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Research Article

GEOGRAPHICAL VARIATION OF ANTIOXIDANT CONSTITUENT IN GARHWAL REGION OF UTTARAKHAND: CHINESE BRAKE FERN (PTERIS VITTATA)

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ABSTRACT

Numerous kind of antioxidants or protecting agent are present in the living body like Glutathione is the master antioxidant produce by the liver and uses free radicals to purify the body. Natural plant consuming antioxidants content is growing the interest for scientific research as well as industrial purposes. Pteridophytes (fern and fern allies) have drawn attention of plants seekers and horticultures since ancient period. Plant of Pteris vittata were collected from different geographical region of Uttarakhand Garhwal. The maximum yield of plant extract was found via the ethanol solvent i.e. 5.07-9.67%. DPV extract exhibited a maximum inhibition of 89.32 relatively closed to 91.96 % inhibition of Ascorbic acid at the concentration of 0.1mg/ml by DPPH radical scavenging assay method. The IC_{50} of the DPV extract and Ascorbic acid was found to be 0.543 and 0.495 mg/ml by same. DPV extract showed a maximum inhibition of 72.33 % relatively close to77.42 % inhibition of BHA at the concentration of 0.8mg/ml by Hydrogen peroxide radical scavenging method. The IC_{50} value of the DPV extract and BHA was found to be 0.279±0.005mg/ml and 0.257±0.002 mg/ml by same. DPV extract showed a maximum inhibition of 84.32% relatively close to 87.96 % inhibition of ascorbic acid at the concentration of 0.8mg/ml by Nitrogen oxide scavenging method. The IC_{50} value of the DPV extract and BHA was found to be 0.233±0.002 mg/ml to 0.218±0.006 mg/ml by same.

KEYWORDS: *Chinese fern, Antioxidant, Scavenging, Phenolic compound, Inhibition.*

INTRODUCTION

Natural antioxidants are required either prevent or cure the disorders caused by free radicals. As folk medicine, the Pteridophytes which constitute fern and ferns allies, have been known to man for more than 2000 years, and also been mentioned in ancient literature [1]. Pteridophytes are original vascular cryptogams, which flourish well in worldly environment. In the world flora of pteridophytes 12,000 species has been identified among which 1,000 species into 70 families and 191 genera are occur in India. *Pteris vittata* was also entitled as the Chinese brake, Chinese ladder brake, or simply ladder brake [2, 3].

Pteridophytes are predictable for the rich diversity of valuable antioxidants. Various works was accomplished towards the medicinal importance of pteridophytes by the researchers. Antioxidants are the chemical which are derived from the plant sources, generally affect health, but are not yet established nutrients. Phenolic rich content plant material simultaneously increasing the interest of market as antioxidant used in the food industry. These natural antioxidants improve the quality and nutritional value of food. Flavonoid related derivatives have remarkable antibacterial, antiviral, anti-inflammatory, anticancer and anti-allergic activities due to its scavenging activity [4].

The investigation was designed to explore the variation of the antioxidant content of whole plant of Chinese Brake, in the different region of Uttarakhand.

MATERIAL AND METHOD

Plant Material:

The investigation was conducted out in the month of August-September, 2018 at Uttaranchal Institute of Pharmaceutical Sciences, Premnagar, Dehradun, Uttarakhand. The fresh whole plant of *P. vittata* was collected from Chamoli, Dehradun, Pauri, Tehri and Uttarkashi. The plant is identified and authenticated in Forest Research Institute, Dehradun, Uttarakhand. The voucher number is 73212(a-e).The specimen is deposited in UIPS, Dehradun. The plant materials were sundried for 15-20 days in the laboratory and transformed into powder.

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Preparation of plant extract:

Air dried plant powder was softened in petroleum ether and stored in tightly closed container. It was rotated on a rotary shaker at 190-220 rpm for one day and the clear filtrate was discarded. Petroleum ether was fully evaporated and collects as powder. Further the powder was extracted out by the different solvent acetone, benzene, ethanol, ethyl acetate and water respectively. By the centrifugation techniques, the resulting extract was obtained as dry residues [5]. All chemical and solvent used during the experiments were analytical grade. The plant extract were entitled consequently as their localities *i.e* Chamoli *P.vittata* (CPV), Dehradun *P.vittata* (DPV), Pauri *P.*vittata (PPV), Tehri *P.vittata* (TPV) and Uttarkashi *P.vittata* (UPV).

