INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com ISSN : 0976-4550

Volume-7, Issue-2, April-June-2016 Coden IJABFP-CAS-USA

Received: 1st Feb 2016 *Revised: 14th Mar 2016* *Accepted: 14th Mar 2016*

Research article

EVALUATION OF MITIGATING EFFECT OF PURIFIED SOLASODINE FROM *SOLANUM MAURITIANUM* AGAINST HYDROGEN PEROXIDE INDUCED OXIDATIVE DAMAGE IN HUMAN ERYTHROCYTES

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ABSTRACT: *Solanum mauritianum* (Solanceae) is an exotic species from South America. *Solanum* species are known for alkaloid content which marks them medicinally important. Knowledge of the secondary metabolites of plant is desirable, not only for the discovery of therapeutic drugs but also it disclose new sources of economically important product. Herbal products have received considerable attention in recent years due to their diverse pharmacological properties, including antioxidant and antitumor potential. Solasodine, the nitrogen analogue of diosgenin, has been reported as potential steroidal precursor for the supplementary source of the commercial synthesis of diverse steroidal drugs. Free radicals are formed during the course of normal metabolic process in the biological system. They are highly reactive molecules due to the presence of unpaired electron. In this juncture, present study was aimed to isolate and purify the alkaloid solasodine from fruits and leaf and to evaluate its antioxidant activity via the assays such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, nitric oxide, hydrogen peroxide, superoxide anion scavenging activities etc. The alkaloid solasodine was isolated from leaves and fruits of the species using non polar to polar solvents and subsequently purified using column chromatography. Remarkable antioxidant activities are shown by the alkaloid solasodine. IC_{50} values were comparable with synthetic antioxidants such as ascorbate, rutin. Moreover, the solasodine exhibited concentration dependent inhibitory activity against the free radicals. Significant scavenging property was noticed especially with DPPH radicals. Antihaemolytic activity of the solasodine was also confirmed from this study. Thus, it can be suggested that solasodine show more protective effects through antioxidant potential against H_2O_2 induced oxidative damage on erythrocytes. So, solasodine can effectively be used as natural antioxidant for the treatment and prevention of lipid peroxidation related disorders.

Key words: *Solanum mauritianum,* solasodine, antioxidant activity, alkaloid, reactive oxygen species.

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INTRODUCTION

Research of herbal antioxidant, has greatly enhanced in recent years due to their multifaceted roles (Sasikumar *et al.*, 2015). Herbals are the unique resource of drugs in traditional systems of medicine, allopathic medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and also as chemical entities for synthetic drugs (Sudha *et al*., 2011). Herbals have been used in traditional treatments for curing many human diseases for time immemorial. They are valuable natural resources and regarded as potentially safe drugs and have been tested for biological, microbicidal and hypoglycemic activity in the modern medicine. Free radicals or ROS are responsible for oxidative stress that can initiate physiopathological abnormalities and chronic disorders like diabetes, neurodegenerative and cardiovascular, inflammation, Alzheimer, Parkinson's and carcinogenesis, which occurs in cell or tissue when imbalance of ROS production to antioxidant capability occurs.

Therefore, much research has focused on plant based antioxidants and on their mechanism of action. In line many plant extracts or secondary metabolites have been screened for antioxidant activity and protection against oxidation induced damages (Thakur *et al*., 2012).

Erythrocytes lack nucleus and hence they can be effectively used for studying the effect of pharmacologically active molecules. These cells are highly susceptible to oxidative damage due to their high polyunsaturated fatty acid (PUFA) content on their membranes and the high cellular concentrations of oxygen (Sana *et al*., 2014).

ROS are largely involved in DNA damage and mutagenesis, and that phytochemicals like alkaloids show an antioxidant potential, it was interesting to evaluate its biological potentialities in different biological models and to determine the concentration threshold of these effects (Abraham *et al.,* 2005). *Solanaceae* is well known and has been screened by researchers for their medical actions. The plants were extensively used to treat severe diseases such as pain, inflammation and fever. Therefore, the aim of the present study was to isolate, purify solasodine from leaves and fruits of *Solanum mauritianum* to evaluate their antioxidant and antihemolytic properties.

