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Article

# Bioactivity evaluation of least explored traditionally acclaimed medicinally potent herb *Nanorrhinum ramosissimum*(Wall.) Betsche

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Present study analysed the therapeutic potential of traditionally acclaimed medicinal herb *Nanorrhinum ramosissimum*, using plant parts extracted with different solvents (10 mg/mL). Shoot extracts exhibited comparatively better antimicrobial properties, in comparison to root extracts. Total phenolic content was estimated, to ascertain its dependency on antioxidant properties of plant extracts. Antioxidant assay revealed promising results in comparison to $IC_{50}$ value of standard ascorbic acid (52.2±0.07 µg/mL), for methanolic extracts of shoot (61.07±0.53 µg/mL and 64.33±0.33 µg/mL) and root (76.705±0.12 µg/mL and 89.73±0.28 µg/mL) for *in vivo* and *in vitro* regenerants respectively. Correlation coefficient $R^2$ values ranged between 0.90-0.95, indicating a positive correlation between phenolic contents and antioxidant activity. Plant extracts were also able to inhibit DNA oxidative damage again indicating their antioxidative potential. Antidiabetic potential was confirmed by alpha amylase inhibition assay where shoot methanolic extracts (*invivo*, *in vitro*) exhibited the best $IC_{50}$ values (54.42±0.16 µg/mL, 66.09±0.12 µg/mL) in comparison to standard metformin (41.92±0.08 µg/mL). Ethanolic extracts of roots (*in vitro, invivo*) exhibited the relative $IC_{50}$ values (88.97±0.32µg/mL,96.63±0.44 µg/mL) indicating that shoot parts had a better alpha amylase inhibition property; thus proving the herb's bioactive potential and its prospective therapeutic source for curing various ailments.

**Keywords**: Branched cancerwort. Antimicrobial activity. Antioxidant activity. Alpha amylase inhibition. DPPH assay.

## INTRODUCTION

Medicinal plants are a significant resource for finding novel remedies for various human health complications (Ghorbani, 2013; Myo *et al*., 2020). *Nanorrhinum ramosissimum* is an important but less explored medicinal of that has been traditionally used in folk medicine in the Indian subcontinent to cure a multiplicity of ailments (Kirtikar, Basu, 2005; Amin *et al.,* 2017). The most popular synonyms for *N. ramosissimum* are *Kickxia ramosissima* and *Linaria ramosissima* and are commonly known as Bhintgalodi, Kanodi (Gujarati), Banwel (Marathi), Branched cancerwort (English) (Pullaiah, Naidu, 2003). The plant habituates countries like India, Pakistan, growing on walls, rocky and stony places, ascending to height of 7,000 ft in the Himalayas (Pandya *et al*., 2012). It is a prostrate perennial herb that grows up to a length of about 40 cms, flowering in the months of August to October. In traditional systems of medicine, the herb is mentioned as a remarkably suitable candidate for curing various diseases e.g. in cases of snake bite the roots and leaves are quite effectual, whereas in fever leaves mixed with black pepper are recommended. The whole plant has also been used traditionally for the management of diabetes, inflammatory disorders such as rheumatism, jaundice, activation of immune system and treatment of urinary stones (Bhandari, 2006; Pandya *et al*., 2012; Jan, Khan, 2016). However, in spite of its numerous therapeutic properties, there are very few scientific reports of evidence regarding the bioactive properties of the herb,

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therefore, it has not yet been included in the modern health care system. The current study was performed to inspect the antimicrobial, antioxidant, alpha amylase inhibitory activities and oxidative DNA damage response of extracts of the shoot and root parts prepared using various solvents (methanol, ethanol, ethyl acetate, petroleum ether, aqueous) from both *in vivo* (field) and *in vitro* regenerants of *N. ramosissimum*. This piece of study could prove to be beneficial in utilizing the remarkable therapeutic potentials of the herb for improving the health of mankind.

## MATERIAL AND METHODS

### Sample preparation

*Nanorrhinum ramosissimum* (Wall.) Betsche (Plantaginaceae) was collected from its natural habitat at Akhnoor, Jammu District, Jammu & Kashmir, India (Latitude = 32°89′ North, Longitude = 74°74’ East and Altitude = 301m). kindly identified by Sh. OP Sharma, IFS, Chief Conservator Forests, Department of Environment, Ecology and Remote Sensing, J&K, India, which served as the *in vivo* plant material. To verify the identity of the plant under study, a vaucher herbarium specimen has been deposited at Janaki Ammal Herbarium, CSIR-Indian Institute of Integrative Medicine (CSIR-IIIM), Jammu, J&K bearing accession No. RRLH23493 (Figure 1). Active shoot tips (0.5 cm length) of the *in vivo* plants were selected as explants for developing the *in vitro* regenerants in the appropriate multiplication medium (Sharma *et al*., 2021).

*In vivo* and *in vitro* shoot and root parts were collected individually, dried in shade and pulverized. The powder (100 mg) was then extracted with five different solvents (10 mL) viz. methanol, ethanol, ethyl acetate, petroleum ether, aqueous) in a conical flask implanted on a shaker (40 rpm) for 48 h at room temperature (25 °C). The extract was recovered and filtered (Whatmann filter paper No. 1). The residue obtained from the first extraction was again extracted twice in the same solvent as described above. The extracts were pooled after three successive extractions and the filtrate recovered was evaporated under reduced pressure at 50 °C using a rotary evaporator in order to obtain the crude extract which was dissolved in dimethyl sulfoxide (DMSO) and used at different concentrations (10-200 µg/mL) for bioactivity analysis.