Qualitative phytochemical analysis:

Highest yield of extract was extracted in the ethanol solvent relatively to others. Therefore ethanol extract was subjected to preliminary qualitative phytochemical investigation for alkaloids, proteins, carbohydrates, flavonoids, cardiac glycosides, saponins, catechins, sugars steroids and triterpenoids, tannins and phenols etc [6].

Quantitative phytochemical analysis:

The phytochemicals constituent was enumerated by the standard procedures.

Determination of total alkaloids (TAS):

1gm of extract was liquefied in 40 ml of 10% acetic acid in ethanol and strained. It was concentrated and add conc. ammonium hydroxide drop wise for the precipitation. The precipitated material was further liquefied with dilute ammonium hydroxide and again concentrated. The resulting residue was alkaloid [5].

Determination of total saponin content (TSC):

2 g of extract liquefied in 20% of aqueous ethanol. The samples was heated for 90 minute and strained out. This procedure revised atleast three times. The resulting solution dissolved with ether, shaken vigorously. The saponin content was recovered by aqueous layer carefully through the mixture of n-butanol and 5% sodium chloride solution. The saponin content was weighed [7].

Estimation of total phenolic content (TPC):

The total phenolic content was estimated by standard procedures with slight modification [8]. 2.5mg of plant sample was dissolved in 10 ml of ethanol and sonicated. There was incubated the solution of plant sample, methanol, and distilled water and Folin-Ciocalteu reagent for 5minute. Add the 10 % sodium carbonate solution and covered through aluminum foil. It was again incubated for 20 minute. The absorbance of the sample was determined using a UV visible spectrophotometer at 765 nm. Gallic acid is used as standard phenolic compound. 100 µg/mL solution of gallic acid was prepared by dissolving 10 mg of Gallic acid monohydrate in 100 mL of ethanol. By diluting the stock solution, obtained different concentration ranging from 10- 80µg/ml. Total phenolic content was stated as µg of gallic acid equivalents (GAE) per ml. A standard calibration curve was drawn by plotting absorbance against concentration. It was found to be linear over this concentration range.

Estimation of total flavonoid content (TFC):

Aluminium chloride technique was slightly modified for the flavonoid estimation [9, 10].

Prepared the mixture of sample solution, methanol, 10% aluminium chloride, 1 M potassium acetate solution and distilled water in test tube and incubated for 30 minutes. The reaction mixture was treated with 1M sodium hydroxide solution. Rutin is used as standard flavonoid compound. 10 mg of rutin dissolved in 10 mL of ethanol to get 100µg/mL. By diluting the stock solution, obtained different concentration ranging from 10- 80µg/ml. The total flavonoid content was expressed in microgram of rutin equivalents (RE) per gram of samples. The absorbance was measured at 415 nm against reagent blank. The calibration curve was drawn by plotting absorbance against concentration. It was found to be linear over this concentration range.

DPPH Radical Scavenging Activity:

The DPPH radical scavenging activity was performed by standard procedures with slight modifications [11, 12].

DPPH (1, 1-Diphenyl –2-picrylhydrazyl) is a kind of stable free radical. Stock solution was prepared the concentration of 0.43mg/ml. Ascorbic acid was used as standard. Prepared the solution of DPPH with sample and control separately. The control contained 0.1ml ethanol in place of the plant sample. The absorbance was measured at 517 nm by UV spectrophotometer [8].

Antioxidant activity was expressed as percentage

$$\% \text{ Antioxidant activity} = [(Ac - As) / Ac] \times 100$$

Ac and As are the absorbance of control and sample, respectively. The IC_{50} was calculated for sample and control by "Figure 3".

Hydrogen peroxide scavenging activity:

The hydrogen peroxide scavenging assay was carried out by standard procedure with minor modification [13].