MATERIALS AND METHODS

Plant material

Shade dried fruits and leaves of *Solanum mauritianum* were collected from Munnar hills of Idukki district, Kerala.

Extraction of Solasodine and Caulophyllumine

40 g of shade dried fruits and leaves of *Solanum mauritianum* were subjected to hot continuous soxhlet extraction separately using non polar to polar solvents viz., chloroform, petroleum ether, ethyl acetate and ethanol. Chloroform and ethanol extracts showed the presence of alkaloid in qualitative screening with dragendorffs reagent test. The intensity of reddish brown colour was found to be high in chloroform fraction and was further lyophilized and purified by using silica column (1 g). The lyophilized fraction was loaded at the top of freshly packed silica column with mesh size 60-120, for isolating the pure compounds. Ethyl acetate and ethanol solvents were used as mobile phase for eluting the lead fractions from the crude alkaloid extract. Bluish and yellowish green fractions were eluted out of the column using ethyl acetate and ethanol respectively. These fractions were further reloaded at the top of freshly packed column for purification. Petroleum ether and Chloroform mobile phase in the ratio 4:1 yielded highly purified bluish fraction. The elution time for bluish fraction was 120 h. Residual weight of the fraction was calculated after drying was 21.3 mg/g. Similarly, chloroform and ethyl acetate (6:4) solvent combination after 168 h resulted purified yellowish fraction. The residual weight was noted as 16.9 mg/g. Purity of the samples was first checked with FTIR. FTIR spectral peaks revealed the functional groups associated with alkaloids. Using proton NMR absorptions peaks, the identity and structure of the compounds were confirmed. Bluish coloured fraction was caulophyllumine-A and yellowish green fraction was identified as solasodine.

Fourier transformed infra red (FTIR) spectra

A Perkin Elmer (Waltham, Massachusetts, USA) was used to obtain Fourier transformed infrared (FTIR) spectra (System 2000) and applied for each pure fraction analysis.

Proton magnetic resonance spectra (1H NMR)

The identification of compounds was confirmed by carrying out 1H-NMR analysis using NMR Joel GIM, EX 270 (400 Hz).

In vitro antioxidant assays

1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay

DPPH radical scavenging was carried by employing the protocol of Villano et al., (2007). Pure samples of 25-150 µg mL^{-1}) were added to methanol solution of DPPH (100 µM). Absorbance was read at 517 nm after 15 min incubating at room temperature. The values were mean ± SD. Vitamin C and rutin was used as synthetic antioxidants.

Reducing power

Reducing power ability of the sample was measured using the protocol of Ferreira et al., (2007) 2.5 mL of sample (25-150 µg mL^{-1}) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide and were incubated for 20 min at 50°C. Further, 2.5 mL of 10% trichloroacetic acid was mixed to the sample to arrest the reaction. Reaction mixture was the centrifuged for 10 min at 2000 g. The supernatant of the reaction mixture (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. Finally, OD the sample was recorded at 700 nm.

Metal chelating

Metal chelating of the sample was examined with the volume 25-300 µg mL^{-1}, 1 mL. Add 0.05 mL of 2 mM $FeCl_2$ and 0.2 mL of 5 mM ferrozine to the mixture. After 10 min incubation at room temperature, OD of the sample was recorded at 562 nm (El and Karakaya, 2004).

Nitric oxide scavenging

Kumaran and Karunakaran, (2006) methodology was adapted for measuring nitric oxide scavenging. 25-150 µg mL^{-1} samples was mixed with 10 mM sodium nitroprusside and incubated for 150 min at 25°C. Add 0.5 mL of Griess reagent (sulfanilamide- 1%, phosphoric acid- 2% and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) to the reaction mixture. Finally, OD of the sample was recorded at 546 nm.