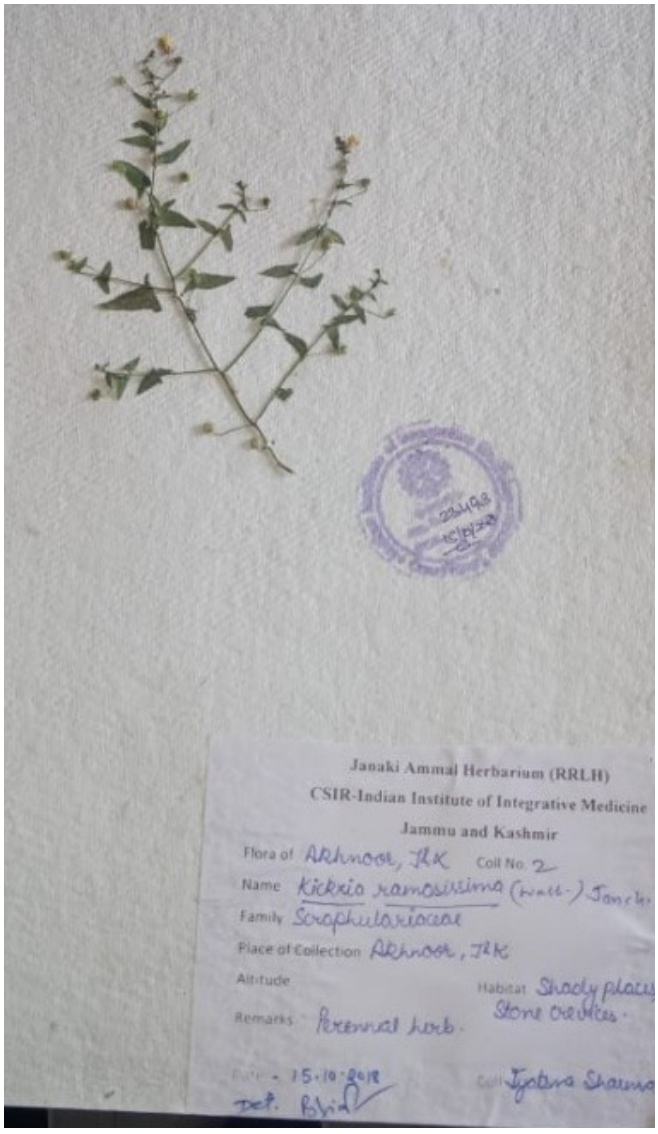


**FIGURE 1 -** Specimen submitted in Janaki Ammal Herbarium CSIR-Indian Institute of Integrative Medicine (CSIR-IIIM), Jammu, J&K bearing accession No. RRLH23493.

### Antimicrobial activity

The extracts prepared from *in vivo* plant materials of *N. ramosissimum* were assayed for their antimicrobial activity against different bacterial strains (gram positive and gram negative) viz. *Bacillus subtilis, Mycobacterium smegmatis, Escherichia coli and Proteus vulgaris*. Antibacterial activity was determined by agar-well diffusion method wherein a suspension containing bacteria 100 µL was spread on

agar medium and wells were punched using a sterile cork borer. Different plant extracts (100 µL) were filled into each well followed by incubation of the petri dishes at 37 °C for 24 h. On completion of the incubation period, the radius of inhibition zones formed was measured and compared with inhibition zone obtained using streptomycin (10 µg/mL) as a reference standard and DMSO (10%) used as a negative control.

### Estimation of total phenolic content

The total phenolic concentration in different solvent extracts was analyzed by Folin-Ciocalteu method with gallic acid as the standard (Ainsworth, Gillespi, 2007). Gallic acid (standard) (400 µL) and sodium carbonate (1.6 mL) were added to Folin-Ciocalteu reagent (2 mL) and repeated similarly with the different plant extracts. The mixture was incubated at room temperature for an hour and the absorbance recorded on a spectrophotometer (Lab India) at 765 nm. The entire estimations were done in triplicates, and the results are expressed as mg gallic acid equivalent (GAE) /g of extract.

### Antioxidant activity

The free-radical scavenging activity of extracts of *N. Ramosissimum* was measured by a decline in the absorbance solution of DPPH (2, 2-Diphenyl-1-picrylhydrazyl)prepared in methanol (400 µg/mL) (Feresin *et al.,* 2002). Stock solution of DPPH (100 µL) was mixed different concentrations of *N.* ramosissimum extracts ranging from 10-200 µg/mL. The solutions were incubated under dark conditions for 20 min. The absorbance was measured at 517 nm. Ascorbic acid was taken as a measure of positive control and percent inhibition of each extract was calculated using the following formula.

### Free radical

$$\text{Scavenging (\%)} = \frac{{}^{*}\text{Absorbance of control} - {}^{*}\text{Absorbance of sample}}{{}^{*}\text{Absorbance of control}} \times 100$$

Using the values of percent inhibition of each extract, $IC_{50}$ value was calculated.

### Total phenolic content vs. antioxidant potential

To analyse the relationship between total phenolic content derived by Folin-Ciocalteu method and antioxidant activity assayed by DPPH assay of the plant extracts, a regression curve was obtained for best fit data and the corresponding $R^2$ value was calculated. The closer the value of $R^2$ to 1.0, the better the fit of the regression curve (Piluzza, Bullitta, 2011).

### Oxidative DNA damage protection activity

In order to study the DNA damage protection activity of plant extracts an experiment was conducted taking DNA isolated from human blood. DNA sample (10 µL) was added in a microcentrifuge tube covered completely by a black paper to avoid any oxidation caused by sunlight/ tubelight. Plant extract (10 µL) was mixed with the DNA sample and incubated for 10 min at room temperature followed by addition of Fenton's reagent (10 µL) to the above mixture, vortexed and incubated at room temperature for an hour under dark conditions. Subsequently, from each tube, 15 µL of the mixture was taken and mixed with loading dye (6x, 3 µL) and loaded on a agarose gel (1%) containing ethidium bromide. Electrophoresis was performed at 50 V until the tracking dye reached 1 cm below the upper edge of the gel. The DNA was observed and photographed on a digital gel doc as per reported protocol (Balakrishnan, Paramasivam, Arulkumar, 2014).

### Alpha amylase inhibitory activity

The assay reaction mixture comprising sodium phosphate buffer (200 µL, 0.02 M), porcine pancreatic alpha amylase enzyme(0.04 units) and the plant extracts at a concentration range of 10 to 200 µg/mL were incubated for 10 min at room temperature. The three-dimensional structure of porcine pancreatic alpha amylase enzyme is extremely similar to that of human pancreatic α-amylase. Various analysis by molecular cloning and also primary structure analysis of porcine pancreatic α-amylase exhibited maximum homology (87.1%) with the sequence of human pancreatic α-amylase sequence among all the other amylases (Anitha, Muralikrishna, 2009).Starch (200 µL)

was added in each test tube and subsequently DNS reagent (3,5- Dinitrosalicylic acid) (400 µL) was added in order to cease the reaction. The mixture was then placed on a boiling water bath for 5 min which was further cooled to room temperature and diluted with distilled water (15 mL). The absorbance of the mixture was recorded at 540 nm. The same was repeated for the controls where plant extracts were replaced by an equal quantity of distilled water and the reference standard used was Metformin (10 to 200 µg/mL). The percent inhibition was calculated according to the following formula (Nanumala, Tulasi, Sujitha, 2015).

$$\text{Alpha amylase inhibitory activity (\%)} = \frac{\text{*Absorbance of control - *Absorbance of sample}}{\text{*Absorbance of control}} \times 100$$

The $IC_{50}$ values were calculated by plotting graphs between percent inhibitions versus log inhibitor.

