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Antioxidant and Diuretic Activity of *Nardostachys Jatamansi* Dc Roots

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ABSTRACT

The aim of present study was to investigate the In-vitro anti-oxidant and In-vivo diuretic activity of Ethyl acetate extract of *Nardostachys jatamansi* DC roots in rats. The preliminary phytochemical investigation was carried out to identify the various chemical constituents present in Ethyl acetate extract. In-vitro antioxidant activity was measured by using hydrogen peroxide scavenging activity, reducing power assay, DPPH scavenging activity. Ethyl acetate extract of *Nardostachys jatamansi* DC roots administered in rats at 100mg/kg, 200mg/kg and 500mg/kg, orally and compared with furosemide (20mg/Kg, intraperitoneally) as the standard. The diuretic effect of the extracts was evaluated by measuring urine volume, electrolyte concentration, pH, density, conductivity, saluretic index, diuretic index and carbonic anhydrase inhibition ratio. The results were analyzed by One Way Analysis Of Variance (ANOVA). The results showed that ethyl acetate extract of *Nardostachys jatamansi* DC roots showed significant Anti-oxidant activity. Extract induced strong diuresis at higher doses and was not accompanied with a reduction in urinary K^+ levels. Further, there was no alkalization of urine. Collectively, these observations suggest that the extract is not acting as potassium-sparing diuretics. Extract showed strong diuretic index, saluretic index, natriuretic index and weak carbonic anhydrase inhibition ratio. The findings concluded that *Nardostachys jatamansi* DC roots extract exhibit anti-oxidant and diuretic activity and further studies are suggested to isolate the active principles responsible for the activity.

Keywords: *Nardostachys jatamansi* DC, Ethyl acetate extract, antioxidant, Diuretic, Urine volume

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INTRODUCTION

Medicinal plants can be important sources of unknown chemical substances with potential therapeutic effects. Besides, the World Health Organization has estimated that over 75% of the world's population still relies on plant-derived medicines, usually obtained from traditional healers, for basic health-care needs. India has about 45000 plant species and among them, several thousands have been claimed to possess medicinal properties. During the process of oxygen utilization in normal physiological and metabolic processes approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals like superoxide, hydrogen peroxide, hydroxyl and nitric oxide radicals. Free radicals are capable of reacting with membrane lipids, nucleic acids, proteins, enzymes and other micro molecules resulting in cellular damage. Free radicals are involved in the development of degenerative diseases. They have also been implicated in the pathogenesis of diabetes, liver damage, nephrotoxicity, inflammation, cancer, cardiovascular disorders, neurological disorders, and in the process of aging. To protect these free radical induced damage, antioxidants are the most popular agents that interactively and synergistically neutralize free radicals. Diuretics are drugs that increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations. Drug-induced diuresis is beneficial in many life threatening disease conditions such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, hypertension, and pregnancy toxaemia. Most diuretic drugs have the adverse effect on quality of life including impotence, fatigue, and weakness [9]. Two widely used diuretics, thiazides and frusemide, the high-ceiling loop diuretic, have been associated with numerous adverse effects, such as electrolyte imbalance, metabolic alterations, and development of new-onset diabetes, activation of the renin-angiotensin and neuroendocrine systems, and impairment of sexual function. Hence, there is a requirement for novel diuretics such as plant-based substances, which are considered to be relatively safe, possessing lower potential for adverse effects. *Nardostachys jatamansi* DC is important plant of the family Valerianaceae. It is commonly known as Indian spikenard and found in Himalayas. In Ayurveda, *Nardostachys jatamansi* is used in treatment of mental disorders, insomnia, hysteria, depressive illness. The plant abounds in sesquiterpenes predominantly; jatamansone and nardostachone. The plant has demonstrated for several pharmacological activities including hepatoprotective, antioestrogenic, antifungal, cardio protective and hypolipidemic. It has protective effect in Parkinsonism, epilepsy, cerebral ischemia and free radical scavenging activity. The significant effect is on the central nervous

system, as diverse pharmacological actions, ranging from sedative to nootropic have been reported. Animal and clinical research with jatamansone, the active principle of the plant, has justified hypno-sedative claim of Ayurveda and also reported to possess antiarrythmic, antiasthamatic, antibacterial activity. *Nardostachys jatamansi* DC is claimed for its diuretic activity but the systematic work was not carried out on this. So, this is to confirm the potential use of *Nardostachys jatamansi* DC roots extracts have diuretic properties in vivo. This study will be a prospective study for the development of new diuretic agents.