Hydrogen peroxide scavenging

Gulcin *et al.*, (2010) protocol was used to evaluate H_2O_2 scavenging potential. 2 mL, 50,300µg mL^{-1} extract was mixed with 0.6 mL of hydrogen peroxide solution (40 mM) in pH 7.4 phosphate buffer. The OD of the reaction mixture was recorded at 230 nm against blank.

Hemoglobin-induced linoleic acid assay

2 ml reaction mixture containing 50-500 µg mL-1 sample, 40 mmol L-1, phosphate buffer with pH 6.5, 0.0016% hemoglobin suspension and 1 mmol L^{-1} linoleic acid emulsion (Bae and Suh, 2007). Reaction mixture was incubated for 45 min at 37°C. Add 2.5 mL of ethanolic solution of hydrochloric acid (0.6%) to the sample for arresting lipid peroxidation. Quantity of peroxidation was measureed via thiocyanate method by recording the OD at 480 nm after adding 100 µl of $FeCl_2$ (0.02 mol/L) and 50 µl of ammonium thiocyanate (0.3 g mL^{-1}).

Preparation of human erythrocytes

Blood samples were collected from consenting volunteers who visited the Medical College, by veni-puncture into lithium heparinized sterile tubes. The blood samples were stored at about 4^oC the collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cell were washed with isosaline (0.85%.pH 7.2) and a 10 % (v/v) suspension was made with isosaline.

Protective role of extracts against H_2O_2 induced hemolysis

Stability role of the alkaloids against H_2O_2 induced hemolysis was evaluated according to the method of Prakash Yoganandam et al., (2010). Different concentrations of the alkaloids (0.5 mL, 50, 100,150 µg-mL^{-1}) were mixed with 2 mL of erythrocyte suspension (4%) and the volume of reaction mixture was made up to 5 mL with phosphate buffered saline. After 5 min incubation at 25°C, 0.5 mL of H_2O_2 solution was added to the reaction mixtures. After 240 min incubation at 25°C, reaction mixtures were centrifuged (2500 g, 10 min). Absorbance of the reaction mixture was recorded at 540 nm.

Statistical analysis

Experimental results are expressed as means ± SD. All measurements were replicated three times. The data were analyzed by an analysis of variance ($p < 0.05$) and the means separated by Duncan's multiple range tests.IC_{50} values were calculated from linear regression analysis.

RESULTS AND DISCUSSION

Reactive oxygen species (ROS) enclose spectrum of diverse reactive molecules such as superoxide anions, hydrogen peroxide, hydroxyl radicals, nitric oxide, peroxynitrite etc. These prooxidants possess multiple roles in living organisms under *in vivo* condition. The major task includes cellular signaling, growth regulation, physiology, and energy synthesis. Meanwhile, they also induce catabolic events like oxidation of biomolecules including DNA and protein which can trigger secondary effects such as mutation, damage target tissues, and result in ageing and apoptotic death. Currently, pharmaceutical industries are in search of natural potential antioxidants in mitigating *in vivo* oxidative stresses and there by degenerative diseases.

Bluish coloured fraction eluted was caulophyllumine-A and yellowish green fraction was identified as solasodine through NMR and FTIR spectra. Inhibitory effects of solasodine on DPPH radical showed a dose dependent response (Table 1). The IC_{50} value of scavenging DPPH radicals was 50.1 ±1.22 µg/ ml.

