 0.83		
120	86.78 ± 1.03	97.24 ± 0.58		

5. References

[1] L. Santosh Vishwakarma, D. Sonawane Rakesh, M. Ramesh, K Goyal, Evaluation of effect of aqueous extract of *Enicostemma littorale* Blume in streptozotocin-induced type 1 diabetic rats. Indian Journal of Experimental Biology. 48 (2010) 26-30.

[2] A. Amos, D. Mc Carty, and P. Zimmet, The rising global burden of diabetes and its complications, Diabetes Care. 21 (1998) 1414-1431.

[3] P J. Kumar and M. Clark, Textbook of Clinical Medicine, Pub: Saunders London. (2010) 1099-1121.

[4] P. Zimmet, C. Cowie, JM. Ekoe, JE. Shaw. Classification of diabetes mellitus and other categories of glucose intolerance, In: International Textbook of Diabetes Mellitus chapter. 3thed, (2004) 3-14.

[5] Ghada Abd El-Moneim Hegazi, In-vitro studies on *Delonix elata* L., An endangered medicinal plant, World applied science journal. 14(5) (2011) 679-686.

[6] Diabetes care, volume 28, Supplement 1 (2005).RS. Yalow, SA. Berson, Immunoassay of endogenous plasma insulin in man, The Journal of Clinical Investigation. 39 (7) (1960)1157–75.

[7] Syamsudin. Standardization of extract of Leucaenaleucocephala (lmk) De Wit seeds by α-glucosidase inhibitor. International Journal of Phytomedicine 2010; 2: 430-435.

[8] Annamalai A, Ponmari1 G, Sathishkumar R,Lakshmi P.T.V. Effect Of Drying Treatment On The Contents Of Antioxidants In Cardiospermum Halicacabum Linn. International Journal of Pharma and Bio Sciences (2011);2(1):304-313.
[9] 9.Ming-HsingHuanga, Shyh-ShyunHuangb, Bor-SenWangc, Chieh-HsiWud,Ming-JyhSheud, Wen-
[10] Chi Houe, Shiang-ShiouLinb, Guan-JhongHuangb.Antioxidant and anti-inflammatory properties of
[11] Cardiospermum halicacabum and its reference compounds ex vivo and in vivo. J Ethnopharmacol(2011);133: 743–750.
[12] Muthumani P, Meera R, Devi P, Mohamed Sheik Arabath S.A, SeshukumarKoduri L.V, SivaramManavarthi.Chemical Investigation Of ToddaliaAsiaticaLinn,AndCardiospermum Halicacabum
[13] Linn. International Journal of Drug Formulation & Research (2010); 1(3): 224-239.
[14] 11.Datta S, Ghosh A, Pal P, Das M, Kar PK. Pharmacognostical, Phytochemical and biological evaluation of Cardiospermum halicacabum. Pharm Sci Bio (2010); 1(1):37-42.
[15] https://archive.org/details/AntiulcerPlantsInPankajOudhiasMedicinalPlantDatabasePart27_pM1m0QU2N6A
[16] K. R. Khandelwal Practical Pharmacognosy. 4th edition. Pune Prakashan., 1998; 149-155.
[17] Trease, Evans. Pharmacognosy.14th ed. London: Harcourt Brace and company; (1996) 14-15.
[18] CK. Kokate, Textbook of Pharmacognosy, 593-597
[19] Karimulla Shaik, Sarath Chandiran I, Jayaveera KN, Antioxidant property of Grewia serrulata DC and its hypoglycemic effect on normal and hyperglycemic rats. journal of pharmacy Research, 6 (2013)813-817
[20] Sarath Chandiran I, Jayaveera KN, Karimulla Shaik Preliminary phytochemical and preclinical toxicity studies of *Grewia serrulata* DC. Drug Invention Today, 5, 2013, 267-274.
[21] Sarath Chandiran I, Jayaveera KN, Karimulla Shaik Antioxidant property of *Grewia serrrulata* DC and its hypoglycemic effect on normal and hyperglycemic rats. Journal of Pharmacy Research, 6, 2013, 813-817.
[22] Sarath Chandiran I, Jayaveera KN, Karimulla Shaik. Investigation of hypoglycemic and hypolipidemic activity of ethanolic extract of Grewia serrulata DC on high fat diet (HFD) - streptozotocin (STZ) induced di abetic rats. Int. J. Res. Pharm. Sci., 5 (1), 2014, 79-84.
[23] Kim, Y.M.; Jeong, Y.K.; Wang, M.H.; Lee, W.Y.; and Rhee, H.I.; 21, 2005, 756-761.
[24] Krentz, A.J.; and Bailey, C.J.; 65, 2005, 385-411.
[25] Watanabe, J.; Kawabata, J.; Kurihara, H.; and Niki, R.; Biosci Biotechnol Biochem. 61, **1997,** 177-178.
[26] Nishioka, T.; Kawabata, J.; and Aoyama, Y.; J Nat Prod. 61, **1998,** l413-l415.
[27] Yao, Y.; Sang, W.; Zhou, M.; and Ren, G.; J Agric Food Chem. 58, **2010,** 770-774.
[28] Yoshikawa, M.; Murakami, T.; Yashiro, K.; Matsuda, H.; Kotalanol, Chem Pharm Bull (Tokyo). 46, **1998,** 1339-1340.
[29] Nishioka, T.; Kawabata, J.; and Aoyama, Y.; J Nat Prod. 61, **1998,** l413-l415.
[30] Dahlqvist, A.; Anal Biochem. 7, **1964,** 18-25. Kwon, G.J.; Choi, D.S.; and Wang, M.H.; J Korean Food Sci Technol. 39, **2007,** 569-574.
[31] JR. Carney, JM. Krenisky, RT. Williamson, J. Luo Achyrofuran, A new Antihyperglycemic dibenzofuran from Southern American Medicinal Plant Achyrocline satureioides, Journal of Natural Product. 65 (2002) 203-205.
[32] A. Andrade-Cetto, H. Wiedenfeld, Hypoglycemic effect of Acosmium panamense bark on Streptozotocin diabetic rats. Journal of Ethno pharmacology. 90 (2-3) (2004) 217-220.
[33] P. Pushaparaj, CH. Tan, BK. Tan, Effects of Averrhoa bilimbi leaf extract on blood glucose and in streptozotocin diabetic rats, Journal of Ethnopharmacol. 72 (2002) 69-76.