### Statistical Methods

The experimental data was collected in triplicates and the results expressed by their mean values and standard error (SE). One way analysis of variance (ANOVA) was performed (SPSS version 17.0) and significance of difference determined according to Duncan's multiple range test (DMRT). p value≤0.05 was considered to be statistically significant.

## RESULTS AND DISCUSSION

*Nanorrhinum ramosissimum* has been in use as a traditional folk medicine to treat microbial infections and constitute potent sources of conventional antimicrobial agents (Amin *et al*., 2017). During the present study, the effectiveness of different solvent extracts (methanol, ethanol, ethyl acetate, petroleum ether, aqueous) of the plant (*in vivo*, *in vitro* regenerants) were analyzed against different pathogens. Zone of inhibition values of the experiment and the results obtained exhibited that most of the solvent extracts possessed good antimicrobial properties (Table I). The inhibition potency of the methanolic shoot extract against tested organisms, was in the order of *E. coli>P. vulgaris>B. subtilis* and no inhibition was found in *M. smegmatis*. In ethanolic extracts the order was *P. vulgaris>E. coli>M. smegmatis>B. subtilis* where the values ranges from 2.63 to 2.90. In ethyl acetate extracts, it was *P. vulgaris>E. coli>B. subtilis*. In petroleum ether, inhibition was only observed in *P. vulgaris* among all the other tested strains whereas in aqueous extract, the order of organisms was *E. coli>P. vulgaris>M. smegmatis*. From all the above results, it can be concluded that shoot extracts are more bioactive where the highest inhibition was obtained with methanolic and aqueous extracts with a zone of inhibition measuring 4.8 and 4.4 mm (*E. coli*) respectively. The lowest inhibition was obtained with ethyl acetate extract in *B. subtilis* (1.8 mm) where as petroleum ether extract exhibited no inhibition in all the organisms tested except *P. vulgaris* (2.03 mm).

**TABLE I -** Antimicrobial activity of plant extracts of *Nanorrhinum ramosissimum*

| Plant part | Extraction solvent | Zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | ***B. subtilis*** | ***M. smegmatis*** | ***E. coli*** | ***P. vulgaris*** |
| **Shoot extract** | Methanol | 2.33±0.33$^{b}$ | N | 4.80±0.12$^{a}$ | 3.93±0.06$^{a}$ |
| | Ethanol | 2.63±0.36$^{a}$ | 2.83±0.08$^{a}$ | 2.83±0.08$^{c}$ | 2.90±0.10$^{b}$ |
| | Ethyl acetate | 1.80±0.11$^{c}$ | N | 1.83±0.08$^{d}$ | 2.90±0.08$^{b}$ |
| | Petroleum ether | N | N | N | 2.03±0.08$^{c}$ |
| | Aqueous | N | 1.86±0.08$^{b}$ | 4.40±0.14$^{b}$ | 2.90±0.05$^{b}$ |

**TABLE I -** Antimicrobial activity of plant extracts of *Nanorrhinum ramosissimum*

| Plant part | Extraction solvent | Zone of inhibition (mm) | | | |
|---|---|---|---|---|---|
| | | ***B. subtilis*** | ***M. smegmatis*** | ***E. coli*** | ***P. vulgaris*** |
| **Root extract** | Methanol | 1.80±0.11[c] | N | 1.76±0.14[b] | 1.30±0.05[a] |
| | Ethanol | 0.86±0.08[d] | 2.83±0.08[a] | 0.86±0.08[c] | 1.48±0.08[b] |
| | Ethyl acetate | 2.73±0.08[a] | N | N | 1.26±0.03[c] |
| | Petroleum ether | 1.90±0.05[b] | 0.90±0.057[c] | N | N |
| | Aqueous | 2.70±0.14[a] | 1.46±0.98[b] | 1.93±0.12[a] | N |

N - no zone of inhibition observed; Values are represented as Mean±SE of three experiments with three replicates per treatment; Means followed by different superscript in each column are significantly ($P \leq 0.05$) differ from each other using Duncan's Multiple Range Test from SPSS version 17.

To the methanolic root extract, the tested strains can be ordered as *B. subtilis>P. vulgaris>E. coli* where as inethanolic extract it is *M. smegmatis>P. vulgaris>B. subtilis=E. coli.* In ethyl acetate extracts, it is *B. subtilis>P. vulgaris* and no inhibition was observed in the other tested strains. In petroleum ether extracts, the order is *B. subtilis>M. smegmatis* and no inhibition was observed in the *E. coli* and *P. vulgaris* where as in aqueous extracts, the trend was *B. subtilis>E. coli>M. smegmatis* while *P. vulgaris* displayed no inhibition. Results obtained from the root extracts indicated that the highest inhibition was obtained in ethyl acetate extract in *B. subtilis* (2.73 mm) where as the lowest inhibition was obtained with ethanolic extract in *E. coli* (0.86 mm). Antimicrobial constituents present in plant extracts have been reported to have certain mechanisms of their action including inhibition of cell wall synthesis, protein/nucleic acid synthesis, inhibition of essential metabolite synthesis and destruction of plasma membrane synthesis by intermingling with microbial cell membrane components (i.e. enzymes, proteins) resulting into their distortion due to the release of protons to the exterior of cell which leads to cell death(Burt, 2004; Gill, Holley, 2006) which could be the probable reason for the antimicrobial activity of *N. ramosissimum* plant extracts.

An estimation of the phenolic content was performed in the plant samples of *N. ramosissimum* using various polar and non-polar solvents in order to analyze its correlation with antioxidant activity. A comparison of phenolic contents obtained with different solvent extracts is shown in Table SI. Amongst all the samples analyzed methanolic extract of *in vivo* shoots exhibited the highest phenolic content (57.14±0.72 µg/mL) followed by ethanolic extract (43.67±0.62 µg/mL) and methanolic extract of the *in vitro* shoot (41.34±0.32 µg/mL).