Though the antioxidant potential of solasodine was found to be significant ($P < 0.05$) but, lower than that of ascorbate. Superoxide radical is a potential biological source of reactive oxygen species. Even though it is a weak oxidant, it generates powerful and toxic hydroxyl radicals and singlet oxygen which contributes oxidative burst in the tissues / cells. The scavenging effect of solasodine on H_2O_2 was dose dependent (50 - 300 µg/ml) as shown in Table 2. The alkaloid displayed strong hydrogen peroxide scavenging activity (IC_{50} 151±0.15 µg/ml) whereas that of the standard, ascorbate exhibited 97.8±0.33 µg /ml ($P < 0.05$). Table 3 shows the concentration dependent response curves for the reducing powers of solasodine (25 - 150 µg/ml). It was found that the reducing power increased with concentration. Significantly higher reducing power was noticed in the sample. The ability of solasodine to scavenge NO radical was determined by percentage inhibition which was found to be 75± 0.63 at concentration 150 µg/ml, where as percentage inhibition of standard rutin at the same concentration were 88. The metal chelating activities of solasodine was also concentration dependent. The absorbance of Fe^{2+}-ferrozine complex was linearly decreased with increasing concentration. The percentage of metal chelating capacity at the concentration of 75µg/ml was found to be 47.8 and that for standard ascorbate was found to be 31.6 at same concentration. Similarly, solasodine showed remarkable activity in hemoglobin-induced linoleic acid assay (Table 4). Significance at 1% level ($p < 0.01$).

Table -1: DPPH and Nitric oxide scavenging assay of the alkaloid solasodine.

		DPPH assay (%)		**NO (%)**		
Concentration (µg/ml)	Solasodine	Ascorbate	Rutin	Solasodine	Rutin	Ascorbate
25	28.3 ± 0.89	33± 0.04	17.5 ± 0. 04	28.4 ± 0.45	37± 0.64	14.5 ± 0. 02
50	50.1± 1.22	46 ± 0.46	22 ± 0. 05	35.6± 0.25	50 ± 0.06	19 ± 0. 15
75	59.2±1.55	53 ± 0. 05	38.6 ± 0.66	47.8± 0.95	60 ± 0. 07	31.6 ± 0.09
100	65.7 ± 2.14	66 ± 1.09	50 ± 0. 11	60.7 ±0.64	69 ± 1.33	42 ± 0. 45
125	78± 4.1	84 ± 0.38	57 ± 1.9	72±0.51	80 ± 0.38	54 ± 0.9
150	80 ± 1.67	88 ± 3.4	58 ± 0.38	75 ± 0.63	88 ± 0.88	55 ± 0.69

Values are mean ± SD. $P < 0.05$%.

Table- 2: Metal chelating and H_2O_2 scavenging assay of the alkaloid solasodine.

		Metal chelating (%)		**H $_2$O $_2$ scavenging (%)**		
Concentration (µg/ml)	**Solasodine**	**Ascorbate**	**Rutin**	**Solasodine**	**Ascorbate**	**Rutin**
50	19 ± 0.22	35± 0.01	18.5 ± 0. 34	32.4 ± 0.05	39± 0.6	11.5± 0. 07
100	30.6± 0.9	48 ± 0.48	24 ± 0. 035	39± 0.95	52± 4.6	20±0. 095
150	47.8±1.25	63 ± 0. 07	33.6 ± 0.09	49.8± 0.15	65±0. 97	29.6±0.39
200	62.7 ± 0.14	73 ± 1.27	47 ± 0. 03	63.7 ±0.6	74± 1.38	38 ±0. 67
250	74± 0.76	81 ± 0.3	52 ± 1	76±0.57	83 ± 0.35	50 ± 0.49
300	77 ± 0.99	83 ± 1.4	56.8 ± 0.08	78 ± 0.63	86 ± 0.88	53 ± 0.64

Values are mean ± SD. $P < 0.05$%

Table -3: Reducing power of the alkaloid solasodine.

		Reducing power (OD)	
Concentration (µg/ml)	Solasodine	Ascorbate	Rutin
25	0.42 ± 0.01	0.58± 0.02	0.29 ± 0. 04
50	0.59± 0.022	0.83 ± 0.06	0.37 ± 0. 05
75	1.4±0.032	1.77± 0. 05	0.86 ± 0.06
100	1.7 ± 0.014	2.1 ± 0.09	0.9 ± 0. 01
125	1.88± 0.031	2.4 ± 0.08	1.3 ± 0.04
150	1.92 ± 0.04	2.56 ± 0.05	1.43 ± 0.038

Values are mean ± SD. $P < 0.05$%.