MATERIALS AND METHODS

Plant material

The roots of *Nardostachys jatamansi* DC is collected from local sources and with the help of expert taxonomist and it was identified by studying its various morphological and microscopic characters and the plant was authenticated by Dr. B. D. Gachande (Associate Professor) of P.G. Department of Botany, Science College, Nanded.

Drugs and chemicals

Ethyl acetate, Ferric chloride (Fine Chem Industries, Mumbai), furosemide (Aventis pharma Ltd), Sodium chloride, Potassium chloride, Trichloro acetic acid (Loba Chemie Pvt. Ltd. Mumbai), Ferric chloride, DPPH, Hydrogen peroxide, (HiMedia Lab. Mumbai).

Preparations of Ethyl acetate extract of *Nardostachys jatamansi* DC roots

The dried powder material was successfully extracted with Ethyl acetate by hot continuous percolation method in Soxhlet apparatus till the solution become colourless. The residue obtained was then utilized for pharmacological screening. Approximately 300g of powdered drug material was extracted using Ethyl acetate in the ratio of 1:3 (w/v) in a Soxhlet apparatus. The extracts obtained and the dried mass was weighed and recorded. The percentage of yield was calculated [15].

(%) yield =Wt. of extract/Wt. of powdered drug× 100

Phytochemical analysis

The Ethyl acetate extract of *Nardostachys jatamansi* DC roots were subjected to preliminary phytochemical screening [16].

Acute toxicity study

Acute toxicity study was carried out on Ethyl acetate extract of *Nardostachys jatamansi* DC roots on male Swiss albino mice. The mice were fasted overnight and the weight of each mouse was recorded just before use. Animals were divided randomly in to ten treatment groups; each

group consisting of three mice; each treatment group received orally the Ethyl acetate extract of *Nardostachys jatamansi* DC roots in a dose of 5, 50, 300, 2000 and 5000 mg/kg. For each dose two groups of animals were used. Animals were kept under close observation for 4 hours after administering the extract, and then they were observed daily for three days for any change in general behaviour and/or other physical activities. Acute toxicity study was done as per OECD, 2006 Guidelines. Hence we selected 200 mg/kg and 500 mg/kg as low and high doses.

In-vitro Anti-oxidant activity

Hydrogen peroxide radical scavenging activity

A solution of H_2O_2 was prepared in phosphate buffer (pH 7.4).). 6 ml of 40 mM H2O2 solution was mixed with 1.0 ml of different concentration (10-100 µg/ml) of extract or standard *Nardostachys jatamansi DC roots.* After incubation at 37°C for 10 minutes absorbance was measured at 230nm. Corresponding blank solutions were taken using phosphate buffer without H2O2. A similar procedure was repeated with distilled water instead of extract which serves as control. Ascorbic acid was used as standard. All the tests were performed in triplicate. The decrease in absorbance indicates increase in free radical scavenging activity. The % scavenging activity was measured by using formula shown below [17].

Reducing power activity

1ml of extract/standard *Nardostachys jatamansi* DC *roots* of various concentrations (100-1000µg/ ml) was mixed with phosphate buffer (2.5ml, pH 7.4) and potassium ferricyanide (2.5ml, 1 %). The mixture was incubated at 50°C for 20 minutes. Aliquots of trichloroacetic acid (2.5ml, 10%) were added to the mixture, then centrifuged at 3000 x g for 10min. Upper layer of the solution(2.5ml) was mixed with distilled water(2.5ml) and freshly prepared ferric chloride solution (0.5ml, 0.1%). Absorbance was measured at 700nm. Corresponding blank solutions were taken containing same solution without ferric chloride. A similar procedure was repeated with distilled water instead of extract which serves as control. Ascorbic acid was used as standard. All the tests were performed in triplicate. Increased in the absorbance of the mixture indicates increased reducing power.