Hydrogen peroxide is an example of oxidizing agent. H_2O_2 (6%v/v) was prepared in phosphate buffer (0.1 M, pH 7.4). Butylated hydroxyl anisole (BHA) was used as standard. The different concentration was prepared in the phosphate buffer and hydrogen peroxide solution. The absorbance was recorded at 230 nm by UV spectrophotometer. Scavenging activity was expressed as percentage inhibition

$$H_2O_2 \text{ scavenging activity } (\%) = (A0 - A1) / A0 \times 100$$

A0 is the absorbance of the control, and A1 is the absorbance of the sample. The IC_{50} was calculated for sample and control by "Figure 4".

Nitric oxide scavenging activity:

The NO scavenging assay was carried out by the use of Griess Illovay reaction [14].

Griess reagent was mixture of 0.1% sulfanilamide, 0.2% phosphoric acid and 0.01% N-(1-naphthyl) ethylene diamine dihydrochloride). Aqueous solution of sodium nitroprusside generates nitrogen oxide (NO) at pH=7 and interact with oxygen molecule to produce stable products (nitrates and nitrite). Sodium nitroprusside in phosphate buffered saline was mixed with different concentration of sample, then dissolved in methanol and incubated at 40° C for 90 minutes. Control was prepared without sample. After the incubation period, add 0.1ml of griess reagent. Ascorbic acid

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was used as standard. The absorbance was calculated at 540 nm.

Scavenging activity was expressed as percentage inhibition

NO scavenging activity (%) = (A0 – A1) /A0 ×100

A0 is the absorbance of the control, and A1 is the absorbance of the sample.

Statistical Analysis:

In this study, antioxidant activity of different concentrations of plant extracts was expressed in mean and standard error of mean. Probability value (P) of less than 0.05 was considered statically significant.

All the determinations were conducted at least three times (n = 3); the statistical mean was calculated with ± SD using Excel 2010.

RESULT

Yield and phytochemical estimation of crude extracts:

Ethanolic extracts of *P.vittata* was appeared as green to dark green. Phytoconstituent was found to be in range of 7.37-.9.67%. Plant extract was exposed the different qualitative test and revealed positive for certain phytochemical tests. Alkaloids, phenolic compounds, flavonoids, saponins and tannins were the secondary metabolites, present in the extract. These compounds play significant role of antioxidant activity of natural resources. Variation in the amount of these constituents directly influences the antioxidant activity of compound [15].

Total alkaloid content and total saponin content:

Alkaloid content of CPV, DPV, PPV, TPV and UPV was found to be in the range of 0.819±0.053, 0.152±0.40, 0.919±0.906, 0.115 ±0.3 and 0.831±0.628 µg /g respectively. Saponins content of CPV, DPV, PPV, TPV and UPV was found to be in the range of 0.595 ±0.71, 0.119±0.185, 0.737±0.503, 0.817±0.125 and 0.541±0.528 µg /g respectively [16].

Total phenol content (TPC):

TPC was calculated from the calibration graph of gallic acid verses sample. Multiple reading was recorded for each sample. The TPC was revealed to be in the huge variation range of 0.411±0.327 to 1.653±0.479 mg GAE/g "Figure 1". Linear regression analysis(y=0.0163x+ 0.0083, R^2=0.0997) was applied to calculate TPC in which y is absorbance at 765nm and x is the amount of gallic acid equivalent (g/ml) per 20gm extract [17, 18].

Total flavonoids content (TFC):

TFC was calculated from the calibration graph of rutin verses sample. Multiple reading was recorded for each sample. The TFC was revealed to be in the huge variation range of 0.346±0.89 to 0.714±0.006 µg RE/ g "Figure 2". Linear regression analysis equation (y=0.015x+ 0.026, R^2=0.9994), was applied to calculate TFC in which y is absorbance at 415nm and x is the amount of rutin equivalent (RE) per 20g extract [19].

DPPH radical scavenging activity:

Ascorbic acid and antioxidant content present in the extract directly react with DPPH radical and produced a yellow diphenyl- β-picryl hydrazine complex. Deviation of discoloration directly proportional to phenolic and flavonoid content "Figure 3" [20, 21].