Table-4: Hemoglobin –induced linoleic acid assay of the alkaloid solasodine.

Concentration (µg/ml)	Solasodine	Ascorbate	Rutin
50	9 ± 0.03	22 ± 0.01	7.5 ± 0.06
100	19.5 ± 0.23	38 ± 0.042	13 ± 0.01
200	48.2 ± 0.071	55 ± 0.012	19 ± 0.03
300	58.25 ± 0.04	67 ± 0.045	27 ± 0.05
400	75 ± 0.041	88.25 ± 0.07	39 ± 0.02
500	78 ± 0.055	91 ± 0.04	45 ± 0.061

Values are mean ± SD. $P < 0.01\%$

Antihaemolytic activity of alkaloids in H_2O_2 induced oxidative damage in erythrocytes

Generally, the formation of free radicals or ROSs is compensated by the activation of antioxidant enzymes such as superoxide dismutase, catalase and gluthathione peroxidase that protect the cells from oxidative stress. The results of the pretreated purified solasodine on human RBC followed by H_2O_2 treatment suggests their protective role against oxidative stress caused by H_2O_2 (Table 5).

Table -5: Antihemolytic assay of the alkaloid solasodine

	% hemolysis
RBC + H2O2	100
Solasodine (50 µg/ml) + RBC	8.25 ± 0.99
Solasodine (50 µg/ml) + RBC +H2O2	13.3 ± 0.08
Solasodine (100 µg/ml) + RBC +H2O2	15.4 ± 0.-04
Solasodine (150 µg/ml) + RBC +H2O2	19.4 ± 0.033
Ascorbate (100 µg/ml) + RBC	4.2 ± 0.01
Ascorbate (100 µg/ml) + RBC +H2O2	9.4 ± 0.07

Values are mean ± SD. $P < 0.05\%$

DPPH is a stable free radical and usually remain unaffected by side reactions, such as metal ion chelation and enzyme inhibition. DPPH solution was deep colour with an absorption maximum at 517 nm. The antioxidants degrade the deep colour by quenching the free radicals through donating hydrogen or electron and change them into bleached product (i.e. 2,2- diphenyl-1-hydrazine, or a substituted analogous hydrazine), which results in to decrease of OD (Amarowicz *et al.,* 2003). The ability of solasodine to scavenge DPPH free radical was found to be increased with concentration and was calculated as percentage inhibition which was found to be 80% at concentration 150 µg/ml, where as percentage inhibition of ascorbate at the same concentration was 88.

The reducing power reflects the electron donating capacity of solasodine, is associated with antioxidant activity. Antioxidant can be reductants and inactive oxidants. The reducing capacity of solasodine can be assayed by the direct reduction of $Fe[(CN)_6]_3$ to $Fe[(CN)_6]_2$. Addition of free Fe^{3}+ to the reduced product leads to the formation of intense Perl's Prussian blue complex, Fe $_4$[Fe (CN-)6]3, which shows strong absorbance at 700 nm. The reductones have the ability to show reducing action. They show antioxidant potential by breaking them via free radical chain by donating hydrogen atom (Dar *et al*., 2014).

In this regarded, increase in Fe^{3+} to Fe^{2+} conversion in presence of solasodine suggests that alkaloid is electron donor and thus can leads to reduction of the oxidized intermediates of lipid peroxidation events. In this assay, depending on the reducing power of antioxidants the yellow colour of the test solution alters to green and blue (Karimi *et al.,* 2010). The reducing capacity of the phytochemical may serve as significant marker of its potential AOX activity.