DPPH (2, 2-dipheny 1, 1-picrylhydrazyl) radical scavenging activity

The reaction mixture (3.0 ml) consists of 1 ml of 0.135mM DPPH solution in methanol was mixed with 1 ml of extract solution of *Nardostachys jatamansi DC roots* and 1.0 ml of methanol. The reaction mixture was vortexed and left in the dark at room temperature for 30 min. The various concentration of extract (10, 20, 40, 60, 80, 100 µg/ml) were prepared. A reaction

mixture without test sample was served as control. The absorbance was measured at 517 nm and (%) inhibition was calculated against control.

(%) Scavenging activity =Control absorbance - Test absorbance / Control absorbance X100

Experimental animals

Male albino Wistar rats weighing 150-200 g were used in the present study. The experimental animals were maintained under standard laboratory conditions in an animal house of Nanded Pharmacy College approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA) (Reg. No. 1613/PO/a/12/CPCSEA) under 12 h light/dark cycle and controlled temperature (24 ± 2°C) and fed with commercial pellet diet and water *ad libitum*. All animals were acclimatized to the laboratory environment for at least one week before the commencement of experiment. The experimental protocol for the study was followed according to the norms of Institutional Animal Ethics Committee.

Diuretic activity in rats (Lipschitz test)

The method of Lipschitz et al [20] was employed for the assessment of diuretic activity. According to this method, the animal should be deprived of food and water for 18 hours prior to the experiment, and were randomly divided into five groups of six animals each as follows:

Group 1(Control) - received saline 25ml/kg p.o.

Group 2(Standard) - received furosemide 20mg/kg p.o.

Group 3(Test) - received Ethyl acetate extract of *Nardostachys jatamansi* DC 100mg/kg p.o.

Group 4(Test) - received Ethyl acetate extract of *Nardostachys jatamansi* DC 200mg/kg p.o.

Group 5(Test) - received Ethyl acetate of *Nardostachys jatamansi* DC 500mg/kg p.o.

Immediately after administration, animals were placed in metabolic cages specially designed to separate urine faecal matter. During the period of study no food, water was made available to the animals. The total volume of urine was collected and measured from control, standard and extract treated groups up to 5 hours of administration. The parameters monitored for the each individual rat were total urine volume and urine concentration of Na+, K+ and Cl-. Concentration of Na+ and K+ were determined using flame photometer while Cl- concentration was estimated titrimetrically using 0.02N AgNO3 with 5% potassium chromate as an indicator. Appearance of brick red precipitate was taken as the end point.

Saluretic activity in rats

Male Wistar rats weighing 150–200 g are used. Three animals per group are placed in metabolic cages provided with a wire mesh bottom and a funnel to collect the urine. Stainless-steel sieves are placed in the funnel to retain feces and to allow the urine to pass. The rats are fed with

standard diet and water ad libitum. 15 hrs prior to the experiment only food is withdrawn not water. Three animals are placed in one metabolic cage. For screening procedures two groups of three animals are used for one dose of the test compound. The test compound is applied orally. Two groups of 3 animals receive orally standard drug (Frusemide). Urine excretion is recorded after 5 hrs. The sodium content of the urine is determined by flame photometry and auto analyzer.

Evaluation of analytical parameter

The urine collected was subjected to determine the concentration of Na+, K+ and Cl^{-} in the urine by flame photometer and titration with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate solution as indicator. Urine pH measured by using electronic pH meter and conductivity by conductivity meter**.** The density of urine was determined by using pycnometer**.**

Evaluation of saluretic index, diuretic index and carbonic anhydrase inhibition ratio

The sum of Na+ and Cl– excretion is calculated as parameter for saluretic activity. The ratio Na+/K+ is calculated for natriuretic activity. Values greater than 2.0 indicate a favourable natriuretic effect. Ratios greater than 10.0 indicate a potassium-sparing effect. The ratio (ion quotient) is calculated to estimate carbonic anhydrase inhibition as

$$CAI = Cl^{-} / Na^{+} + Cl^{-}$$

Carbonic anhydrase inhibition can be excluded at ratios between 1.0 and 0.8. With decreasing ratios slight to strong carbonic anhydrase inhibition can be assumed.

Statistical Analysis

The results were expressed as a mean ± S.E.M. The differences were compared using One Way Analysis of Variance (ANOVA) and subsequently followed by Bonferroni´s test.