Hydrogen peroxide scavenging activity:

Hydrogen peroxide is oxygen containing species involved in the certain cellular activities like phagocytosis, cell growth and synthesis of biological compound. Hydrogen peroxide can cross cell membranes rapidly. Hydrogen peroxide scavenging activities of the extract were present in "Figure 4" [13, 22].

Nitric oxide scavenging activity:

Nitric oxide is potent chemical mediators which regulate the certain activities like smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity etc. It is a diffusible free radical. The scavenging of nitrogen oxide is present in "Figure 5" [14, 22].

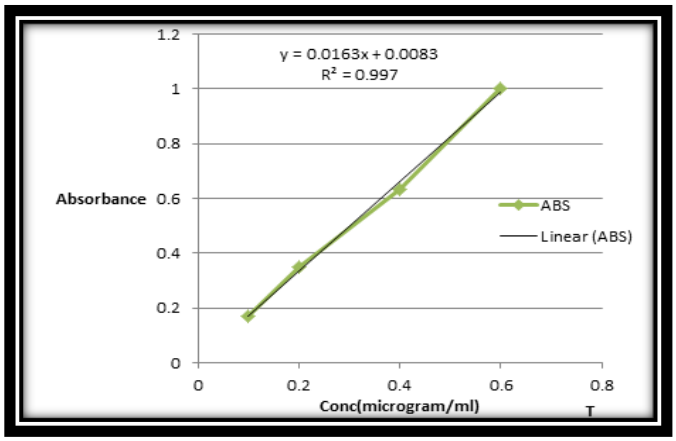

Fig. 1: Standard curve between concentration of Gallic acid (µg/ml) and absorbance

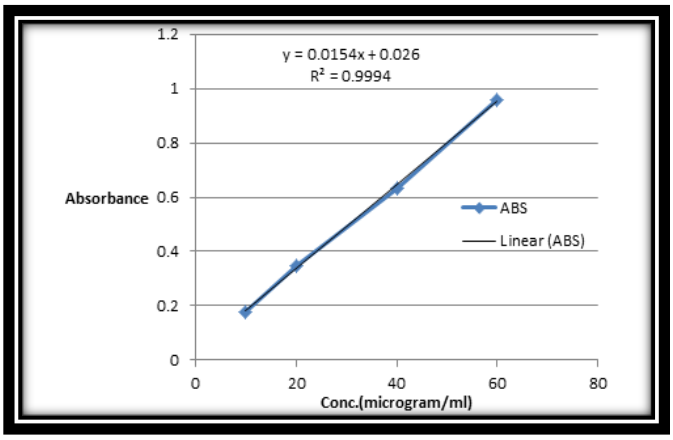

Fig. 2: Standard curve between concentration of Rutin (µg/ml) and absorbance

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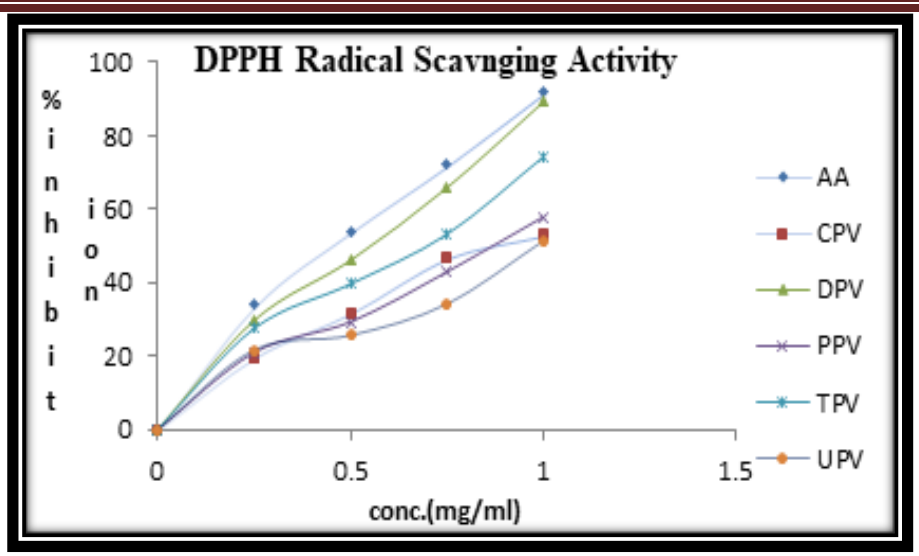