**TABLE SI -** Total phenolic content of different solvent extracts from *in vitro* and *in vivo* shoot and root parts of *N. ramosissimum*

| Extraction Solvent | Total phenolic content±SE ( mg GAE/gm extract) | | | |
|---|---|---|---|---|
| | Shoot | | Root | |
| | ***In vitro*** | ***In vivo*** | ***In vitro*** | ***In vivo*** |
| **Methanol** | 41.34±0.32 | 57.14±0.72 | 36.44±0.12 | 21.34±0.12 |
| **Ethanol** | 36.67±0.22 | 43.67±0.62 | 26.64±0.56 | 18.47±0.19 |

**TABLE SI -** Total phenolic content of different solvent extracts from *in vitro* and *in vivo* shoot and root parts of *N. ramosissimum*

| Extraction Solvent | Total phenolic content±SE ( mg GAE/gm extract) | | | |
|---|---|---|---|---|
| | Shoot | | Root | |
| | *In vitro* | *In vivo* | *In vitro* | *In vivo* |
| **Petroleum ether** | 31.89±0.12 | 38.89±0.65 | 32.69±0.11 | 12.49±0.32 |
| **Aqueous** | 31.89±0.16 | 39.89±0.52 | 21.89±0.11 | 10.59±0.13 |
| **Ethyl acetate** | 14.51±0.08 | 29.41±0.05 | 16.31±0.14 | 16.21±0.43 |

Values are represented as mean±SE of three observations; (GAE-Gallic acid equivalent)

Disorders like liver cirrhosis, atherosclerosis, cancer, diabetes and other neurodegenerative diseases are caused due to the generation of free radicals by metabolic activities in the human body, caused by reactive oxygen species which can be averted by using various antioxidants. This becomes particularly important when endogenous factors cannot ensure a complete control and protection of the organism against reactive oxygen species. In such cases, the need for exogenous antioxidants as a supplementation arises, to keep us safe from the oxidative processes (Kanatt, Chander, Sharma, 2007). The remedial potential of medicinal plants in reducing such free radical induced tissue injury, confirms that many plants have antioxidant activities that can be therapeutically useful (Piluzza, Bullitta, 2011). Since, herbal constituents have the potential to inhibit the oxidation caused by these free radicals, the antioxidant activity of the different extracts of *N. ramosissimum* were also investigated. DPPH assay is one of the methods to investigate the antioxidant action of plant constituents (Koleva *et al.,* 2002). The spectrophotometric quantification of the level of DPPH scavenging in *N. ramosissimum* extracts (shoot, root parts) was conducted in order to assess their free radical scavenging potential. An analysis of the results obtained, indicates that there is a rise in the scavenging effect of *N. ramosissimum* extracts with an increase in its concentration. The methanolic *in vivo* and *in vitro* shoot extract at a concentration of 200 µg/mL exhibited maximum free radical scavenging capacity (96.50±0 µg/mL, 95.0±0 µg/mL respectively) and ethanolic *in vivo* shoot extract (95.06±0.06 µg/mL) at 200µg/mL. Standard ascorbic acid had an $IC_{50}$ value of 52.2±0.07 µg/mL, while amongst all the samples analysed, methanolic extract of *in vivo* shoot samples showed the least and most suitable $IC_{50}$ value (61.07±0.53 µg/mL) as comparable to the standard followed by the *in vitro* shoot extract of the same solvent (64.33±0.33 µg/mL) (Table S II). It can be inferred from the results obtained that the methanolic extract of *in vivo* shoot is most appropriate in comparison to the standard as an antioxidizing agent where as in root samples, both methanolic (90.20%) and ethanolic (90.50%) extracts of *in vivo* samples exhibited the maximum inhibition with $IC_{50}$ value of 76.70±0.12 µg/mL and 80.90±0.18 µg/mL respectively in comparison to the standard (Table S II). Among the root extracts, methanolic extracts of *in vivo* samples exhibited maximum inhibition as shown in Table S III but it is less than that of the shoot samples

**TABLE SII –** DPPH assay of different solvent (Methanol, ethanol, ethyl acetate, petroleum ether and aqueous) extracts of shoot parts of both *in vitro* and *in vivo* samples of *Nanorrhinum ramosissimum*

| Extraction Solvent | Concentration | *In vivo* sample | | *In vitro* sample | | Standard | |
|---|---|---|---|---|---|---|---|
| | (µg/mL) | % inhibition | IC50 (µg/mL) | % inhibition | IC50 (µg/mL) | % inhibition | IC50 (µg/L) |
| **Methanol** | 10 | 91.03±0.03 | 61.07±0.53 | 91.03±0.03 | 64.33 ±0.33 | 96.37±0.65 | 52.2±0.07 |
| | 20 | 92.96±0.61 | | 91.5±0.00 | | 96.90±0.16 | |
| | 40 | 92.93±0.03 | | 93±0.00 | | 97.21±0.22 | |
| | 60 | 94±0 | | 93.066±0.06 | | 97.40±0.53 | |
| | 80 | 95.03±0.03 | | 94±0 | | 97.73±0.53 | |
| | 100 | 96±0 | | 94.5±0 | | 98±0.21 | |
| | 150 | 96.46±0.03 | | 94.96±0.03 | | 98.1±0.17 | |
| | 200 | 96.5±0 | | 95±0 | | 98.2±0.13 | |
| **Ethanol** | 10 | 91.033±0.03 | 66.24±0.94 | 88.506±0.006 | 74 ±0.94 | | |
| | 20 | 91.5±0.00 | | 88.51±0.01 | | | |
| | 40 | 93±0 | | 89.5±0.00 | | | |
| | 60 | 93±0 | | 90±0 | | | |
| | 80 | 94.033±0.03 | | 90.13±0.0 | | | |
| | 100 | 94.5±0.00 | | 90.5±0.00 | | | |
| | 150 | 95±0.00 | | 91±0.00 | | | |
| | 200 | 95.066±0.06 | | 91.03±0.04 | | | |
| **Ethyl acetate** | 10 | 89±0.00 | 69.25±0.09 | 85.5±0.00 | 80.44 ±0.18 | | |
| | 20 | 89±0.00 | | 86±0.00 | | | |
| | 40 | 90±0.00 | | 86.803±0.003 | | | |
| | 60 | 90.3±0.003 | | 88±0 | | | |
| | 80 | 90.3±0.00 | | 88.033±0.003 | | | |
| | 100 | 90.50±0.003 | | 89.50±0.006 | | | |
| | 150 | 91±0.00 | | 90.133±0.03 | | | |
| | 200 | 91±0.00 | | 90.5±0.003 | | | |
| **Petroleum ether** | 10 | 87.06±0.066 | 89.02±0.55 | 90.03±0.03 | 92.658 ±0.35 | | |
| | 20 | 87.53±0.033 | | 90±0.00 | | | |
| | 40 | 87.56±0.066 | | 90.303±0.003 | | | |
| | 60 | 88.06±0.066 | | 90.71±0.005 | | | |
| | 80 | 88±0.00 | | 92±0 | | | |
| | 100 | 89.5±0.00 | | 92.223±0.01 | | | |
| | 150 | 90±0.00 | | 92.5±0 | | | |
| | 200 | 90.13±0.057 | | 93.03±0.03 | | | |
| **Aqueous** | 10 | 85.5±0 | 76.09±0.49 | 87.033±0.03 | 90.48 ±0.56 | | |
| | 20 | 86.04±0.04 | | 87.5±0.00 | | | |
| | 40 | 86.8±0.00 | | 87.5±0.00 | | | |
| | 60 | 88±0 | | 88±0.00 | | | |
| | 80 | 88.03±0.30 | | 88.033±0.033 | | | |
| | 100 | 89.503±0.03 | | 89.506±0.006 | | | |
| | 150 | 90.03±0.033 | | 90±0.00 | | | |
| | 200 | 90.166±0.03 | | 90.20±0.003 | | | |