RESULTS AND DISCUSSION

In traditional medicine system, many plants and herbs are claimed to have diuretic and antioxidant efficacy without any significant scientific study. *Nardostachys jatamansi* DC is used traditionally as folk medicine to treat a number of illnesses including some disorders where its diuretic potential is claimed to be useful. However, there is no scientific evidence in diuretic activity of Roots of *Nardostachys jatamansi* DC. Hence present study was selected for evaluation of its antioxidant and diuretic activity and its effect on electrolytes level. The extract was prepared with ethyl acetate.

Phytochemical analysis

Preliminary phytochemical evaluation of extracts was carried out for the determination of phytoconstituents [16] and showed presence of tannins, resin, protein, amino acids. The results pertaining to this investigation were presented in Table 1.The determination of various physicochemical parameters i.e. total ash, acid insoluble ash, water soluble ash, loss on drying, alcohol soluble extractive and water soluble extractive values were calculated as per Indian Pharmacopoeia. The results are tabulated in the Table 2.

Table 1. Preliminary Phytochemical analysis of ethyl acetate extract of *Nardostachys jatamansi* DC roots

Chemical constituents	Ethyl acetate Extract
Flavonoids	–
Coumarins	–
Proteins and amino acids	+
Starch	–
Tannins	+
Resins	+

(+) present, (-) absent

Table 2. Physicochemical Parameters

Sr.no.	Parameters	% w/w
1	Ash value	5.7
	a) Total Ash	2.1
	b) Acid insoluble ash	1.9
	c) Water soluble ash	
2	Loss on drying	3.3
3	Extractive valuea) ethyl acetate soluble	4

Acute toxicity study

Acute oral toxicity study was carried out for extracts of *Nardostachys jatamansi* DC roots as per OECD guideline No. 423 (Acute Oral Toxicity-Class method) which reveals LD_{50} is greater than 5000mg/kg. Such study is useful for interpreting safety of test product and to calculate safe dose for experimental purpose. Accordingly 1/10 of LD_{50}; that is $\leq$ 500mg/kg dose of extracts was considered for pharmacological screening as maximum dose.

In-vitro Anti-oxidant activity

Oxidative stress is commonly associated with most of the diseases and disorders, so it was thought to evaluate antioxidant property of *Nardostachys jatamansi* DC roots as a supportive study and diuretic activity as main study.

Hydrogen Peroxide Scavenging Activity of ethyl acetate extract and ascorbic acid

Hydrogen peroxide itself is not very particularly reactive with most biologically important

molecules, but is an intracellular precursor of hydroxyl radicals which is very toxic to the cell. The decreased absorbance of reaction indicates increased reduction of hydrogen peroxide and hydroxyl radical production. Ethyl acetate extract shows 62.85 percent scavenging activity at 100µg/ml conc. while standard ascorbic acid at 100µg/ml conc. shows 80.95 percent scavenging activity (Table 3).

Table 3. Hydrogen Peroxide Scavenging Activity of ethyl acetate extract and ascorbic acid

Sr. No.	Conc.	Absorbance of et. acetate extract	% Inhibition due to et. acetate extract	Absorbance of Standard (A.A.)	% Inhibition due to Standard (A.A.)
1	10µg/ml	0.701±0.10	16.54	0.51±0.74	38.21
2	20µg/ml	0.598±0.0036	28.80	0.44±0.0049	47.38
3	40µg/ml	0.514±0.02	38.80	0.33±0.006	60.59
4	80µg/ml	0.389±0.260	53.69	0.23±0.044	73.80
5	100µg/ml	0.312±0.61	62.85	0.16±0.48	80.95

Each value represents the mean ±S.E.M. (n=6)

Reducing Power Assay of ethyl acetate extract and Ascorbic acid

In reducing power assay, the presence of antioxidants in the sample reduced Fe3+/ ferricyanide complex to the ferrous form. This reducing capacity of compounds could serve as an indicator of potential antioxidant properties and increase in absorbance could indicate an increase in reducing power [23]. Absorbance of ethyl acetate extract at higher concentration that is 100µg/ml is nearer to 20µg/ml of standard (Table 4).