Fig. 3: DPPH radical scavenging (%) inhibition versus concentration (mg/mL) of standard and extracts

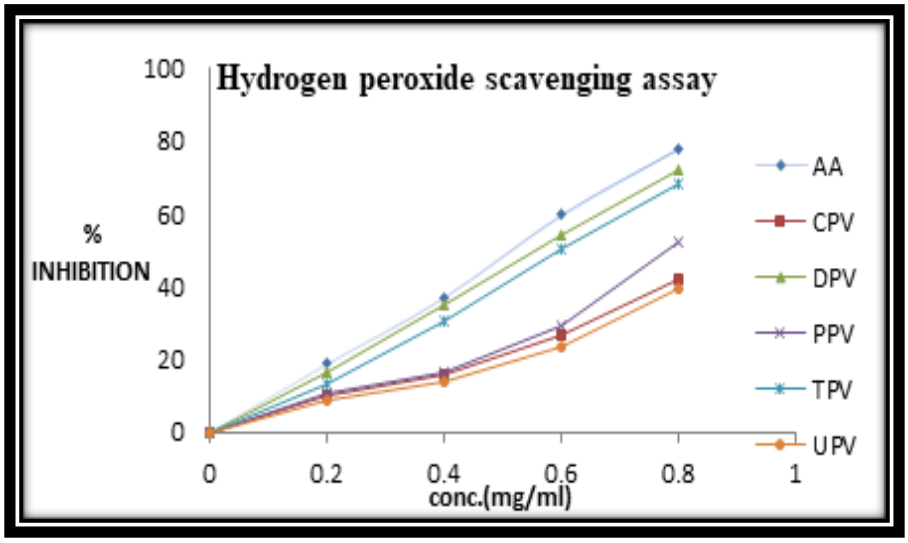

Fig. 4: Hydrogen peroxide scavenging (%) inhibition versus concentration (mg/mL)

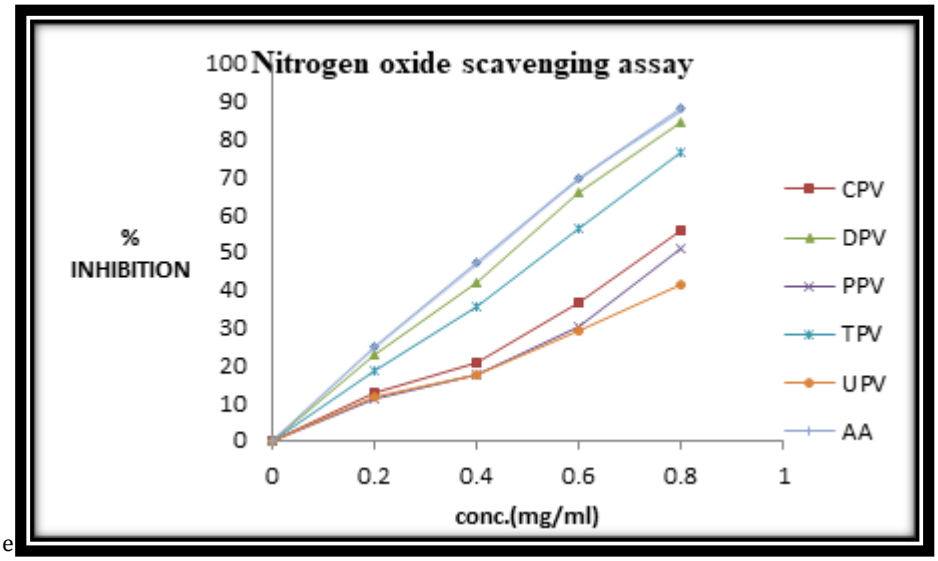

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Fig. 5: Nitric oxide scavenging (%) inhibition versus concentration (mg/ml)

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DISCUSSION

The term phenolic and flavonoid compound represent a wide range of plant substances tend to be water-soluble. Phenolic compounds are all aromatic, so that they all show sharp absorption in the UV region of the spectrum. In addition, phenolic compounds characteristically exhibit bathochromic shifts in their spectra in the presence of alkali. Antioxidant quality is a measure of the effectiveness of the antioxidant(s) present as a pure compound or a mixture [15, 18, 19, 23-26].