Values are represented as mean ± SE of three observations

**TABLE SIII -** DPPH assay of different solvent (Methanol, ethanol, ethyl acetate, petroleum ether and aqueous) extracts of root parts of both *in vitro* and *in vivo* samples of *Nanorrhinum ramosissimum*

| Extraction Solvent | Concentration | *In vivo* sample | | *In vitro* sample | | Standard | |
|---|---|---|---|---|---|---|---|
| | (µg/mL) | % inhibition | IC50 (µg/mL) | % inhibition | IC50 (µg/mL) | % inhibition | IC50 (µg/L) |
| **Methanol** | 10 | 85.503±0.03 | 76.705±0.12 | 87±0.00 | 89.73 ±0.28 | 96.37±0.65 | 52.2±0.07 |
| | 20 | 86.033±0.03 | | 87.5±0.00 | | 96.90±0.16 | |
| | 40 | 86.8±0.00 | | 87.50±0.00 | | 97.21±0.22 | |
| | 60 | 88±0.00 | | 88±0.00 | | 97.40±0.53 | |
| | 80 | 88±0.00 | | 88.03±0.03 | | 97.73±0.53 | |
| | 100 | 89.50±0.003 | | 89.5±0.00 | | 98.0±0.21 | |
| | 150 | 90±0.00 | | 90.00±0.00 | | 98.1±0.17 | |
| | 200 | 90.20±0.006 | | 90.2±0.00 | | 98.2±0.13 | |
| **Ethanol** | 10 | 85.5±0.00 | 80.90±0.18 | 86±0.00 | 104.368 ±0.21 | | |
| | 20 | 86±0.00 | | 87.04±0.04 | | | |
| | 40 | 86.803±0.03 | | 87±0 | | | |
| | 60 | 88.04±0.04 | | 87.5±0.003 | | | |
| | 80 | 88±0.00 | | 87.5±0 | | | |
| | 100 | 89.50±0.006 | | 88.066±0.03 | | | |
| | 150 | 90±0.00 | | 88±0.00 | | | |
| | 200 | 90.5±0.00 | | 90±0.00 | | | |
| **Ethyl acetate** | 10 | 86±0.00 | 104.13±0.56 | 84.5±0.00 | 110.50 ±0.36 | | |
| | 20 | 87.04±0.04 | | 84.50±0.00 | | | |
| | 40 | 87±0.00 | | 85±0.00 | | | |
| | 60 | 87.50±0.003 | | 85.6±0.00 | | | |
| | 80 | 87.5±0.00 | | 86.04±0.04 | | | |
| | 100 | 88.033±0.03 | | 86±0.00 | | | |
| | 150 | 88.06±0.06 | | 86.5±0.00 | | | |
| | 200 | 90±0.00 | | 88.03±0.00 | | | |
| **Petroleum ether** | 10 | 84.5±0.00 | 110.55±0.30 | 84.5±0.00 | 118.73 ±0.48 | | |
| | 20 | 84.5±0.00 | | 84.50±0.003 | | | |
| | 40 | 85±0.00 | | 84.5±0.00 | | | |
| | 60 | 85.6±0.00 | | 85±0.00 | | | |
| | 80 | 86.04±0.04 | | 85±0 | | | |
| | 100 | 86±0.00 | | 85.6±0.017 | | | |
| | 150 | 86.5±0.00 | | 86.03±0.03 | | | |
| | 200 | 88±0 | | 87±0.4 | | | |
| **Aqueous** | 10 | 84.5±0.00 | 119.121±0.16 | 84±0.00 | 126.50 ±0.26 | | |
| | 20 | 84.50±0.00 | | 84±0.00 | | | |
| | 40 | 84.5±0.00 | | 84.04±0.04 | | | |
| | 60 | 85±0.00 | | 84.506±0.006 | | | |
| | 80 | 85±0.00 | | 84.5±0.00 | | | |
| | 100 | 85.606±0.003 | | 84.50±0.002 | | | |
| | 150 | 86±0.00 | | 85±0 | | | |
| | 200 | 87±0.00 | | 86±0 | | | |

Values are represented as mean ± SE of three observations

Various available reports indicate that there exists a correlation between total phenolic content and antioxidant potential but it may not always be true, since non-phenolic components can also be responsible for the antioxidant potential shown by different plant extracts (Kim *et al.,* 2003; Djeridane *et al.,* 2006; Tabart *et al.,* 2007). The highest phenolic content was obtained in the methanolic extract that could be attributed to its high polar nature and also having good capacity for solubility to phenols (Zhao *et al.,* 2006). In order to study the phenolic dependency of antioxidant potential for *N. ramosissimum*, the phenolic estimation was done by the method as discussed previously(Ainsworth, Gillespie, 2007). To estimate its dependency, different plots (linear, quadratic and exponential) were plotted. From the results obtained it can be inferred that there is a positive correlation between these two values for different extracts as depicted from the linear graphs obtained (*in vitro* shoot extract $R^2 = 0.9007$, *in vivo* shoot extract $R^2 = 0.919$, *in vitro* root extract $R^2 = 0.952$, *in vivo* root extract $R^2 = 0.932$). Their linear relationship curve gave the best fit with $R^2$ values ranging from 0.90 to 0.95. Previous reports also support our inference that there is a correlation between phenolic content and antioxidant activity. Numerous other components like reducing carbohydrates, ascorbates, carotenoids, terpenes, and tocopherols as well as the synergistic outcome amongst them could also possibly add on to the antioxidant activity (Babbar *et al*., 2011; Piluzza, Bullitta, 2011).