Table 4. Reducing Power Assay of ethyl acetate extract and Ascorbic acid

Extract	Absorbance at 700 Nm				
	10µg/ml	20µg/ml	40µg/ml	80µg/ml	100µg/ml
Ethyl acetate extract	0.687±0.001	0.703±0.003	0.721±0.005	0.798±0.001	0.837±0.007
Standard	0.729±0.007	0.813±0.001	0.958±0.003	0.980±0.004	0.988±0.006

Each value represents the mean ±S.E.M. (n=6)

DPPH radical scavenging assay of ethyl acetate extract and Ascorbic acid

Proton radical scavenging action is one mechanism for oxidation. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, suggesting that antioxidant activity of CB extract is due to its proton donating ability and it showed concentration dependent DPPH scavenging activity. Ethyl acetate extract shows 65.26percent scavenging activity at 100µg/ml conc. while standard ascorbic acid at 100µg/ml conc. shows 79.88 percent scavenging activity. By all these antioxidant methods it provides evidence that *Nardostachys jatamansi* DC roots has a good potential for antioxidant activity (Table 5).

Table 5. DPPH radical scavenging assay of ethyl acetate extract and Ascorbic acid

Sr. No.	Conc.	Absorbance of et. acetate extract	% Inhibition due to et. acetate extract	Absorbance of Standard (A.A.)	% Inhibition due to Standard(A.A.)
1	10µg/ml	0.973±0.10	22.66	0.823±0.74	34.57
2	20µg/ml	0.849±0.052	32.51	0.648±0.0049	48.48
3	40µg/ml	0.736±0.0051	41.49	0.401±0.006	68.12
4	80µg/ml	0.501±0.072	60.17	0.342±0.044	72.81
5	100µg/ml	0.437±0.0085	65.26	0.253±0.48	79.88

Each value represents the mean ±S.E.M. (n=6)

Effect of Ethyl acetate extract of *Nardostachys jatamansi* DC roots on urine volume and diuretic index in rats

This study investigated the diuretic potential of extracts of *Nardostachys jatamansi* DC roots. The results showed that the highest dose (500mg/kg) of extract of roots possesses strong diuretic activity when given orally in a single dose. The findings suggest effect of three different doses of extract (100 mg/kg, 200 mg/kg, and 500 mg/kg) of *Nardostachys jatamansi* DC roots is probably mediated through its ability to cause a significant increase in urine volume, sodium and potassium excretion, without interfering with other parameters related to renal functions (Table 6).

Table 6. Effect of Ethyl acetate extract of *Nardostachys jatamansi* DC roots on urine volume and diuretic index in treated rats

Treatment	Urine volume (ml/5hr)	Diuretic index
Normal control	2.75± 0.05	1.00
Standard Frusemide (20 mg/kg p o)	5.87± 0.025	2.13
Ethyl acetate extract (100 mg/kg)	3.45± 0.05*	1.25
Ethyl acetate extract (200 mg/kg)	4.65± 0.05**	1.69
Ethyl acetate extract (500 mg/kg)	5.13± 0.035**	1.86

Each value represents the mean ±S.E.M. (n=6), **P*<0.05, ** *P*<0.001 compared with control and $^{\#}$p>0.05 compared with standard.

Effect of Ethyl acetate extract of *Nardostachys jatamansi* DC roots on urine volume and electrolyte excretion in rats

Ethyl acetate extract of *Nardostachys jatamansi* DC roots induced strong diuresis and was not accompanied with a reduction in urinary K^+ levels. Further, there was no alkalization of urine. Collectively, these observations suggest that the extract is not acting as potassium-sparing diuretics. In this study, both urinary Na^+ and K^+ levels were increased without any significant alteration in the Na^+/K^+ ratio [25]. The diuresis induced by the extract of *Nardostachys jatamansi* DC roots was strong and the intensity was similar to that of frusemide and accompanied by

marked increases in both urinary Na^+ and K^+ levels at higher doses. These features strongly suggest that the extracts may act as a loop diuretic [26] (Table 7).

Table 7. Effect of Ethyl acetate extract of *Nardostachys jatamansi* DC roots on urine volume and electrolyte excretion in rats

Treatment	Urine volume (ml/5hr)	Na^+(mEq/L)	K^+(mEq/L)	Cl^- (mEq/L)
Normal control	5.05± 0.05	90.59± 0.16	28.52±0.32	146±1
Frusemide(20mg/kg po)	9.1± 0.1	199.98±1.75	65.17±0.2	221.5±1.5
Ethyl acetate extract (100mg/kg po)	5.75±0.05*	94.83±0.23*	31.18±0.42*	163.1±1.9*
Ethyl acetate extract (200mg/kg po)	6.05±0.15*	97.67±0.28*	34.85±0.24*	174.1±0.9*
Ethyl acetate extract (500mg/kg po)	7.05±0.05**	111.07±0.22**	37.9±0.55*	231.9±2.9*#

Each value represents the mean ±S.E.M. (n=6), *P<0.05, ** P<0.001 compared with control and # p>0.05 compared with standard.