NO radical inhibition assay proved that solasodine is a potent scavenger of RNS like nitric oxide. In this assay sodium nitroprusside generates nitric oxide which form nitrite when reacts with oxygen. The solasodine inhibits nitrite formation by competing with oxygen to react with nitric oxide directly and also to inhibit its synthesis. Scavengers of NO compete with oxygen leading to reduced production of nitric oxide (Orech *et al.,* 2005). The ability of solasodine to scavenge nitric oxide radical was determined by percentage inhibition which was concentration dependent and was found to be 75% at concentration 150 µg/ml, where as percentage inhibition of standard rutin at the same concentration were 88.74%. Ferrozine can quantitatively form complexes with Fe^{2+} but in the presence extracts able of ion chelation, the complex formation is disrupted resulting in a reduction in the red colour of the complex. The measurement of the rate of reduction of the colour, therefore allows estimation of the chelating activity of the coexisting chelator. The absorbance of Fe^{2+}- Ferrozine complex was linearly decreased in concentration dependent manner. The data obtained from the present results reveal that the solasodine demonstrate an effective capacity for iron binding, suggesting its action as peroxidation protector through iron binding capacity. In this assay, the solasodine and standard compound interfered with the formation of ferrous complex with the reagent ferrozine, suggesting that it has chelating activity and possess the capacity to captures the ferrous ion before ferrozine. The ion chelating activity of the solasodine may be attributed due to the presence of endogenous chelating agent (Senevirathne *et al.,* 2006). The metal chelating activities of solasodine were concentration dependent. The percentage of metal chelating capacity at the concentration of 100 µg/ml was found to be 30.6% for solasodine and that for standard ascorbate, it 48% and at 300 µg/ml 77% and 83% respectively.

The H_2O_2 scavenging ability of solasodine and ascorbic acid is shown in (Table 2). H_2O_2 scavenging activity of solasodine at 300µg/ml was found to be 78 *%* and for ascorbate at the same concentration was 86 %. The solasodine was capable of scavenging hydrogen peroxide in dose dependant manner. H_2O_2 is a weak oxidizing agent that inhibits the oxidation of essential thiol (-SH) groups directly by few enzymes. Many of its deleterious effects are due to their ability to rapidly cross the cell membrane and once inside the cell, it can probably react with Fe^{2+} or possibly Cu^{2+} ions to form hydroxyl radicals (Nabavi *et al.,* 2009). From the results, it can be interpreted that solasodine was capable to scavenge hydrogen peroxide in dose dependant manner.

Yu-Ling Ho *et al*., (2012) compared *in vitro* antioxidant properties with total phenolic contents among wetland medicinal plants in Taiwan. Sheng-Yang Wang (2004) evaluated antioxidant activity of extracts from *Calocedrus formosana* leaf, bark, and heartwood. Sasikumar *et al.,* (2015) proved protective role of alkaloids of *Amaranthus viridis* against hydrogen peroxide induced oxidative damage in RBC. These results were lower that of solasodine. Vijayaraghavan *et al.,* (2013) screened phytochemicals with antioxidant activity of *Chromolaena odorata* and *Annona squamosa*. Joshan and Rawal (2012) made comparative evaluation of antioxidant and antihemolytic capacities of Indian medicinal plants using multiple antioxidant assays. Antioxidant and antihemolytic potential of solasodine of the present study was more significant compared that of raw Pepion fruit (*Solanum muricatumaiton*) (Sudha *et al.,* 2011). These comparisons suggest that solasodine can be better antioxidant for removing hydrogen peroxide or free radicals and thus protecting living organisms from degenerative diseases or may be used in promotion of longevity of food products.

CONCLUSION

In conclusion, the *in vitro* experimental analysis like DPPH radical, superoxide anion, hydrogen peroxide scavenging and reducing power demonstrate that solasodine of *Solanum mauritianum* might have significant antioxidant potentials. Herbal antioxidants offer great cancer chemopreventive effects or efficacies through these free radical mediated scavenging properties. Further works are warranted to confirm the obtained results in terms of *in vivo* animal or cell line experiments.

Conflict of interest

The authors have no conflict of interest.

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ISSN : 0976-4550

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