The effectiveness of *N. ramosissimum* extracts in DNA protection was investigated against free radicals induced by action of $H_2O_2$ which indicated that all the extracts analysed possessed significant DNA damage protective potential against free radicals. DNA bands (Figure 2) were obtained in the test samples with induced oxidation. Studies have revealed that high concentrations of extra and intra-cellular glucose lead to oxidative stress during diabetes in general. Oxidative stress coexists with a diminution in the antioxidant significance which can boost the detrimental effects of free radicals (West, 2000). Supplementation with non-toxic antioxidants could provide a chemoprotective role in diabetes (Bajaj, Khan, 2012). It has also been reported that oxidative injury could be enhanced during diabetes and can become a precursor for various cardiovascular disorders (Siti, Kamis, Kamsiah, 2015). Treatments with various antioxidants can be helpful in managing such diseases due to their free radical scavenging activity and if the antioxidant is of natural origin than it will be an added benefit due to its lesser side effects.

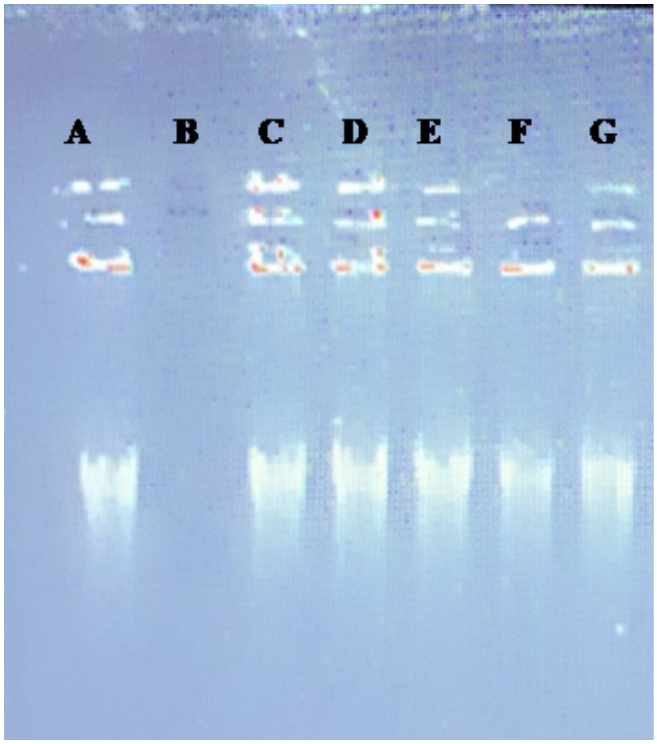


**FIGURE 2 -** DNA damage protection assay: A- DNA (human blood), B- DNA + Fenton's reagent, C- DNA + Fenton's reagent + aqueous plant extract, D- DNA + Fenton's reagent + ethanolic plant extract, E- DNA + Fenton's reagent + methanolic plant extract, F- DNA + Fenton's reagent + petroleum ether plant extract, G- DNA + Fenton's reagent + ethyl acetate plant extract.

Alpha amylase is an enzyme which plays an important role in the process of breakdown of long chain carbohydrates to glucose and is therefore involved in digestion and aids in intestinal absorption of the ingested food. It has been reported that inhibitors of alpha amylase are the key agents for the treatment of diabetes (Anagnostopoulou*et al.,* 2006). Moreover, there are reports that *N. ramosissimum* has been widely used in ancient times for the cure of diabetes, therefore, in order to confirm its antidiabetic potential the alpha amylase inhibition test was performed. The results of percentage alpha amylase inhibitory activity exhibited by the plant extracts and standard Metformin at a concentration 200

µg/mL showed a 73.59% inhibitory effect on the alpha-amylase activity with an $IC_{50}$ value of 41.92±0.08µg/ml (Figure 3). The methanolic *in vitro* shoot extract at 200 µg/mL showed a maximum alpha amylase inhibition activity (59%) as compared to other extracts (Table S IV, Figure 3). Methanolic *in vivo* shoot samples exhibited a maximum inhibition percentage at equal concentration of 200 µg/mL (57.62%) with the lowest $IC_{50}$ value of 54.42±0.16 µg/mL. All the plant extracts showed a promising alpha amylase inhibitory activity in a dose-dependent manner. Among the root extracts, ethanolic (47%) and methanolic (46%) extracts of *in vivo* samples exhibited maximum inhibition at a concentration of 200 µg/mL with $IC_{50}$ values of 96.63±0.44 µg/mL and 100.96±0.28 µg/mL respectively (Table S V, Figure 3). On comparing the $IC_{50}$ values of each extract, it was found that the most promising value that is comparable to the control (41.92±0.08 µg/mL) was obtained in the methanolic shoot extracts (*in vitro* 54.42±0.16 µg/mL; *in vivo* 66.09 ± 0.12 µg/mL) which is attributed to the presence of more flavonoids and glycosides in the herb as has been earlier reported (Amin *et al.,* 2017).