Effect of ethyl acetate extract of *Nardostachys jatamansi* DC roots on urine volume, pH, density, conductivity

Doses of ethyl acetate extract (100 mg/kg and 200mg/kg) of *Nardostachys jatamansi* DC roots have decreased the urine pH as compared to normal control group. However, the (500 mg/kg) of *Nardostachys jatamansi* DC roots extract has increased the urine pH as compared to normal control group. Different doses of extract (100mg/kg, 200mg/kg, and 500 mg/kg) of Nardostachys jatamansi DC roots and Standard Frusemide (20 mg/kg) shows similarity as compared to normal control group have not produced significant change in density of urine as compared to normal control group but the treatment significantly increased the urine conductivity as compared to normal control group (Table 8).

Table 8. Effect of ethyl acetate extract of *Nardostachys jatamansi* DC roots on urine volume, pH, density, conductivity of treated rats

Treatment	Urine volume (ml/5hr)	pH	Density (gm/ml)	Conductivity (mS/cm)
Normal control	5.05± 0.05	7.38±0.015	0.97±0.005	12.46±0.035
Frusemide(20mg/kg po)	9.1± 0.1	6.29±0.01	0.98±0.005	17.43±0.02
Ethyl acetate extract (100mg/kg po)	5.75±0.05*	6..87±0.025	0.98±0.001	16.88±0.015
Ethyl acetate extract (200mg/kg po)	6.05±0.15*	7.28±0.02#	0.97±0.001	15.04±0.025*
Ethyl acetate extract (500mg/kg po)	7.05±0.05**	7.53±0.07#	0.98±0.001	13.07±0015*

Each value represents the mean ±S.E.M. (n=6), *P<0.05, ** P<0.001 compared with control and # p>0.05 compared with standard.

Effect of ethyl acetate extract of *Nardostachys jatamansi* DC roots on saluretic, Natriuretic index and carbonic anhydrase inhibition activity in rats

Result also reveals that 500mg/kg dose of ethyl acetate extract of *Nardostachys jatamansi* DC roots have saluretic index greater than saluretic index of normal control group. Extract at different doses have greater CAI ratios than normal control group and standard group. It indicates that ethyl acetate extract have good saluretic activity at 500mg/kg and at different doses have weak carbonic anhydrase inhibition. Ethyl acetate extracts showed ratio Na+/K+ more than 2.0 and less than 10.0 which indicates favourable natriuretic activity and no potassium sparing effect. Increase in dose of extract cause acidification of urine. Further, the onset of the diuretic activity of extract was extremely rapid as observed with clinically used synthetic loop diuretics. The results are tabulated in Table 9.

Table 9. Effect of ethyl acetate extract of *Nardostachys jatamansi* DC roots on saluretic, Natriuretic index and carbonic anhydrase inhibition activity in treated rats

Treatment	Natriuretic index	Saluretic index	CAI index
Normal control	3.17	1.16	1.22
Frusemide (20mg/kg po)	2.10	1.78	0.83
Ethyl acetate extract (100mg/kg po)	3.06	1.08	1.47
Ethyl acetate extract (200mg/kg po)	2.8	1.14	1.31
Ethyl acetate extract (500mg/kg po)	2.93	1.37	1.25

CONCLUSION

Ethyl acetate extract was tested for its antioxidant activity and it was proved. Ethyl acetate extracts showed presence of sesquiterpenes and they may be responsible for strong diuretic activity. Loop diuretics are clinically used in patients with salt and water overload due to conditions such as pulmonary oedema, heart failure ascites and hypertension. Loop diuretic type of mode of action of the different extracts of *Nardostachys jatamansi* DC roots indicate that it may be useful as a non toxic natural therapeutic agent in the treatment of such conditions by traditional practitioners; although further mechanism based investigation is needed to confirm exact mechanism.

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