The finely divided powder of the plant of the fern *P. vittata* was exposed to cold percolation extraction using a Soxhlet apparatus. As a result, acetone, benzene, ethanol, ethyl acetate and aqueous extracts were obtained. Only ethanol extracts were examined further for antioxidant activity. A number of assays were conducted to analyze antioxidant activities of the extracts.

Total alkaloid content and total saponin content:

Among all of extract, DPV and TPV revealed the maximum content of alkaloid and saponins. Alkaloids are the widely available phytochemicals responsible for the anticancer and antimicrobial activities. Saponins are applicable as a suitable option for phytochemicals to defend plant against several pathogens.

Total phenol content (TPC):

DPV extract was found the highest amount of TPC i.e. 1.605317±0.4794 mg GAE / g. Hydroxyl group of phenol play important role in the scavenging activity of plant.

Total flavonoids content (TFC):

DPV extract was found the highest amount of TFC i.e. 0.7143±0.0064 µg RE/ g. Like phenolic, flavonoids have positive effect on human health [18, 19, 24].

DPPH radical scavenging activity:

DPV extract was showed a maximum inhibition of 89.32% relatively close to of 91.96 % inhibition of ascorbic acid at the concentration of 0.1mg/ml. IC_{50} of the DPV extract and ascorbic acid was found to be 0.543±0.002mg/ml and 0.495±0.0005 mg/ml. Percentage inhibition of other was found to be in range of 51.44 to 74.33%. IC_{50} of other was found to be in the range of 0.871±0.001 to 1.006±0.007 mg/ml [20, 21, 25-27].

Hydrogen peroxide scavenging activity:

DPV extract showed a maximum inhibition of 72.33 % relatively close to77.42 % inhibition of BHA at the concentration of 0.8mg/ml. The IC_{50} value of the DPV extract and BHA was found to be 0.279±0.005mg/ml and 0.257±0.002 mg/ml. Percentage inhibitions of others were found to be in the range of 39.44 to 68.33%. IC_{50} of others were found to be in range of 0.301 ±0.001-0.553±0.001mg/ml [13, 25-27].

Nitric oxide scavenging activity:

DPV extract showed a maximum inhibition of 84.32% relatively close to 87.96 % inhibition of ascorbic acid at the concentration of 0.8mg/ml. The IC_{50} value of the DPV extract and BHA was found to be in the range of 0.233±0.002 mg/ml to 0.218±0.006 mg/ml. Percentage inhibitions of others were found to be in the range of 41.44%-76.33%. IC_{50} of others were found to be in the range of 0.259±0.0052 to 0.499±0.001 mg/ml [22, 25, 26].

CONCLUSION

Uttarakhand is rich source of natural sources. Alkaloid, saponins, phenolic and flavonoid constituent are possessing efficient antioxidant activity in natural resources [23, 26-30].

It is unfortunate that these forms were discounted group of plants in biodiversity, in spite of their economic value is familiar in worldwide. We summarized the outcomes that antioxidant activity of chinese brake fern was strongly revealed by ethanol extract of Dehradun and Tehri district. It is very much supportive in the era of new drug investigation for the cancer and tumors disease. Therefore cultivation should be supported as a medicinal plant for the development of health care products for aging and chronic disease [27-30]. The plant extract was obtained from different graphical region showed the strong presence of natural antioxidant. It is concluded that Dehradun and Tehri District possessing more favorable condition for the cultivation of Chinese bracken fern. This work will provide a foundation for further isolation of natural antioxidant constituent especially Dehradun and Tehri district of Uttarakhand.

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Authors Contribution statement:

Dr.Nardev Singh and Kiran Dobhal perceived of the presented idea. Kiran Dobhal developed the theory and performed the computations. Dr. Arvind Semwal verified and encouraged the analytical methods. All authors discussed the results and contributed to the final manuscript. Kiran Dobhal carried out the experiment and wrote the manuscript with support of other authors.

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