**TABLE SIV -** Alpha amylase inhibition assay of different solvent (Methanol, ethanol, ethyl acetate, petroleum ether and aqueous) extract of shoot parts of both *in vitro* and field samples of *Nanorrhinum ramosissimum*

| Extraction Solvent | Concentration | *In vivo* sample | | *In vitro* sample | | Standard | |
|---|---|---|---|---|---|---|---|
| | (µg/mL) | % inhibition | IC50 (µg/mL) | % inhibition | IC50 (µg/mL) | % inhibition | IC50 (µg/mL) |
| **Methanol** | 10 | 44±0.00 | 54.42±0.16 | 42±0 | 66.09 ±0.12 | 48±0 | 41.92 ±0.12 |
| | 20 | 50.8±0.00 | | 46.03±0.04 | | 56.04±0.04 | |
| | 40 | 51±0.00 | | 51±0 | | 66±0 | |
| | 60 | 52.04±0.04 | | 52.03±0.03 | | 67.13±0.066 | |
| | 80 | 52.80±0.003 | | 52.8±0.23 | | 68±0 | |
| | 100 | 54±0.00 | | 55.04±0.12 | | 71.5±0.30 | |
| | 150 | 57.03±0.03 | | 56.04±0.32 | | 72.4±0 | |
| | 200 | 57.62±0.02 | | 59±0.03 | | 73.59±0.0 | |
| **Ethanol** | 10 | 44±0.00 | 70.58±0.09 | 33.5±0.00 | 83.75 ±0.23 | | |
| | 20 | 46.03±0.03 | | 33.9±0.00 | | | |
| | 40 | 49±0.00 | | 36.03±0.03 | | | |
| | 60 | 49.9±0.24 | | 37±0.00 | | | |
| | 80 | 50±0.00 | | 40.04±0.04 | | | |
| | 100 | 50.5±0.01 | | 42±0.00 | | | |
| | 150 | 53.03±0.00 | | 43±0 | | | |
| | 200 | 54±0.00 | | 44±0 | | | |
| **Ethyl acetate** | 10 | 16.8±0.00 | 108.388±0.03 | 17.2±0.00 | 122.02 ±0.20 | | |
| | 20 | 17.2±0.00 | | 17.2±0.00 | | | |
| | 40 | 17.20±0.00 | | 17.20±0.00 | | | |
| | 60 | 19.2±0.00 | | 19.2±0.00 | | | |
| | 80 | 19.6±0.00 | | 19.6±0.03 | | | |
| | 100 | 20±0.00 | | 20.03±0.03 | | | |
| | 150 | 20±0.00 | | 20±0.00 | | | |
| | 200 | 24±0.00 | | 24±0.00 | | | |

**TABLE SIV -** Alpha amylase inhibition assay of different solvent (Methanol, ethanol, ethyl acetate, petroleum ether and aqueous) extract of shoot parts of both *in vitro* and field samples of *Nanorrhinum ramosissimum*

| Extraction Solvent | Concentration | *In vivo* sample | | *In vitro* sample | | Standard | |
|---|---|---|---|---|---|---|---|
| | (µg/mL) | % inhibition | IC50 (µg/mL) | % inhibition | IC50 (µg/mL) | % inhibition | IC50 (µg/mL) |
| Petroleum ether | 10 | 33.5±0.00 | 90.14±0.12 | 23±0.00 | 96.122 ±0.04 | | |
| | 20 | 34±0.00 | | 23.04±0.04 | | | |
| | 40 | 36.04±0.04 | | 25.6±0.00 | | | |
| | 60 | 36.8±0.00 | | 27±0.00 | | | |
| | 80 | 39.6±0.00 | | 27.5±0.00 | | | |
| | 100 | 40.03±0.03 | | 28±0.00 | | | |
| | 150 | 40.11±0.01 | | 29±0.00 | | | |
| | 200 | 43±0.00 | | 32±0.00 | | | |
| Aqueous | 10 | 20.4±0.00 | 89.4681±0.01 | 21.6±0.00 | 123.60 ±0.010 | | |
| | 20 | 21.6±0.0 | | 22±0.00 | | | |
| | 40 | 22±0 | | 22±0.0.00 | | | |
| | 60 | 22.13±0.02 | | 22±0.00 | | | |
| | 80 | 23±0 | | 23±0.00 | | | |
| | 100 | 23.30±0.003 | | 23.30±0.00 | | | |
| | 150 | 25.6±0.00 | | 25.603±0.00 | | | |
| | 200 | 26±0.00 | | 28±0.00 | | | |

Values are represented as mean ± SE of three observations

**TABLE SV -** Alpha amylase inhibition assay of different solvent (Methanol, ethanol, ethyl acetate, petroleum ether and aqueous) extract of root parts of both *in vitro* and field samples of *Nanorrhinum ramosissimum*

| Extraction Solvent | Concentration | *In vivo* sample | | *In vitro* sample | | Standard | |
|---|---|---|---|---|---|---|---|
| | (µg/mL) | % inhibition | IC50 (µg/mL) | % inhibition | IC50 (µg/mL) | % inhibition | IC50 (µg/mL) |
| Methanol | 10 | 38.03±0.03 | 100.96±0.28 | 38.07±0.03 | 102.53 ±0.49 | 48±0 | 41.92 ±0.12 |
| | 20 | 39.04±0.04 | | 38.03±0.03 | | 56.04±0.04 | |
| | 40 | 39.6±0 | | 39±0.00 | | 66±0 | |
| | 60 | 40.06±0.03 | | 39.60±0.00 | | 67.13±0.066 | |
| | 80 | 40.10±0.00 | | 40.06±0.03 | | 68±0 | |
| | 100 | 43±0 | | 42.03±0.03 | | 71.5±0.30 | |
| | 150 | 44.06±0.03 | | 44.03±0.03 | | 72.4±0 | |
| | 200 | 46±0 | | 45±0.00 | | 73.59±0.00 | |

**TABLE SV -** Alpha amylase inhibition assay of different solvent (Methanol, ethanol, ethyl acetate, petroleum ether and aqueous) extract of root parts of both *in vitro* and field samples of *Nanorrhinum ramosissimum*

| Extraction Solvent | Concentration | *In vivo* sample | | *In vitro* sample | | Standard | |
|---|---|---|---|---|---|---|---|
| | (µg/mL) | % inhibition | IC50 (µg/mL) | % inhibition | IC50 (µg/mL) | % inhibition | IC50 (µg/mL) |
| **Ethanol** | 10 | 39.033±0.03 | 96.63±0.44 | 35.03±0.03 | 88.97 ±0.32 | | |
| | 20 | 39.033±0.03 | | 36±0.00 | | | |
| | 40 | 40.06±0.03 | | 38±0.00 | | | |
| | 60 | 42±0.00 | | 39.033±0.06 | | | |
| | 80 | 43.03±0.03 | | 41±0.00 | | | |
| | 100 | 44.04±0.04 | | 42.07±0.03 | | | |
| | 150 | 45.03±0.03 | | 44.03±0.03 | | | |
| | 200 | 47±0.00 | | 46±0.00 | | | |
| **Ethyl acetate** | 10 | 16±0.00 | 110.89±0.46 | 17.2±0.00 | 106.74 ±0.14 | | |
| | 20 | 16.803±0.003 | | 18±0.00 | | | |
| | 40 | 17.603±0.003 | | 19.17±0.02 | | | |
| | 60 | 17.6±0.00 | | 19.6±0.00 | | | |
| | 80 | 18±0.00 | | 20±0.00 | | | |
| | 100 | 19.603±0.003 | | 20±0.00 | | | |
| | 150 | 20±0.00 | | 21±0.00 | | | |
| | 200 | 23±0.00 | | 24±0.00 | | | |
| **Petroleum ether** | 10 | 30±0.00 | 119.23±0.009 | 27±0.00 | 113.15 ±0.05 | | |
| | 20 | 30±0.00 | | 27±0.00 | | | |
| | 40 | 31±0.00 | | 28±0.00 | | | |
| | 60 | 31±0.00 | | 28±0.00 | | | |
| | 80 | 31.50±0.00 | | 29.03±0.03 | | | |
| | 100 | 33±0.00 | | 31±0.00 | | | |
| | 150 | 34±0.00 | | 35±0.00 | | | |
| | 200 | 37±0.00 | | 37±0.00 | | | |
| **Aqueous** | 10 | 17.2±0.00 | 112.12±0.03 | 17.2±0.00 | 120.46 ±0.07 | | |
| | 20 | 17.20±0.00 | | 17.20±0.00 | | | |
| | 40 | 18±0.00 | | 18±0.00 | | | |
| | 60 | 19.2±0.00 | | 19.2±0.00 | | | |
| | 80 | 19.60±0.003 | | 19.60±0.00 | | | |
| | 100 | 20±0.00 | | 20±0.00 | | | |
| | 150 | 20±0.00 | | 20±0.00 | | | |
| | 200 | 23±0.00 | | 24±0.00 | | | |

Values are represented as mean ± SE of three observations

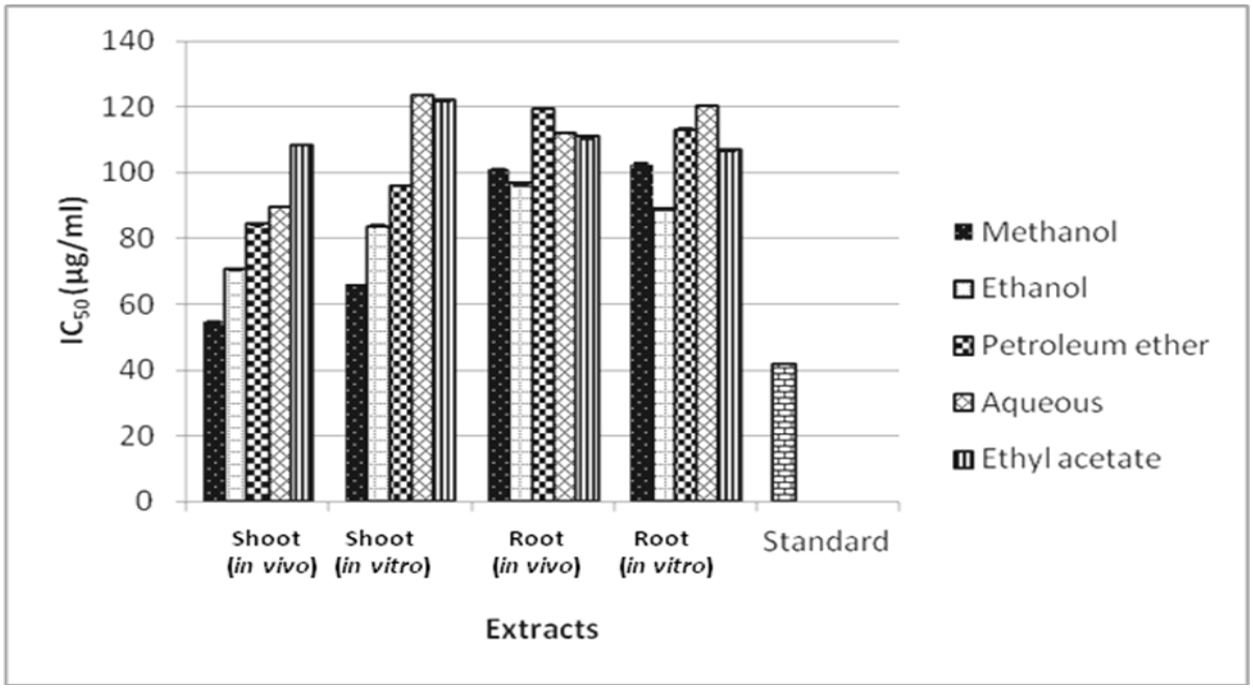


**FIGURE 3 -** Alpha amylase inhibition assay of different extracts (Methanol, ethanol, ethyl acetate petroleum ether, aqueous) of shoot and root parts of *in vivo* and *in vitro* plant samples. The data represents mean of three experiments ±SE.

## CONCLUSION

The results of the study indicated the potential of various extracts obtained from polar and non polar solvents of *Nanorrhinum ramosissimum* for their substantial antimicrobial, antioxidant, DNA damage protection and α-amylase inhibitory activities. The results obtained signify that in general the methanolic extracts emerged as the best solvent followed by ethanolic extracts of *N. ramosissimum*. Shoot extracts exhibited more antimicrobial potential against the tested strains in comparison to root extracts. Highest phenolic concentration as well as antioxidant potential was obtained in the methanolic shoot extract and on correlating the results obtained, it can be concluded that phenols do have a role in the antioxidant attribute of the herb. Antioxidant activity was also confirmed from the DNA damage assay as plant extracts were potentially able to protect the DNA damage. Antidiabetic potential of the herb has also been demonstrated and the best results were obtained in methanolic shoot extracts of the herb. Significant differences were not observed between the extracts of *in vivo* and *in vitro* regenerants indicating the possibility of large scale cultivation of herb under controlled conditions for its commercial usage. The result of the present study supports the traditional ayurvedic conception that the herb has a substantial potential in management of various human diseases.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest

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